Radioisotopes and Radiation in Entomology

Proceedings of a Symposium,
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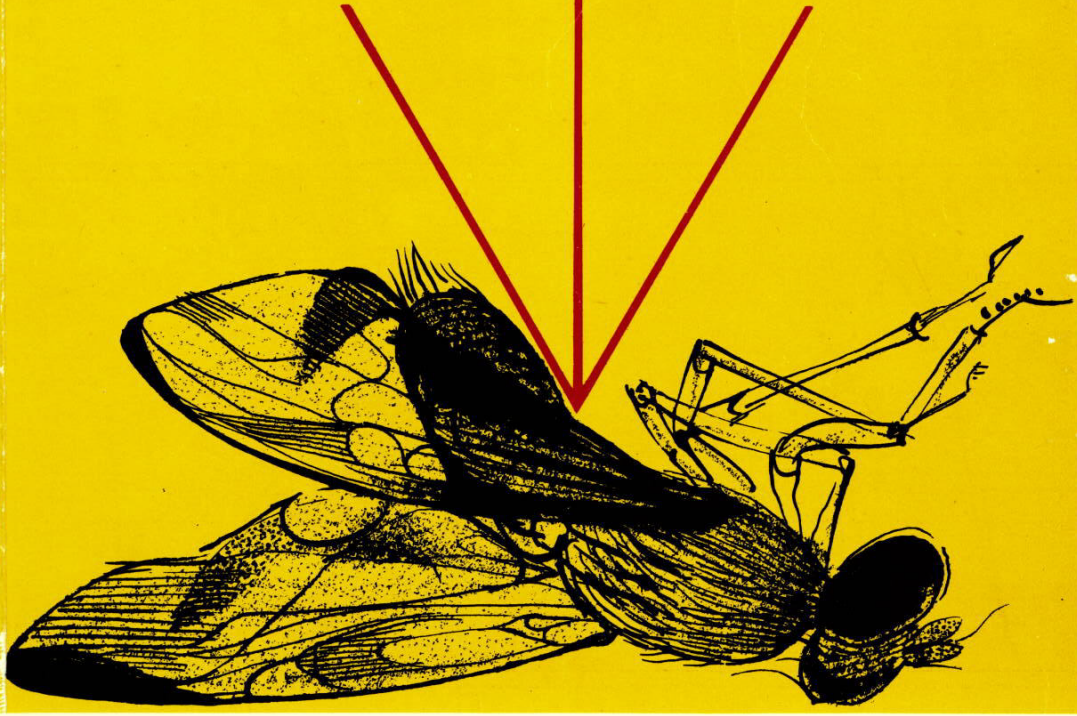

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RADIOISOTOPES AND RADIATION IN ENTOMOLOGY

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FOREWORD

One of the most serious problems affecting the world's food supply is the enormous direct and indirect damage done by insects and other pests to agricultural crops and livestock. Each year the losses to agriculture caused by insects alone amount to thousands of millions of dollars; but in many regions of the world the true losses must be measured, not in money, but in terms of the misery, disease and loss of productivity that a serious food shortage entails. These losses can be diminished only by controlling or eliminating insects and other pests.

For some considerable time man has been fighting constantly against the innumerable insects which attack crops, livestock and health. Millions of tons of chemical poisons are used in this battle each year. But the use of these weapons has introduced new problems. Insects have, for instance, shown a capacity for developing resistance to these toxic substances so that new insecticides constantly have to be developed. Furthermore, the chemicals used are often toxic to animals and even to man himself. Long-lasting insecticide residues on consumable crops therefore present a potential hazard to public health.

Although radioisotopes are not a universal panacea, they play a unique role in opening several new avenues for research and an approach to new control methods. Research with radioisotopes improves understanding of the physiology and behaviour of insects and of their biochemical processes. This knowledge is essential for the development of better control techniques and more effective insecticides.

In radiation man has found a new and additional weapon for eliminating insect populations. The direct killing effect of radiation can, for instance, be used in the disinfestation of stored products. Another effect of radiation is the induction of sterility or lethal mutations which has already proved to be of extreme practical value, especially when used on insect populations already reduced to small numbers by insecticides.

The Proceedings now published give the record of the Symposium on Radioisotopes and Radiation in Entomology, held in Bombay at the invitation of the Indian Government. It was the first meeting organized by the IAEA in Asia. It discussed the above-mentioned problems and showed that radioisotopes and radiation are tools of real proved value of even greater potential than had previously been realized. It is hoped that the proceedings will be a valuable source of information to agricultural scientists and authorities.

The Agency's sincere thanks are due to the Indian Government, who so generously provided facilities and thus greatly assisted the Agency in the organization and conduct of the Symposium.

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OPENING ADDRESS

delivered by

Dr. H. J. BHABHA, Chairman, Indian Atomic Energy Commission, Bombay

Mr. Chairman, Chief Minister, Ladies and Gentlemen,

It is my very pleasant duty today to welcome on behalf of the Government of India the representatives of the International Atomic Energy Agency, which in co-operation with the Indian Atomic Energy Commission has organized this Symposium, and the many distinguished scientists who have come from many countries to participate in it. It seems to me particularly fitting that the Chief Minister of Maharashtra should be with us today to inaugurate this Symposium, since Bombay is today the largest scientific centre in the country. There are institutions in this city which have made important contributions to science since the beginning of this century, historic contributions in the field of medical research and the prevention of disease, there is an institute which is the national centre for fundamental research and one of our largest national laboratories, and then there is the Atomic Energy Establishment at Trombay with which we are particularly concerned, and which is by far the largest scientific centre in the country, being comparable in the number of its scientific workers and the scale of its operations to practically all the other national laboratories put together.

The theme of this Symposium is radioisotopes and radiation in entomology. In a historic sense, the radioisotope technique ushered in a new era in our scientific thinking. It is new in terms of historical development. I believe I am right in saying that in entomology this unique tool found its first application in 1931 when lead-212 arsenite was used to investigate the permeability of the gut wall of the silk worm. During the three decades since that pioneering effort a multiplicity of problems not only in entomology but in almost every field of human endeavour has been scanned by this incredibly versatile technique. In their scope and applicability they encompass a wide spectrum of conditions and situations relating to insects. As a result of such applications we have been able to obtain an insight into the bioecology of insects, modes of dispersal, migration and transmission of diseases, physiology and biochemistry, problems of biosynthesis, mode of action of insecticides and pesticides, toxicological properties of insecticides, and questions concerning the entomological control as well as radiogenetical and radiobiological phenomena of insects.

One of the tasks of the Indian Atomic Energy Commission is to make these tools available to scientific workers in the universities and scientific institutions all over the country. The tools are radioisotopes, radiation sources, instrumentation for measuring radiation and tests on radioisotopes, especially electronic instrumentation. May I, therefore, take a few minutes of your time to indicate what we have been doing in this direction. Perhaps a short time-table of events may be of interest in this connection.

In August 1956 our first reactor, Apsara, went into operation. In April 1957 we set up the Isotope Division at Trombay with an initial staff of only three people. In February 1958 the first samples of phosphorus-32 and sulphur-35 were delivered, processed, as in so many other cases, in temporary laboratory space. In May 1958, the Isotope Laboratory, a temporary one, was ready for occupation. In February 1959, the first sample of iodine-131 for medical use was delivered. In January 1960, the first samples of gold-198 for medical use were delivered, and regular production of phosphorus-32, sulphur-35, iodine-131, gold-198, iron-59, chromium-51 and sodium-24 was begun in quantities sufficient to meet the demands

in this country. In July this year, the CIR, a reactor of 40 MW, went into operation, and in November we produced our first samples of labelled compounds. The present total strength of the Isotope Division is about 70 people, and laboratories are in the process of design which will allow all isotopes necessary for various purposes to be produced.

It is also of interest to see the extent to which the use of these materials has increased in this country. For example, only 9 samples of phosphorus-32 totalling 9 mc were used in 1958, 20 samples totalling 91 mc in 1959, and this year 83 samples have already been used totalling 530 mc. These operations are, of course, very small compared with what takes place in many other countries, but what I think is of interest is the rate of growth. Similarly, in the case of sulphur-35, 2 mc only were used in 1958 compared with some 91 mc this year. Iodine-131 went up from 50 mc last year to 758 mc this year, while gold-198 rose from 10 mc last year to 1260 mc. It is interesting to note that, as a result of the production that has been undertaken here, the total import of radioisotopes has decreased, though the total use has increased very rapidly. This is because we have been able more and more to supply all the needs of this country even before any isotopes have been produced in the CIR. When the CIR goes into full operation in a few months time, we shall have one of the largest isotope producers in the world, about which I shall say a few words presently.

In the case of labelled compounds, we have just made a beginning and therefore, while the use of Indian-produced labelled compounds is increasing, imports are also increasing in a marked way, showing the growing use of labelled compounds. It is also of some interest to see how these various radioisotopes were used in India. Of the samples sent out, 176 were used for research, 41 for medical applications, 12 in education and only 4 in industry. This clearly shows that Indian industry has not yet realized the importance of radioisotopes for itself. I said some time earlier that in the CIR we will have an important radioisotope producer. In the CIR, the outer irradiation rods will give us an irradiation index, that is to say, a neutron flux times volume, of about 30 compared with 1.5 which we have in our first reactor Apsara, and compared with 24 which BEPO provides in the United Kingdom. Therefore, already we will have a very considerable amount of isotope-producing ability. What is also important for certain types of isotope is the intensity of flux, and here the maximum neutron-flux in units of $10^{11}/cm^2$ s is 390 in the CIR, compared with only 12 in BEPO at Harwell and 23 in Apsara. Radiocobalt is, as you know, an important isotope, and the CIR in full operation can produce about 20000 c/yr of high-activity radiocobalt, i.e. with an activity of 30 c/g, and of low-activity cobalt we shall be able to produce as much as 500000 c with an activity of 3 c/g. Therefore, assuming that a medical source requires 1000 c of high-intensity radiocobalt, we could produce some 20 sources p.a. for medical use in the country and, for industrial purposes, something like 100000 sources p. a. It is, of course, our intention to provide radiocobalt and other radioisotopes to various institutions in India for many different purposes including research in agriculture and, naturally, on subjects connected with this Symposium. It may interest you to know that we are also building a facility at Trombay in the used-fuel-elements storage-bay, which will allow us to irradiate samples with the used fuel elements from the reactor, and we propose to undertake research into the effects of radiation on the preservation of food and fruits, among other subjects. There are also radiocobalt irradiation facilities being constructed.

The third tool I mentioned is electronic instrumentation, and in this we supply practically all that is used by our own workers. We will soon be in a position to supply all the expanding needs of this country for electronic instrumentation for biological investigations.

Perhaps I may be permitted to say a few words about some of the work being done at Trombay on the use of radioisotopes in entomological problems about which you will, no

doubt, hear in greater detail. Though conventional methods of disinfestation are to some extent adequate in controlling some of the insect pests in stored grains, certain species of insects like the lesser grain borer, the Khapra beetle and the fig moth have been observed to cause untold damage to stored grains in spite of fumigation. In the case of the fig moth, *Ephestia cautella*, the larva is the pest, and therefore the sterile-male technique should be an effective method of controlling it inside warehouses and storage silos. The other two species mentioned here could be eradicated by exposing the grains to radiation. Besides the irradiation facilities under construction, which I have just mentioned, mobile units are also under consideration, and could be used at the sites of grain entry into the country and wherever large warehouses are situated. Spices form an important export commodity, and disinfestation of spices by conventional methods has not been found to be very effective for various reasons. Studies on the effect of gamma-radiation on different spices are in progress. Studies conducted on the leather beetle, which does considerable damage to stored leather, have indicated that low-level radiation could successfully be employed in controlling this insect pest. As soon as the gamma-irradiation facility at the CIR is installed, it is proposed to evaluate the economics of this method and, if found feasible, the facilities of the Trombay Establishment could be extended to industries interested in the disinfestation of insect pests from stored leather.

Work has been done on the control of mosquitoes, fruit-flies and stem borers, but I will not dwell on this. I would only like to mention one problem which is of interest to this country because of its silk industry. Statistics on the yearly production of silk based on 1958 figures show that only 2530000 lb are produced in India as against 44120000 lb in Japan, which is a much smaller country. This wide disparity can be reduced to a large extent by adopting improved methods in sericulture. The Biology Division is considering a project in this connection designed to improve the quality as well as quantity of silk-production. The technique of initial processing of cocoons is very important, as you know, for maximizing the quantity of their silk covering. The properties of a silk fibre depend to a great extent on the method of killing the pupae in the cocoons as well as in the removal of moisture. The conventional methods for killing and drying at present in vogue in this country are not only time-consuming and laborious but produce deformation of the cocoon covering, besides denaturing the sericin. In addition to these defects the fibres exhibit impairment of their winding properties and certain other physico-chemical characteristics. All these contribute to a decrease in the yield of raw silk. In order to overcome these shortcomings, experiments are planned involving the use of high-energy radiation sources for killing the worm. When we visited Tashkent a few months ago we saw, if I am not mistaken, sources of several hundred thousand curies being used precisely as a method of killing the worm in the cocoon, instead of the more conventional method.

Ladies and gentlemen, I will not take up more of your time. May I say that we are most happy that this Symposium is being organized by the International Atomic Energy Agency in India on this occasion. We hope that the arrangements will be found satisfactory. If there are any shortcomings we trust you will make allowances for them, because we are still on improvised premises. We are building an auditorium with all modern facilities to house some 800 to 1000 people at the Tata Institute of Fundamental Research at Colaba, and an auditorium for 1200 people is to be built at Trombay. But these facilities are not yet ready and therefore you are, I am sure, being put to considerable inconvenience. We trust that you will excuse this. I hope that more such symposia will be organized in this country and I can assure you that we will be most happy to co-operate with the International Atomic Energy Agency in such undertakings.

PART I

USE OF RADIOISOTOPES AS TRACERS

SECTION 1

ECOLOGY AND GENERAL BIOLOGY

RADIOISOTOPES IN ECOLOGICAL AND BIOLOGICAL STUDIES OF AGRICULTURAL INSECTS

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Abstract — Résumé — Аннотация — Resumen

Radioisotopes in ecological and biological studies of agricultural insects. Atomic energy used for research and control of agricultural and livestock insect pests has proved to be of very great value. Radioisotopes are an excellent tool in carrying out detailed and accurate ecological studies of insect pests required for effective control. With this information, economic control of insects can be carried out by hitting the weakest links and most vulnerable points using crop practices, quarantines, killing or sterilizing techniques, and other control-methods on a co-ordinated basis.

Radioisotopes have been of value in labelling and studying the dispersal and movement, life history, and behaviour of underground insects, orchard and forest insects, honey bees, and cotton- and food-crop insects. They have been helpful in elucidating the role of plant-sucking insects particularly with regard to plant virus transmission. Ecological studies have been carried out on radioisotope-tagged flies, ticks, mosquitoes and other insects which transmit diseases or are important pests of cattle and other domestic animals. Use of radioisotopes has contributed to the study of parasites and predators of agricultural and livestock pests and disease vectors. They have been of value in ecological studies on food chains, nutrition cycles, and population studies.

New and additional biological studies are suggested using radioisotopes and radiation. Certain plant-insect relationships can be explored using radioisotopes, for example in studying trace elements which sometimes form the basis of plant resistance to insect attack. They can be used in studying pollination of plants by insects. Host-parasite relationships of domestic-animals and insects can be studied using radioactivity to elucidate life histories and to locate the radioactive parasites in the host animals at intervals of time. Radioactivity can be used to locate egg-laying and overwintering sites of various insects. Radioisotopes and radiation provide excellent tools in research providing ecological data required in successful control by co-ordinated use of irradiation and sterilization, insecticides, special chemicals and hormones, new genetic developments including abnormal sex-ratio and sterility-producing genes, and natural control with parasites and predators.

Les radioisotopes dans les études écologiques et biologiques sur les insectes nuisibles. L'emploi de l'énergie atomique s'est révélé extrêmement précieux pour étudier et combattre les insectes qui nuisent à l'agriculture et à l'élevage. Les radioisotopes sont très utiles dans les études écologiques précises et détaillées qu'il faut faire si l'on veut lutter efficacement contre ce fléau. Les renseignements ainsi recueillis permettent d'agir aux moindres frais en frappant les insectes aux points les plus faibles et les plus vulnérables, selon un plan coordonné comprenant pratiques agricoles, quarantaines, techniques d'extermination et de stérilisation, etc.

Les radioisotopes se sont révélés utiles pour le marquage et l'étude de la dispersion et des déplacements, de l'évolution et du comportement des insectes vivant sous terre, des insectes nuisibles aux arbres fruitiers et aux forêts, des insectes mellifères, des charençons, etc. Ils ont aidé à préciser le rôle des insectes phytophtyres, notamment en ce qui concerne la transmission des virus. L'auteur a fait des études écologiques sur des mouches, tiques, moustiques et autres insectes qui transmettent des maladies ou nuisent beaucoup au bétail ou à d'autres animaux domestiques. L'emploi des radioisotopes a facilité l'étude des entomophages et parasites des animaux qui nuisent à l'agriculture et à l'élevage ou qui transmettent des maladies. Les radioéléments se sont révélés utiles dans les études écologiques sur les chaînes alimentaires, les cycles bromatologiques et les études de population.

L'auteur propose d'entreprendre des études biologiques nouvelles et complémentaires au moyen des radioisotopes et des rayonnements. L'emploi des radioisotopes permet d'explorer certains rapports entre les plantes et les insectes, par exemple d'identifier les oligoéléments qui parfois sont indispensables aux plantes pour pouvoir résister aux attaques des insectes. On peut s'en servir également pour étudier la pollinisation des plantes par des insectes. En recourant à la radioactivité, on peut étudier les rapports entre les animaux hôtes et les insectes parasites en suivant l'évolution des parasites radioactifs et leurs mouvements dans l'organisme hôte à intervalles réguliers. La radioactivité permet de découvrir les lieux de ponte et d'hibernation de divers insectes. Les radioisotopes et les rayonnements constituent d'excellents moyens de recherche, car ils fournissent les données écologiques voulues pour un programme de lutte comportant l'utilisation coordonnée de l'irradiation et de la stérilisation, d'insecticides, de produits chimiques spéciaux et d'hormones, de nouveaux phénomènes génétiques y compris l'apparition de gènes produisant une proportion anormale des deux sexes et la stérilité, et de l'élimination naturelle au moyen des parasites et des entomophages.

Радиоизотопы в экологических и биологических исследованиях сельскохозяйственных вредителей. Использование атомной энергии для исследований и борьбы с сельскохозяйственными вредителями и паразитами домашнего скота оказалось очень ценным. Радиоизотопы являются прекрасным средством для проведения всестороннего и точного экологического изучения вредных насекомых в целях эффективной борьбы с ними. Располагая такими данными, можно вести экономичную борьбу с насекомыми, поражая самые слабые и наиболее уязвимые места путем правильного выращивания урожая, карантинов, методов уничтожения и стерилизации и других способов борьбы на скоординированной основе.

Радиоизотопы оказались ценными в мечении и изучении распространения и передвижения, биологии и поведения почвенных вредителей, садовых и лесных вредителей, пчел, хлопковых и зерновых вредителей. Они помогли выяснить роль растительных сосущих вредителей и особенно передачу растительных ядов. Экологические исследования проводились на меченных радиоизотопами мухах, клещах, москитах и других насекомых, которые переносят болезни или являются вредными паразитами крупного рогатого скота и других домашних животных. Использование радиоизотопов способствовало изучению паразитов скота, сельскохозяйственных вредителей и переносчиков болезней. Они оказались ценными в экологических исследованиях пищевых цепей, циклов питания и популяций.

В работе предлагаются новые и дополнительные биологические исследования с использованием радиоизотопов и радиации. Некоторые виды связи между растением и насекомым могут быть изучены с помощью радиоизотопов. Например, они могут использоваться для изучения метящих элементов, которые иногда способствуют сопротивляемости растения воздействию насекомых. Они могут использоваться при изучении опыления растений насекомыми. Взаимосвязь между домашним животным и паразитом может изучаться с помощью радиоизотопов для того, чтобы выяснить биологию и определять местонахождение паразитов в животном через определенные промежутки времени. Радиоактивность может использоваться для определения места откладывания яиц и мест зимовки различных насекомых. Радиоизотопы и радиация оказались прекрасным средством для исследования экологических данных, необходимых для успешной борьбы с вредителями путем одновременного использования облучения и стерилизации, инсектицидов, специальных химикалиев и гормонов, новых генетических достижений, включая анормальное соотношение полов и стерильные гены и обычную борьбу с паразитами и вредителями.

Los radioisótopos en los estudios ecológicos y biológicos sobre insectos dañinos. El empleo de la energía atómica en las investigaciones sobre los insectos que atacan los cultivos y el ganado y en la lucha contra los mismos ha resultado de gran utilidad. Los radioisótopos constituyen un medio excelente para realizar estudios detallados y precisos sobre los insectos dañinos, estudios que son necesarios para combatirlos eficazmente. Los datos así obtenidos permiten obrar en las mejores condiciones económicas, atacando los insectos en sus puntos más débiles y vulnerables, combinando las prácticas agrícolas, las cuarentenas, las técnicas de exterminación o esterilización y otros procedimientos de lucha.

Los radioisótopos han demostrado ser útiles para marcar los insectos que viven bajo tierra, los insectos de huertos y bosques, las abejas y los gorgojos, y estudiar su dispersión, desplazamiento, evolución y comportamiento. Han contribuido a la determinación del papel de los fitoftirios, especialmente en lo referente a la transmisión de los virus de las plantas. Se han llevado a cabo estudios ecológicos mediante la marcación con radioisótopos de moscas, ácaros, mosquitos y otros insectos que transmiten enfermedades o constituyen plagas importantes del ganado y de otros animales domésticos. El empleo de los radioisótopos ha facilitado el estudio de los entomófagos y parásitos de los cultivos y del ganado, así como de los vehículos de enfermedades. Han resultado útiles para las investigaciones ecológicas sobre cadenas alimentarias, ciclos de nutrición y estudios sobre población.

El autor sugiere que se realicen nuevos estudios biológicos mediante el empleo de radioisótopos y radiaciones. Utilizando radioisótopos, es posible explorar ciertas relaciones entre plantas e insectos, identificando, por ejemplo, los elementos trazadores que las plantas suelen necesitar para poder resistir al ataque de los insectos. Pueden emplearse para estudiar la polinización de las plantas por los insectos. Se pueden analizar las relaciones entre los animales domésticos huéspedes y los insectos parásitos siguiendo la evolución de los parásitos radiactivos y sus desplazamientos en el organismo huésped a intervales regulares. Asimismo es posible utilizar la radiactividad para determinar los lugares en que diversos insectos suelen poner sus huevos e invernar. Los radioisótopos y las radiaciones constituyen excelentes medios de investigación, ya que facilitan los datos ecológicos necesarios para establecer un programa de lucha basado en el empleo coordinado de la irradiación y la esterilización, los insecticidas, los productos químicos y las hormonas especiales, los caracteres genéticos peculiares comprendida la aparición de genes que originen índices sexuales anormales y esterilidad, y la eliminación natural con ayuda de parásitos y entomófagos.

I. Introduction

Insects cause enormous losses to agricultural and forest crops and products. No reliable estimates are available but the annual loss and damage in the United States is estimated at 4 billion dollars, and in the United Kingdom as the work of 51000 skilled farm-workers. The Food and Agriculture Organization of the United Nations has estimated that over 10% of the world's stored-grain crop is lost annually to insects, representing 50% of the products in world trade [5]. Half of the stored grain is lost in some tropical areas. One million tons of stored grains are lost annually to insects in India [35]. In the southern United States, losses of 9 wt.% per month and of 50 wt.% per summer for stored corn are reported [33] [42].

The efforts of man in combatting agricultural pests by use of toxic chemicals are frequently thwarted by the development of insecticide resistance by the insects, or by the establishment of new pests, such as mites, when predators and parasites are killed by insecticides. Every potential method for combatting these insect pests must be utilized when we recognize the rapid population increases of the human race and the necessity for producing more food. Use of insecticides must be made more efficient and effective, and above all, new and different methods of insect control must be discovered and used.

Man has made serious problems for himself by the methods he has developed for supplying his food. In clearing land, draining swamps and using other methods, he has concentrated the production of selected food-plants and domestic animals. He has often carelessly introduced plant and animal pests, sometimes without the natural parasites and predators which prevent production of enormous numbers in the native home of the insects. With a concentrated food-supply frequently developed for specialized yields, without consideration of insect pests, the crops and animals are highly vulnerable. Uninformed use of insecticides may present greater problems. Use of atomic energy opens several avenues of approach to new control-methods, all of which require knowledge of the biology and ecology of the pest insects. It furnishes us with a tool of real proven value and with a potential even greater than has at present been realized.

The use of radioisotopes in entomology has expanded and progressed rapidly. In reviewing the field in 1950 there were 40 published references [69], in 1957 over 500, and in 1960 well over 1000 available references represent an incomplete coverage. Reviews on the uses of radioisotopes in entomology cover the general area and also specialized fields [2—4] [8] [25] [37] [46] [63] [66—69] [81—84] [92] [106] [114]. About half the published research applies to agricultural entomology and most of the remainder to medical entomology and related fields.

The elimination of the highly destructive cattle pest—the screwworm fly—from Curaçao and from the southeastern United States [16] [77] [82] is a truly great accomplishment that results in a saving of over $10000000 worth of cattle per year. This was accomplished by the use of radiation from the radioisotope Co^{60}. Many other outstanding accomplishments have resulted due to the use of radioisotopes in the field of entomology. Full advantage should be taken of this new opportunity to study and control insects.

The objectives of this report are (a) to summarize the research completed or in progress on the use of radioisotopes in biological and ecological studies of insects of agricultural and veterinary importance, and (b) to suggest new avenues of approach for insect-control using radioactive materials.

II. Radioisotopes used in entomology

About 43 different radioisotopes have been used in studies involving insects (Table I). These studies include labelling of insects, irradiation for killing or sterilizing insects, labelling the organisms transmitted by insects, physiological and toxicological studies, labelling of insecticides, marking insecticide sprays, and studying accumulation of fission products or other elements in the environment. In addition, a number of stable isotopes have been used for various purposes such as deuterium, H^2.

TABLE I

LIST OF RADIOISOTOPES USED IN ENTOMOLOGY

H^3		
C^{14}	Zn^{65}	Cs^{137}
$Na^{22\ 24}$	$As^{74\ 76}$	Ba^{140}
P^{32}	Br^{82}	La^{140}
S^{35}	Rb^{86}	Ce^{144}
$Cl^{36\ 38}$	$Sr^{89\ 90}$	Pr^{144}
K^{42}	Y^{91}	Ta^{182}
Ca^{45}	Zr^{95}	Ir^{192}
Sc^{46}	Ru^{106}	Au^{198}
$Mn^{52\ 54\ 56}$	Ag^{110}	Po^{212}
$Fe^{55\ 59}$	Cd^{115}	Pb^{212}
Co^{60}	Sb–	Ra^{226}
Cu^{64}	I^{131}	Th–

In selecting suitable radioisotopes for use, particularly for tagging insects, the following factors must be considered:

(a) the type of emission—beta, gamma, or alpha,

(b) the energy of radiation,

(c) the half-life of the radioisotope,

(d) biological accumulation and retention in the insect (if labelled internally) and the biological half-life,
(e) toxicity to the insect,
(f) the form of the radioelement (liquid, metal, etc.), and
(g) the ease of handling, labelling, and detecting the radioisotope.

The radioisotopes which have been used successfully for labelling or marking insects for ecological and biological studies include Na^{24}, P^{32}, S^{35}, Cl^{36}, Ca^{45}, Sc^{46}, Co^{60}, Zn^{65}, Sr^{89}, I^{131}, Ce^{144}, Ir^{192}, Au^{198}, Ra^{226}, and Th^{-}. Various radioisotopes have been found to be toxic in certain insects such as Ca^{45}, Fe^{59}, Rb^{86}, Ag^{110}, Cd^{115}, and I^{131}, while others are not retained such as Ca^{45} and Zr^{95}, or are unsuitable for other reasons such as C^{14}, S^{35}, Cl^{36} and I^{131}. The use of one radioisotope for one species of insect may be very suitable while for another it may be impossible. The most commonly used radioisotopes for ecological studies are beta-emitters P^{32} and Sr^{89}. These have many advantages such as suitable half-lives, effective incorporation and retention in the insect, low toxicity, ease of detection and handling, and relatively low cost. For many insect-ecology studies, the beta-emitters are most suitable since they are easier to handle and to detect. Those which have been used effectively include P^{32}, S^{35}, Cl^{36}, Ca^{45}, Sr^{89}, and Ce^{144}. (Ce^{144} also has weak gamma-emission). They do not permit location of insects from a suitable distance with the quantities usually used for labelling. However, for locating insects underground, in and under debris, and under bark and in trees, a strong gamma-emitter is required. The gamma-emitters successfully used for tagging insects include Sc^{46}, Co^{60}, Zn^{65}, I^{131}, Ir^{192}, Ta^{182}, Au^{198}, Ra^{226}, and Th^{-}. Some of these radioisotopes also give off beta (or alpha) radiation.

Insects have been externally irradiated with X-rays, gamma-rays, beta and fast-beta-radiation from cyclotrons and betatrons, alpha-particles and neutrons, and with mixed radiation from fission products. Radioactive isotopes have been metabolically incorporated or placed internally in insects and have resulted in internal gamma, beta and alpha-radiation.

(a) Methods of labelling insects with radioisotopes

Radioisotope-tagging is an efficient technique for marking and rapidly identifying large numbers of many insects with a minimum of labour and cost. A variety of agricultural, forest, and animal pest-insects have been made radioactive to study their dispersal and behaviour. Many methods have been developed for marking insects with radioisotopes. These include the contamination of food, of growing medium, of external surface of the insect, or injection. Insect food has been made radioactive by soaking the food, by injecting radioisotopes into animal-hosts, by contaminating drinking-water, milk, sugar solutions, and by injecting insect food-plants or growing them in radioactive material. Insects have been contaminated by growing them in a medium which has been made radioactive, such as organic substrate for flies, water for mosquitoes and other aquatic insects, in animals made radioactive for screwworm flies, and radioactivated plants. Many methods have been used for external contamination including dipping, painting, spraying, attaching clips or wires of radioactive materials, and treating the surface with wetting or adhesive agents to make the radioisotopes stick. Radioisotopes have been injected or perfused as liquid, or inserted as a piece of metal or other substance into the body of the insect. In addition, insects have been exposed to radioactive gases which are taken up and accumulated in the body.

(b) Methods of detection

Radioactive insects are detected by a variety of methods. The most commonly used method for detecting beta and gamma-radiation is the use of a portable field survey Geiger

counter (ionization chamber). Insects are also brought into the laboratory and checked with a proportional counter or other instruments. In the field an inexpensive method is to use photographic plates or dental X-ray film strips for detecting labelled insects. This requires later development of the film. For gamma-detection, a scintillation counter or "scintillometer" is also used with good results. A complex system using nuclear emulsion plates has been developed for showing alpha-particle tracks when using thorium. This also required dissection and ashing of specific body-organs of insects.

III. Ecological studies

No effective control of insects is possible without knowledge of the biology and ecology of the insects. The most successful method of gaining control of important insects is to understand their entire life history, food habits, dispersal, mating, behaviour, parasites, predators and other aspects of their biology. Then economic control can be carried out by capitalizing on the weakest link or most vulnerable point, using quarantines, environmental control, crop practices, insecticide treatment, irradiation sterilization, baits, traps, and predators or parasites, or a combination of these. Studying the biology of many insects frequently involves prolonged field studies and requires efficiency of techniques and time-saving methods.

Radioisotopes have been of value in labelling and studying the dispersal and movement, life-history and behaviour of underground insects, orchard and forest insects, honey bees, and cotton and food crop insects. They have been helpful in elucidating the role of plant-sucking insects, particularly with regard to plant virus transmission. Ecological studies have been carried out on radioisotope-tagged flies, ticks, mosquitoes and other insects which transmit diseases or are important pests of cattle and other domestic animals. Use of radioisotopes has contributed to the study of parasites and predators of agricultural and livestock pests and disease vectors. They have been of value in ecological studies of food chains, nutrition cycles, and in population studies.

The ecological and biological studies on agricultural insects using radioisotopes are reviewed and some additional or new suggestions are presented.

(a) Forest insects

Forest insects have been tagged to determine dispersal and movement, overwintering sites, longevity, and location of the insects at given times (Table II). They have been labelled in a variety of ways for dispersal studies. Englemann spruce beetles were dipped in a I^{131} alcohol solution which gave good radioactivity for a relatively short period [39]. White pine weevils were tagged with Co^{60} nitrate dissolved in cellulose acetate and acetone to form an adhesive attached to the wings of the beetle. Of 64 beetles tagged, 33% were alive after two months, the same as untagged controls. The tagged insects could be detected from a distance of 2.7 m [110]. The Douglas fir beetle was labelled with P^{32} by feeding P^{32} in sugar solution [45]. White pine weevils tagged with Sc^{46} in 20% alcohol could be detected at 1.5 m. The longevity, flight range, direct uninterrupted flight range, and practical control methods were determined from the releases [55]. The southern pine beetle was labelled with Ir^{192} and released in isolated beetle-infested areas [107]. Female European pine-shoot moths *Rhyacionia buoliana* were labelled with Co^{60} by painting the radioisotope on the abdomen [56]. The labelled insects could be located at 1 m. Of the 104 moths released, 42.9% were recovered. The moths stayed in the pine stand where they were released and dispersed normally to 46 m per flight. The moths were decimated by birds and other predacious animals. The radiation from 25 to 50 μc of Co^{60} did not affect the moths adversely. The leaf-miner moth

TABLE II

DISPERSAL OF RADIOISOTOPE MARKED FOREST INSECTS

Insect	Isotope	No. released	Recovery and longevity	Maximum dispersal (km)	Authority
White Pine Weevil *Pissodes strobi*	Co^{60}	64	33% in 60 d	—	[110]
" "	Sc^{46}	1600	60—70% up to 8.5 months	0.22	[55]
Douglas Fir Beetle *Dendroctonus pseudotsugae*	P^{32}	—	—	—	[45]
Southern Pine Beetle *Dendroctonus frontalis*	Ir^{192}	20000	—	—	[107]
Englemann Spruce Beetle *Dendroctonus engelmanni*	P^{32} I^{131}	19000	5% up to 14 d	—	[39]
" "	—	—	—	4.8	[17]
European Pine Shoot Moth *Rhyacionia buliana*	Co^{60}	104	42.9	0.046	[56]
White grubs	Ta^{182}	Movement in soil		—	[107]

Phyllocnistis labyrinthella was labelled in the adult stage by feeding P^{32} in sugar water. The adults could be located at 0.5 m. When 200 moths were released, they dispersed and were later located mostly underground where they had hibernated. They were located from 5 August to 30 September, when the experiment ended [111].

(b) ORCHARD INSECTS

Orchard-insect dispersal has been studied using radioisotopes (Table III).

Peach orchards are seriously damaged by the plum curculio *Conotrachelus nenuphar*. This beetle was successfully labelled with P^{32}, Co^{60}, Zn^{65}, Sr^{89}, and I^{131} [101] [102]. Sr^{89}, fed to the beetles in plant tissue or solution, was the best radioisotope for tagging. Of 473 beetles released, 193 recoveries were made at an average distance of about 25 m and up to a max. of 123 m from the release site. Hibernation of the beetles was studied and they were collected up to 287 d after activation and release. The cherry fruit fly *Rhagoletis cingulata* was made radioactive by feeding the adult flies P^{32} in sugar solution [72]. Of 2010 radioactive flies released, 39 were recovered, 37 within 139 m and a maximum of 287 m for a period up to 6 weeks after activation. Oriental fruit flies *Dacus dorsalis* were made radioactive by growing larvae in carrot medium containing P^{32}, and by feeding adults on a sugar solution containing P^{32}. Both techniques produced good radioactivity of the adult flies for over 40 d [103].

The Mediterranean fruit fly *Ceratitis capitata*, marked with P^{32}, dispersed nearly 3.2 km, and 7% of 944 marked flies were recovered 1.2 km and 2.4 km away. A marked male was recovered 32 km away, of which 14.4 km were open sea [28].

About 20000 fruit flies *Drosophila melanogaster* were made radioactive by feeding P^{32} spread on fermenting figs. The flies dispersed from a garbage dump 2.24 km into the direction of the prevailing wind to fig orchards. In another release, the flies flew 3.84 km in 2 d [115]. The walnut husk fly, *Rhagoletis completa*, was labelled by spraying an attractant, corn

TABLE III

DISPERSAL OF RADIOACTIVE MARKED ORCHARD INSECTS

Insect	Isotope	No. released	Longevity	Maximum dispersal (km)	Authority	
Plum curculio						
Conotrachelus nenuphar	P^{32}	62	up to 120 d	0.11	[101]	[102]
,, ,,	Co^{60}	705	up to 8.5 months	0.27	,,	,,
,, ,,	Zn^{65}	23	up to 120 d	0.041	,,	,,
,, ,,	Sr^{89}	175	3 d	0.123	,,	,,
,, ,,	I^{131}	86	—	0	,,	,,
Oriental Fruit Fly						
Dacus dorsalis	P^{32}	—	up to 40 d	—	[103]	
Fruit Fly						
Drosophila melanogaster	P^{32}	20000	—	3.84	[115]	
Mediterranean Fruit Fly						
Ceratitis capitata	P^{32}	944	—	32.0	[28]	
Cherry Fruit Fly						
Rhagoletis cingulata	P^{32}	2010	up to 42 d	0.287	[72]	
Walnut Husk Fly						
Rhagoletis completa	P^{32}	15% of natural population	—	14.4	[15]	

protein hydrolysate and P^{32} on branches of foliage in a walnut orchard. About 15% of the natural population of flies were P^{32}-labelled and were shown to disperse up to 1.4 km in 3 weeks. The fly eggs deposited in walnut fruit were found to be radioactive [15].

(c) UNDERGROUND INSECTS

Tracing the dispersal and underground movement of soil insects such as wireworms and cutworms requires the use of a gamma-emitting radioisotope to be detected through several centimeters of soil. Adult wireworm beetles *Agriotes sp.* were first tracked [113] using a Ra^{226} disc below the wing of the beetle. For tagging the immature stages, an internal tag is required which remains and is not excreted. This was partially solved by inserting a Co^{60} wire in the larvae [7] [53] to tag a species of wireworm and two species of cutworms to study their response to moisture, food, and temperature. In Russia the movement of soil-inhabiting insects has been studied [79] by radioactive tagging. The most successful technique [51] employs a gold-plated Co^{60}-wire attached to the wireworm. This was used in studying the effects of insecticides in reducing movement and in killing wireworms. A very interesting automatic Geiger device has been made [57] [108] which continuously follows, tracks and records the position of the wireworm through 11.4 cm of soil.

A species of white grub infesting forest trees was tagged [107] with the gamma-emitter Ta^{182}. The radioisotope was introduced as a wire into the grub, and movements of the larvae through the soil were studied.

(d) CROP INSECTS AND GRASSHOPPERS

Various species of crop insects have been made radioactive to study their dispersal, movement, behaviour, and longevity.

Many plant-sucking insects have been made radioactive by making plants radioactive with P^{32} and allowing the leaf hoppers, psyllids, aphids, and plant bugs to suck up plant sap. Most of these studies have been concerned with the transmission of plant disease instead of dispersal. Bean aphids and green peach aphids became radioactive from feeding on broad-bean and sugar-beet plants watered with $Na_3P^{32}O_4$ solution. The movement of the aphids *Myzus persicae* and *Aphis fabae* in the field and their relation to spreading sugar-beet yellows was studied [19]. Mealybugs were fed on P^{32} radioactive cacao seedlings [31], in studies relating to mealybug behaviour and the control of virus disease of cacao in Ghana.

In a study of coccinelid beetle larval-behaviour [14], small wires of Ta^{182} were glued to the prothorax of newly-hatched larvae, increasing the weight by 24%, but the behaviour while crawling on plants was apparently normal.

The larvae of the lepidoptera *Panaxia dominuta* and *Arctia caja* were fed [74] on plants treated with S^{35}. The larvae were radioactive and, after 6—8 weeks, the resulting adults showed increased radioactivity. The labelling was successful for studying population size, larval death-rates, and dispersal activity.

The cotton boll weevil *Anthonomus grandis* was tagged by dipping the adult beetles in a water solution of Co^{60} chloride containing a wetting agent (Tergitol 7). The resulting adults [9] showed an average radioactivity of 4690 counts/min before washing and 3523 counts/min afterwards. A cotton-plant stem was immersed in Co^{60} solution and the leaves and especially the cotton in the immature bolls became radioactive. Large numbers of boll weevils have been marked for use in field studies.

The mealworm, *Tenebrio molitor*, and tobacco hornworm, *Protoparce quinquemaculata*, were fed or injected with $As^{76}{}_2O_3$, and cabbage and turnip maggot flies were activated [89] with P^{32} by applying P^{32} around turnip plants. The cabbage maggot, *Hylemya brassicae*, adults were fed P^{32}-labelled sugar solution with excellent results. Spraying adults with P^{32} was not a suitable procedure [44]. Of the 1 854 radioactive adults released, only two were recovered. Larvae of the cabbage butterfly, *Pieris rapae crucivora*, were fed cabbage smeared with Ca^{45} and P^{32}. Large quantities of Ca^{45} were excreted. Ca^{45} was found in the blood of the adults, and was found deposited in white scales of the wings of males. Nematodes and fungous growth from the larvae were radioactive [118].

Adult stinkbugs *Eurygaster* labelled with Co^{60} or P^{32} were released, and migration to forests for overwintering occurred in the autumn [2]. The bugs migrated 0.5—3.0 km W and 10—12 km E to a forest at a rate of 0.5 km in 24 h. After overwintering they congregated; some dispersed up to 15 km. Adult grain moths, *Hadena basilinea*, were attracted to lights and then fed [2] on attractant solution containing P^{32} or Co^{60}. Larvae of the moths fed on radioactive grain or were sprayed with radioisotope solution. The adult female moths dispersed in a radius of 2 km and the males 3 km. The grain moth parasite flies *Pseudogonia cinerascens* were attracted to baits where they were labelled [2] with P^{32} or Co^{60}. They then dispersed up to 19 km, indicating greater mobility of the parasite than the host moth.

Two species of grasshoppers, *Melanoplus m. mexicanus* and *Camnula pellucida*, were made radioactive [52] by feeding them on P^{32}-sprayed plants. At first, the rate of loss of P^{32} from excretion was high, but a low level was retained for 28 d through the nymph to adult stage. The dispersal of these species of grasshoppers was studied and 20000 nymphs and adults were fed on wheat seedlings sprayed with 0.5 mc of P^{32} in 50 ml. When released on bare cultivated fields, they showed no ability to orient or move toward a food supply. The average

rate of movement was 6.4 m/h at 70°F, and after 6d, the total movement of up to 220 m was random but for a response to wind direction [100] [108]. The grasshopper *Melanoplus m. mexicanus* was made radioactive [12] by feeding P^{32} in bran and molasses. About 8000 3rd and 4th instar nymphs and 7500 adults were released in a field of alfalfa, timothy, and weeds. 20 d after releasing the nymphs, 43% were recaptured at 9.2 m, 39% at 18.4 m, and 18% at 27.6 m from the release-point. At 18d after releasing the adults, 56% were recaptured at 9.2 m, 36% at 18.4 m, and 9% at 27.6 m from the release-point.

The locusts, *Locusta migratoria* and *Locustana pardalina*, were made radioactive with P^{32} in the nymph and adult stages by feeding P^{32}-treated bran or hydroponic corn or grass. Adults were also sprayed and could be detected at a distance of 25.4 cm after 2 weeks, but no dispersal studies were carried out [75]. It is suggested that locusts be fed or treated with Sc^{46}, Co^{60} or other gamma-emitting radioisotopes so that they could be located at a distance at night to rapidly determine their dispersal and migration.

(e) Bees and ants

Studies have been carried out with honeybees to determine their flight range, mixture with other hives, territoriality, and relationships between the workers, drones, and the queen. Drone honeybees were shown to take C^{14}-labelled glucose in syrup through a screen from worker bees in another cage, even when syrup was available in the cage containing drones [88]. The workers contained an average of 9420 counts/min per bee, and they passed syrup through the screen to drone-bees that accumulated an average of 456 counts/min per drone.

The continual exchange of nourishment between worker bees was studied [87]. It was shown that exchange of sugar syrup containing P^{32} was very rapid. After 27h, 43—60% of the bees of a hive contained considerable P^{32} which was distributed from only a few workers. After 48h, 100% of the larvae in the hive were radioactive.

Exchange of nourishment and dispersal were studied [34] using radiogold Au^{198}. When fed as a colloidal suspension in syrup, 100% of the bees were labelled in 24h and it was possible to detect the foraging bees at a distance of 10—60 cm with a scintillometer. In a larger experiment with 40000 bees, 90% were marked in 12h, and 100% after one day. The exchange of food in the hive was rapid. Some bees of one hive were discovered in nearby hives. The maximum dispersal of marked foraging bees was 1.1 km from the hive. A comparison was made of marking bees with P^{32} and a genetic marker "cordovan" in showing dispersal of foraging honeybees [80]. The P^{32} was fed in syrup and was taken up by nearly all bees in the hives. The P^{32}-labelled bees were found 1.6 km E and 2.6 km NE, and the cordovan bees 1.6 km E and 1.1 km NE.

Queen bees, which have to be checked at intervals in hives, have been tagged by an external paint-marker containing 0.5μc of Co^{60} per queen. The queen is quickly located in the hive by a Geiger counter, and the method is effective. The possible radiation damage was not determined [99].

Mn^{52}, Mn^{54}, and Mn^{56} have been used to study the accumulation of manganese by social wasps [21] [23]. Manganese was accumulated by larvae, extensively lost at pupation, but adults were found to accumulate it particularly in the midgut epithelium, greatly in excess of any known metabolic usage. Ba^{140} was accumulated in hornets and ants even when exposed to infinitesimal amounts [21] [24]. This suggested their use as detectors for radioactive barium leaks not otherwise detectable. Ba^{140} decays to lanthanum which in turn goes to cerium. Such decay-chains offer means of studying non-metabolites in organisms by selection and introduction of the proper precursors.

In studies on food transmission between ants, single workers in several laboratory colonies of five species were fed I^{131} in honey. The workers fed first, passed it on to other workers

and finally to larvae and queens [117]. There was limited food transmission in *Pogonomyrmex badius* to rapid transmission and colony saturation in *Crematogaster lineolata* [43]. In mounds of the ant *Lasius minutus* P^{32}-labelled honey was passed to other workers and larvae within 8 h. Ants in adjacent mounds became radioactive indicating that single colonies consist of one or more mounds connected below the surface of the ground [73]. Honeydew-gathering ants were studied by making aphids radioactive from a P^{32}-injected thistle plant [91].

(f) Insects of veterinary importance

Pest insects bother domestic animals, reduce weight gains and milk yields, destroy or damage hides, and kill the animals directly or indirectly. Insects transmit such animal diseases as anaplasmosis, cattle fever, encephalitis, anthrax, filariasis, trypanosomiasis, and other diseases.

Radioactive labelling of medically important insects for dispersal studies was initiated in 1949 [26] [60]. Since that time extensive studies have been carried out with this technique.

The dispersal of radioactive houseflies has been studied by several groups. The data have been compiled [68] and show that the houseflies fly a maximum distance of 32 km and, frequently, 15 km. Blowfly populations disperse up to 45 km, and various other flies have been found to disperse widely.

The primary screwworm *Callitroga hominivorax* was made radioactive [98] by rearing larvae in a meat medium containing P^{32}, or in wounds of living sheep or goats injected with P^{32}. The emerged adults were well marked, and 10000 were released for field studies. Five species of blackflies were marked [50] with P^{32} in Saskatchewan. Colonies of radioactive larvae and pupae were found over 0.4 km downstream from the point of release, and an adult was recaptured 90 m from the stream. Blackfly pupae of *Simulium venustum* were tagged [65] with P^{32} at Churchill, Manitoba, by moistening them with a solution containing 0.3 μc P^{32}/ml.

About 90000 eye gnats, *Hippelates pusio*, were fed P^{32} in honey and released in two tests, 0.8 and 1.6 km distant from a small town. In both tests [41] there was almost complete penetration of the small town on the day of release. Tagged eye-gnats were collected over 1.6 km from the release-site in 3.5 h.

Mosquitoes are also severe pests and transmit diseases to domestic animals. Eleven species of mosquitoes have been labelled, and dispersal studies carried out in North America, Africa, and South America. Various species of mosquitoes have been labelled by growing the larvae in a radioisotope or by feeding the adults on animals or plants injected with radioisotope, or by external marking of the insect by dipping, spraying or painting the radio-element. The radioisotopes used include P^{32}, S^{35}, Sr^{89}, Ce^{144}, and Th^{-}. Mosquitoes have also been grown in many other radioisotopes for physiological studies. *Culex pipiens* larvae have accumulated Sr^{90}, Ru^{106}, Cs^{137}, and Ce^{144} which are important fission products [54]. The use of "biocomplexon" or EDTA, a chelating agent (sodium salt of ethylene-diamine tetraacetic acid), caused a 100% increased accumulation of Sr^{90}, but decreased accumulation of Ru^{106}, Cs^{137}, and Ce^{144}.

The studies on dispersal of mosquitoes have been summarized [68]. Many studies have been carried out on the behaviour of mosquitoes. At Churchill, Canada, during radioactive-labelling studies [70] the abundant pest mosquito, *Aedes communis*, was shown to consist of two races. One race was shown not to bite human beings. This has a potential importance in natural control by introducing and substituting a non-biting race or strain into an ecological niche for a harmful pest. This replacement could be carried out concurrently with the introduction of radiation-sterilized males of the biting race.

Lone star ticks *Amblyomma americana* were made radioactive by soaking in P^{32}. The addition of a wetting agent did not increase uptake or retention but did reduce survival, so that wetting agents were not recommended [76]. Ticks *Ixodes sp.* were tagged with Ce^{144} by bathing them in the radioisotope solution for 5 min [97].

The nymphs of the bugs *Triatoma infestans* and *Panstrongylus megistus* were marked with thorium by injection into the abdomen, and by feeding on a pidgeon previously inoculated with Th. The resulting adult bugs were radioactively labelled [6]. Blood-sucking bugs *Triatoma protracta* were successfully labelled by anesthetizing them with CO_2 and applying Ce^{144} to the thorax [97].

IV. Biological control by predators and parasites

Biological control of insects by predator insects eating injurious pest insects is important; for example, lady-bird beetles have been used economically to control scale insects in California. Field observation to prove the importance of certain predators in controlling injurious insects is frequently a tedious job, especially if the insects are nocturnal, underground, or inconspicuous. When a pest insect is radioactively labelled, it leaves a radioactive record in the predator after being eaten. Chinese praying mantids became highly radioactive [69] when fed on fruit flies and yellow-fever mosquitoes labelled with P^{32}. Aphids which had fed on a P^{32}-labelled thistle plant became radioactive. They were eaten by various aphid predators including three species of lady-bird beetles, a spider, and syrphid fly larvae. The predator efficiencies were calculated, based on the amount of radioactivity, which indicated the relative numbers of aphids consumed [91].

Cotton aphids *Aphis gossypii* were fed S^{35}-labelled Systox to determine the effect of this insecticide in killing aphid predators [1]. Three species of syrphid larvae were highly susceptible, and mortality rates of five species of coccinellid larvae ranged from 100% to 3.7%, while adults were not susceptible except for 1 species. The radioisotope showed the amount of Systox taken up by the predators. Radioactive eggs and larvae of the codling moth *Carpocapsa pomonella* were exposed to predation, and predators were later collected in the area [83]. Coccinelids, pentatomids, nabids, chrysopids, mirids, thrips, ants, anystid and *Atomus* mites, and spiders had fed on the eggs or larvae and become radioactive. Spruce budworm larvae were made radioactive from feeding on balsam-fir foliage treated with P^{32}. In a laboratory study 21 species of spiders fed on the budworm larvae [83]. Large dytiscid beetles became radioactive after feeding on P^{32}-labelled larval arctic mosquitoes [71]. Frogs and several species of spiders became radioactive from eating labelled adult mosquitoes, and several species of water beetles and bugs, dragon fly, damsel fly, and other aquatic insects became radioactive after eating labelled mosquito larvae [11] [70] [112]. This technique was made quantitative [90] so that the exact number of mosquito larvae eaten by a predator could be counted. Radioactive blackfly larvae were put in a stream [50] and were fed upon by various predatory stream-inhabiting insects, which became radioactive.

P^{32}-tagged mosquito larvae decreased in numbers with an increase in predator numbers [11] and with an increase of percentage of predators that were radioactive from consuming radioactive prey.

The commensal relationships between several species of honeydew gathering ants and their aphid "cows" were studied [91] by making aphids radioactive on a P^{32}-treated thistle plant. The ants became radioactive after collecting honeydew by "milking" the aphids. They carried the honeydew to the ant colony with the result that the colony ants became radioactive. The territorial relations of the ants were also studied.

Radioisotopes are of value in studying the parasitic relationships of parasite to host. A species of mite, *Pimeliaphilus podapolypophagus*, was found to be parasitic rather than

commensal in habit when the mites became radioactive [36] after feeding on roaches which had been fed on radioactive NaCl. Parasitic *Habrobracon* wasps became radioactive when actively parasitizing P^{32}-labelled hosts. The ingested P^{32} had no effect on the longevity of the parasites [58]. Mermithid nematode parasitic worms emerging from radioactive arctic-mosquito larvae were radioactive, and enabled specific nematode worms to be identified under field conditions [66].

Polyhedral virus suspended in C^{14}-labelled alanine and glycine was injected into silkworm larvae [18]. The beta-radiation inhibited-virus multiplication, and 40% of the larvae developed to adults. The radioactive polyhedral bodies gave 1300 cpm/ml and the virus particles liberated from the polyhedral bodies gave 3800 cpm/ml.

V. Population studies

Efficient tagging and ease of detection of insects by using radioisotopes make accurate studies possible on the abundance and size of natural populations. This is done by releasing a known number of radioactive, labelled insects in an area, followed by collecting and trapping, and then calculating the percentage of marked recaptures to unmarked insects. By knowing the original number of released tagged insects and the daily mortality rates, the natural population can be calculated. Mortality rates can be determined during any stage of the life history, or at any specific time using radioisotope tagging. It has a special value in evaluating effectiveness of control by chemical insecticides or biological methods. Longevity and mortality rates have been ascertained with radioisotopes for some of the insects reported in Tables II and III. Population sizes of broods and generations emerging have also been determined for certain mosquitoes [70] [96].

Estimation of population size and population mortality during specific periods is possible using the radioactive marking and release and recapture method. A total of 1227 larvae of the moth *Panaxia dominula* were labelled by feeding on deadnettle *Lamium* which had been grown in water-culture containing S^{35}. The late larval and pupal mortality in colonies under natural conditions were estimated by the recapture method [30]. The total larval population was estimated by multiplying the number of radioactive larvae released by the total number of adults caught, divided by the radioactive adults caught. Of 4038 adults sampled, 18 were radioactive, which means that the larval population was 198185 to 225210 with an adult population of 23000, with a late larval and pupal mortality of 88—90%. In a second colony when 1210 labelled larvae were released and the mean number of adults was 77, the mortality was estimated at 94%. The method indicates that estimates can be made in wild populations using the radioactive-labelling technique.

Population estimates of mosquito larvae were made by using P^{32}-marked and recaptured mosquito larvae in temporary and permanent pools [116]. When 4300 marked larvae were released in a permanent pool, recaptures indicated a recovery ratio of 0.2396 and an estimated population of 17900 ± 1423 standard error. In a temporary pool after release of 1855 marked larvae the recovery ratio was 0.01585 and an estimated population of 117000 ± 24740. It was determined that a large error is associated with a low recovery ratio. The technique and analysis are easy to use and analyse, but it was apparent that large numbers of larvae should be tagged, that the experiment should be of short duration, and many samples taken.

VI. Insect transmission of disease

Use of radioisotopes in studying insect transmission of animal and plant diseases has already yielded valuable results; this is a most promising and fertile, although relatively unexplored,

field. Epidemiological and transmission studies can be made with radioactive-tagged insect vectors or tagged viral, bacterial, protozoan, or nematode pathogens.

Radioisotopes have proved useful in studying the transmission of plant diseases by insect vectors. Po^{212} was used [59] as a tracer to show the quantity of material removed from a plant by a feeding aphid *Myzus persicae* and also the amount of ingested material that was returned to the plant during the next feeding period. The two-spotted spider mite *Tetranychus bimaculatus* and its eggs became radioactive [104] when reared on bean plants grown in P^{32}. The potato psyllid *Paratrioza cockerelli* is an important sucking insect which transmits the "psyllid yellows" disease. The psyllid nymphs and adults became radioactive after feeding on plants containing P^{32}, but normal plants did not become radioactive from being fed on by the radioactive psyllids [93]. The pineapple mealybug *Pseudococcus brevipes* transmits striping, spotting, and wilt disease of pineapple. Mealybugs fed on agar gel containing P^{32} became radioactive, and transferred P^{32} to plain agar gels and to plants by their oral secretions [27]. Tarnished plant bugs transmit a number of plant diseases. With P^{32} as a tracer, it was found [47] [48] that when feeding, the volume of oral secretion injected into the tissues of a host plant was from 0.05 to 0.25 lambda. Green peach aphids fed on P^{32}-treated tobacco plants became radioactive, and injected radioactive saliva which was translocated by the tobacco leaf. The aphids also excreted radioactive honeydew and faeces. The saliva and honeydew may cause part of the injury to tobacco plants [78].

The nematode *Wuchereria bancrofti* became highly radioactive in *Culex fatigans*, and *Setaria digitata* in *Armigeres obturbans* when these mosquitoes were treated [40] in the larval stage with P^{32}. The radioactive mosquitoes fed on infected animals and the nematodes became radioactive, which will be of value in studying the early development of the filarial infections in definitive hosts.

VII. Radiation

The effects of radiation on insects have been studied with regard to mutations and chromosome phenomena, cell division, embryology, growth, physiology, metamorphosis, reproduction, and behaviour. These effects have been practically applied to controlling insects, controlling vector-borne pathogens, and sterilizing foods infested with insects. Insects have also been used extensively as a biological dosimeter of radiation.

It is difficult to summarize the effects of radiation on insects because the result may be retardation, inhibition, or enhancement of development, metabolism, or reproduction, or even death.

There are many practical uses for radiation in agriculture. These may be listed as follows:

(a) Radiation for developing new genetic characteristics of value for control such as high male-sex-ratio strains, lethal factors, characteristics that are dominant which may be introduced into a natural population and will result in mortality, such as wingless, sterile, or other characters.

(b) Irradiation-sterilization of males. The practicality has been completely demonstrated in the screwworm fly elimination in Curaçao and the southeastern United States [16] [77] [82]. This success has stimulated research on the potential of male sterilization in the oriental fruit fly *Dacus dorsalis* [13], the Mediterranean fruit fly *Caratitis capitata* and the melon fly *Dacus cucurbitae* [109], the codling moth *Carpocapsa pomonella* [83], the white pine weevil *Pissodes strobi* [64], the tsetse fly *Glossina morsitans* [94], and mosquitoes [38] and other insects [29].

(c) Irradiation killing of stored insects in agricultural products, especially in packaged foods. The use of gamma-radiation from Co^{60} and fission products has been well studied with a large number of species of stored-products insects [10] [31] [61] [62] [68] [92] [95] [105]. Units for irradiating food have been designed and tested.

(d) Radiation control of wood-boring insects in certain wood, including furniture, offers some promise [20].

(e) Use of radiation has proven to be highly effective in detecting insect infestation in grain [85], coffee [44], and in other foods or agricultural products.

VIII. New and additional uses of radioisotopes

New methods of insect control are being initiated that offer excellent promise in control and elimination by using insects against themselves. These methods include the following:

(a) Release of irradiation-sterilized males. This has already been proved with the elimination of the screwworm fly in Curaçao and the southeastern United States.

(b) Use of chemicals and hormones to cause sexual sterility or deficient characteristics.

(c) Release of insects infected with parasites or pathogens particularly to destroy progeny and contaminate the environment.

(d) Release of insects with special genetic characteristics including lethals, sterile hybrids, high male-sex-ratio, deficient characters and mutants.

(e) Substitution or replacement of harmless races or strains into ecological habitats or niches of pest species.

Radiation is required in sterilizing males and in producing genetic mutants and characters. In all these methods where the insects are not killed as with insecticides, but are released to eliminate or control themselves, detailed ecological and population data are required. Methods using radioisotopes have been reviewed briefly which are required to provide the ecological data to make this type of control possible. The use of methods (a) to (d) has been reviewed [77] and the theoretical statistics of population reduction have been determined using data with the cotton boll weevil. The substitution or replacement method was based on a discovery of a non-biting race of mosquito almost identical to a biting race of the same species. The difference in biting habits was discovered by use of P^{32} in labelling 3 million of the mosquitoes [70]. These new methods of control can be used together in various combinations, or concurrently with insecticide control. Use of a sterile strain which has been selected to become insecticide resistant would give it dominance in a normal susceptible population and speed up its elimination.

Many other uses of radioisotopes in agricultural entomology are awaiting study. Certain plant-insect relationships can be explored using radioisotopes, for example in studying trace elements which sometimes form the basis of plant resistance to insect attack. They can be used in studying pollination of plants by insects. Domestic-animal and insect host-parasite relationships can be studied, using radioactivity to elucidate life histories and to locate the radioactive parasites in the host animals at intervals of time. Radioactivity can be used to locate egg-laying and overwintering sites of various insects.

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DISCUSSION

G. B. VIADO (Philippines): In your remarks on labelling the migratory locust, I presume you were referring to the solitary stage?

D. W. JENKINS: As a matter of fact I was quoting Dr. Huque, who told me of his plans to label the solitary stage and to determine what happens on transition to the migratory stage.

P. J. DEORAS (India): It may be recalled that in India, some twenty years ago, individuals of *Schistocerca gregaria* were successfully converted from the solitary to the gregarious stage by means of rotating machines; the conversion appeared to be induced by rotary motion.

M. S. QURAISHI (Pakistan): Dr. Jenkins showed us slides of a fly attacked by *Empusa muscae*. This is of particular interest to us as we have been doing some work with this parasitic fungus and have found that DDT-resistant strains of housefly were completely eliminated by the fungus, whereas normal strains were less affected. In collaboration with the Chemical Research Division of CSIR we worked on the isolation and identification of the metabolic products of this fungus. To date we have been able to identify three different compounds: one is succinic acid which is, of course, a metabolic product of several fungi; another is an oil with a sweet aroma, on the constitution of which work is being done; and the third compound is found only in traces with succinic acid, and has some insecticidal properties. Now I think that if the mode of action of *Empusa muscae* were further studied it would help us not only in identifying this compound but also in discovering its mode of action in killing the flies. At one time it was believed that the fungus kills only by ramifications of the mycelium, which become so extensive that the physiology of the fly is disturbed, but the discovery that certain of the metabolic compounds are insecticidal would, it seems to me, throw a new light on the situation. Perhaps isotopes might be helpful here.

D. W. JENKINS: This report you have given concerning possible selective effectiveness against resistant houseflies is extremely interesting. My own particular interest in *Empusa* was prompted by the consideration that, by disseminating heavily-labelled fungi—now possible with the aid of a suitable radioisotope—it would be possible to determine how widely the material is spread, through autoradiographs on suitably-placed X-ray film, and to check the effectiveness of the flies in disseminating the radioactive fungi through the population on release. Radioisotope technique would also be extremely valuable for showing the method of entry of the fungi into the insect, and so on. Altogether, I think that radioisotopes could be extremely valuable in this field.

M. S. QURAISHI: I should like to add one further comment. In Karachi we know that large numbers of flies breed in sewage pipes, encouraged by the thick consistency of the sewage, which has been brought about by failure of the water supply to keep pace with the recent enormous growth of population. In addition, many of the manholes are broken, providing easy access for oviposition. In some pipes there are larvae breeding by the hundred thousand. For some time past I have thought it might be a feasible idea to introduce *Empusa muscae* into the pipes, and if we can now make the fungus radioactive in order to trace its movement and pattern of establishment, there might be a real possibility of control here. Perhaps Dr. Jenkins would comment on this proposal.

D. W. JENKINS: I have no specific comments, but I think it would be very interesting to try this. There have, actually, already been enough studies with *Empusa muscae* to show that it has a real potential, and I might mention that a symposium was held on the use of various parasites, pathogens, and so on in Washington, D.C., in February of this year, the proceedings of which will be available through the American Institute of Biological Sciences. A large number of specialists participated and there was quite a lot of discussion on *Empusa*.

A. R. GOPAL-AYENGAR (India): Could Dr. Jenkins tell us whether any work is being done with radioisotopes in the United States on bookworm and book lice?

D. W. JENKINS: I know of none that has been done.

ПРИМЕНЕНИЕ РАДИОАКТИВНЫХ ИЗОТОПОВ ПРИ ИЗУЧЕНИИ ВОПРОСОВ ЗАЩИТЫ РАСТЕНИЙ

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Abstract — Résumé — Аннотация — Resumen

The use of radioisotopes and radiation in the field of plant protection. Extensive investigations are being carried out in the Soviet Union with a view to working out methods of using radioisotopes and ionizing radiation for the solution of theoretical problems, and for carrying out practical work in the field of plant protection.

Ionizing radiation is used for its effects on microorganisms, crop seeds, and insects. By means of the effects of ionizing radiation on microorganisms, strains of entomopathological fungi (*Beanveria bassiana* and *Aspergillus flavus*) of increased virulence have been produced. Sterilizing doses for stored products pests have been worked out and used as the basis for the design of a gamma de-insector.

The use of isotopes as tracers has made it possible to follow the dynamics of the movement of insecticides within plants and within the organisms of pests; to make a comparative evaluation of toxic agents having a systemic action; and to ascertain the duration of the toxic characteristics of such agents in plants and agricultural produce, which is very important for defining the safe time-limits for using toxic agents on agricultural crops. For studying the selective action of the 2,4-D herbicides, the concentrations of herbicides as governed by additions of mineral oils were determined.

The labelling of toxic chemicals with short-lived isotopes makes it possible to test the degree of effectiveness, for various crops, of airborne and ground techniques of dusting and spraying and also to ascertain the amount of toxic chemicals required per unit of area in relation to the crop grown, and to determine the effectiveness of various systems of dusting and spraying.

By means of marking agricultural pests with radioisotopes it is possible to study the migration of large-scale grain-crop pests and their parasites, to define the reservoir areas, the size of the populations, etc. The method also makes it possible to study the food cycles, to locate predatoriness and parasitism among insects, and also the preferences of predatory and parasitic insects with regard to their victims and hosts. Further advances along the lines described will hasten the solution of urgent problems connected with plant protection.

Emploi des radioisotopes et rayonnements pour la protection des végétaux. L'Union soviétique effectue d'importantes recherches en vue de mettre au point des méthodes permettant d'utiliser les radioisotopes et les rayonnements pour résoudre les problèmes théoriques et pratiques relatifs à la protection des végétaux.

Les rayonnements ionisants sont employés pour agir sur les micro-organismes, les semences de céréales et les insectes. En exposant les micro-organismes à l'action de rayonnements ionisants, l'auteur a obtenu des souches de champignons entomopathogènes (*Beanveria bassiana* et *Aspergillus flavus*) d'une virulence accrue. Il a déterminé les doses nécessaires à la stérilisation des espèces nuisibles vivant dans les silos à grains, et ces doses ont servi de base à l'étude d'un irradiateur gamma destiné à la lutte contre les insectes.

L'emploi de radioindicateurs a permis de déterminer la dynamique de la diffusion des insecticides dans les plantes et dans les organismes des espèces nuisibles, d'évaluer par comparaison les qualités des poisons agissant sur l'ensemble de l'organisme et d'établir la durée pendant laquelle ces poisons conservent leurs propriétés toxiques dans les végétaux et les produits agricoles, ce qui revêt une grande importance pour fixer des délais à observer lorsque les insecticides sont appliqués aux céréales. En étudiant les effets sélectifs des herbicides du type 2,4-D, l'auteur a également déterminé les concentrations de ces produits en fonction des diverses quantités d'huile minérale qui leur sont ajoutées.

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Le marquage des agents toxiques au moyen d'isotopes de courte période permet d'évaluer l'importance du traitement à appliquer aux diverses cultures par épandage aérien ou terrestre, de déterminer les quantités appropriées de produits toxiques à utiliser par unité de surface pour une culture donnée et de vérifier l'efficacité des pulvérisateurs de divers modèles.

Le marquage des espèces nuisibles aux cultures agricoles permet d'étudier les migrations massives de ces espèces et de leurs parasites, de découvrir les endroits où ils vivent en colonies, de déterminer l'importance des populations, etc. En outre, on peut ainsi examiner les problèmes des cycles alimentaires, étudier l'entomophagie et le parasitisme chez les insectes, et déterminer la préférence dont les entomophages et les parasites font preuve dans le choix de leurs victimes. Le développement ultérieur des recherches dans le sens indiqué hâtera la solution des problèmes les plus urgents que pose la protection des végétaux.

Использование радиоактивных изотопов и радиации в области защиты растений. В Советском Союзе в широком плане проводятся исследования по разработке методов использования радиоактивных изотопов и ионизирующих излучений при разрешении теоретических вопросов и практических задач в области защиты растений.

Ионизирующие излучения применяются для воздействия на микроорганизмы, семена сельскохозяйственных культур, насекомых. Путем воздействия ионизирующими излучениями на микроорганизмы были получены штаммы энтомопатогенных грибов (*Beauveria bassiana* и *Aspergillus flavus*) с повышенной вирулентностью. Установлены стерилизующие дозы для амбарных вредителей, которые положены в основу проектирования гамма-дезинсектора.

Применение изотопов в качестве индикаторов позволило проследить за динамикой распространения инсектицидов в растениях и организмах вредителей, производить сравнительную оценку качества ядов системного действия, устанавливать длительность сохранения токсических свойств ядов в растениях и сельскохозяйственной продукции, что имеет особо важное значение при определним допустимых сроков обработки ядами сельскохозяйственных культур. При изучении избирательного действия гербицидов 2,4-Д были установлены и гербицидные концентрации в зависимости от различных добавок минеральных масел.

Внесение в ядохимикаты изотопов с коротким периодом полураспада (в индикаторных количествах) позволит проверять качество обработки ядами различных культур при применении авиационного и наземного методов опрыскивания, а также устанавливать потребные нормы расхода ядохимикатов на единицу площади в зависимости от обрабатываемой культуры и определять эффективность работы опрыскивателей различных систем.

Метод маркировки радиоактивными изотопами вредителей сельскохозяйственных растений позволил изучить миграции массовых вредителей зерновых культур и их паразитов, определять места их резервации, численность популяций и т.д. Кроме того, метод дает возможность исследовать вопросы пищевых циклов, устанавливать хищничество, паразитизм среди насекомых, а также предпочитаемость у хищников и паразитов в выборе хозяина и жертвы. Дальнейшее развитие работ в указанных направлениях ускорит разрешение наиболее актуальных проблем в защите растений.

Empleo de los radioisótopos y de las radiaciones en la esfera de la protección de los vegetales. En la Unión Soviética se llevan a cabo amplios estudios con miras a establecer métodos que permitan emplear los radioisótopos y las radiaciones ionizantes para la solución de una serie de problemas teóricos y prácticos en la esfera de la protección de los vegetales.

Las radiaciones ionizantes se utilizan para irradiar microorganismos, semillas de cereales e insectos. Por exposición de microorganismos a las radiaciones ionizantes, se obtuvieron cepas de hongos entomopatógenos (*Beauveria bassiana* y *Aspergillus flavus*) de virulencia acrecentada. Se determinaron las dosis necesarias para la esterilización de especies nocivas que se desarrollan en los silos de cereales, lo cual permitió deseñar un desinsectador de rayos gamma.

El empleo de los radioisótopos como trazadores permitió estudiar la dinámica de la difusión de los insecticidas en las plantas y en los organismos de estas especies nocivas, evaluar por comparación las cualidades sistémicas de los agentes tóxicos, establecer la duración del período durante el cual éstos conservan sus propiedades tóxicas en los vegetales y en los productos agrícolas; este factor reviste considerable importancia para conocer los límites de tiempo que se han de respetar en la

aplicación de estos venenos a los cereales. Al estudiar la acción selectiva de los herbicidas del tipo 2,4-D, se determinaron asimismo las concentraciones de estos productos en función de las cantidades de aceite mineral añadidas.

La marcación de los agentes tóxicos con radioisótopos de corto período de semidesintegración permite determinar el grado de eficacia de los métodos de pulverización aérea y terrestre para distintos cultivos, así como la cantidad óptima de veneno por unidad de superficie en función del cultivo tratado y la eficacia de distintos modelos de pulverizadores.

El método que consiste en marcar con radioisótopos las especies nocivas permite estudiar las migraciones en masa de estos organismos, de sus parásitos, localizar los sitios en que forman colonias, la densidad de éstas, etc. También pueden estudiarse así los ciclos alimenticios, determinar la entomofagia y el parasitismo de los insectos, e igualmente la preferencia de los entomófagos y de los parásitos al escoger sus víctimas. La ampliación de estas investigaciones siguiendo esta dirección acelerará la solución de los problemas más urgentes que plantea la protección de los vegetales.

В Советском Союзе использование атомной энергии в мирных целях является государственной задачей. Исследования в этом направлении ведутся широким фронтом, охватывая различные области науки.

В области сельского хозяйства исследования по использованию атомой энергии возглавляет координационный Совет при Всесоюзной сельскохозяйственной академии имени В. И. Ленина. В планах Совета значительное место отводится исследованиям по применению радиоактивных изотопов и излучений в области защиты растений, имеющей чрезвычайно важное значение для сельского хозяйства страны.

В этих исследованиях ведущую роль выполняет Всесоюзный институт защиты растений.

Основными направлениями исследований в области использования радиоактивных изотопов и излучений в защите растений являются:

1. Применение радиоактивных изотопов в качестве индикаторов при изучении вопросов токсикологии насекомых, грызунов и интоксикации растений.
2. Определение остаточных количеств ядохимикатов в сельскохозяйственной продукции.
3. Изучение избирательного действия гербицидов.
4. Определение качества обработки сельскохозяйственных растений пестицидами при наземном и авиационном методах опрыскивания.
5. Изучение вопросов биологии вредных грызунов, насекомых, а также паразитов и хищников последних.
6. Использование радиоактивных излучений для воздействия на организмы в целях стимулирования процессов развития полезных и угнетения вредных организмов.

В исследованиях, относящихся к первым трем направлениям и имеющих производственное значение, используются преимущества метода радиоактивных индикаторов.

Радиоактивные изотопы в качестве индикаторов применяются при изучении проникновения и динамики распространения инсектицидов внутрирастительного действия в растениях, а также различных токсикантов в насекомых и грызунах [1]. Так, например, методом радиоавтографии была установлена картина проникновения инсектицидов в растения при нанесении их на листья или введении их через корни.

В институте был разработан метод сравнительной оценки инсектицидов, имеющих производственное значение.

Исследуемые по этому методу инсектициды вводятся в почву или наносятся на часть листьев растений. По истечении некоторого времени из листьев растений

приготовляются экстракты различными растворителями — водой, эфиром, ацетоном, спиртом и др., — которые в дальнейшем анализируются хроматографическим методом. Радиометрический анализ хромотограмм экстрактов, приготовленных через различные промежутки времени от начала опыта, позволят установить зависимость поступления яда в растение и его разложения от времени. Типичная зависимость накопления в растения неразложившегося токсиканта или образовавшихся токсических соединений изображена на рис. 1; для системного яда тина меркаптофоса — кривая I и для контактного яда типа паратион — кривая II. Максимумы этих кривых сдвинуты относительно друг друга. Причем максимум для ядов, обладающих системным действием, наступает раньше, чем для ядов контактного действия. Величина этих максимумов также различна. Если принять максимум кривой (I) за 100%, то для кривой (II) этот максимум будет составлять 17-20% от максимума для токсиканта системного действия.

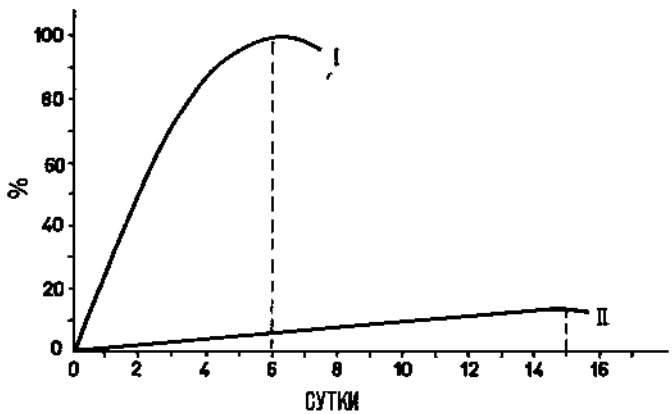

Рис. 1

Типичная зависимость накопления токсикантов в растениях:
I — яд системного действия; II — яд контактного действия

Дальнейшие исследования инсектицидов системного действия проводятся над растениями, корни которых помещаются в водные растворы, содержащие питательные вещества (по Кнопу) и испытуемые токсиканты. В опытах к водным растворам добавляются инсектициды, концентрация которых по отношению к общему объему раствора, составляет 0,05% с удельной радиоактивностью 1 мккюри на миллилитр раствора. По истечении шести суток, что находится в пределах максимума накопления токсиканта, установленного предыдущим опытом, корневая система растений обмывается в проточной воде и переносится в питательный раствор, не содержащий инсектицидов. Затем, методом радиохроматографии производится анализ экстрактов.

Такой метод позволяет установить длительность сохранения токсикантов в растении, т.е. стабильность этих токсикантов.

На рис. 2 приведена типичная кривая, показывающая зависимость содержания токсиканта в растении от времени. Эта кривая была получена с помощью хромотографического и радиометрического методов анализа опытных растений.

Одновременно с введением токсиканта в раствор проводился биологический контроль токсического действия инсектицида. С этой целью на листья растений подсаживались тли (*Myzodes persicae Sulz*) с определенной периодичностью.

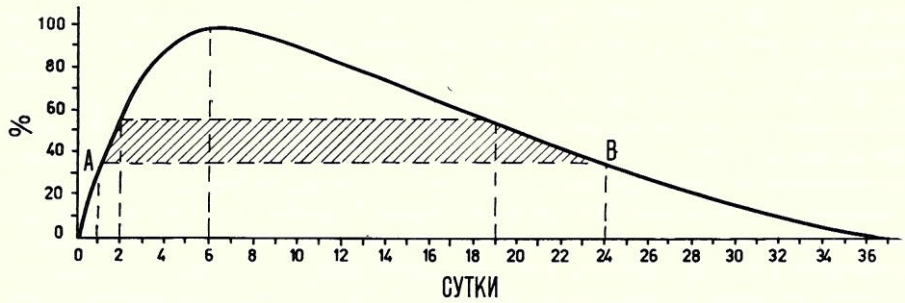

Рис. 2

Зависимость сохранения токсичности инсектицидами системного действия
АВ — токсический уровень

При данной концентрации раствора начало гибели насекомых происходит в интервале от 1 до 2 суток. Этому времени соответствует содержание поступившего токсиканта, достигающее от 35 до 45% максимума, изображенного на рисунке. В дальнейшем концентрация инсектицида в растении превышала токсическую дози-

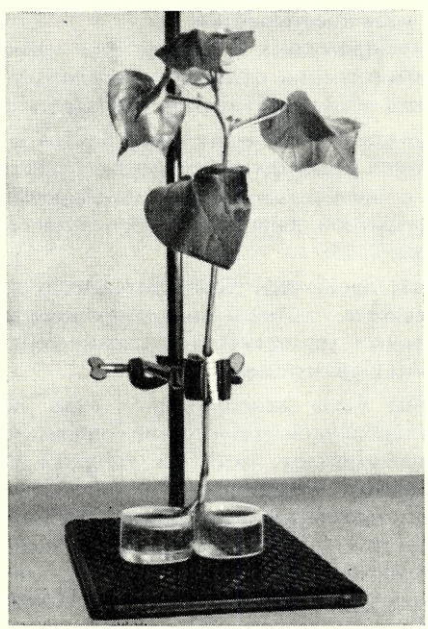

Рис. 3

Схема опыта для изучения выделения токсикантов корневой системой растения

ровку и только на 19-24 сутки концентрация инсектицида, вследствие его разложения, уменьшалась ниже токсического уровня АВ.

Однако, как показывал хромотографический анализ наличне убывающего неразложившегося инсектицида наблюдалось после 35 суток.

Убыль в растении токсиканта может быть отнесена за счет его распада вследствие процессов метаболизма, а также за счет выведения токсиканта корневой системой, что подтверждается следующим опытом. У нормального растения (Рис. 3) отмывалась от почвы корневая система и корни осторожно разделялись на две части. Затем эти корни помещались в два сосуда с питательным раствором Кнопа. В один из сосудов доливался 0,05%-ный раствор инсектицида, синтезированный с радиоактивным изотопом фосфора. Затем через каждые 30 минут из сосуда, где был только питательный раствор (без радиоактивных добавок) брались пробы, которые проверялись на радиометрической аппаратуре. Оказалось, что через час в этом растворе начала обнаруживаться радиоактивность, которая увеличивалась со временем. Через двое суток корни растения извлекались из сосудов, и в сосуд, в котором первоначально не было радиоактивности, вводились корни другого растения, на которое были подсажены тли. Через трое суток 90% тлей погибло. Это показывает, что корнями первого растения был введен в сосуд неразложившийся токсикант, который в дальнейшем поступил во второе растение и достиг токсической концентрации.

Приведенные данные показывают, что применение радиоактивных изотопов в качестве индикаторов дает возможность исследовать скорость проникновения ядов в растения, локализацию их в отдельных органах и длительность сохранения токсичности.

Изучение этих процессов дает возможность получить характеристику токсического действия ядов, особенно ядов нового синтеза.

Применение ядов, синтезированных с одним или несколькими изотопами, в сочетании с методом хромотографии позволяет исследовать те изменения, которые претерпевают токсиканты в результате процессов жизнедеятельности растений.

Большое значение в практике применения инсектицидов в защите растений имеет вопрос остаточных количеств ядохимикатов в сельскохозяйственных продуктах.

Опыты, проведенные с препаратами ядов, синтезированных с радиоактивным изотопом фосфора типа паратион, дали возможность установить скорость процессов гидролиза этих препаратов [2—5].

Эти процессы гидролиза происходят более интенсивно внутри растения, нежели на его поверхности, вследствие участия в данном процессе ферментов. Определение скорости гидролиза позволяет установить допустимые сроки предуборочной обработки растений для борьбы с вредителями.

Радиоактивные изотопы были использованы также при изучении вопросов избирательного действия гербицидов. Исследования, проведенные с гербицидом 2,4 Д, синтезированным с радиоактивным изотопом углерода C^{14}, дали возможность изучить процессы дыхательного обмена растений, обработанных гербицидом 2, 4Д. Были также установлены стимулирующие развитие растений дозы и гербицидные концентрации для препарата 2,4Д. Стимулирующие концентрации гербицида 2,4Д равны 0,0001% для неустойчивых растений и 0,005% для пшеницы и кукурузы. Гербицидные концентрации (2,4Д) равны 0,005% для неустойчив ыхрастений и 0,1% для пшеницы и кукурузы.

Для наиболее рационального практического использования препаратов в борьбе с вредными насекомыми необходимо всестороннее изучение токсических свойств

различных ядов. Разработка наиболее эффективных способов применения инсектицидов требует выяснения механизма их действия на вредных насекомых. Первым этапом в решении этой задачи должно быть исследование путей проникновения и динамики распределения в различных органах и тканях насекомых инсектицидов и продуктов их разложения.

Использование методов радиоактивных изотопов может значительно приблизить нас к пониманию указанных задач.

Опытами, проведенными с инсектицидом контактного действия роданоорганического соединения

$$ClCH_2CH_2OCH_2CH_2SCN,$$

а также с инсектицидом кишечного действия типа триэтилизотиофосфата

$$\begin{matrix} C_2H_5O \\ C_2H_5O \end{matrix} \!\!\rangle\, P - SC_2H_5 \;\; \begin{matrix} \\ \| \\ O \end{matrix}$$

была установлена динамика проникновения этих ядов в различные органы насекомых. В этих опытах в качестве объекта исследования служила азиатская саранча *Locusta migratoria L.*

Таблица 1

ДИНАМИКА РАСПРЕДЕЛЕНИЯ ИНСЕКТИЦИДОВ КИШЕЧНОГО И КОНТАКТНОГО ДЕЙСТВИЯ

Анализируемые ткани органов насекомого	Количество радиоактивных распадов из расчета 1 мг веса ткани									
	В живых обьектах, вскрытых после нанесения препарата на кутикулу через:					В живых обьектах, вскрытых после введения препарата через:				
	15 мин.	30 мин.	45 мин.	60 мин.	В мертвых через 24 часа	15 мин.	30 мин.	45 мин.	60 мин.	В мертвых через 24 часа
Зоб	0	0	0	2	9	1875	1275	804	591	73
Жевательный желудок	0	0	0	4	18	516	800	507	301	53
Пищеварительный желудок	0	0	0	6	19	309	578	317	237	82
Гемолимфа	11	26	42	46	54	3	17	27	38	49
Задняя кишка:										
I участок	0	6	9	27	75	21	67	75	91	125
II участок	0	0	5	21	51	0	14	31	75	96
III участок	0	0	0	11	21	0	0	0	25	38
Сердце	9	19	33	39	46	2	21	28	32	48
Нервная цепочка	0	2	9	13	37	0	3	11	17	31
Головной ганглий	0	0	0	3	7	0	0	0	2	11
Жировое тело	0	3	11	12	49	0	5	15	23	44
Половые органы ♂	0	0	3	5	39	0	0	5	9	38
Половые органы ♀	0	0	5	7	50	0	0	8	12	40
Мышцы бедра	0	0	0	0	7	0	0	0	2	11

Из приведенных данных видно, что препарат контактного действия, проникая через кутикулярные слои, попадает в гемолимфу, затем разносится с гемолимфой по организму насекомого и распределяется в органах и системах насекомого, концентрируясь в большей степени в органах с преимущественной функцией обмена веществ. С течением времени распространение препарата становится повсеместным, но преимущественная локализация токсиканта в наиболее физиологически активных органах сохраняется.

Следует отметить, что несмотря на отсутствие токсиканта в пищеварительной системе, токсикант появляется в переднем участке задней кишки. Этот факт может быть объяснен активной деятельностью выделительной системы — мальпигиевых сосудов — освобождающей организм от конечных продуктов обмена веществ.

Токсикант кишечного действия, введенный в насекомое, после поступления в кишечный тракт также быстро проникает в гемолимфу и распространяется по отдельным органам.

Данные о локализации токсикантов и продуктов их распада в отдельных органах при летальном эффекте могут служить сравнительными показателями эффективности действия различных ядов.

Дальнейшие более глубокие исследования в этом направлении должны сочетаться с весьма тонкими физиолого-биохимическими методами исследования.

В практике применения инсектицидов против вредных насекомых существенное значение имеет определение качества обработки сельскохозяйственных культур, установление оптимальных норм расходов инсектицидов и сравнительных характеристик опрыскивателей (машин) разных систем в целях разработки наиболее рациональной и экономичной системы обработки инсектицидами растений.

Для решения этих вопросов в СССР использован новый метод, основанный на применении радиоактивных изотопов, обладающих сравнительно коротким периодом полураспада, например, радиоактивного изотопа фосфора.

Метод заключается в том, что в рабочую жидкость, содержащую токсикант, вводятся небольшие добавки растворимого препарата. После тщательного перемешивания берутся пробы, в которых определяется удельная радиоактивность рабочей смеси, отнесенная к единице объема. После опрыскивания опытных участков инсектицидом растений, последним сообщается радиоактивность в индикаторных количествах. По величине радиоактивности, отнесенной к единице поверхности растения, и известной удельной радиоактивности пробы вычисляются микроколичества инсектицидов, осевших на различный частях растений или на почве. Применение бета-излучателей с малой энергией излучения, например типа S^{35}, позволяет определять раздельно концентрации инсектицидов на нижней и верхней поверхностях листьев, что чрезвычайно важно при детальной оценке качества обработки сельскохозяйственных растений.

Проведенные опыты по обработке различных культур (пшеницы, картофеля и др.) с применением указанного метода показали значительную неравномерность распределения ядохимикатов (рис. 4) как по ярусам растения, так и в горизонтальном направлении на обработанной площади. Этим вызывается необходимость внесения коррективов в конструкции машин и методы обработки.

По сравнению с существующими методами указанный метод является наиболее чувствительным и точным при определении микроколичеств инсектицидов. Метод дает также возможность производить сравнительную оценку удерживаемости фунгицидов на семенах при предпосевной обработке и на растениях в зависимости

от погодных условий и применения различных закрепителей (полимеров), смачивателей (поверхностно активных веществ).

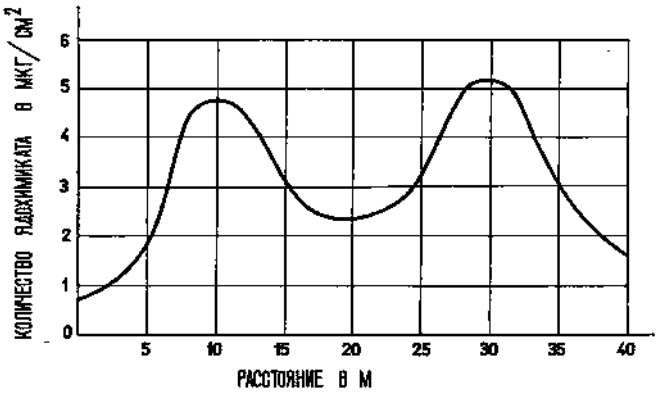

Рис. 4

Кривая, характеризующая распределение инсектицидов при обработке растений с помощью самолетов

Разработка эффективных мероприятий по борьбе с вредными насекомыми, составление прогнозов на массовое размножение вредителей сельскохозяйственных культур и установление карантинных зон опасных вредителей невозможно без всестороннего изучения биологии вредителей. Для ряда массовых вредителей многие вопросы их биологии являются недостаточно изученными. К числу таких вопросов относятся вопросы миграций насекомых, установление численности популяций, определение ареалов распространения вредителей, мест их резерваций в период зимовки и т. д.

Изучение указанных вопросов старыми методами не обеспечивает тех требований, которые предъявляются современными условиями исследований. Так, например, метод окраски насекомых различными красящими или люминесцирующими, составами является весьма трудоемким и не обеспечивает достоверности результатов наблюдений, вследствие сложности обнаружения окрашенных насекомых в природе. В связи с этим возникла необходимость в разработке новых, более совершенных методов исследования.

Наиболее перспективным в этом отношении является метод маркировки насекомых радиоактивными изотопами. Метод заключается в том, что опытным насекомым сообщается радиоактивность путем погружения их в радиоактивный раствор или питания радиоактив нойпищей. Маркированные насекомые расселяются в естественных условиях и затем диффузно распределяются среди насекомых, находящихся в природе. И по поведению меченых насекомых можно судить и о тех насекомых, которые находятся в природе.

По истечении времени, определяемого условием опыта, обнаружение маркированных насекомых производится с помощью специальных полевых радиометров, разработанных институтом, или же путем анализа ручных сборов в лаборатории на радиометрической аппаратуре. Для маркировки бабочек и паразитических мух в полевых условиях был применен метод самомаркировки их с использованием фото- и хемотаксисов. В опытах по маркировке бабочек, ведущих ночной образ жизни,

применялись специальные лампы, окруженные тканью, нижний край которой был погружен в радиоактивный раствор с добавками питательных и ароматических веществ. Привлеченные запахом и светом насекомые при соприкосновении с тканью, смоченной раствором, или питании этим раствором приобретали радиоактивность.

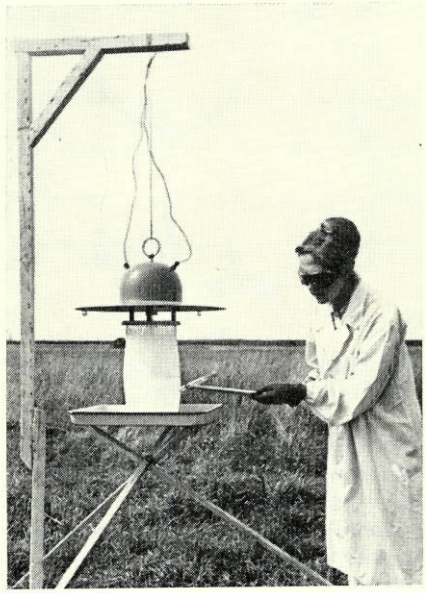

Рис. 5

Внесение радиоактивного изотопа в питательный раствор для самомаркировки насекомых

На рис. 5 изображен момент внесения в питательный раствор радиоактивного изотопа.

Отлов насекомых производился с помощью автоматических электроловушек, расставленных в поле на различных расстояниях в зависимости от условий .опыта. На рис. 6 изображены автоматические электроловушки, установленные для отлова насекомых.

Метод самомаркировки (*Hadena sordida Ekh*) и ее паразитов (*Pseudogonia cinerascens Rom* и *Menisus agnatus Grov*) позволил установить размеры миграции этих насекомых, вопросы дополнительного питания их и т. д. В результате проведенных исследований было показано, что бабочки способны разлетаться в безветренную погоду на различные расстояния от 1—3 и более км. Однако самцы являются более подвижными. Паразиты мухи (*Pseudogonia cinerascens Rom*) могут совершать полеты, превосходящие в 2—3 раза по дальности полеты бабочек. Установлено также, что паразиты в стадии имаго питаются на сорной растительности.

Применяя маркировку растений различными радиоактивными изотопами было установлено преимущественное питание этих паразитов сорными растениями (сурепка обыкновенная — *Barbarea vulgarih* (*R*) *Br*; горчица полевая — *Sinapis arvensis L*).

Метод маркировки картофельного жука (*Leptinotarsa decemlineota Say*) был применен также с целью определения плотности заражения посевов картофеля.

Рис. 6
Электроловушки для отлова насекомых

На рис. 7 изображен момент обнаружения маркированных жуков на посевах картофеля. Маркировка жуков радиоактивным изотопом желом (F^{59}), дающим жесткое гамма-излучение, позволяет вести наблюдения над жуком, находящимся в почве на глубине до 5-10 см. Помимо защиты растений в СССР метод маркировки был применен рядом авторов, в исследованиях с насекомыми — разносчиками инфекционных заболеваний (мухи, комары и т. д.) [6—11].

Метод маркировки насекомых радиоактивными изотопами, как показывают предварительные исследования, должен найти применение также при изучении

Рис. 7
Обнаружение маркированных колорадских жуков на посевах картофеля

вопросов, связанных с развитием биологического метода борьбы. К числу этих вопросов относятся: установление пищевых циклов, определение хищничества, паразитизма среди насекомых, а также определение предпочитаемости хищниками и паразитами хозяина и жертвы.

Большое значение приобретает использование радиоактивных излучений для целей защиты растений. В институте широким фронтом ведутся работы по облучению микроорганизмов, растений и насекомых. Для этих целей используются специальные установки, в которых в качестве источника применяется радиоактивный кобальт, активностью в 50 и 900 грамм-эквивалент радия (см. рис. 8).

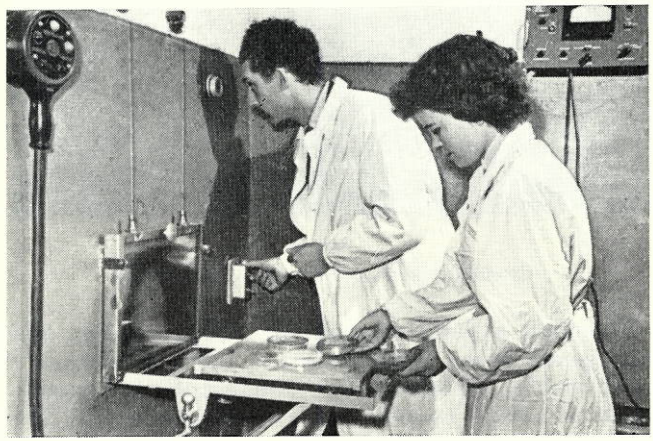

Рис. 8
Подготовка опыта по облучению энтомопатогенных микроорганизмов на гамма-установке

Путем облучения энтомопатогенных грибов (*Beauveria bassiana* и *Aspergillus flavus*) дозами порядка 1000—4000 рентген были получены новые штаммы этих грибов с повышенной вирулентностью. Эти штаммы испытывались в полевых условиях путем инфецирования массовых вредных насекомых (*Eurygaster integriceps put*). Штамм (*Beauveria bassiana*) дал резкое повышение смертности насекомых по сравнению с контролем [12].

Наряду с этим установлены необходимые дозы для половой стерилизации амбарных вредителей (*Calandra granaria* Z, *Calandra orysae* Z), которые находятся в интервале 7000—10000 рентген. Эти дозы положены в основу проектирования стационарных установок гамма-дезинсекторов для крупных зернохранилищ. Вопросу лучевой дезинсекции зерна посвящен ряд работ [13, 14, 15, 16, 17].

Приведенные данные и намеченные перспективы далеко не охватывают всех возможностей, связанных с применением радиоактивных изотопов и излучений в области защиты растений.

В целях дальнейшего успешного развития работ в этой области необходимо совместное усилие и координация исследований научных учреждений различных стран мира.

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DISCUSSION

F. P. W. WINTERINGHAM (United Kingdom): I was most interested in Dr. Andreev's experiment in which the root system was divided between two reservoirs. Could he, firstly tell me what labelled insecticide was used, and how rapidly radioactivity appeared in the second reservoir? The second part of my question is motivated by the fact that some years ago we did some experiments in which we labelled plants with S^{35} supplied by S^{35}-labelled

sulphate as the sole source of sulphur. The object of the experiment was to label some monocotyledonous seedlings with a view to placing them out in the open to study their survival in competition with rival strains. Our big difficulty was the surprising rapidity with which the sulphur not only was taken up by the root system but returned to the soil after transplanting, and I should be interested to know the rapidity with which this occurred with Dr. Andreev's insecticide.

S. V. ANDREEV: The insecticide used was mercaptophos. As regards the rapidity of appearance in the second reservoir, the first traces were detected after 15 min, and by the time an hour had elapsed the activity had become quite pronounced.

I might add that this experiment has great practical significance in connection with the use of systemic insecticides under field conditions. For example, cotton plants, as you may know, are planted in two's or three's. When insecticide is applied either from the air or from ground apparatus, one or more plants may happen to by masked by their neighbours, yet the insecticide has been shown to move into the masked plant through the interwoven root systems. This fact has important bearings on the use of systemic insecticides.

D. F. HEATH (United Kingdom): Are systemic insecticides also excreted from the roots after leaf treatment as they are after root treatment? In some experiments about 9 years ago Parks and I showed that, when the plants were grown hydroponically and the leaves were treated, Schradan and a number of non-insecticidal compounds were not excreted from the roots of brassica seedlings, nor some water-soluble related compounds from turnip seedlings. No experiments were carried out with compounds of the Systox type of insecticide.

S. V. ANDREEV: I will describe an experiment which illustrates penetration of insecticides into the root via the leaves of neighbouring plants. If, say, 6 or 10 plants are placed in one pot, fairly close together so that good interlacing of the roots is assured, and the leaves of one plant, separated from the rest, are then soaked in an active insecticide solution, activity will be observed in neighbouring plants after a certain interval. This is clear proof of root excretion, I think.

P. B. CORNWELL (United Kingdom): I should like to ask Dr. Andreev, and in fact the meeting generally, whether any work has been done using radioactively-labelled plant viruses.

S. V. ANDREEV: We have tried to use labelled viruses or spores of certain fungi, one of the *Tilletia* species in particular. Unfortunately, however, the results were not conclusive owing to the difficulty of achieving satisfactory concentrations of activity in either viruses or spores. I think therefore that such experiments must await the evolution of more efficient methods of labelling.

J. HALBERSTADT (IAEA, Scientific Secretary): Labelling of viruses has been done by Lydia Sverak in the chemical laboratory of the University of Vienna. Her work, using P^{32}- and C^{14}-labelled viruses and substrates, has been published in *Atompraxis*.

D. W. JENKINS (United States of America): There are, in fact, at least a dozen different viruses and several bacteriophages that have been labelled, using a variety of radioisotopes. The first labellings of an animal virus—an influenza virus actually—and of a bacteriophage were done in 1950, the former by Graham and McClellan. Several studies with plant viruses have also been carried out. Hamilton, in 1935, reported some work with *Myzus persicae* in connection with virus transmission from infected plants. Stanley, in 1942, was one of first to label tobaccco mosaic, In all, there are probably about twenty papers now available on bacteria-labelling using various methods.

P. PÉLEGRIN (France): I have two questions I should like to put to Dr. Andreev on a somewhat different subject: is irradiation of food permitted by the laws of the USSR and, if so, are irradiated commodities obtainable through normal commercial channels?

S. V. ANDREEV: There is no law prohibiting irradiation. With regard to commercial availability, I think it is true to say that no market in irradiated commodities for human consumption exists yet; we are still in the experimental stages of food sterilization by irradiation.

A. R. GOPAL-AYENGAR (India): This question is also on another subject: I wonder if Dr. Andreev could tell us something about the silkworm irradiation work that is going on in Tashkent to improve the quality of silk? We are particulary interested in this matter.

S. V. ANDREEV: Numerous experiments are in progress on the irradiation of cocoons. As you are aware, the killing of the chrysalides in the cocoons is normally achieved by suffocation through exposure to the sun's rays. The disadvantage of this method is the deleterious effect on the quality of the silk. We have found recently that powerful sources of gamma-rays are capable of yielding the desired result without this disadvantage, and also more quickly than with thermal treatment.

THE CHAIRMAN (K. K. Nair, India): Could you tell us the dose used?

S. V. ANDREEV: I think I am right in saying that it was in the range 7000—10000 r. The work is, of course, not being done at our establishment but at the sericulture institute in Tashkent.

P. J. DEORAS (India): Can Dr. Andreev tell us of any work done where radioisotopes are used for tagging bacteria and protozoa to study, for example, the deformation of blood due to plague, and the movement of malarial parasites in mosquitoes?

S. V. ANDREEV: Some work on mosquitoes has been done in the Crimea, but I do not think it has been published yet.

MARQUAGE RADIOACTIF DES FOURMIS DANS LES PLANTATIONS D'ANANAS

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Abstract — Résumé — Аннотация — Resumen

Radiolabelling of ants in pineapple plantations. Radiolabelling offers an ideal method of studying certain structural aspects of ant-cochineal associations (*Pheidole megacephala* [F] and *Pseudococcus brevipes*) in pineapple plantations. The special feature of radiotracers is that, contrary to the usual methods, it is possible with them to visualize exactly the dimensions of the nests and the ground their influence extends to, without disturbing the ecological and demographic equilibrium.

Marquage radioactif des fourmis dans les plantations d'ananas. La méthode de marquage radioactif convient parfaitement pour étudier certains aspects structuraux des associations fourmis-cochenilles (*Pheidole megacephala* [F] et *Pseudococcus brevipes*) dans les plantations d'ananas. L'apport original des radiotraceurs réside dans le fait qu'ils permettent, à l'encontre des méthodes habituelles, de visualiser sans ambiguïté et sans perturber l'équilibre écologique et démographique, les dimensions des nids et des territoires tenus sous leur dépendance.

Радиоактивное мечение муравьев на ананасовых плантациях. Метод радиоактивного мечения полностью подходит для изучения некоторых аспектов структуры сообщества муравьи-червецы (*Pheidole megacephale* [муравьи] и *Pseudococcus brevipes*) на ананасовых плантациях. Основное значение радиоактивных индикаторов состоит в том, что в противоположность обычным методам они позволяют, точно и не нарушая экологического и демографического равновесия, вести наблюдение над размерами гнезд и территориями их распространения.

Los trazadores radiactivos aplicados a las hormigas en las plantaciones de ananás. Los indicadores radiactivos pueden aplicarse con éxito al estudio de ciertos aspectos estructurales de las asociaciones de hormigas y cochinillas (*Pheidole megacephala* [F] y *Pseudococcus brevipes*) en las plantaciones de ananás. La originalidad del método basado en los indicadores radiactivos reside en el hecho de que, a diferencia de los procedimientos usuales, permite determinar sin ambigüedad y sin perturbar el equilibrio ecológico y demográfico, las dimensiones de los nidos y los radios de acción de sus poblaciones.

Introduction

L'association fourmis-cochenilles peut être considérée comme le facteur écologique le plus important qui favorise le développement et l'essaimage des colonies de *Pseudococcus brevipes* Ckll dans les plantations d'ananas. Cette cochenille provoque l'apparition sur la plante d'une maladie de flétrissures dite «Wilt de l'ananas», dont la cause ne nous est pas encore connue. On ne connaît pas bien les modalités selon lesquelles les fourmis interviennent, ni la liste exacte des espèces susceptibles de former des associations avec *P. brevipes*. Des observations montrent que certaines fourmis (*Pheidole*, *Camponotus*) construisent au pied des ananas des abris aériens ou hypogées à l'intérieur desquels elles entretiennent de véritables élevages de cochenilles dans des conditions favorables à un développement rapide de la colonie. Chaque nid de fourmis tient sous sa dépendance plusieurs pieds d'ananas.

L'envahissement progressif d'une parcelle par les cochenilles est ainsi étroitement lié au développement et à l'extension des colonies de fourmis associées. L'étude de l'espace occupé par une même colonie est rendue particulièrement délicate par le fait que, le plus

souvent, plusieurs colonies de la même espèce occupent des territoires adjacents et également par la présence de plusieurs nids secondaires éloignés du nid principal et difficiles à relier entre eux.

Le marquage radioactif des fourmis constitue sans aucun doute la seule méthode rapide, efficace et élégante pour résoudre ce problème. Le principe en est simple: le marquage étant effectué au niveau du nid principal, on détermine avec un détecteur de radiations la présence ou l'absence de fourmis radioactives au niveau des nids secondaires et des pieds d'ananas contaminés. On a ainsi les dimensions de l'espace occupé par la colonie. Si le rayonnement émis par le radioisotope est pénétrant, il sera possible de déceler la présence des fourmis qui se déplacent dans la couche superficielle du sol et, par là même, de visualiser les galeries qui relient les ananas entre eux.

Dans cette étude préliminaire, nous nous sommes fixés pour but la mise au point d'une technique de marquage radioactif des fourmis rencontrées couramment dans des plantations et sans préjuger de leur rôle dans la dispersion des cochenilles.

La première partie de ce rapport est essentiellement d'ordre méthodologique. Dans la seconde partie, nous donnerons les résultats de quatre expériences effectuées sur le terrain et nous discuterons des potentialités de la méthode pour les recherches envisagées.

Méthodologie

Toutes les expériences ont été faites dans la «Plantation de la presqu'île» située sur le terrain de l'Institut d'études et de recherches tropicales (IDERT) situé près d'Abidjan (Côte-d'Ivoire). Le terrain a été replanté dans les premiers jours de juin 1960; la qualité du sol est médiocre, mais la position enclavée de cette plantation au milieu de la forêt favorise une haute densité en fourmis parmi lesquelles plusieurs espèces sont susceptibles d'entrer en association avec les cochenilles. Les dimensions de la surface plantée sont 55 m sur 35 m, et les pieds d'ananas sont distants les uns des autres de 80 cm.

Technique de radiomarquage

Notre but étant de marquer le plus grand nombre d'individus d'un même nid sans en modifier ni les structures ni l'équilibre biologique, nous avons opéré par voie nutritionnelle en déposant à proximité de celui-ci une coupelle contenant un petit volume (5 à 10 cm^3) d'une solution du radiotraceur dans un mélange à volume égal de miel et d'eau. Les solutions titraient 0,86 mc/cm^3 pour les espèces de petite taille et 0,46 mc/cm^3 pour celles de grande taille. Après 24 h, les prélèvements effectués à proximité des nids nous ont montré que la quasi-totalité des fourmis étaient radioactives à un taux suffisant pour que les repérages sur le terrain se fassent sans ambiguïté.

Radioélément

Nous avons choisi le radiophosphore-32, fourni par le Commissariat à l'énergie atomique sous forme de phosphate monosodique en solution isotonique. L'activité de la solution-mère était de 2 mc/cm^3 à la réception.

Les considérations suivantes ont fixé notre choix:

a) Forme chimique facilement assimilable par les organismes vivants;

b) Emission de radiations bêta d'énergie maximum 1,7 MeV, donc aisément détectables avec un tube GM à fenêtre mince;

c) Période de désintégration de 14,3 jours assurant une décontamination des zones d'expériences.

Il va sans dire que le phosphore-32 n'est pas le seul radiotraceur qui puisse servir pour le marquage des fourmis.

MATÉRIEL ÉLECTRONIQUE

Nous avons utilisé le détecteur de radiations Philips PW 4014 muni d'une sonde GM d'épaisseur de fenêtre 2 mg/cm^2 et d'un écouteur auriculaire. L'appareil fonctionne avec une pile de 3 V qui assure une marche continue de 20 h.

Résultats expérimentaux

EXPÉRIENCE N° 1

Nous avons d'abord choisi de travailler avec *Pheidole megacephala* (*F*), espèce abondamment répandue dans la plantation et qui vit en association avec *Pseudococcus brevipes*. Le nid principal est relié à des nids secondaires situés dans les massifs radiculaires des

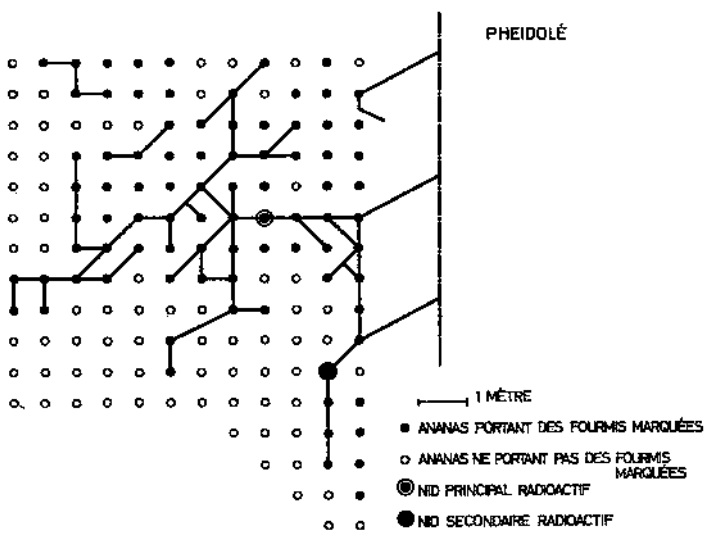

Fig. 1
Expérience n° 1.

ananas par des galeries généralement couvertes. La solution radioactive a été déposée près du nid principal. Un prélèvement effectué après 24 h indique que 100% des fourmis sont marquées. Nous avons décelé la présence de fourmis radioactives sur quatre-vingt sept pieds d'ananas également répartis autour du nid principal. Les plantes contaminées sont reliées les unes aux autres par un réseau dense de galeries plus ou moins superficielles. Toutefois, la présence de fourmis marquées sur des pieds apparemment isolés laisse à penser qu'il existe également des galeries profondes. Trois chemins parallèles qui partent de la zone contaminée et s'enfoncent sous le couvert forestier pourraient constituer des voies de contamination permanente. Nous avons acquis la certitude que la structure des galeries ainsi que les dimensions de l'espace colonisé se modifient dans le temps; cette question mérite d'être l'objet d'une étude ultérieure approfondie.

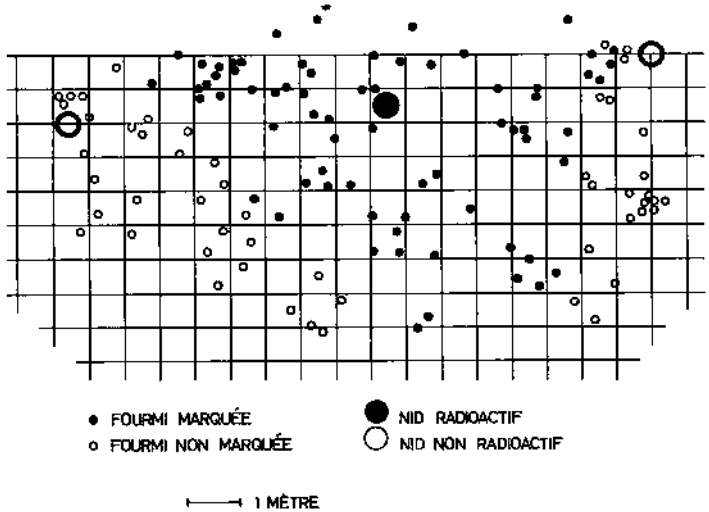

Fig. 2
Expérience n° 2.

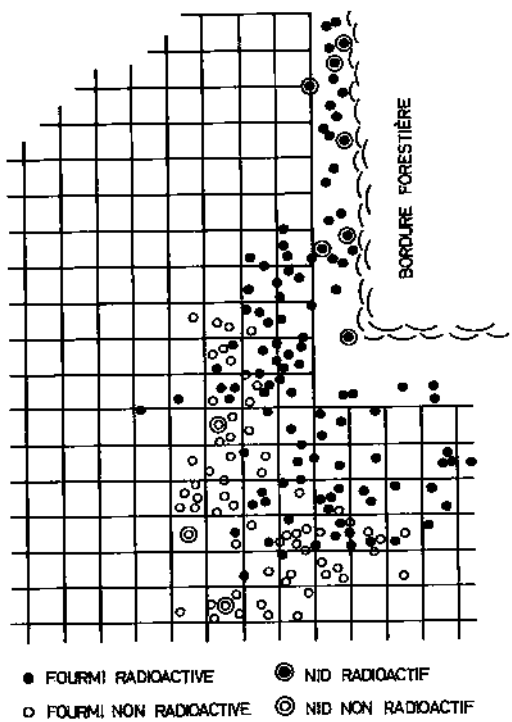

Fig. 3
Expérience n° 3.

EXPÉRIENCES N° 2 ET 4

Il s'agit dans les deux cas d'une myrmicinée actuellement en cours d'identification. Elle ne semble pas entrer en rapport direct associatif avec la cochenille de l'ananas, mais il n'est pas exclu qu'elle joue un rôle dans la biocœnose du champ. La population d'un nid est peu nombreuse; ce dernier, assez profondément enterré, débouche en une sortie étroite s'évasant en un entonnoir d'une dizaine de centimètres de diamètre. Les nombreux débris d'insectes trouvés à proximité immédiate ne laissent aucun doute sur les habitudes de chasse de cette espèce. Nous avons analysé au détecteur toutes les fourmis trouvées dans un rayon de 7 à 8 m autour du nid radioactif et noté leurs coordonnées. L'ensemble des résultats figure sur les graphiques 2 et 4. On y remarque que chacun des nids possède un territoire de chasse bien délimité sans interpénétration de l'un par l'autre.

EXPÉRIENCE N° 3

Fourmi noire, en cours d'identification, assez fréquente dans la plantation de la presqu'île. Le marquage radioactif a été fait au niveau du nid principal situé à l'angle de la bordure forestière (voir le graphique 3). Dans un périmètre de quelques mètres, nous avons noté la présence de neuf autres nids, dont six étaient occupés par des fourmis marquées. Il est à noter que ces derniers sont tous situés entre le couvert forestier et la première rangée de pieds d'ananas. En surface, ces fourmis circulent individuellement ou par petits groupes. Les territoires ne sont pas aussi strictement limités que dans les cas précédemment rapportés. Les communications entre les divers nids sont vraisemblablement souterraines. A remarquer que l'absence totale d'individus dans la partie supérieure du graphique est sans doute en relation avec la présence dans cette partie de la plantation d'une importante colonie de *Pheidole* étudiée dans l'expérience n° 1.

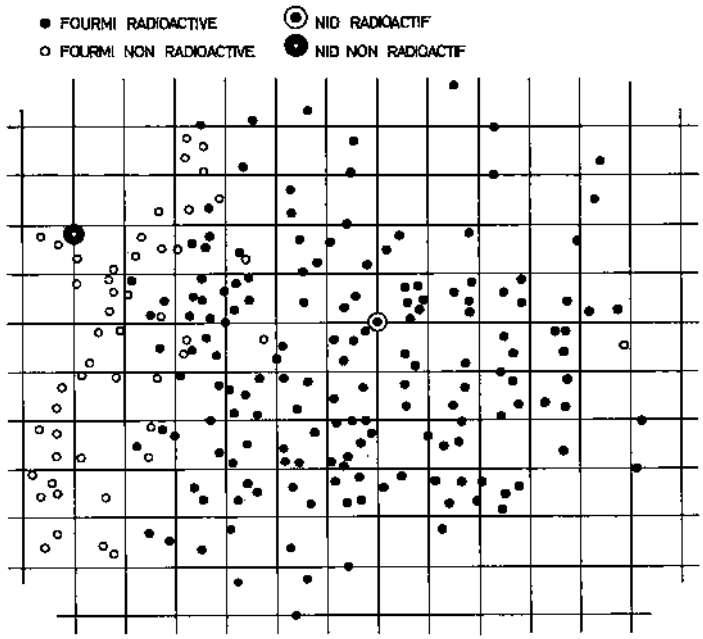

Fig. 4
Expérience n° 4.

DISCUSSION

W. KLOFT (Federal Republic of Germany): In connection with this very interesting paper by Dr. Mortreuil, I should like to recall that Gösswald and I (*Zool. Beiträge*, **5** (1960) 519—556) have made a number of contributions to this subject, using radiotracers. In particular, I would point out that there are big differences between the various subfamilies. For example, in the primitive subfamily *Ponerinae* the workers take up food on an individual basis for their own nourishment only, and in this case marking of the whole nest would therefore not be possible by Dr. Mortreuil's method. With *Pheidole* and *Camponotus* on the other hand, it works very well. I would also like to point out that there are a number of species in which different colonies maintain an extremely active interchange of nutriment with one another. For example, with species of the genus *Formica* we have been able to demonstrate the exchange of labelled nutriment over distances of 200—300 m.

It is also a fact that individuals from different colonies (even from different species) exchange food when they meet in the open. This act of offering food here represents a "peace-offering", and is also a potential source of error in studies of population movement and behaviour. As I said, however, the method described is an excellent one for work with *Pheidole*.

F. P. W. WINTERINGHAM (United Kingdom): Perhaps the very fact of being something of an outsider on such subjects as the use of tagged insects suggests to me an important point which I rather suspect is not receiving all the attention it should. On the one hand, we are stressing the great potentialities of using tagged specimens for studying the habits and behaviour of insects, and there is no doubt that such applications will increase. On the other hand, however, we ascribe great value and importance to the adverse effects of absorbed radiation on the longevity, fertility, survival, etc. of insects. All the studies discussed, however—and this is the point—make the sweeping assumption that the radioactive tag itself has no effect.

Although it is my *guess* that the radiation doses received by the insects during most ecological studies have been within the safe ranges, are we *sure* of this? I am open to correction, but it does seem to me that in many cases no attempt has been made to estimate the radiation dose received by the insects during the experiment. There are then insufficient grounds for assuming that the radioactive label itself has had no effect on behaviour.

May I suggest, therefore, that a little more attention be given to the radiation dose received? Let us not take it too much for granted that the radioactive label itself is without significant effect under all conditions.

P. B. CORNWELL (United Kingdom): I am entirely in agreement with Dr. Winteringham's suggestion that we should pay more attention to studying the effects of the label on the behaviour and subsequent physiology of the insect. Many labelling studies are designed to investigate mobility in the first instance; I think one should here attempt to carry out, alongside the field studies, some experiments to determine whether, in fact, there is any effect on mobility even under laboratory conditions.

Of course, in tagged-insect studies carried out over a long term in the field, the insect is subject to chronic rather than acute irradiation; and we do know that chronic irradiation has less adverse effects. I would think that at very low levels of irradiation we are mainly concerned with genetic aberrations, rather than any major upset in physiology.

There is a possibility, not yet fully investigated, of using an alternative technique as a labelling process: it has been suggested that we should attempt to label insects with inactive elements which, owing to their high cross-section for neutron capture can be subsequently activated — an indirect use of the radiotracer technique. We would not, of course, use

detection-equipment in the field to study distribution; with samples taken from the field, however, it would be possible to assess the proportion of labelled insects and carry out population studies in this way. This technique obviates any criticism that the label adversely affects the insect by irradiation, but it would still be necessary to ensure that the inactive element has no toxic properties.

D. W. JENKINS (United States of America): In answer to Dr. Winteringham's remarks on the effects of radiation on the insect and the extent to which this has been studied, the best work I know of on the subject is an excellent study of P^{32} and *Drosophila* carried out at Brookhaven by R. C. King. This analyses the amount of radiation received by the various tissue areas, and gives the total internal irradiation. I think that it fully covers the subject as far as *Drosophila* is concerned.

With regard to the effects of radiation on behaviour, longevity and fecundity of insects, we carried out some laboratory experiments with mosquitoes to try and determine such effects before proceeding to our extensive field work. We found that there was a certain threshold above which there were discernible effects. Therefore all our experiments were carried out using a level of activity, as I recall, less than this threshold by a factor of about 10.

I believe that very detailed studies of this kind were carried out in connection with the screwworm project. Perhaps Dr. Weidhaas could comment on these.

D. E. WEIDHAAS (United States of America): At Orlando, as I recall, some quite detailed studies on the screwworm flies were carried out by Bushland and others to determine whether there was any effect of the radiation dose used—6000 r, I believe—on longevity, fecundity, and behaviour. I am not familiar with the details, but the result was that no effects were observed at the dose stated.

F. P. W. WINTERINGHAM: As a matter of fact I myself now recall a case of this type of experiment. I think that Dr. Lindquist did an experiment using a duplicate technique. He marked what I believe were houseflies with P^{32} and made a study of their migration from the point of release; at the same time he marked other specimens with a fluorescent dye, which provided an alternative method of detection. As far as I recall he was unable to detect any significant difference in migration.

However, I think the point I was trying to make is still valid—and I must say I was very glad to hear Dr. Jenkins mention that there was a threshold not exceeded in experiments at his laboratory. Radioisotope experiments have been and are being made, however, in which no indication of the dose is given at all. We must not get too much into the habit of assuming that, whatever the dose or isotope used, there will be no effect on behaviour. I suspect that in some experiments there definitely must be such an effect. As you know, we use radioisotopes for quite a different purpose, and we have to be very careful, even in our physiological and biochemical studies, not to exceed a dose which will most certainly affect the well-being of the insect.

S. V. ANDREEV (Union of Soviet Socialist Republics): Before using radioisotopes for studies of the biological characteristics of insect pests we carried out exhaustive tests on the effects of chronic irradiation on the insect organism. These tests were performed under both laboratory and field conditions, and showed that the amount of radiation finding its way into the body of the insects was only a fraction of a microcurie and had no detectable effect on behaviour, fertility or longevity.

P. PÉLEGRIN (France), who presented the paper: As will be seen from Dr. Mortreuil's paper, the level of activity of the solution was 2 mc/cm^3. I do not know whether or not this would represent a radiation hazard for the ants; it depends, of course, on how much radiation they absorb. At all events I imagine the author was bearing this factor in mind.

SECTION 2

LABELLED INSECTICIDE STUDIES

METABOLISM OF ORGANOPHOSPHATE INSECTICIDES BY PLANTS: A REVIEW

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Abstract — Résumé — Аннотация — Resumen

Metabolism of organophosphate insecticides by plants: a review. A thorough knowledge of the fate of insecticides on or in plants is necessary for their safe use in the control of phytophagous pests. To evaluate the potential hazard of residues to consumers of treated crops, "residue dissipation curves" for thousands of insecticide-crop-environment combinations have been established. The analytical methods employed include almost all forms of bioassay, chemical and radiochemical techniques which can be adapted to microgram amounts of the insecticide. In many cases the insecticide is absorbed and subjected to the diverse biochemical processes of the plant. Radioisotopes provide the best method for studying the metabolic fate because of the rapidity and sensitivity of analysis and the ability to provide a "balance sheet" for the total residue dissipation due to physical loss and chemical changes. Slight structural changes in these compounds can result in a great change in the toxicity to mammals. The insecticide may disappear within a few hours after application to plants, but toxic metabolites may persist for weeks or even months. Specific analytical procedures for the compound administered may not detect metabolites of toxicological significance.

The studies on the metabolism of inorganic, botanical and chlorinated hydrocarbon insecticides in plants have generally been made without the use of radioisotopes. It is only with the organophosphates that radiolabelled compounds have been fully, and even dramatically, utilized. These radiotracer studies have been a major factor in the rapid development of the background knowledge essential to the safe use of systemic insecticides.

The current knowledge of the metabolic fate of insecticides in plants will be reviewed with particular attention to the synthetic routes available for labelling organophosphates with P^{32}, and the use of these compounds in metabolism studies.

Etude du métabolisme des organophosphates insecticides dans les plantes. Pour utiliser sans danger les insecticides dans la lutte contre les insectes phytophages, il faut savoir exactement ce que deviennent les insecticides répandus sur les plantes ou absorbés par elles. Afin d'évaluer les risques que la présence de résidus comporte pour les consommateurs des végétaux traités, on a établi des «courbes de dispersion des résidus», pour des milliers de combinaisons insecticide-plante-milieu.

Les méthodes analytiques employées comprennent presque tous les genres de techniques d'analyse biologique, chimique et radiochimique applicables à des quantités d'insecticide de l'ordre du microgramme. Souvent l'insecticide est absorbé et soumis aux différents processus biochimiques de la plante. L'emploi des radioisotopes constitue le meilleur moyen d'étudier les cycles du métabolisme en raison de la rapidité de l'analyse, de la sensibilité de la méthode et de la possibilité de dresser un bilan de la dispersion totale des résidus par perte de matière et transformations chimiques. De légers changements de structure intervenant dans ces composés peuvent en modifier considérablement la toxicité pour les mammifères. Il se peut que l'insecticide disparaisse quelques heures après avoir été administré aux plantes, mais que des métabolites toxiques persistent pendant des semaines, voire des mois, et que des méthodes spécifiques d'analyse du composé administré ne suffisent pas pour déceler des métabolites toxiques.

Le métabolisme des insecticides — produits minéraux ou végétaux ou hydrocarbures chlorés — dans les plantes a généralement été étudié sans faire appel aux radioisotopes. On n'a utilisé les composés marqués pleinement, et même d'une manière spectaculaire, que pour les organophosphates. Les études faites au moyen des radioindicateurs ont notablement contribué au développement rapide des connaissances de base nécessaires pour employer les insecticides systémiques sans danger.

L'auteur fait le point de connaissances sur les cycles du métabolisme des insecticides dans les plantes, compte tenu notamment des procédés de synthèse dont on dispose pour marquer les organophosphates au phosphore-32 et de l'emploi de ces composés dans les recherches sur le métabolisme.

Метаболизм органофосфатных инсектицидов у растений: Обзор. Исчерпывающие знания о судьбе инсектицидов, находящихся на растениях или внутри их, необходимы для их безопасного использования в борьбе против питающихся растительной пищей вредителей. Для оценки потенциальной опасности, которую представляют собой остатки инсектицидов для потребителей обработанного ядом урожая, установлены „кривые утечки остатков" для тысяч комбинаций окружающей среды обработанного инсектицидом урожая. Применяемые аналитические методы включают почти все формы определения силы биологического препарата, химические и радиохимические методы, которые могут применяться к выражаемым в микрограммах количествам инсектицида. Во многих случаях инсектицид поглощается и подвергается в растении отличным друг от друга биохимическим процессам. Радиоизотопы дают наилучший метод для изучения метаболической судьбы инсектицида вследствие быстроты и чувствительности анализа и способности обеспечивать „баланс" для общей утечки остатков благодаря физической потере и химическим изменениям. Незначительные структурные изменения в этих соединениях могут привести к большому изменению в токсичности для млекопитающих животных. Инсектицид может исчезнуть в течение нескольких часов после применения к растениям, но токсичные метаболиты могут сохранять свое действие в течение недель и даже месяцев. Специфические аналитические процедуры для применяемого соединения могут не обнаружить метаболитов токсилогического значения.

Исследования метаболизма неорганических, ботанических и хлорзамещенных углеводородных инсектицидов в растениях в основном проводились без использования радиоизотопов. Меченые радиоактивные соединения были полностью и даже драматически использованы только с органофосфатами. Эти исследования с радиоактивными индикаторами являются главным фактором быстрого накопления исходных данных, имеющих существенное значение для безопасного использования инсектицидов определенных систем.

Существующие знания в области метаболической судьбы инсектицидов в растениях будут рассмотрены с особым вниманием по отношению к имеющимся синтетическим способам мечения органофосфатов фосфором-32 и использованию этих соединений в изучении метаболизма.

Estudio panorámico del metabolismo de los insecticidas de fosfatos orgánicos en las plantas. Para poder utilizar sin riesgos los insecticidas en la lucha contra los insectos fitófagos, es necesario conocer en detalle el destino de los insecticidas aplicados a las plantas o absorbidos por las mismas. A fin de evaluar los riesgos que la presencia de residuos entraña para los consumidores de productos alimenticios que han sido sometidos a tratamiento, se han determinado "curvas de dispersión de los residuos" para millares de combinaciones insecticida-planta-medio ambiente. Los métodos analíticos empleados abarcan casi todas las técnicas químicas, radioquímicas y biológicas capaces de ser aplicadas a cantidades de insecticida del orden del microgramo. En muchos casos, el insecticida es absorbido y participa en los diversos procesos bioquímicos de la planta. Los radioisótopos ofrecen el método más adecuado para estudiar los ciclos metabólicos, debido a la rapidez y sensibilidad del análisis y a la posibilidad de establecer un balance de la dispersión total de los residuos desaparecidos como consecuencia de pérdidas materiales y de transformaciones químicas. Ligeros cambios de estructura en esos compuestos pueden traducirse en una considerable modificación de su grado de toxicidad para los mamíferos. El insecticida puede muy bien desaparecer pocas horas después de ser aplicado a las plantas; en cambio, es posible que los metabolitos tóxicos subsistan durante semanas e incluso meses, y que los métodos analíticos concretos aplicados al compuesto que se haya administrado no permitan detectar metabolitos de importancia toxicológica.

El metabolismo que sufren en las plantas los insecticidas minerales, vegetales y a base de hidrocarburos clorados se ha estudiado sin recurrir a los radioisótopos. Solamente con los fosfatos orgánicos se han empleado los compuestos marcados en gran escala, y a veces con resultados espectaculares. Estos estudios con trazadores han contribuido poderosamente al rápido desarrollo de los conocimientos básicos esenciales para utilizar sin riesgos los insecticidas sistémicos.

Se examinan los conocimientos actuales sobre los ciclos metabólicos de los insecticidas en las plantas, prestándose especial atención a los procedimientos de síntesis que pueden utilizarse para marcar los fosfatos orgánicos con ^{32}P, así como al empleo de estos compuestos en los estudios sobre metabolismo.

Introduction

A thorough knowledge of the fate of insecticides on or in plants is necessary for their safe use in the control of phytophagous insects. The potential hazard of residues to consumers of insecticide-treated crops is estimated from a combined consideration of the amount of insecticide used and the method of application, the rate of residue dissipation and the chronic toxicological data available on the compound.

Almost all forms of bioassay, chemical and radiochemical techniques that can be adapted to microgram amounts of insecticides have been used in residue analysis. Radioisotopes provide the best method for studying the metabolic fate of the insecticide because of the rapidity and sensitivity of analysis and the ability to provide a total accounting of the radiolabelled residue dissipated by physical loss and chemical changes. Radiolabelled compounds are also advantageous for evaluating residues when other suitable analytical methods are not available or when a number of metabolites must be simultaneously determined.

In many cases the insecticide is absorbed and subjected to the diverse biochemical processes of the plant. The products formed are sometimes different from those resulting from the usual procedures of chemical degradation of the insecticide. Both the rate and route of metabolism may vary with plant species and conditions altering the physiological activity of the plant. Slight structural changes in the insecticide effected by plant metabolism can result in a great change in the toxicity to mammals, or in the anticholinesterase activity as with the organophosphates. The insecticide may virtually disappear within a few hours of application to plants, but toxic metabolites may persist for weeks or even months. Analytical procedures, specific for the compound administered, may not detect such metabolites.

Many reviews on insecticide metabolism and translocation in plants [1—15] provide background information for the present discussion.

I. Metabolism of insecticides other than the organophosphates

Data are available on the persistence of almost all the insecticides on a variety of crops. The studies on the metabolism of inorganic, botanical and chlorinated hydrocarbon insecticides in plants have generally been made without the use of radioisotopes. Exceptions are a brief study on C^{14}-benzene hexachloride metabolism in wheat seedlings [16] and experiments on the biosynthesis of such botanical insecticides as nicotine [17] [18] and pyrethrins and cinerins [19].

II. Synthesis of radiolabelled organophosphates

Radiolabelled insecticides have been fully, and even dramatically, utilized in studies on the metabolism of the organophosphates in plants. These radiotracer studies have been a major factor in the rapid development of the background knowledge essential to the safe use of systemic insecticides. As a direct result of the need for information on the metabolism of the organophosphates in plants and other organisms, considerable progress has been made in preparing radiolabelled compounds of high specific activity.

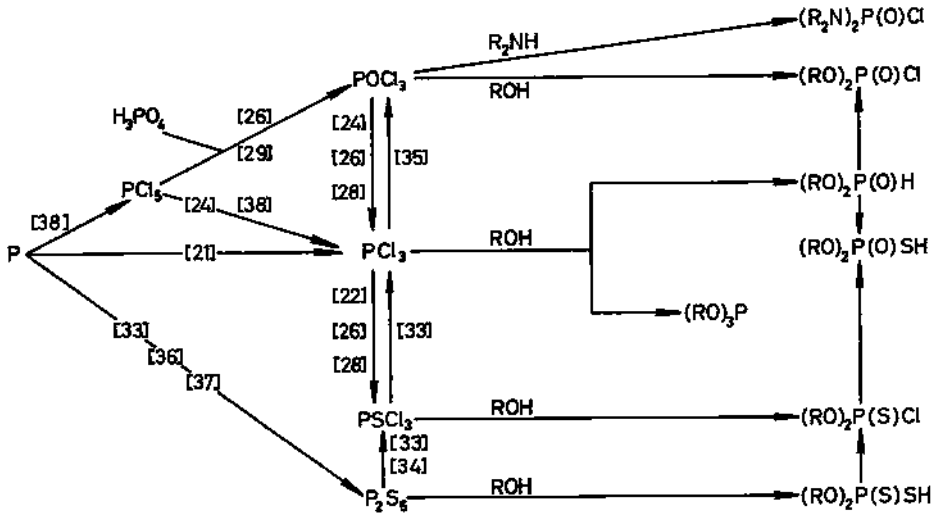

Fig. 1

Routes of synthesis for inorganic and organic P^{32} intermediates useful in preparing radiolabelled organophosphate insecticides (modified from Mühlmann and Schrader [20] with numbers referring to the literature

Certain of the routes for interconversion of the radiolabelled intermediates are given in Fig. 1. This scheme is modified from one presented by MÜHLMANN and SCHRADER [20]. Many of these P^{32} compounds are commercially available. Neutron irradiation has been used for direct labelling of red phosphorus [21], phosphorus trichloride [22] and phosphorus pentasulphide [23]. This method is often inconvenient, may yield mixed labelling, and the specific activity of the compounds prepared from these irradiated intermediates is usually not greater than 5 mc/g. As a starting material, P^{32}-labelled phosphoric acid offers the advantage of being readily available at a high specific activity. It can be converted by rather laborious procedures via iron, calcium or silver salts to phosphorus oxychloride [24—28], or more simply by reacting the phosphoric acid directly with phosphorus pentachloride [26] [29]. Other methods of limited applicability can be used to prepare organophosphates from phosphoric acid or phosphorus pentoxide without going through chlorinated phosphorus intermediates [30] [31]. Isotope exchange can also be utilized with P^{32}-labelled phosphoric acid to label inefficiently thiophosphoryl chloride [32] [33], but readily form P^{32}-labelled phosphorus pentasulphide [33]. The efficiency of this latter isotope-exchange procedure provides a very convenient starting material for preparing many radiophosphorus intermediates [33].

The actual reaction conditions for converting the inorganic to organic intermediates, as shown in Fig. 1, and subsequently to the insecticides can be readily interpreted from the literature cited in Table I. Specific activities well above 300-mc/g compound can be obtained via P^{32}-labelled phosphoric acids, so that amounts of radiolabelled compounds in the 10^{-9} to 10^{-10}-g region can be readily detected. Extreme caution must be exercised in the synthesis of compounds of such high specific activity to ascertain that the radiolabel is on the molecule intended and that the isotope does not accelerate the rate of certain chemical changes that are being followed in metabolism studies, nor lead to formation of derivatives that would not be formed by the same compound lacking the radioisotope.

III. Metabolism of organophosphate insecticides in plants

The studies involving radiolabelled materials are summarized in Table I. At least a dozen completed studies are not included in Table I as they are not published in readily accessible form but, rather, have been carried out in industrial laboratories or on contract with these laboratories where the results have had limited distribution. In most cases, the P^{32} isotope was used. The purity and even the structure of the radiolabelled compounds used in certain of these studies is questionable. Distillation, crystallization and preparative column chromatography, along with infra-red spectral studies, have been used in purifying the radiolabelled compounds, with paper chromatography reserved as a check on the purity attained.

Many of the absorption and translocation studies are difficult to interpret as total radioactive counts or radioautographs were utilized, and no attempt was made to distinguish between the insecticide administered and its hydrolysis products. Such studies are only valid when the *in vivo* stability of the molecule is established and critically considered in the experimental design. The studies on absorption and translocation have contributed greatly to the concept of what type of molecules will penetrate plant roots and cuticular epidermis and the method of formulation to allow the molecule either to penetrate or remain on the surface of the plant.

Hydrolysis studies are useful in evaluating the persistence of the insecticide in plants for both insect control and as a potential residue hazardous to man and animals consuming the treated crop. The hydrolysis products have been shown in many cases, and are otherwise generally presumed, to be non-toxic. The extent of hydrolysis of the organophosphate has been almost routinely evaluated by aqueous-organic extractions designed to leave the hydrolysis products in the water and the unhydrolysed compounds in the organic phase. Chloroform is usually the solvent chosen. This procedure is adequate when due consideration is accorded to the organo-soluble hydrolysis products or the plant derivatives formed from them. Carboxylic rather than phosphoric ester hydrolysis may yield products extracting into chloroform if the hydrogen-ion concentration of the aqueous phase is not suitably adjusted. Special precautions are also necessary when P^{32}-labelled phosphoric acid is formed on the degradation of the insecticide in the plant, since it is partially reincorporated into plant fatty-phosphoric esters, such as lecithin, which are organosoluble.

Non-hydrolysed metabolites have been separated and tentatively identified by their antiesterase activities, partitioning properties and R_f-values in selected paper-chromatographic systems. All too frequently the presumed characterization of products has been made without isolation of amounts sufficient for adequate spectral and degradation examination. The nature of these non-hydrolysed metabolites is of critical importance in designing a residue analysis method to evaluate the hazard of the treated crop. Many cases are known where a sensitive specific analytical method for the administered compound failed to detect residues when the plants were still toxic to insects and mammals because of these non-hydrolysed metabolites.

The great variation with plant species and physiological state in the absorption, translocation and metabolism is evident from the studies presented in Table I. The increasing number of organophosphorus compounds found to be active as nematocides, fungicides and herbicides will result in further metabolism studies on an even greater variety of molecules. It seems surprising that with all the work on relatively complex molecules, little information is available on the plant metabolism of the simple trialkyl phosphites, phosphates and thiophosphorus compounds or of their mono- or diester-hydrolysis products. Economic necessity for studying the pesticides is undoubtedly the explanation, but much could be learned by the use of the simpler model compounds. It is hoped that more frequent

attempts will also be made to study the plant enzymes effecting these metabolic reactions, rather than merely to confine the studies to establishing the products formed *in vivo*. A recent study [99] has shown that Parathion hydrolysis and oxidation are catalysed by the enzyme peroxidase in the presence of a suitable hydrogen donor.

The plant metabolism of organophosphates is accomplished primarily through oxidation and hydrolytic attack. Phosphorothionate groupings are oxidized to their corresponding phosphate analogues (compounds 13, 22, 24, 25 and 30, plus limited evidence on 15, 26, 31 and 32 from radiotracer studies and 27 from anticholinesterase studies). Sulphides are oxidized rapidly to sulphoxides and more slowly to sulphones (compounds 7, 8, 19—22, 24, 25 and 29). Certain *N,N*-dimethyl phosphoramides are oxidatively demethylated, probably through intermediate *N*-oxide and *N*-methylol derivatives (compounds 33—41). Enzymatic hydrolysis in plants is effected by cleaving the acid anhydride bond in phosphates, phosphorothiolates and phosphorothionates (i.e., in most, if not in all the compounds listed in Table I), the phosphorus-oxygen-alkyl ester grouping (compounds 3 and 13 and probably compound 30), and carboxylic ester (compounds 2 and 15) and carboxylic amide (compound 13) groupings. Many compounds, in particular the *O,O*-dimethyl phosphorus derivatives, are readily degraded to phosphoric acid which is reincorporated into such plant derivatives as lecithin (compounds 7—9, 10, 12, 13, 15, 24, 25 and possibly 30 and 41). One case is reported of a presumed enzymatically catalysed isomerization in plants of a phosphorothionate to a phosphorothiolate (compound 29).

The reaction of the organophosphate on the plant surface has been extensively studied with radiolabelled materials [45] [46] [48] [58] [76] [100] and others. The rate and extent of these surface reactions are affected by the temperature, intensity and quality of ultraviolet light, the nature of the plant surface and type of insecticide deposit. Volatilization, isomerization of phosphorothionates, oxidation of phosphorothionates, sulphides and sulphoxides, and various hydrolytic reactions are involved. More polar and less polar non-hydrolysed derivatives may be formed. Phosphorothionates are frequently converted to very potent anticholinesterase agents.

Unexpected chemical changes may occur with the insecticide prior to its deposition on or in the plant. Hydrolysis of phosphorothionates in the technical material or in formulation may result in a reattack of the phosphorothioic ion on the original molecule or a formulation constituent to yield toxic derivatives [101] [102]. Alkyl exchange can occur between the organophosphate and certain formulation constituents [102]. When a site susceptible to alkylation, such as a binary sulphur or a ternary nitrogen, is present on the molecule, self-alkylation may occur to yield highly toxic products [103] [104]. Each of these reactions must be considered along with the metabolic changes in the chemical effected by the plant.

IV. Summary

Studies in which radiolabelled compounds have been used to investigate insecticide metabolism in plants are briefly reviewed. No reference is made to the voluminous literature on insecticide residues and metabolism in plants where radiotracer techniques have not been utilized. Radioisotopes provide the most certain method for establishing the metabolic pathway of an insecticide. Such information on metabolism is essential for insuring the efficient and safe use of insecticides.

ACKNOWLEDGEMENTS

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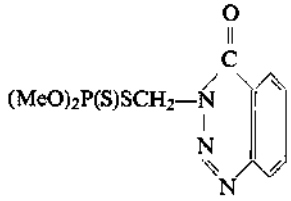
TABLE I

RADIOTRACER STUDIES ON THE METABOLISM OF ORGANOPHOSPHATE INSECTICIDES BY PLANTS

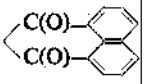
(Abbreviations: Me = methyl, Et = ethyl, Ph = phenyl, antiChE = anticholinesterase agent or cholinesterase inhibitor)

No.	Structure and name	Absorption and translocation	Hydrolysis products	Non-hydrolysed metabolites
1	$(MeO)_2P(O)OCH = CCl_2$ DDVP	pea [39]	pea [39]	
2	$(MeO)_2P(O)OC(Me) = CHC(O)OMe$ Phosdrin or OS 2046 alpha and beta isomers studied separately	Bryophyllum, cucumber, pea [41]	pea [40] [41]: bean, Bryophyllum, corn, cucumber [41]: plants; $(MeO)_2P(O)OH$? and $(MeO)_2P(O)OC(Me) = CHC(O)OH$? [41]: pea; $(MeO)_2P(O)OH$ and $(MeO)_2P(O)OC(Me) = CH—C(O)OH$ [42]	None in plants [40] [41]
3	$(MeO)_2P(O)OC(Me) = CHC(O)OH$ Carboxylic acid hydr. prod. of alpha Phosdrin		pea; $(MeO)_2P(O)OH$ and $(MeO)(HO)P(O)OC(Me) = CHC(O)OH$ [42]	
4	$(MeO)_2P(O)OCHBr \cdot CCl_2Br$ Dibrom		plants?; $(MeO)_2P(O)OH$, $BrCl_2C \cdot CHO$, $Cl_2HC \cdot CHO$, and complex amino acid conjugates [43]	plants?; $(MeO)_2P(O)OCH = CCl_2$ [43]
5	$(MeO)_2P(O)CHOH \cdot CCl_3$ Dipterex, Dylox, Bayer L 13/59	pea [39]	pea [39]	
6	$(MeO)_2P(O)CHOC(O)Me$ with CCl_3 on CH, acetylated Dipterex	pea [39]	pea [39]	
7	$(MeO)_2P(O)SC_2H_4SEt$ methyl isosystox, thiol isomer of meta-Systox		many plants; $(MeO)_2P(O)OH$, H_3PO_4 and lecithins [44]	many plants; $(MeO)_2P(O)SC_2H_4S(O)Et$ and $(MeO)_2P(O)SC_2H_4S(O_2)Et$ [44]
8	$(MeO)_2P(O)SC_2H_4S(O)Et$ sulphoxide of methyl isosystox		cabbage, potato; $(MeO)_2P(O)—OH$, H_3PO_4 and lecithins [44]	cabbage, potato; $(MeO)_2P(O)SC_2H_4S(O_2)Et$ [44]

9	$(MeO)_2P(O)SC_2H_4S(O_2)Et$ sulphone of methyl isosystox		cabbage, potato; $(MeO)_2P(O)OH$, H_3PO_4 and lecithins [44]	
10	$(MeO)_2P(S)OPh—NO_2—4$ methyl Parathion	many plants [45]	many plants [45]: hydrangea; H_3PO_4 and unident. metabolites [45]	
11	$(MeO)_2P(S)OPh—S(O)Me—4$ Bayer 25198	cotton [46]	cotton [46]	
12	 Guthion, Gusathion, Bayer 17147	cotton [47]	cotton; hydr. prod's including phosphatides [47]	cotton; two strongly lipophilic unident. metabolites, of unknown toxicity, but no apparent P = O Guthion [47]
13	$(MeO)_2P(S)SCH_2C(O)NHMe$ Dimethoate, Rogor	corn, cotton, pea, potato [48]	corn, cotton, potato; $(MeO)_2P(O)OH$, $(MeO)_2P(S)OH$, $(MeO)(HO)P(S)CH_2C(O)NHMe$, and $(MeO)_2P(O)SCH_2C(O)OH$ [48]: pea; as above plus H_3PO_4 [48]	corn, cotton, pea, potato $(MeO)_2P(O)SCH_2C(O)NHMe$ [48]: bean, lettuce; $(MeO)_2P(O)SCH_2C(O)NHMe$? and a less polar antiChE-agent [49]
14	$(CH_3O)_2P(S)SCH_2C(O)NHEt$ CL 18,706	cotton [48]	cotton [48]	
15	$(MeO)_2P(S)SCH(CH_2C(O)OEt)\cdot C(O)OEt$ Malathion	bean [50] [51]	bean; $(MeO)_2P(S)OH$, H_3PO_4, $(MeO)_2P(S)SH$?, $(MeO)_2P(S)SCH(CH_2C(O)OH)\cdot(CO)OH$ and $(MeO)_2P(S)SCH(CH_2C(O)OEt?)\cdot C(O)OH$ [51]	bean: $(MeO)_2P(O)SCH(CH_2C(O)OEt?)\cdot C(O)OEt$ [51]
16	$(EtO)_2P(O)SC_2H_4N(Et)_2$ Amiton	cotton, lemon [52]	cotton, lemon [52]	none in plants [52]

17	$(EtO)_2P(O)SC_2H_4N(Et)_2 \cdot HX$ salts of Amiton (Tetram or Chipman R-6199 is hydrogen oxalate salt)	cotton; citrate, dimethyl sulphate, oxalate, picrate, phthalate, *p*-toluene sulphonate, trichloroacetate salts [52]: lemon; dimethyl sulphate, hydrochloride, oxalate, *p*-toluene sulphonate salts [52]: orange; oxalate salt [52]: cacao; *p*-toluene sulphonate salt [53]	cotton, lemon; oxalate salt [52]: cacao; *p*-toluene sulphonate salt [53]	none in plants [52] [53]
18	$(EtO)_2P(O)SC_2H_4S(Me)Et \cdot MeSO_4$ dimethyl sulphonium salt of isosystox	cotton, lemon [54]		cotton; unidentified metabolite [54]
19	$(EtO)_2P(O)SC_2H_4SEt$ and $(EtO)_2P(S)OC_2H_4SEt$ Systox Demeton (mixed isomers)	cotton [55] [56]: lemon [57]	many plants; field residue data [58]: plants; $(EtO)_2P(O)OH$?, alcohols? [58]	apple, orange, walnut; $(EtO)_2P(O)SC_2H_4S(O)Et$, $(EtO)_2P(O)SC_2H_4S(O_2)Et$?, $(EtO)_2P(S)OC_2H_4S(O)Et$ plus $(EtO)_2P(S)OC_2H_4S(O_2)Et$ and/or $(EtO)_2P(O)OC_2H_4S(O)Et$ [58]
20	$(EtO)_2P(O)SC_2H_4SEt$ isosystox, thiol isomer of Systox or Demeton	cotton, lemon [52] [54]: bean, lemon [60]: potato, tobacco [61]: bean, borage, mustard [63]: apple, bean, coleus [64]: lettuce, nettles [66]: alfalfa, cotton, sugar beet [68]: cotton [69]	cotton [52]: bean, cotton, orange [58]: potato, tobacco [61] [62]: bean, borage, mustard [63]: apple, bean, coleus [64]: lettuce, nettles [66]: alfalfa, cotton, sugar beet [68]: cotton [69]	orange; $(EtO)_2P(O)SC_2H_4S(O)Et$, $(EtO)_2P(O)SC_2H_4S(O_2)Et$(58): cotton; $(EtO)_2P(O)SC_2H_4S(O)Et$, $(EtO)_2P(O)SC_2H_4S(O_2)Et$ [54] [65] [67]: Brassica, nettles, sugar beet, turnip; 3 unidentified metabolites [59]:bean, lettuce; unidentified metabolite(s) [60]; potato, tobacco; unidentified "more toxic" compounds [61]: bean, borage, mustard; 2 unidentified metabolites [63]: lettuce, nettles; $(EtO)_2P(O)SC_2H_4S(O)Et$ plus 1 unidentified oxidative metabolite plus 2nd unidentified metabolite [66]
21	$(EtO)_2P(O)SC_2H_4S(O)Et$ sulphoxide of isosystox	cotton, lemon [54]	cotton [54]	cotton; $(EtO)_2P(O)SC_2H_4S(O_2)Et$ [54]

22	$(EtO)_2P(S)OC_2H_4SEt$ Systox or Demeton	cotton, orange [58]: bean, lemon [60]: turnip [66]: many plants [70]	bean, cotton, orange [58]	orange; $(EtO)_2P(S)OC_2H_4S(O_2)Et$, $(EtO)_2P(O)OC_2H_4S(O)Et$ [58]: plants; unidentified metabolite(s) [59]: bean, lemon; unidentified metabolite(s) [60]: cotton; $(EtO)_2P(S)OC_2H_4S(O)Et$ plus $(EtO)_2P(S)OC_2H_4S(O_2)Et$ and/or $(EtO)_2P(O)OC_2H_4S(O)Et$ [65]: turnip; 3 unidentified metabolites [66]
23	$(EtO)_2P(S)OC_2H_4N(Et)_2$ thiono isomer of Amiton	cotton, lemon [52]	cotton, lemon [52]	
24	$(EtO)_2P(S)SCH_2SEt$ Thimet or Phorate	many plants [37]: alfalfa, cotton, sugar beet [68]: alfalfa, cotton, lemon [69]: bean [71]: pea [72]	many plants [37]: alfalfa, cotton, sugar beet [68]: cotton [69]: bean; $(EtO)_2P(S)SH$, $(EtO)_2P(S)$—OH, $(EtO)_2P(O)OH$, H_3PO_4, unknown (maybe de-ethylated deriv.) [71]; pea [72]	cotton; $(EtO)_2P(S)SCH_2S(O)Et$, $(EtO)_2P(S)SCH_2S(O)_2Et$, $(EtO)_2$—$P(O)SCH_2S(O)Et$, $(EtO)_2P(O)S$—$CH_2S(O_2)Et$ [37]: pea; $(EtO)_2S$—$P(S)SCH_2S(O)Et$, $(EtO)_2P(S)$—$SCH_2S(O_2)Et$ [37]: alfalfa, cotton, lemon; as above on cotton [69]: bean; as above on cotton [71]
25	$(EtO)_2P(S)SC_2H_4SEt$ Disyston, Dithiodemeton, Ekatine	cotton [46]: alfalfa, cotton, sugar beet [68]: alfalfa, bean, cotton, lemon [69]: pineapple [74]	cotton [46]: alfalfa, cotton, sugar beet [68]. alfalfa, cotton [69]: cotton; diethyl phosphoric acids?, H_3PO_4?, phospholipids [69]: cotton, tomato and 10 other plants [75]	alfalfa, bean, cotton, lemon; $(EtO)_2P(S)SC_2H_4S(O)Et$, $(EtO)_2$—$P(S)SC_2H_4S(O_2)Et$, $(EtO)_2P(O)S$—$C_2H_4S(O)Et$, $(EtO)_2P(O)SC_2H_4S(O_2)Et$ [69]: cotton, tomato and 10 other plants; as above [75]: cotton; $(EtO)_2P(S)SC_2H_4S(O)Et$, $(EtO)_2P(S)SC_2H_4S(O_2)Et$ [73]
26	$(EtO)_2P(S)SSP(S)(OEt)_2$? impurity in Delnav	bean, cotton, tomato [76]	bean, cabbage, cotton, tomato [76]	bean, cabbage; more polar derivatives, possibly phosphorothiolates [76]
27	$(EtO)_2P(S)OPh—NO_2—4$ Parathion	many plants [45]: apple [77]	many plants [45]: grape and lemon leaf juice [45]	
28	$EtOP(S)(OPh—NO_2—4)_2$ impurity in Parathion	plants [45]	plants [45]	

29	$(EtO)_2P(S)OPh—S(O)Me—4$ Bayer 25141	cotton [46]	cotton [46]	cotton; $(EtO)_2P(S)OPh—S(O_2)—Me—4$, $(EtO)_2P(S)OPh—SMe—4$ (trace), $(EtO)(EtS)P(O)OPh—S(O)Me—4$ (major), $(EtO)(EtS)P(O)OPh—S(O_2)Me—4$ [46]
30	$(EtO)_2P(S)O—N$  Bayer 22408	cotton [78]	cotton: $(EtO)(HO)P(O)O$-naphthalimido?, H_3PO_4 incorporated into plant products? [78]	cotton: $(EtO)_2P(O)O$-naphthalimido [78]
31	$(EtO)_2P(S)S$  impurity in Delnav	bean, cotton, tomato [76]	bean, cabbage, cotton, tomato [76]	bean, cabbage; more polar derivative, probably $(EtO)_2P(O)S$-*p*-dioxene-2 [76]
32	$(EtO)_2P(S)S$  $(EtO)_2P(S)S$ Delnav-studies on separate and combined *cis* and *trans* isomers	bean, cotton, tomato [76]	bean, cabbage, cotton, tomato [76]	bean, cabbage; more polar derivatives, more active antiChE agents, possibly phosphorothiolates [76]
33	$EtOP(O)(NMe_2)_2$	turnip [66]	turnip [66]	turnip; $EtOP(O)(NMe_2)(NHMe)$? $EtOP(O)(NHMe)_2$? [66]
34	$MeHNP(O)(NMe_2)_2$	turnip [66]	turnip [66]	turnip; $(MeHN)_2P(O)NMe_2$? [66]
35	*i*-$PrHNP(O)(NMe_2)_2$	turnip [66]	turnip [66]	turnip; *i*-$PrHNP(O)(NHMe)—(NMe_2)$? [66]
36	*n*-$BuHNP(O)(NMe_2)_2$	turnip [66]	turnip [66]	turnip; *n*-$BuHNP(O)(NHMe)—(NMe_2)$?, *n*-$BuHNP(O)(NHMe)_2$? [66]
37	$(Me_2N)_3P(O)$ impurity in Schradan	bean [35]; turnip [66]; sugar beet, strawberry [79]	bean [35]; turnip [66]; sugar beet, strawberry [79]; plants [80]	bean; more and less polar derivative(s), some containing organically bound formaldehyde [35]: turnip; $(Me_2N)_2P(O)NHMe$? [66]: sugar beet; $(Me_2N)_2P(O)NMe \cdot CH_2OH$? [79]: plants; unidentified intermediate [80]

38	$(Me_2N)_2P(O)F$ Dimefox, Hanane	pea [35]: bean [81]	pea [35]	pea: less and probably also more polar derivative(s), some containing organically bound formaldehyde and antiChE activity [35]
39	$(MeHN)(Me_2N)P(O)OP(O)(NMe_2)_2$ demethylated Schradan		turnip [66]	
40	$(Me_2N)_2P(O)OP(O)(NMe_2)_2$ and $(Me_2N)_2P(O)OP(O)NMe_2$ $(Me_2N)_2P(O)O$ Schradan or OMPA, technical	brussel sprout, hop, sugar beet, strawberry [82]	brussel sprout, hop, sugar beet, strawberry [82]	
41	$(Me_2N)_2P(O)OP(O)(NMe_2)_2$ Schradan or OMPA	sugar beet, strawberry [79]: marrow [86]: bean, cabbage, hop, pea, strawberry [87] [88]: groundnut [90]: bean [57] [91] [96]: lemon, orange [92]: borage, mustard [93]: apple, bean, chrysanthemum, coleus [94] [95]: cotton [97]	clover, turnip [66]: sugar beet, strawberry [79]: plants [80]: bean [88] [91] [96]: lettuce [89]: groundnut [90]: lemon, orange [92]: borage, mustard [93]: apple, bean, chrysanthemum, coleus [94] [95]: plants; $(Me_2N)_2P(O)$—OH, $(Me_2N)(Me_2NO)P(O)OH$? plus non-chloroform extractibles [66]: cotton, $(Me_2N)_2P(O)OH$?, H_3PO_4? [97]: many plants; $(Me_2N)_2P(O)OH$? [98]	plants; general reviews [83—85]: bean; unidentified plant metabolite(s) [35]: bean, brussel sprout, clover, turnip; $(MeHN)(Me_2N)P(O)OP(O)(NMe_2)_2$ [66]: clover; $(MeHN)(Me_2N)P(O)OP(O)$—(NMe_2) plus antiChE agents [66]: plants; $(Me_2NO)(Me_2N)P(O)OP$—$(O)(NMe_2)_2$, $(HOCH_2 \cdot MeN)$—$(Me_2N)P(O)OP(O)N(Me_2)_2$, $(MeHN)(Me_2N)P(O)OP(O)$—$(NMe_2)_2$ [66]: plants; no non-hydrolysed intermediates [80]: bean; antiChE agent(s) [91]

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DISCUSSION

P. J. DEORAS (India): What is Dr. Casida's opinion on the use of radioisotopes in insecticides of plant origin, such as pyrethrum? Many of these insecticides disintegrate very rapidly in the presence of daylight, and I wonder to what extent radioisotopes could be used in tracing their metabolism in the plant.

J. E. CASIDA: Radiolabelled studies on insecticides of plant origin have been restricted to biosynthesis investigations on nicotine, cinerins and pyrethrins. These radiolabelled compounds, along with radiolabelled allethrin, have been used in detoxication studies with insects but to the best of my knowledge have not been studied with plants. The actual degradation products of pyrethroids, rotenone and alkaloids other than nicotine used as insecticides have not been completely established. It would certainly be interesting to use C^{14}-tagged compounds to establish the mechanism of the rapid breakdown of these compounds in the presence of sunlight.

G. W. RAHALKAR (India): I am presuming that the work that you have reported was carried out with labelled isotopes. Do you get a similar metabolic pattern with non-labelled compounds?

J. E. CASIDA: In our studies we always cross-check the radiotracer studies with bioassay, enzyme assay, chromatography and spectrophotometric studies. We have no reason to suspect that different metabolic pathways exist for labelled and non-labelled insecticides and many reasons to believe that they are identical. The P^{32}-labelled insecticides are not the same compound after decay, but the proportion of radiolabelled insecticide used is almost infinitely small compared with the "cold" carrier, and when it decomposes by decay we no longer detect the molecule. I certainly do not recommend using radiolabelled compounds as a sole technique, but believe that the method should be simultaneously followed up by every other one available.

J. DE WILDE (Netherlands): Is it known whether incident light influences the breakdown of insecticides within the plant tissues? I am referring particularly to photosynthesis and the resulting changes in redox potential within the tissues. It seems to me that this could well influence the oxidative breakdown of a plant.

J. E. CASIDA: The rates of absorption, translocation and degradation of absorbed insecticide, and the rate of breakdown of surface insecticide residues are definitely affected by the quantity and quality of the available light. Effects are both indirect—alteration of

the physiology of the plant—and direct—by catalysing certain reactions of the insecticide. I am unaware of any *in vivo* plant studies designed to correlate redox potential with insecticide degradation. A related study we recently conducted showed that plant peroxidase enzymes *in vitro* coupled with a suitable hydrogen donor would catalyse both the oxidation and the hydrolysis of Parathion. This could be interpreted in terms of some mechanism, but it does not seem to deal directly with the redox potential.

J. HALBERSTADT (IAEA, Scientific Secretary): Dr. Casida mentioned that in his absorption and translocation studies he had difficulty in distinguishing between the insecticide administered and its breakdown products. I should like to ask him whether he has ever used one of the analytical isotope-dilution techniques, for instance the extremely useful isotope-derivative technique, to distinguish at least between the original insecticide still present and the total of its radioactive hydrolysis products. In my own experience this has been very helpful.

J. E. CASIDA: The difficulties referred to are those of distinguishing *in situ* whether the radiolabel is associated with the original compound or with a metabolite. Isotope-dilution and derivative techniques would require removing the radiolabelled compound from its position *in vivo* and thereby contaminating it with radiolabelled materials from adjacent cells or parts of cells. The techniques you mention are indeed extremely useful, but I cannot quite see how they can be readily applied to the localized *in vivo* fate of the insecticide.

THE CHAIRMAN (A. R. Gopal-Ayengar, India): May I ask a question myself? To what extent do these insecticides and their detoxication products affect the genetic make-up of either the insect or the plant itself?

J. E. CASIDA: As a partial answer, I may recall that there were certainly some insecticides on the market that had a carcinogenic effect. There are other compounds used as insecticides which, if applied at low levels throughout the developmental stages of the insect, prevent it from reproducing normally. I should not care to say whether this is a genetic change or not. Most insecticides act so quickly, and the recovery of survivors is so complete and rapid, that I doubt whether these compounds cause genetic changes in the surviving insects other than by selection-pressure leading to resistance.

METABOLISM OF SYSTEMIC AND OTHER RECENT INSECTICIDES IN ANIMALS

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Abstract — Résumé — Аннотация — Resumen

Metabolism of systemic and other recent insecticides in animals. Organophosphates undergo several activation and detoxication processes in insects and mammals. Activation mechanisms include such oxidative processes as conversion of phosphorothioates to corresponding phosphates, thiophenyl oxidation of Baytex (*O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate) to the sulphoxide and sulphone derivatives, thioether oxidation of Systox (*O,O*-diethyl-*O*-[and *S*]-2-[ethylthio] ethyl phosphorothioates) and phorate (*O,O*-diethyl *S*-ethylthiomethyl phosphorodithioate) to sulphoxide and sulphone Derivatives, and amide oxidation of Schradan (octamethylpyrophosphoramide) and Dimefox (bis [dimethylamino] fluorophosphine oxide) to the *N*-oxide derivative followed by rearrangement to the *N*-methoxide or methylol compounds. Carboxylic ester hydrolysis serves as an activation mechanism for Butonate (*O,O*-dimethyl 2,2,2-trichloro-1-*n*-butryloxyethyl phosphonate) and Acetyl-Dipterex (*O,O*-dimethyl 2,2,2-trichloro-1-acetoxy-ethyl phosphonate) to form the more toxic compound Dipterex (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphonate). The dehydrochlorination of Dipterex forming DDVP (*O,O*-dimethyl 2,2-dichlorovinyl phosphate) is an activation mechanism in houseflies but absent in mammals. The isomerization of a phosphorothioate to the *S*-alkyl derivative is operative in plants, but such an activation process has not been reported in animals. The mono- and di-carboxylic acid hydrolytic products of Dimethoate (*O,O*-dimethyl *S*-[*N*-methylcarbamoylmethyl] phosphorodithioate) and Malathion (*O,O*-dimethyl-*S*-[1,2-dicarbethoxyethyl] phosphorodithioate) may undergo decarboxylation *in vivo* to form potentially toxic materials. Activation is dependent upon the substituents surrounding the phosphorus atom and the particular biological system.

Detoxication mechanisms of organophosphates include destructive hydrolysis at the P—O—C, P—S—C, P—C, P—N, or P—O—N bonds. Other hydrolytic processes occur at groups not linked to the phosphorus atom. Hydrolysis of the ethyl ester moieties of the diethyl succinate nucleus of Malathion occurred in mammals. The N-methyl amine group of Dimethoate was cleaved resulting in the carboxy derivative of Dimethoate. The nitrophenol group of Parathion was reduced to the non-toxic Aminoparathion. Cleavage of the coumarinyl ring of Co-Ral occurs in alkali and possibly in mammals.

Insecticide metabolism studies are basic in understanding the selective toxicity of insecticides, resistance mechanisms, residue problems, and mode-of-action concepts. Radioisotopes have been indispensable analytical tools in increasing the wealth of knowledge concerning the metabolism of insecticides.

Le métabolisme des insecticides systémiques et d'autres insecticides nouveaux chez les animaux. Les insecticides organiques phosphorés subissent dans les insectes et les mammifères plusieurs processus d'activation et de perte de toxicité. Parmi les mécanismes d'activation, il y a lieu de signaler les processus d'oxydation tels que la transformation des thiophosphates en phosphates correspondants, l'oxydation du groupe thiophényle du Baytex (thiophosphate de *O,O*-diméthyl-*O*-[4-(méthylthio)-*m*-tolyle]) avec formation de dérivés de sulfones et sulfinones, l'oxydation du thioéther du Systox (thiophosphate de *O,O*-diéthyl-*O*-[et *S*]-2-[éthylthio] éthyle) et du Phorate (dithiophosphate de *O,-O* diéthyl-S-éthylthiométhyle) avec formation de dérivés de sulfones et sulfinones, et l'oxydation des groupes amide du Schradan (octaméthyl-pyrophosphoramide) et du Dimefox (oxyde de bis [diméthylamino] fluorophosphine) avec formation de dérivés de *N*-oxyde, suivie d'un réarrangement en composés de *N*-méthoxyde ou de méthylol. L'hydrolyse de l'ester carboxylique est un mécanisme d'activation du Butonate (phosphonate de *O,O*-diméthyl-2,2,2-trichloro-1-*n*-butryloxyéthyle) et de l'Acétyl-Dipterex (phosphonate de *O,O*-diméthyl-2,2,2-trichloro-1-acétoxyéthyle) pour former le Dipterex,

composé plus toxique (phosphonate de *O,O*-diméthyl-2,2,2-trichloro-1-hydroxyéthyle). La perte d'HCl du Dipterex, qui donne lieu à la formation du DDVP (phosphate de *O,O*-diméthyl-2,2-dichlorovinyle) est un mécanisme d'activation que l'on observe chez les mouches communes mais qui n'existe pas chez les mammifères. L'isomérisation d'un thiophosphate avec formation du dérivé de *S*-alcoyle intervient dans les plantes, mais aucun processus d'activation de ce genre n'a été signalé chez les animaux. Les acides mono et di-carboxyliques formés par hydrolyse du Diméthoate (dithiophosphate de *O,O*-diméthyl-*S*-[*N*-méthylcarbamoylméthyle]) et du Malathion (dithiophosphate de *O,O*-diméthyl-*S*-[1,2-dicarbéthoxyéthyle]) peuvent subir une décarboxylation *in vivo* pour former des substances potentiellement toxiques. L'activation dépend des produits de remplacement qui entourent l'atome de phosphore et du système biologique considéré.

Parmi les mécanismes de perte de toxicité des insecticides organiques phosphorés, il convient de mentionner l'hydrolyse destructive aux unions P—O—C, P—S—C, P—C, P—N ou P—O—N. D'autres processus hydrolytiques interviennent aux groupes non liés à l'atome de phosphore. Chez les mammifères, on a observé l'hydrolyse des parties d'ester éthyliques du noyau de diéthyl-succinate du Malathion. Le groupe *N*-méthylamine du Diméthoate a été scindé et a donné le dérivé carboxylique du Diméthoate. Le groupe nitrophénol du Parathion a été réduit à l'aminoparathion non toxique. La scission du cycle coumarinyle de Co-Ral se produit en milieu alcalin et probablement chez les mammifères.

Les études sur le métabolisme des insecticides sont essentielles pour la compréhension de la toxicité sélective de ces agents, des mécanismes de résistance, des problèmes des résidus et des modalités d'action. Les radioisotopes constituent des moyens analytiques indispensables pour l'accroissement des connaissances relatives au métabolisme des insecticides.

Метаболизм общеизвестных и других новых инсектицидов у животных. Органофосфаты у насекомых и млекопитающих подвергаются нескольким процессам активации и детоксикации. Механизм активации включает такие окислительные процессы, как превращение фосфоротиатов в соответствующие фосфаты, тиофенилового окисления Бейтекса (*O,O*-диметил *O*-[4-(метилтио)-*m*-толил] фосфоротиат) в производные окиси сернистого алкила и сульфона, окисления тиоэфира Систекса (*O,O*-диэтил *O*(и *S*)-2-(этилтио) этил фосфоротиат) и фората (*O,O*-диэтил *S*-этилтиометил фосфородитиат) в производные окиси сернистого алкила и сульфона, а амидного окисления шрадана (октаметилпирофосфорамид) и димефокса (bis (диметиламино) окись флуорофосфина) в производное *N*-оксид с последующим превращением в *N*-метилат или соединения метилола. Гидролиз карбоксилового эфира служит в качестве активирующего механизма для Бутоната (*O,O*-диметил 2,2,2,-трихлоро-1-*n*-бутрилоксиэтил фосфонат) и Ацетил-Диптерекс (*O,O*-диметил 2,2,2-трихлоро-1-ацетоксиэтилфосфонат) для образования более токсичного соединения Диптерекса (*O,O*-диметил 2,2,2-трихлоро-1-гидроксиэтил фосфонат). Дегидрохлорирование Диптерекса с образованием DDVP (*O,O*-диметил 2,2-дихлоровенил фосфат) является активирующим механизмом у домашних мух, но они отсутствуют у млекопитающих. Изомеризация фосфоротиата в производное *S*-алкил является действенной в растениях, но такой процесс активации не зафиксирован у животных. Продукты диметоата моно- и дикарбоновой кислоты (*O,O*-диметил *S*-(*N*-метилкарбамоилметил) фосфородитиат) и малатиона (*O,O*-диметил-*S*-(1,2-дикарботоксиэтил) фосфородитиат) могут подвергаться в организме декарбоксилации для образования потенциально токсичных веществ. Активация зависит от заместителей, окружающих атом фосфора, и от соответствующей биологической системы.

Механизмы детоксикации органофосфатов включают деструктивный гидролиз при связях P—C—C, P—S—C, P—C, P—N или P—O—N. У групп, не связанных с атомом фосфора, имеют место другие гидролитические процессы. У млекопитающих наблюдался случай гидролиза половин этиолового эфира ядра этиловой соли малатиона. В результате карбокислового производного диметоата *N*-метил-аминовая группа не исчезла. Нитрофеноловая группа паратиона восстанавливалась до нетоксичного аминопаратиона. Расщепление кумаринилового кольца Ca-Ral встречается у щелочи и, возможно, у млекопитающих.

Изучение метаболизма инсектицида является основой понимания селективной токсичности инсектицидов, механизмов устойчивости, проблем остатков и понятий об образах действий.

Радиоизотопы являются необходимым аналитическим средством в приобретении знаний по метаболизму инсектицидов.

Metabolismo de los insecticidas sistémicos y otros insecticidas recientes, en los animales. Los compuestos orgánicos fosforados sufren diversos procesos de activación y de pérdida de toxicidad en los insectos y en los mamíferos. Entre los mecanismos de activación, cabe mencionar los procesos de oxidación, como la transformación de los tiofosfatos en los fosfatos correspondientes, la oxidación del grupo tiofenilo del "Baytex" (tiofosfato de *O,O*-dimetil *O*-[4-(metiltio)-*m*-toluilo]), con formación de los derivados sulfóxido y sulfona, la oxidación del grupo tioéter del "Systex" (etiltiofosfato de *O,O*-dietil *O*-(y *S*)-2-(tioetilo)) y del "Phorate" (ditiofosfato de *O,O*-dietil *S*-etiltiometilo) en los sulfóxidos y sulfonas derivados, y la transformación de la amida del Scharadan (octometilpirofosforamida) y del "Dimefox" (óxido de bis (dimetilamino) fluorofosfina) en los derivados *N*-oxídicos por oxidación seguida por un reordenamiento molecular para formar compuestos del tipo *N*-metóxido o metilol. La hidrólisis del ester carboxílico sirve como mecanismo de activación del "Butonate" (fosfonato de *O,O*-dimetil 2,2,2-tricloro-1-*n*-butiloxietilo) y del "Acetil-Dipterex" (fosfonato de *O,O*-dimetil 2,2,2-tricloro-1-acetoxietilo) para formar el compuesto "Dipterex" (fosfonato de *O,O*-dimetil 2,2,2-tricloro-1-hidroxietilo) más tóxico. La pérdida de cloro e hidrógeno en el "Dipterex" con formación de DDVP (fosfato de *O,O*-dimetil 2,2-diclorovinilo) constituye un mecanismo de activación que se observa en la mosca común, pero no en los mamíferos. La isomerización del tiofosfato en su derivado *S*-alcoilado se produce en las plantas, pero ningún proceso de activación de este tipo se describió en los animales. Los ácidos mono y dicarboxílicos formados por hidrólisis del "Dimethoate" (ditiofosfato de *O,O*-dimetil *S*-(*N*-metilcarbamoilmetilo)) y del "Malathion" (ditiofosfato de *O,O*-dimetil-*S*-(1,2-dicarbetoxietilo)) pueden sufrir una descarboxilación *in vivo* para formar sustancias potencialmente tóxicas. La activación depende del sistema biológico considerado y de los sustituyentes que rodean al átomo de fósforo.

Entre los procesos de pérdida de toxicidad de los compuestos orgánicos fosforados se cuentan la hidrólisis destructiva de los enlaces P—O—C, P—S—C, P—C, P—N o P—O—N. Otros procesos hidrolíticos tienen lugar en grupos que no están ligados al átomo de fósforo. En los mamíferos se ha observado la hidrólisis de la parte ester etílico en el núcleo de dietilsuccinato del "Malathion". El grupo *N*-metilamina del "Dimethoate" se separa dando un derivado carboxílico del "Dimethoate". El grupo nitrofenol del "Parathion" se reduce al aminoparatión, no tóxico. La rotura del anillo del cumarinilo del Co-Ral se produce en medio alcalino probablemente en los mamíferos.

Los estudios del metabolismo de los insecticidas son fundamentales para comprender la toxicidad selectiva de estos agentes, los mecanismos de resistencia, los problemas de residuos, y los modos de acción. Los radioisótopos constituyen medios analíticos indispensables para aumentar el caudal de conocimientos relativos al metabolismo de los insecticidas.

The toxicological and pharmacological properties of an insecticide are more complicated when insects and mammals are capable of converting the material to more toxic metabolites or to materials slightly less toxic than the parent compound. In many instances, the metabolic products can be synthesized biologically but not synthetically. Differences in mammalian and insect metabolic pathways of an insecticide provide clues fo the future development of selective toxicants. A knowledge of the molecular configuration as related to toxicity to insects and mammals, differences in biological activation and detoxication mechanisms in insects and mammals, and physiologically important enzymes of insects and mammals is basic for an understanding of poisoning processes, and fundamental to the preservation of health of man and his allies.

Radioisotopes have been indispensable analytical tools for increasing the wealth of knowledge concerning the animal metabolism of insecticides, particularly organophosphates. Organophosphate metabolism studies were stimulated by DuBois *et al.* [39] and Gardiner and Kilby [42] demonstrating that Schradan required metabolic activation to be an effective

antiesterase agent *in vivo*. Several reviews have been published recently on the metabolism of organophosphates in insects, mammals, and plants [27] [41] [63] [65] [76] [93]. Most metabolism studies have been conducted using radiotracer techniques; phosphorus-32 and sulphur-35 have been the predominant isotopes incorporated into the organophosphate molecule. Radioisotopes have aided greatly in elucidating intoxication and detoxication processes, and typical biochemical mechanisms will be discussed below, with specific systemic and non-systemic insecticides as illustrations.

Malathion

The selective toxicity of Malathion (*O,O*-dimethyl-*S*-[1,2-dicarbethoxyethyl] phosphorodithioate) has been explained on the basis of differences in activation and detoxification mechanisms in insects and mammals [31] [32] [55] [56] [70]. In early work with Malathion, the oxygen analogue (Malaoxon) was suggested as the probable oxidative metabolite having marked *in vivo* anticholinesterase activity and toxicity [55] [63] [69] [70]. The *in vivo* formation of Malaoxon by insects and rats was shown conclusively by KRUEGER and O'BRIEN [51] and MITCHELL and ARTHUR [66] using high-activity P^{32}-labelled Malathion [29] and column and paper-chromatographic techniques. Malaoxon production was considerably lower in rats than in the German cockroach (*Blattella germanica* L.), housefly (*Musca domestica* L.), or boll weevil (*Anthonomus grandis* Boh.) [66], and the same biological relationship existed between mice and three species of insects [51]. O'BRIEN [70] suggested that the selectivity exhibited by Malathion for the American cockroach (*Periplaneta americana* L.) over the mouse was due to the balance of oxidative and hydrolytic processes.

Malathion metabolism in rats, cows and poultry was more complex than in the American cockroach and, in vertebrates, involved hydrolysis of the P—S and S—C bonds as well as the carboxylic ester groups of the diethylsuccinate moiety (Fig. 1) [55] [56]. Hydrolysis of the P-O-methyl bond forming desmethyl Malathion or desmethyl Malaoxon was suspected, based on indirect evidence [66]. Following oral administration of Malathion to rats, monomethyl phosphoric acid was isolated from the urine. This acid was probably formed by hydrolysis of desmethyl Malaoxon and not by further degradation of dimethyl phosphoric acid, since the dimethyl derivative was recovered from the urine of rats as the administered chemical [7] [78].

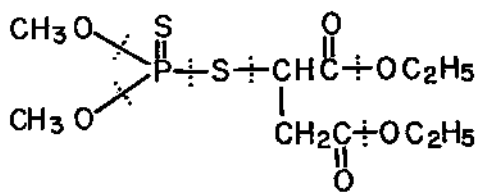

Fig. 1
Malathion sites susceptible to hydrolysis by phosphatases or carboxyesterases [51] [55] [56] [66]

Hydrolysis of the ethyl ester groups of the diethylsuccinate moiety is of significance, since it involves cleavage of groups not linked directly to the phosphorus atom. The resulting ionic products could undergo decarboxylation and rearrangement to form neutral phosphate esters [66]. Thus, Malathion may be degraded to nontoxic ionic products that can be further metabolized to potentially toxic derivatives. Apparently, this metabolic scheme is not operative to a significant extent either in insects or mammals.

The activating mechanism of Malathion is phosphorothioate oxidation; phosphatases and carboxyesterases serve as detoxication processes (Fig. 1). The possibility that introduction of a carboxyester group into an organophosphate anticholinesterase would confer selective toxicity towards insects as compared to mammals was investigated by O'BRIEN *et al.* [71]. Acethion (*O,O*-diethyl *S*-carboethoxymethyl phosphorodithioate) was almost 100 times more toxic to the housefly than to the mouse [71], and the phosphorodithioate was degraded more rapidly to acethion acid by insect tissues than by mouse liver [52]. Selective toxicity was attributed to variations in carboxyesterase activity of insects and mammals [71].

Dimethoate

Dimethoate (*O,O*-dimethyl *S*-[*N*-methylcarbamoylmethyl] phosphorodithioate) possesses animal systemic activity against ecto- and endoparasites of mammals [16] [37] [43] [44]. The metabolism of Dimethoate by insects and mammals is analogous to that of Malathion, although the two phosphorodithioates are structurally quite different.

Dimethoate was rapidly metabolized and excreted as water-soluble products by rats and lactating dairy cows [33]; hydrolysis occurred at the methyl-phosphate, phosphate-sulphur, sulphur-carbon, and carbonyl-nitrogen bonds forming non-toxic products (Fig. 2), but

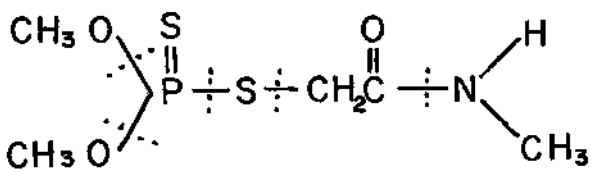

Fig. 2
Dimethoate sites susceptible to hydrolysis by phosphatases and amidase [18] [33] [47]

phosphorothioate oxidation was not demonstrated for the parent material. Similar degradation patterns have been observed in sheep treated with Dimethoate [34]. Cows treated orally or intramuscularly with Dimethoate excreted about 90% of the administered dose in the urine within 24 h of treatment; the metabolic degradation products were isolated and characterized as dimethyl phosphoric, -thioic and -dithioic acids [47]. Amidase was the predominant esterase hydrolysing Dimethoate in mammals [33]. Although the oxygen analogue was not identified from cows treated with Dimethoate [33], a material more toxic to the stable fly (*Stomoxys calcitrans* (L.)) than Dimethoate was present in the blood of cows treated orally, intramuscularly, or intravenously [87]. The oxygen analogue was isolated from rats and mice, and found to be the effective antiesterase agent [18] [52].

The carboxy derivative of Dimethoate formed by hydrolysis of the *N*-methyl group underwent decarboxylation *in vivo* resulting in *O,O*-dimethyl *S*-methyl phosphorodithioate [18] which was more polar but less toxic than Dimethoate [33]. The radioactive *S*-methyl compound was formed by rats and German cockroaches, and was isolated and characterized by its co-chromatographic behaviour with non-radioactive parent compound on silica gel (Fig. 3) [18].

Dimethoate was more stable in insects than in rats [18] or mice [52]. About 65% of the chloroform-soluble radioactive materials from insects was residual Dimethoate, while the excreta of orally and dermally treated rats contained a much smaller percentage of the compound [18]. Rats were 50 to 75 times more efficient than houseflies, German cockroaches,

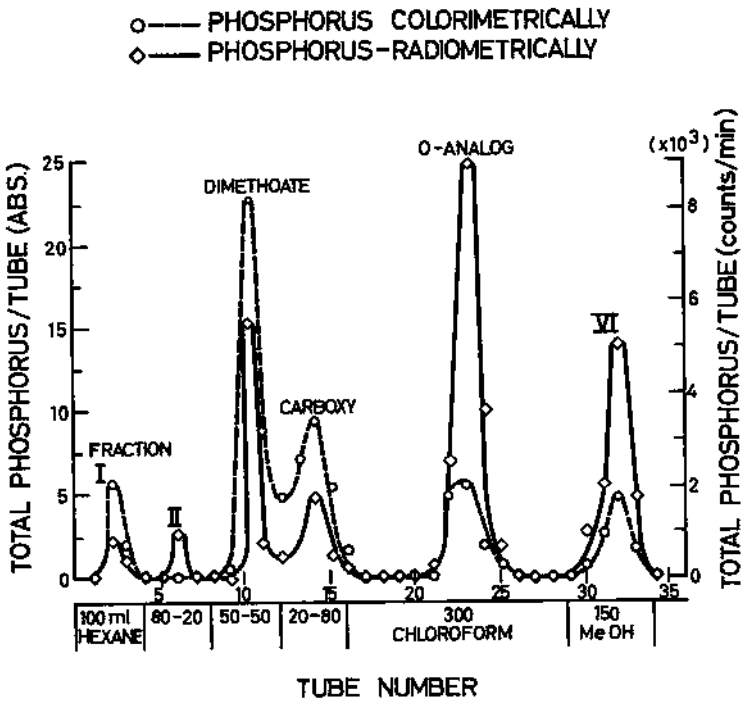

Fig. 3

Co-chromatography on silica gel of Dimethoate and known carrier-materials with the chloroform-soluble metabolites recovered from German cockroaches, (Fraction I = *O,O*-dimethyl *S*-methyl phosphorodithioate [18])

or boll weevils in degrading Dimethoate to other metabolites, especially to the carboxy and desmethyl derivatives. The oxygen analogue was produced in larger quantities by houseflies than by rats or cockroaches. Injection of 0.5μg of Dimethoate per gram of housefly, American cockroach, or mouse resulted in 32, 16, and 89% degradation, respectively, in 0.5 h [52]. Activation and degradation systems were present in both mammals and insects but the activation system was predominant in insects. The greater stability of Dimethoate and the more rapid formation of the oxygen analogue in insects as compared to mammals appeared to be a plausible explanation of selective toxicity [18] [52].

Dipterex and related derivatives

The metabolism of P^{32}-labelled Dipterex (*O,O*-dimethyl [2,2,2-trichloro-1-hydroxyethyl] phosphonate) was investigated in rats, a dog, houseflies [6] and a cow [83]. The low toxicity of Dipterex to mammals was attributed to phosphonate hydrolysis by serum esterases and elimination of the trichloro-portion of the molecule in the urine as trichloroethyl glucuronide [6]. Dipterex was rapidly degraded by a cow; 66% of the administered dose was eliminated in the urine by 12 h post-treatment [83]. In the cow, the major metabolic pathway of Dipterex was not by rupture of the P—C bond but by hydrolysis of the *O*-methyl ester linkage or modification of the 2,2,2-trichloro-1-hydroxyethylphosphonate portion of the molecule [83].

In rats, a dog or a cow, there was no evidence that the toxicity of Dipterex was due to dehydrochlorination and rearrangement to the more active antiesterase, DDVP (*O,O*-dimethyl 2,2-dichlorovinyl phosphate).

The dehydrochlorination of Dipterex to form the more toxic DDVP was demonstrated in houseflies using P^{32}-labelled phosphonate and paper chromatographic techniques (Fig. 4) [64]. The dehydrochlorination reaction served as an activation mechanism for this phosphonate, since DDVP was considerably more toxic to insects and mammals than Dipterex [6]. Apparently, this enzymatic dehydrochlorination occurred in houseflies [64] but not in mammals [6] [7]. If this biochemical mechanism is specific for insects but not for mammals, then it can be responsible for selectivity. It is interesting to note that this activation process of Dipterex is the same as the defence mechanism of resistant houseflies against DDT which involves a dehydrochlorination of DDT to form the relatively non-toxic DDE [75] [94] [95] [96].

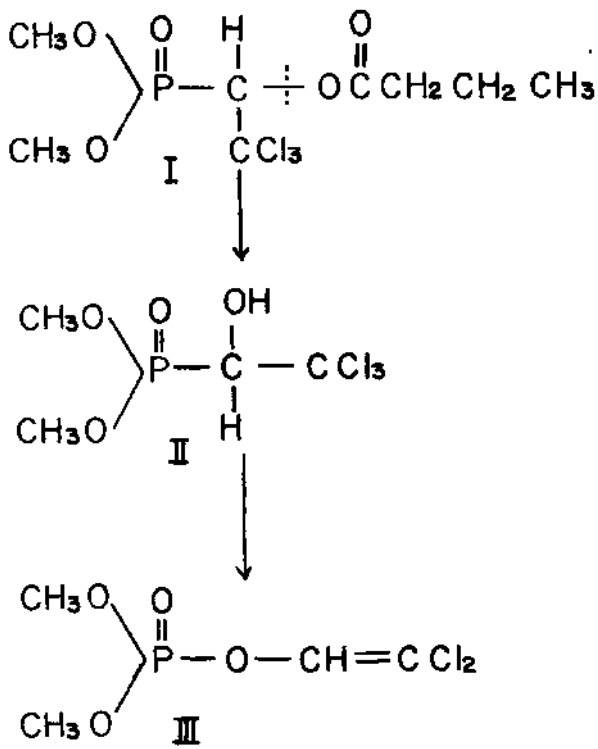

Fig. 4
Carboxylic ester hydrolysis of Butonate (I) yields Dipterex (II) [7] which may be dehydrochlorinated by houseflies forming DDVP (III) [64]

The acetyl derivative of Dipterex (*O,O*-dimethyl 2,2,2-trichloro-1-acetoxyethyl phosphonate) and the Butonate derivative (*O,O*-dimethyl 2,2,2-trichloro-1-*n*-butryloxyethyl phosphonate) [6] [7] when compared with Dipterex are less toxic to mammals but about equally toxic to houseflies. These two derivatives were hydrolysed *in vivo* by rats and insects at the carboxylic

ester group to form Dipterex as an intermediary metabolite [6] [7]. Hydrolysis of the carboxylic ester group of Butonate was more complete in the American cockroach and housefly than in rats [7]. Carboxylic ester hydrolysis increased the anticholinesterase activity and toxicity of Butonate and Acetyl-Dipterex, while this hydrolytic process rendered Malathion non-toxic.

Co-Ral

Co-Ral (*O,O*-diethyl *O*-[3-chloro-4-methylumbelliferone] phosphorothioate) is an effective animal systemic insecticide when applied dermally [88] [92] but ineffective when administered orally [1] [58]. Following oral administration, Co-Ral was rapidly degraded to water-soluble metabolites and excreted in the urine and faeces of rats [53] [103] and steers [48]. Co-Ral was absorbed very poorly through the intestinal tract of steers [48]; 35% of an oral dose of 10 or 20 mg/kg was eliminated in the faeces within 7 d of treatment as compared to 38% eliminated in the urine. Rats also eliminated a large amount of the administered dose in the faeces [103]. Co-Ral was absorbed slowly through the skin of rats, a cow, a goat [50], steers [48] and bulls [85], and the radiolabelled materials absorbed in the blood were water-soluble metabolites [48] [85]. Thus, the effectiveness of dermal applications of Co-Ral against the cattle grub, *Hypoderma lineatum* (DeVill), in the gullet of cattle [20] cannot be explained on the basis of Co-Ral transport by the blood. When Co-Ral was injected into steers at 5 to 10 mg/kg, 85% or more of the injected dose had moved from the site of injection within 3 d of the treatment [23]. Minute quantities of the oxygen analogue of Co-Ral were isolated from muscle tissue. About 3% of the injected Co-Ral equivalents were accounted for in the faeces [23]. From 2 to 6% of the Co-Ral applied dermally was eliminated in the urine of cattle [85].

The selective toxic action of Co-Ral has been explained by differences in activation and deactivation processes in insect and mammalian tissues [72]. Phosphorothioate oxidation was the only activation mechanism in mammals [50] [103]; the production of Coroxon, the oxygen analogue of Co-Ral, was also pronounced in the housefly [72] [103] but was less extensive in the cattle grub [72]. The liver was primarily responsible for Co-Ral metabolism; activation and degradation systems were operative in the liver of the ox, but the degrading system was more potent [72]. In insects susceptible to Co-Ral, such as the housefly and cattle grub, there was an activation but no degradation system [72]. When P^{32}-labelled Co-Ral was applied topically at 25 mg/kg to the housefly, stable fly, or German cockroach, about 50% of the applied material was absorbed within 4 h of treatment [103]. From 91 to 99% of the non-hydrolysed radioactive materials was characterized as unchanged Co-Ral; the remainder was the oxygen analogue. The significance of Coroxon production in insects is not quite clear, since Co-Ral and Coroxon are about equally toxic to the housefly [86] [103] and other insects [103].

Degradation of Co-Ral to non-toxic products was more rapid in mammals than in insects. In mammals, hydrolysis occurred at the P-C-coumarinyl bond and less extensively at the ethoxy linkage (Fig. 5) [50] [103]. Opening of the pyrone ring of Co-Ral occurred in alkali [46] [50], and the pH (8.0—8.5) of cow urine was conducive for the formation of open-ring Co-Ral [50]. Opening of the pyrone ring was not a suspected degradative mechanism in rats [103] or poultry [35] [36]. Apparently, de-ethylation occurred before and after Coroxon formation [50].

Co-Ral metabolism was investigated in laying-hens following dermal or oral administration [35] [36]. Co-Ral was rapidly metabolized to water-soluble products and no organic solvent-soluble materials were detectable in internal tissues within 3 d of dermal application

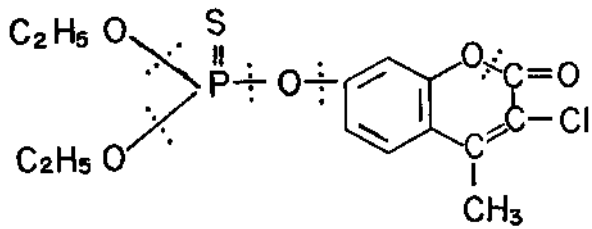

Fig. 5
Co-Ral sites susceptible to hydrolysis in biological systems [50] [103]

at 50 mg/kg. Radioactive materials excreted in the faeces consisted of residual Co-Ral, the oxygen analogue, diethylphosphoric acid, and diethylphosphorothioic acid. Some Co-Ral was completely degraded to phosphoric acid that was incorporated into normal phosphorus-containing metabolic products (Table I). Radioactive phospholipids, ribose nucleic acid, and desoxyribose nucleic acid were isolated from the liver [35]. These naturally occurring phosphorus-containing metabolites were also isolated from the liver and kidney of bulls treated with Co-Ral; P^{32} was an integral part of the normal metabolites [85]. Laying-hens receiving Co-Ral at 100 ppm in the feed eliminated 79% of the Co-Ral equivalents in the faeces by 28d of post-treatment; over 85% of the excreted materials were hydrolytic products [36].

TABLE I

FRACTIONATION OF THE RADIOACTIVE MATERIALS FROM FAECES AND LIVER OF HENS DUSTED WITH CO-RAL AT APPROX. 50 mg/kg

Tissue or Excreta	Days after Treatment	Per cent present as * —				
		Acid Soluble Compounds	Phospholipids	RNA **	DNA **	Residue
Liver	3	35.8	25.4	36.8	1.6	0.4
Liver	7	23.9	28.5	43.1	4.5	0
Faeces	3	46.6	45.2	7.4	0	0.8
Faeces	7	44.2	23.4	12.7	9.1	10.6

* Fractionation procedure of Schneider [90].
** Ribose nucleic acid and desoxyribose nucleic acid, respectively.

The low toxicity of Co-Ral to mammals or poultry may be attributed to poor absorption through the skin or digestive tract and to rapid hydrolysis of the organophosphate. Co-Ral was absorbed quite readily through the cuticle of insects; degradative processes were slow, but activation was rapid. Thus, the selectivity of Co-Ral is probably due to differences in absorption, activation, and detoxication processes in insects, mammals and poultry.

Ronnel

Ronnel (*O,O*-dimethyl *O*-2,4,5-trichlorophenyl phosphorothioate) administered to cattle by oral drench or bolus at 100 mg/kg was almost 100% effective for controlling cattle grubs [2] [45] [59] [82] [89]. About 50% of a 100 mg/kg dose of Ronnel administered to a cow

was eliminated in the urine by 7 d of post-treatment [78]. Although the presence of the oxygen analogue was not demonstrated *in vivo* [78], its formation by rat liver was established *in vitro* [15]. Ronnel was hydrolysed at the *P-C*-methyl and the *P-O*-phenyl bonds by rats and a dairy cow [78]. In houseflies, hydrolysis of Ronnel was predominantly at the phenyl group, while in rats, hydrolysis occurred mostly at the *P-C*-methyl group. This difference in hydrolytic attack of Ronnel by insects and mammals may serve to explain selective toxicity [78]. This explanation of selective toxicity was substantiated with other dialkyl aryl phosphorothioates [79]. Alkyl-phosphate hydrolysis was an alternate detoxication mechanism in mammals, when the dosage was too great to be metabolized through hydrolysis of the aryl-phosphate bond. Very little alkyl-phosphate hydrolysis occurred in American cockroaches. The lower alkyl-phosphate hydrolysis in cockroaches and houseflies as compared to rats may contribute to selective toxicity, but does not explain toxicity differences between different species of mammals [79].

Ruelene

Ruelene (4-*tert*-butyl-2-chlorophenyl methyl methylphosphoramidate) possesses promising parasiticidal and systemic insecticidal activity in livestock [60] [91] [101]. The *in vivo* degradation of Ruelene is complex and involves enzymatic attack at the 3 different ester groupings (Fig. 6). Ruelene was highly unstable in sheep; almost complete hydrolysis

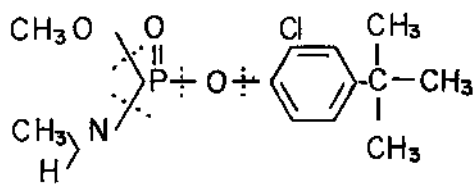

Fig. 6
Ruelene sites susceptible to hydrolysis in biolocical systems [15] [21] [98]

occurred within 24 h of treatment [98]. Hydrolysis occurred at the *P-N*-methyl, *P-O*-methyl, and *P-C*-phenyl bonds to form a large number of metabolites. The complete metabolic scheme was not established in sheep [98] [100] or poultry [21] but the following metabolites were isolated and identified: *O*-methyl phosphoric acid, 4-*tert*-butyl-2-chlorophenyl methyl phosphoric acid, *O*-methyl *N*-methyl amidophosphoric acid, and 4-*tert*-butyl-2-chlorophenyl methylamidophosphoric acid. 6, possibly 9, metabolites were isolated from sheep and poultry excreta using anion-exchange chromatography [80] and paper chromatography.

P^{32}-labelled Ruelene and a polymer formulation of Ruelene were administered orally to sheep at 50 mg/kg [100]. The polymer decreased the hydrolytic rate of Ruelene and decreased the amount of absorption through the intestine; consequently, there was a decrease in Ruelene residues in internal tissues such as muscle, liver, kidney, and fat. The faeces from sheep treated with the polymer formulation of Ruelene contained more Ruelene equivalents than the faeces from sheep treated with the non-poylmer formulation (Table II). A polymer formulation of an insecticide may limit its usefulness for cattle-grub control, but increase its effectiveness in controlling the multitude of livestock pests that breed in faeces, and in controlling gastrointestinal parasites. The polymer formulation of Ruelene was as effective as commercial formulations for controlling several species of gastrointestinal parasites of sheep [101].

TABLE II

CUMULATIVE PERCENTAGE OF RUELENE EXCRETED IN URINE AND FAECES OF SHEEP TREATED ORALLY AT 50 mg/kg

Days after treatment	Urine		Faeces	
	Polymer treatment	Non-polymer treatment	Polymer treatment	Non-polymer treatment
1	16.5	50.9	12.6	7.8
2	22.8	63.9	22.2	9.3
3	35.7	65.3	26.4	10.8
4	37.8	66.0	39.8	13.2
5	37.8	67.1	47.5	14.6
6	38.1	68.0	49.3	15.0
7	38.2	68.9	50.3	16.0

Ruelene was rapidly metabolized by laying-hens receiving the toxicant in the diet for 7 d at 100 ppm [21]. When hens were returned to normal feed for 3 d, toxic residues had dissipated from several tissues. Ruelene was degraded to phosphoric acid that was incorporated into normal phosphorus-containing metabolites, particularly in bone tissue, liver and kidney. Only 29% of the Ruelene consumed in the feed was eliminated in the faeces during the 21-d experimental period. The complete degradation of Ruelene to phosphoric acid and the utilization of this acid in the normal metabolic pool probably accounted for the small percentage of the Ruelene equivalents eliminated in the excreta [21]. Six metabolites were isolated from the excreta; *O*-methyl phosphoric acid and 4-*tert*-butyl-2-chlorophenyl methyl phosphoric acid were tentatively identified [21].

The absorption and metabolism of Ruelene was studied in 19 species of insects [19]. In general, Diptera degraded Ruelene more completely than lepidoptera, followed by coleoptera, then hemiptera. A large portion of the absorbed Ruelene was not extractable from the cuticular fraction. Ruelene was absorbed more readily by the American cockroach than the German cockroach, but the absorbed material was more stable in the German roach. This animal systemic insecticide was considerably more stable in insects [19] than in sheep [98] [100] or poultry [21], and metabolism was less extensive in insects as judged by the limited number of metabolites resolved by paper chromatography.

Baytex

Baytex or Bayer 29493 (*O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate) is a promising animal systemic insecticide [16]. Structurally, Baytex is similar to Systox and Phorate (Thimet), and its oxidative and hydrolytic metabolites are analogous to those of the aliphatic phosphorothioates [54] and phosphorodithioates [12]. About 80% of the Baytex administered orally to rats at 100 mg/kg or administered intraperitoneally at 10 mg/kg per day for 10 d was eliminated in the urine and faeces [10] [17]. More than 90% of the P^{32}-materials in the urine and faeces were the hydrolytic products, dimethyl phosphoric and dimethyl phosphorothioic acids. Hydrolysis of the *P-O*-methyl bond probably accounted for the unidentified ionic product isolated by anion exchange chromatography. The hydrolysis of Baytex by rats receiving multiple intraperitoneal injections followed a peculiar pattern in that the percentage of hydrolytic products decreased in the urine as the number of Baytex

doses increased. Perhaps there was an over-saturation of the enzymes necessary for complete hydrolysis [17].

Baytex underwent thiophosphate and thiophenyl oxidation forming 5 oxidative metabolites in rats, houseflies, German cockroaches, and boll weevils [10] [17]. Oxidative products were produced by rats and insects; hydrolysis was more rapid in rats. The characterized oxidative metabolites were the oxygen analogue, the sulphoxide and sulphone of the parent compound, and the sulphoxide and sulphone of the oxygen analogue (Fig. 7). The percentage of oxidation

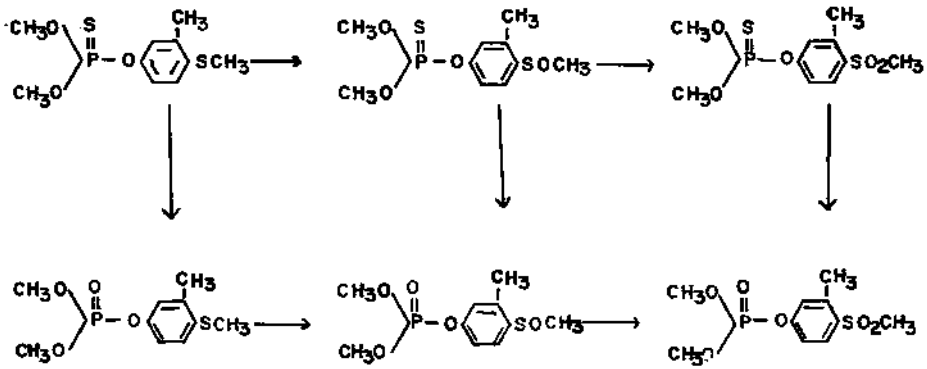

Fig. 7
Oxidative metabolites of Baytex produced by insects and rats [10] [17]

products was variable between insect species; the percentage in the urine and faeces was also quite variable (Table III). More efficient oxidation and greater stability of Baytex in insects as compared to rats probably accounts for the selective toxicity of this material.

TABLE III

FRACTIONATION OF BAYTEX OXIDATION PRODUCTS BY CELITE PARTITION CHROMATOGRAPHY

Biological System	Time after Treatment	Per cent of Chloroform-Soluble Radioactivity*					
		Peak I	Peak II	Peak III	Peak IV	Peaks V&VI	MeOH
Rat (urine)**	1 d	6.5	3.7	1.3	0	87.4	1.1
„ „	2 d	2.2	3.6	2.7	0	86.5	5.0
„ „	3 d	2.2	7.7	21.4	6.4	57.4	4.9
Rat (faeces)	1—3 d	22.4	0	53.5	0	14.3	9.8
Housefly**	1 h	63.5	5.5	7.8	18.3	4.9	0
Housefly	4 h	44.3	5.7	3.3	18.6	26.3	1.8
German Roach	1 h	91.1	0	0	6.0	3.0	0
German Roach	4 h	52.7	.5	3.1	31.5	11.9	.3
Boll Weevil	4 h	48.6	20.5	18.4	8.5	4.0	0

* (I) Bayer 29493, (II) the sulphoxide of Bayer 29493, (III) the oxygen analogue of Bayer 29493, (IV) the sulphone of Bayer 29493, (V) the sulphoxide of the oxygen analogue, and (VI) the sulphone of the oxygen analogue. MeOH fraction represents unidentified metabolites.
** Rats treated orally at 100 mg/kg; insects treated topically at 25 mg/kg.

There was no evidence that the *S*-methyl isomerization product of Baytex was formed *in vivo* as was demonstrated for Bayer 25141 (*O,O*-diethyl *O-p*-[methylsulphinyl] phenyl phosphorothioate) in plants [11]. *S*-alkyl isomers are generally formed by heating phosphorothioates such as Parathion [57] [61] and Systox [40], but the activation reaction has not been demonstrated in insects or mammals. However, the possibility exists that the reaction is a new activation process in these biological systems.

Bayer 22408

Bayer 22408 (*O,O*-diethyl *O*-naphthaloximido phosphorothioate) is a new phosphorothioate that compares favourably in toxicity to mammals with the least toxic of the organophosphates and is highly effective against many species of insects [13]. Bayer 22408 was metabolized to its oxygen analogue, ethyl phosphoric acid, diethyl phosphoric, and diethyl phosphorothioic acids by rats and several species of insects [14]. The degradation of Bayer 22408 by insects and rats did not differ in the number of metabolites but in the amount of each metabolite formed. Bayer 22408 was stable in insects; most of the absorbed material was recovered as administered. Bayer 22408 was degraded rapidly by rats to water-soluble phosphoric acids, which were eliminated primarily in the urine. The differences among the cumulative percentages of radioactivity eliminated in the faeces from rats treated orally at 100 mg/kg subcutaneously at 500 mg/kg or dermally at 1000 mg/kg were negligible. About 25% of the administered dose was eliminated in the faeces within 7 d of treatment, regardless of the route of administration. More ethyl and diethyl phosphoric acid was present on the first day following treatment of rats orally or subcutaneously than on the second and third days (Table IV). In comparison with other phosphorothioates, Bayer 22408 was unusual in this respect [9] [78]. Since a large amount of Bayer 22408 was eliminated in the faeces of mammals, it may have a practical use for controlling pests of livestock that breed in faeces [14].

TABLE IV

HYDROLYTIC PRODUCTS OF RADIOACTIVE BAYER 22408 ISOLATED FROM RAT URINE AND CHARACTERIZED BY ANION-EXCHANGE CHROMATOGRAPHY

Treatment	Days after Treatment	Per cent present as —	
		$(C_2H_5O)_2P(O)OH$	$(C_2H_5O)_2P(S)OH$
Oral (100 mg/kg)	1	35.4	64.6
,,	2	20.3	79.7
,,	3	17.1	82.9
Subcutaneous (500 mg/kg)	1	31.9	68.1
,,	2	25.3	74.7
,,	3	21.8	78.2
Dermal (1000 mg/kg)	1	21.2	78.8
,,	2	21.1	78.9

P^{32}-labelled Bayer 22408 was applied dermally as a 0.5% emulsion to two Holstein dairy cows [22]. Detectable quantities of the intact insecticide were isolated from milk for the first 6 d of post-treatment, and Bayer 22408 equivalents in the milk were about 10 times higher than the actual Bayer 22408 (Table V). No oxygen analogue of the parent compound was isolated from milk, but it was the predominant non-hydrolysed product in the faeces (Table VI). Less than 1% of the radioactivity applied dermally appeared in the milk, while

about 35% appeared in the faeces [22]. The faecal metabolites were toxic to stable fly larvae but not housefly larvae. The possibility that insecticides might be excreted in milk *in vivo* but would undergo degradation during the first few days *in vitro* can be discounted for such a compound as Bayer 22408, since it was stable in milk under *in vitro* conditions for 14 d [97].

TABLE V

BAYER 22408 AND METABOLITES EXCRETED IN THE MILK OF DAIRY COWS TREATED DERMALLY WITH ONE QUART OF A 0.5% EMULSION PER COW

Days after Treatment	ppm Bayer 22408 in Milk based on —		
	Total P^{32} Materials	Acetonitrile-Soluble Radioactivity	Actual Bayer 22408*
0.25	0.086	0.062	0.053
1	.241	.094	.074
2	.364	.057	.044
3	.365	.039	.023
5	.613	.036	.026
7	.376	.017	.010

* Characterization by co-chromatography on a Celite partition column.

TABLE VI

CELITE PARTITION AND ANION EXCHANGE CHROMATOGRAPHY OF THE ACETONITRILE AND WATER-SOLUBLE RADIOACTIVE MATERIALS RECOVERED FROM THE FAECES OF COWS TREATED WITH BAYER 22408

Days after Treatment	Celite-Acetonitrile			Anion-Water	
	Bayer 22408	O-analogue	MeOH*	$(C_2H_5O)_2P(O)OH$	$(C_2H_5O)_2P(S)OH$
1	4.5	11.4	0.5	17.2	40.6
3	6.7	8.8	.1	26.9	43.0
5	5.3	8.2	.2	18.5	53.3

* Represents unidentified products.

Bayer 22408 was formulated as a polymer and administered orally to sheep at 50 mg/kg [99]. Of the P^{32} administered, a greater percentage was eliminated in the faeces and a smaller percentage in the urine of the sheep treated with the polymer formulation than was excreted in the urine and faeces of the sheep treated with the non-polymer formulation. The ratio of the diethyl phosphoric acid and diethyl phosphorothioic acid in the excreta of sheep was about the same as for rats [14]. The 22408 eliminated in the faeces of sheep was resistant to weathering for at least 20 d under natural conditions. Leaching of the phosphoric acids from the faeces occurred but the Bayer 22408 remained in the faeces. This finding might be of significance in controlling fly larvae in faeces or in controlling infective stages of roundworms [99]. The effectiveness of Bayer 22408 against several species of gastrointestinal parasites has been demonstrated [101], but this phosphorothioate does not possess animal systemic properties [14].

Other organophosphates

This discussion on activation and detoxication mechanisms of systemic and non-systemic organophosphates would not be complete without mentioning two other biochemical mechanisms. The phosphoramides, Schradan and Dimefox, and the phosphorothioate, Parathion, are not animal systemics but their activation and detoxication processes are unique. Most of the metabolism studies with Schradan were conducted before radioisotope techniques became a common laboratory-tool [4] [24] [67] [102]. P^{32}-labelled Schradan and Dimefox were prepared and their metabolism was investigated in insects and mammals [8]. The American cockroach and rats converted both phosphoramides to the more potent anticholinesterase agents, the *N*-oxides of the parent compounds. Whether the *N*-oxide rearranges to the *N*-methoxide [25] [26] or to the methylol derivative [68] has not been established conclusively. The liberation of a formaldehyde-yielding material in this oxidative and rearrangement reaction resulted in the formation of heptamethylpyrophosphoramide before hydrolysis of the P-O-P bond. Hydrolysis of Dimefox liberates HF [73]. Schradan was more stable to hydrolysis than Dimefox in either biological system [8]. It has been postulated that susceptibility of insects to Schradan depends on activation of the compound within nerve-tissue and that, in non-susceptible species, the rate of conversion in the fat body is so rapid that little or no unconverted compound reaches the nerve tissues [67]. Possible pathways for the hydrolytic breakdown of Schradan that could account for its ineffectiveness against many insects have not been found.

The toxicity of Parathion to the American cockroach was due to its conversion to Paraoxon [62]. In susceptible houseflies, Para-oxon accumulated to a greater extent than in resistant strains [74]. P^{32}-labelled Parathion was converted to Para-oxon by a cow but, in *in vitro* studies utilizing rumen fluid, this metabolite was not a major constituent. Parathion follows two metabolic detoxification pathways, hydrolysis of the P-O-phenyl bond and reduction of the *p*-nitro group forming amino Parathion. The reduction takes place before or after Parathion oxidation and the reduced Parathion is relatively innocuous [3]. This detoxification mechanism appears unique for *p*-nitrophenyl phosphates.

The metabolism of other P^{32}-labelled organophosphates in mammals that has been investigated includes Delnav (2,3,-*p*-dioxane dithiol*S*,*S*-bis [*O*,*O*-diethyl] phosphorodithioate) [9] [30] [81], Phosdrin (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate) [28], and Diazinon (*O*,*O*-diethyl *O*-[2-isopropyl-6-methyl-4-pyrimidryl] thiophosphate) [84]. Although these pesticides are not animal systemics, they are of interest as contact insecticides for use on mammals and forage crops [5] [38] [49] [77].

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DISCUSSION

F. P. W. WINTERINGHAM (United Kingdom): I should like firstly to congratulate Dr. Arthur on his constructive interpretation of insecticide metabolism, especially in relation to selective toxicity. At the same time, I hope that he will forgive me if I expand a little on one of his statements. In discussing the Dipterex-DDVP metabolism in insects, I think he drew an analogy between the removal of HCl from DDVP and the removal of HCl from DDT. I don't think Dr. Arthur would mean that the processes are identical, because in actual fact the enzyme responsible for DDT dehydrochlorination is—most unfortunately!—not the same as that dehydrochlorinating Dipterex. If it were so, we might expect Dipterex to be more toxic to those DDT-resistant houseflies which possess high concentrations of DDT dehydrochlorinase than to DDT-susceptible flies. There are indeed reasons for expecting that an enzyme capable of dehydrochlorinating the lipophilic DDT would not be capable of similarly dehydrochlorinating Dipterex.

B. W. ARTHUR: Thank you for your elucidation. I am still not quite clear, however, as to why DDT dehydrochlorination does not attack Dipterex.

F. P. W. WINTERINGHAM: I think the answer to that almost certainly lies in the very high degree of specificity of enzymes. There are many enzymes which, of course, are hydrogen-activating—the dehydrogenases and so on—but they are highly specific in the substrates they attack. Now, the enzyme which attacks DDT is, I think, fairly specific for the DDT-type molecule. There is not only a big difference between DDT and Dipterex, even in terms of solubility in the phases in which they are likely to finish up in the insect, but the dehydrochlorination of Dipterex of course also involves a molecular rearrangement. I suspect that the enzymes which bring about these two reactions are quite different.

J. E. CASIDA (United States of America): The reported DDVP in insects could be attributed to the method of work-up, since the spontaneous dehydrochlorination could have yielded the amount that was recovered. Dibrome, on the other hand, will react with sulphydro compounds to give DDVP. This has now been reported for a number of systems, but apparently a non-enzymatic debromination is involved.

We have been working on the enzymes that break down DDVP, carrying out a strictly enzymological study to find out how many enzymes are concerned. There are at least seven acting by breaking off the methyl, the dichlorovinyl, taking the aldehyde to dichloroethanol. taking the desmethyl to dichlorovinylphosphate, cleaving that to phosphoric acid. And in a few cases some of these demethylating esterases are sensitive to other phosphates—the dephosphoramidating esterases, for example.

CL^{36}-DIELDRIN IN MICE

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Abstract — Résumé — Аннотация — Resumen

Cl^{36}-dieldrin in mice. The distribution of Cl^{36}-Dieldrin injected intravenously into mice (L.A.C. grey strain) was studied. Extraction of Cl^{36}-Dieldrin from tissues proved difficult, and experimental techniques are described in detail.

Shortly after injection, high concentrations were found in the liver and brain, but the compound rapidly dispersed and, 24 h after injection, was mainly in the fatty tissues. Very little was excreted. Some was probably metabolized. An attempt is made to relate these findings to the toxic effects.

La dieldrine marquée au chlore-36 chez la souris. L'auteur a étudié la distribution de la dieldrine marquée au chlore-36 injectée par voie intraveineuse dans des souris (variété grise L.A.C.). Il s'est révélé difficile d'extraire la dieldrine marquée au chlore-36 des tissus des sujets; l'auteur décrit en détail les méthodes employées à cette fin.

Peu de temps après l'injection, on a observé des concentrations élevées dans le foie et le cerveau; cependant, le composé s'est dispersé rapidement, et, 24 h après l'injection, il était contenu principalement dans les tissus adipeux. Il en a été rejeté très peu par excrétion. Il est probable qu'une partie de la substance était métabolisée. L'auteur tente d'établir une relation entre ces résultats et les effets toxiques.

Диелдрин-Cl^{36} в мышах. Было изучено распределение введенного внутривенно мышам (L.A.C. серый вид) диелдрина-Cl^{36}. Извлечение диелдрина-Cl^{36} из ткани оказалось трудным, и в докладе детально описываются использованные для этого методы.

Вскоре после введения высокие концентрации вещества были обнаружены в печени и мозге, однако соединение быстро рассеялось и через 24 часа после введения находилось в основном в жировых тканях. Очень небольшая часть была выделена. Часть, вероятно, метаболизировалась. Была сделана попытка согласовать эти выводы с токсическим действием.

Distribución del dieldrin-^{36}Cl en los ratones. El autor ha estudiado la distribución del dieldrin-^{36}Cl administrado por vía intravenosa a ratones (cepa gris L.A.C.). Resultó difícil extraer el insecticida marcado de los tejidos de los animales; el autor describe detalladamente los métodos utilizados con tal fin.

Poco tiempo después de la inyección se hallaron concentraciones elevadas del insecticida en el hígado y el cerebro, pero el compuesto se dispersó rápidamente y 24 h después de la inyección se encontró principalmente en los tejidos adiposos. Muy poca cantidad fue excretada y una pequeña parte de la sustancia fue metabolizada. El autor procura establecer una relación entre estos resultados y los efectos tóxicos.

I. Introduction

Dieldrin (1:2:3:4:10:10-hexachloro-6:7-epoxy-1:4:4a:5:6:7:8:8a-octahydro-1:4-endo-5:8-exodimethanonaphthalene) is a widely used insecticide, toxic to mammals. Many cases of human poisoning have been reported [1]. PATEL *et al* [2], for example, have described twenty. The mechanism of action is unknown. It seemed possible that a knowledge of the distribution and fate of Dieldrin *in vivo* would help us to understand the mechanism, but such studies were hampered by difficulties in estimating low concentrations in animal tissues, and by the impossibility of establishing the presence of metabolites, which might be important. This situation was changed when the Shell Chemical Company made available supplies of Dieldrin labelled uniformly with Cl^{36}.

This paper describes some preliminary experiments with Cl^{36}-Dieldrin. Mice were chosen as experimental animals, because they are the smallest readily available species which show the typical syndrome of convulsions. Complete absorption of the compound into the system was ensured by injecting it intravenously.

II. Compounds and Methods

Dieldrin

A commercial specimen was recrystallized twice from ethanol. The product melted at 175.5°C, over a range of less than 0.5°.

Cl^{36}-dieldrin

The specimen was a gift from Shell Research Ltd., Sittingbourne, Kent, U. K. All experiments were carried out on one specimen, of specific activity 95.4 μc/g.

Mice

Most experiments were carried out on female LAC grey mice, of 25—30 g weight. A few were carried out on female Swiss albino mice.

Administration

Dieldrin was injected intravenously by the tail vein as a 1% solution in glycerol formal. Trials showed that doses were accurate to about 2%.

Extraction of Cl^{36}-dieldrin from tissues

Blood was obtained by decapitating mice, and running the blood into a weighing bottle containing heparin. A known volume was used for an estimation. Faeces and urine were collected from a group of 5 mice over a 24-h period after injection. The separation of faeces from urine was imperfect. The extraction methods used varied as shown below.

(a) Brain, blood, heart, kidney, lung, muscle and faeces

These were ground with 20 times their weight of Na_2SO_4 anhydrous, and placed in a Soxhlet thimble. Unlabelled Dieldrin, 50 mg in 3 ml *n*-hexane, was run into the contents of the thimble (to brain, exactly 22.1 mg in 2 ml were added). The Dieldrin was then extracted for 2—3 h with *n*-hexane in a Soxhlet apparatus, and the extract concentrated to about 2 ml.

(b) The gastro-intestinal tract

The gastro-intestinal tract was divided into three parts: large intestine, small intestine, and stomach with duodenum. Each was macerated for 5 min with 50 ml acetone in a homogeniser with 30 mg of carrier Dieldrin. The acetone extract was separated by filtration and evaporated down to 5 ml, 20 ml of light petroleum (80—100°C) were added, and the mixture evaporated down in turn to 2—3 ml, thus removing the acetone. The residue was diluted with 20 ml of light petroleum, shaken with a few ml of water, and the layers separated by centrifugation. The petroleum layer was removed and evaporated to near dryness, and the residue dissolved in *n*-hexane. The separation with water was necessary because solids and a small aqueous layer separated out when the acetone was removed.

(c) Skin

The skin was shaved and boiled with acetone for several hours. The acetone extract was worked up as under *(b)*.

(*d*) *Fat*

Fat was dissolved in boiling *n*-hexane and filtered.

(*e*) *Urine*

Urine was extracted with *n*-hexane, and the extract concentrated to 10 ml.

(*f*) *Carcass*

This was the residue left after the organs listed had been removed. The carcass was macerated with acetone and left several hours at 4°. The acetone was filtered off, and worked up as under (*b*).

(*g*) *Whole mice*

Each mouse was homogenized with about 150 ml of water for 5 min in a Townson and Mercer bottom-drive macerator. The whole homogenate was then transferred to a flask with about 1.2 liters of acetone and 1 g (accurately weighed) of carrier Dieldrin, and the mixture refluxed for 3 h. The acetone was filtered off, and the filtrate boiled down to about 300 ml. Light petroleum (300 ml, BP 100—120°) was added, and boiling continued to expel the acetone. The petroleum layer was separated, and the petroleum boiled off as completely as possible. The residue was dissolved in about 100 ml of *n*-hexane.

ESTIMATION OF CL^{36}-DIELDRIN

Three methods were used:

(a) For all but whole mice and extracts from brains, the *n*-hexane extracts obtained were diluted to 10 ml with *n*-hexane (carcass samples to 50 ml) and counted in a liquid counting tube. The sensitivity was 3—4 cpm/μg of Cl^{36}-Dieldrin, depending on the particular tube used. Enough counts were recorded to give a standard error on the counts equivalent to 0.5 to 1 μg of Cl^{36}-Dieldrin.

(b) Each *n*-hexane extract from a whole mouse was extracted once with an equal volume of methyl cyanide to separate the Dieldrin from fat. The methyl cyanide was evaporated to dryness, whereupon Dieldrin crystallized out. The Dieldrin was recrystallized in turn from *n*-hexane and ethanol (the solvents must be used in this order), dried at 110° for 15 min, weighed, and its MP determined. The MP-range was usually 1—3°, and the yield 0.2—0.35 g, i.e. 20—35% of carrier Dieldrin. The Dieldrin was dissolved in 10 ml of *n*-hexane, counted in a liquid counting tube, and the quantity of Cl^{36}-Dieldrin in the mouse calculated on the assumptions usually made in isotope dilution experiments, i.e. that the only labelled compound appearing in the purified Dieldrin was Cl^{36}-Dieldrin, and that all the Cl^{36}-Dieldrin was extracted from the tissue.

(c) Cl^{36}-Dieldrin in brain extracts was estimated as AgCl^{36}. The Dieldrin had, therefore, to be separated from any inorganic or water-soluble chlorides and from fat, and its chlorine content converted to inorganic chloride. Adaptations of well-known methods were used. The extract was diluted with ether, and washed with water to remove inorganic chlorides. The ether was removed by evaporation and the residue dissolved in *n*-hexane and methyl cyanide (about 4 ml of each). The *n*-hexane was extracted 3 times with methyl cyanide, a process which extracts most of the Dieldrin but leaves most of the fats in the hexane [3]. The methyl cyanide was evaporated, and the residue refluxed with 5 ml *sec*-butanol and 0.2 g metallic Na for 2½ h to obtain the Cl^{36} as inorganic chloride [4]. The mixture was cooled, 3 ml of water added, and the butanol evaporated in an air stream at 100°. The residue was made slightly acid with HNO_3. It still contained some interfering compounds, which

were extracted with ether. Agar (1 ml of a 0.1% solution) was added, followed by 5 ml of 0.1 $N AgNO_3$. The AgCl precipitate was filtered through a sintered glass planchette, porosity 3, washed with 0.02 $N HNO_3$ (50 ml), a little water, and acetone, and dried at 150° for 30 min. The planchette was cooled, and the precipitate weighed, and counted with an end-window counter. The carrier Dieldrin added was enough to give 50 mg of AgCl. As the planchettes were 7 cm^2 in area, self-absorption was negligible during counting. The concentration of Agar was critical, and each solution had to be adjusted to give a suitable precipitate — fine enough to spread over the planchette, but not so fine that it ran through. The precipitation and counting procedure were tested using HCl containing HCl^{36}. In 18 gravimetric estimations, the recovery of AgCl was 100 ± 1%, and the standard deviation on the counts was 1.98%. When, however, Cl^{36}-Dieldrin and carrier were added to a sodium-sulphate brain-mixture in the Soxhlet thimble before extracting, the recoveries of Dieldrin chlorine as AgCl varied from 60 to 95%. The major cause of this variation was the presence of compounds during the precipitation which altered the state of the precipitate so that variable amounts passed through the planchettes. This was corrected for from the weights of AgCl actually obtained and those obtained theoretically from the known weight of carrier. 17 determinations were made with these corrections. The cpm/μg of Cl^{36}-Dieldrin were 20.4 ± 1.5 (SD). The method therefore gave six times more counts/μg Cl^{36}-Dieldrin than did liquid counting.

As the standard error was fairly high, the method was not more accurate than liquid counting when the quantity of Cl^{36}-Dieldrin exceeded about 10 μg. One was able, however, to show the presence of about 0.1 μg of Cl^{36}-Dieldrin with certainty since the background of the end-window counter was only about 7.5 counts/min. With liquid counting the background was higher, and about 1 μg was required to give a definite positive count.

Metabolites

The experimental procedures are described later.

III. Results

LD_{50} of dieldrin and signs of poisoning

The intravenous LD_{50} in female mice (LAC greys) of a 1% solution of unlabelled Dieldrin was 15.25 mg/kg, with fiducial limits of 14.25—16.32 mg/kg as calculated by the method of Weil [5].

Mice usually died within 30 min of receiving a lethal dose, but two of the ten deaths observed took place over an hour after injection. Doses of 10 mg/kg and above caused brief clonic convulsions within about 10 min. In severe cases tonic convulsions were observed. Death took place during or immediately after tonic convulsions. It never followed one convulsion only, but ensued after 2, 3 or 4 convulsions. One hour after injection most mice appeared fairly normal. After 16 h they were indistinguishable from normal mice.

For work with Cl^{36}-Dieldrin 12 mg/kg was the dose chosen. This rarely killed, but always caused at least one convulsion.

The effects of a second injection were studied briefly. Mice were given 12 mg/kg intravenously, and then a further dose intravenously, 80 min later. Only 5 mice were treated in this way. They seemed to be at least as resistant as untreated mice. Thus of the two mice given a second dose of 10 mg/kg neither convulsed, the one given 13 mg/kg convulsed three times rather mildly and, of the two given 17 mg/kg, one died after 31 min and the other was killed 23 h later, when it was prostrate. The syndrome was different in the 3 mice which convulsed. Convulsions were never violent, but there was a continuous

shaking movement throughout the convulsive period. All 5 mice had received well above the acute LD_{50} (22, 25 and 29 mg/kg), but only one died rapidly. Thus in mice there is no marked cumulative action of intravenous Dieldrin.

DISTRIBUTION EXPERIMENTS

(a) Brain and blood

These were estimated at various times after injection, with the results shown in Fig. 1. The concentration in the blood fell rapidly to about 0.2 μg/ml. The concentration in the brain rose to a maximum of about 16 μg/g within 10 min of injection, fell roughly linearly for 2 h and then stayed substantially constant at about 1—2 μg/g for a day.

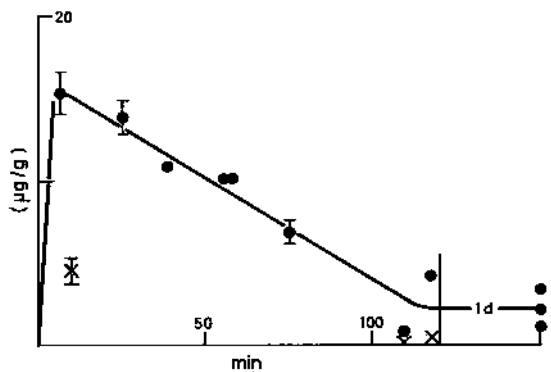

Fig. 1

Cl^{36}-Dieldrin in brain and blood

The concentrations of Dieldrin were determined at different intervals after injecting 12 mg/kg intravenously

● = brain, × = blood, I = standard errors

(b) Other tissues

The concentrations found at various times after injection are shown in Table I. The compound was fairly evenly distributed. Only two aspects appear of interest: the liver concentration was initially high, and remained high for over an hour; and the total recoveries fell rapidly to about 70%.

(c) Urine and faeces

Dieldrin in the urine and faeces was collected from 5 mice in the first 21 h after injection. The faeces contained 6.6 μg/g and the urine 1.6 μg/g. (The urine content may have been leached out of the faeces). These tiny concentrations cannot explain the low recoveries.

(d) Whole mice

Losses may well arise in the piece-meal determination of a compound from many organs, so attempts were made to extract Cl^{36}-Dieldrin from whole mice as already described. From 4 mice killed 10 min after injection, recoveries of 90.0, 93.9, 94.4 and 95.9% were obtained, and from one mouse killed 24 h after injection 92.4%. These recoveries are a marked improvement on those recorded in Table I. They suggest that the Soxhlet extraction method is not really satisfactory. The recoveries were still not complete. When Cl^{36}-Dieldrin

TABLE I

Cl^{36}-DIELDRIN IN MOUSE TISSUES AT VARIOUS TIMES AFTER INJECTION OF 12 mg/kg INTRAVENOUSLY

Organs	Time after injection (min.)		
	6	80	1440
	No. of mice		
	2	3	3
	μg/g of tissue		
brain	16, 14	4.0—7.4	about 2
liver	35, 43	16—22	6—10
lungs	35, 19	7—11	0— 6
kidneys	12, 18	7— 9	3— 7
heart	24, 27	6—11	0
stomach & duodenum	6, 5	3— 6	1— 6
small intestine	8, 8	5—10	5— 8
large intestine & colon	3, 3	4— 7	2— 4
muscle	11, 9	4— 5	3— 6
fat	—	25*	—
skin	2, 2	4— 9	18—19
carcass	8.4, 9.6	8—14	10—12
% recovery of Cl^{36}	82, 89	57—82	61—73

* 1 mouse only.

Quantities are given in μg/g and the extreme ranges are given.

and carrier were added to a homogenate of mouse in water, and the homogenate worked up in the usual way, recoveries of 100.7 and 102.0% were obtained, i.e. quantitative within counting errors. The slightly low recoveries could not, therefore, be attributed either to impurities in the Cl^{36}-Dieldrin, or to interference from natural products. Either Cl^{36}-Dieldrin remained bound to solid matter, or a few per cent was metabolized rapidly.

METABOLITES

The Cl^{36}-Dieldrin was first shown to be at least 98% pure by an isotope dilution technique. Thus when Cl^{36}-Dieldrin was recrystallized with carrier from ethanol the specific activity was 100.5% of the theoretical value. The mother liquors were then evaporated to dryness, and the specific activity of the residue determined to be 102% of the theoretical value. Both results were 100% within the standard errors of the counts, and there was no significant difference between the specific activity of the Dieldrin purified by crystallization and the residue in the mother liquors after crystallization. Cl^{36}-Dieldrin was also chromatographed by the method of MCKINLEY *et al.* [6]. One major peak, $R_f = 0.36$, contained 98% of the total Cl^{36}. Traces of faster and slower moving compounds were found.

Metabolites were looked for in the livers, brains, fat and faeces (all pooled) from 4 mice 21 h after an intravenous injection of 10 mg/kg. The complex extraction procedure shown in Fig. 2 was used. The tissues were extracted in turn with decreasingly polar solvents so that Cl^{36}-compounds of any polarity from that of chloride ion (oil-insoluble, water soluble) to

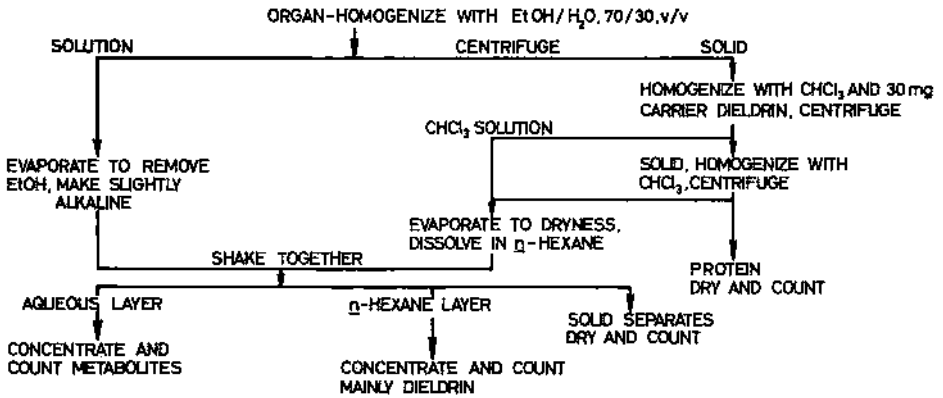

Fig. 2
Extraction procedure to distinguish metabolites from Dieldrin

Dieldrin itself (oil-soluble, water insoluble) were likely to be extracted. The solvents were then evaporated, and the residues dissolved in *n*-hexane and 0.1 *N* NaOH, which were shaken together, separated, and counted. Dieldrin cannot be extracted from *n*-hexane in measurable quantities by 0.1 *N* NaOH, whereas chloride ion and some other possible metabolites can be. Cl^{36} in the caustic fraction therefore shows the presence of metabolites. Some metabolites may not be extracted from *n*-hexane by aqueous NaOH. The results therefore give the minimum degree of metabolism. The residue of the tissue after extraction (mainly protein) was also counted. These counts may represent either Dieldrin or metabolites. The results are shown in Table II. There is evidence that some metabolites are produced. The counts were low, and one cannot extrapolate from the few tissues etc. studied to the whole animal. There is also a possibility that the 'metabolite' counts are misleading, for reasons given in the discussion. No quantitative estimate can therefore be made of the fraction of Dieldrin metabolized.

TABLE II

Cl^{36}-METABOLITES (?) IN MICE 21 h AFTER INJECTION

Organ etc.	*n*-hexane (Dieldrin)	water (metabolites)	protein (?)
brain	1.0	0.2	0.0
liver	5.0	0.8	0.23
fat	16.3	0.8	0.5
faeces	6.6	2.7	0

The standard errors on the counts were equivalent to about 0.2 μg/g.
The concentrations are expressed as μg of Cl^{36}-Dieldrin/g in all cases. Three fractions — *n*-hexane (Dieldrin); water (metabolites); and protein (?) are distinguished.

As only 93% of the Dieldrin injected was recovered from whole mice 10 min after injection, it was possible that some was rapidly metabolized to volatile products such as methyl chloride, and that these were excreted in the expired air. To investigate this possibility

two mice were injected, and placed immediately in a small cylindrical chamber through which air was aspirated at about 150 ml/min. The air stream was stripped of CO_2, water and metabolites in a complex trap immersed in liquid O_2. Low concentrations of compounds are not readily condensed from air, but it was hoped that the CO_2 and water would act as carriers. Collection was continued for 20 min. The contents of the trap were then dissolved in acetone at —80°, and the solution warmed to room temperature and counted. Less than 0.05% of the Cl^{36} given as Cl^{36}-Dieldrin was found in the acetone. Thus very little, if any, volatile metabolites were formed.

IV. Discussion

From these preliminary results some tentative conclusions can be drawn.

Shortly after injection the blood concentration falls rapidly. A high concentration is found in the liver, and a rather lower one in the brain. The concentration initially in the blood is probably much above saturation, so that Dieldrin may well be precipitated in the blood. The initial high concentrations in some organs rich in fine capillaries may therefore be due to nothing more than removal of solid Dieldrin from the blood stream by a filtration process. Later, the Dieldrin is transported from these tissues to more fatty ones: fat itself, skin (subcutaneous fat was included in skin samples), and large intestine. The only surprising feature is the transfer of Dieldrin from the brain, which is rich in lipids. This suggests that much of the Dieldrin in brain is initially deposited as solid in capillaries, and does not enter the brain cells. Very little Dieldrin is excreted.

Recoveries of Dieldrin were incomplete. Later work carried out by Dr. Vandekar in our laboratories on rats suggests that this was because Dieldrin is difficult to extract from animal tissues, and not because it had been decomposed. Some evidence for metabolism was obtained. It is possible that some Cl^{36}-Dieldrin was adsorbed on colloids in the aqueous layer, and so was recorded as 'metabolites', but it is probably that a few per cent of an intravenous dose were metabolized in the first day after injection. Dr. Vandekar, has shown conclusively that a metabolite of Dieldrin is excreted in the bile of treated rats. This metabolite is insoluble in *n*-hexane but soluble in ethanol and water. No volatile metabolites were expired in the first 20 min after injection.

The effects of successive doses on mice suggests that the gross concentration of Dieldrin in the brain is not directly related to toxic effects. Second injections were performed about 80 min after first injections when, from Fig. 1, it appears that the concentration of Dieldrin in the brain was at about half its maximum value. The mice were at least as resistant to the second as to the first dose, although the concentration after the second dose was presumably higher than after the first. This may imply that resistance to Dieldrin is developed rapidly. I think it more likely that much of the Dieldrin found in the brain shortly after injection is not in the brain cells, but is present as solid. This hypothesis would explain why symptoms mount in intensity after a single dose while the brain concentration is decreasing—Dieldrin may still be diffusing continuously into the cells during this period—and would also explain why symptoms are very much more prolonged after a second injection.

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DISCUSSION

J. E. CASIDA (United States of America): Do your results bear any relation to those of Hosein who reported that certain betaine esters accumulated in rodent brain during the Dieldrin syndrome?

D. F. HEATH: It is plain that the action of Dieldrin is on the central nervous system but, as I mentioned, it is possible that the action is not on the brain but is at the spinal level, or that only certain parts of the brain are affected directly. Until very much more is known one cannot say how the betaine ester accumulation is related to Dieldrin poisoning. For example, a convulsion started at one level of the CNS may then affect reflexly other levels, and these secondary effects may cause the accumulation of betaine esters. This suggestion—which may well be incorrect—is made only to show that the relationship may be highly complex.

F. P. W. WINTERINGHAM (United Kingdom): The most recent news I have —indirectely— from Hosein is that he is unable to confirm his original suggestion about gamma-betaine, one reason for this being that he was not dealing with the betaine derivative he thought. May I now ask a question of my own? Did Dr. Heath investigate the possibility of isotope exchange between organic Cl^{36} and tissue chlorides? He did mention, I believe, the possibility of methyl chloride being a metabolite. I would suggest that if that were formed it is very likely that the Cl^{36} as methyl chloride would indeed exchange with tissue chlorides. Many years ago my colleagues and I studied the metabolism of methyl bromide labelled with Br^{82}, and under certain conditions the speed with which the organic Br^{82} exchanged with tissue bromides was quite spectacular.

D. F. HEATH: No, I did not investigate that possibility. Chlorine in Dieldrin itself probably exchanges only very slowly. I agree with Dr. Winteringham that if methyl chloride was formed, some activity would be lost by exchange. Volatile chlorides were only collected for the first 20 min and perhaps there would not be much exchange in this time. Even if exchange was complete, plainly very little CH_3Cl^{36} was formed as the total decomposition of Dieldrin was so low—certainly under 8% in 24 h.

J. E. CASIDA: There have been reports, although I doubt whether any of them have been published, that Dieldrin administered to animals is recovered in part as Aldrin or an Aldrin-like material. Do you feel that by adding cold carrier Dieldrin, and then recrystallizing to constant melting point, you obtained the same results as if you had recrystallized to constant specific activity? If a trace of "Aldrin-like" material had been present, would your procedure have differentiated it from Dieldrin?

D. F. HEATH: In one case I did recrystallize to constant activity with no change in the result. A few μg of Aldrin-like material might, however, recrystallize indefinitely with 1 g of carrier Dieldrin; this was not investigated. Your suggestion does not, of course, explain the low recoveries by other methods; both Aldrin and Dieldrin would be recovered, and recorded as Aldrin.

METABOLISM OF RADIO-LABELLED SYSTEMIC INSECTICIDES IN ANIMALS

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Abstract — Résumé — Аннотация — Resumen

Metabolism of radio-labelled systemic insecticides in animals. A summary is presented of research work on the metabolism of radio-labelled systemic insecticides in animals, with details on experimental procedures and important results. Analyses of samples of blood, urine, faeces, meat and milk are made by various methods in order to trace the fate of an insecticide or its associate residues in an animal. Results are cited for numerous insecticides. Studies are also included in which radioisotopes are used to aid research on the mode of action of insecticides and repellents, metabolism of insect repellents, and insecticide resistance problems.

Métabolisme des insecticides systémiques marqués chez les animaux. L'auteur donne un aperçu de recherches faites sur le métabolisme des insecticides systémiques marqués chez les animaux et fournit des précisions sur les techniques adoptées pour les expériences. On a analysé des échantillons de sang, d'urine, de fèces, de viande et de lait par diverses méthodes pour suivre l'insecticide ou ses résidus dans l'organisme de l'animal. L'auteur indique les résultats obtenus pour un grand nombre d'insecticides. Il fait également mention de travaux dans lesquels on a eu recours aux radioisotopes pour étudier le mode d'action des insecticides et des insectifuges, le métabolisme des insectifuges et les problèmes relatifs à la résistance aux insecticides.

Метаболизм меченных радиоизотопами общих инсектицидов в животных. В докладе дается краткое описание исследовательской работы по метаболизму меченных радиоизотопами общих инсектицидов в животных, а также подробно излагаются экспериментальные методы и важные результаты. При помощи различных методов проводятся анализы образцов крови, мочи, экскрементов, мяса и молока с тем, чтобы проследить за инсектицидом или связанными с ним остатками. В докладе даются результаты для многих инсектицидов. В доклад включены также работы, в которых радиоизотопы использовались для облегчения исследований по способу действия инсектицидов и средств, отпугивающих насекомых, по метаболизму средств, отпугивающих насекомых и по проблемам сопротивления инсектицидам.

Metabolismo de los insecticidas sistémicos marcados con sustancias radiactivas, en los animales. Esta memoria contiene una reseña de las investigaciones realizadas en los animales acerca del metabolismo de los insecticidas sistémicos marcados con sustancias radiactivas; se completa con detalles sobre los procedimientos experimentales aplicados y con los resultados más importantes. Recurriendo a diversos métodos, se analizaron muestras de sangre, orina, heces, carne y leche, a fin de estudiar el comportamiento de un insecticida o de sus residuos en el organismo animal. Se exponen los resultados obtenidos con numerosos insecticidas. Se incluyen también referencias a estudios en que se usaron radioisótopos para facilitar las investigaciones sobre el modo de acción de insecticidas y los insectífugos, el metabolismo de estos últimos, y los problemas de resistencia a los insecticidas.

The use of radio-labelled systemic insecticides in animals has made possible the solution of many otherwise unresolvable problems. We are able to determine with a very high degree of accuracy the residues of the insecticides found in meat or milk. Residues as low as a few parts per thousand million have been determined. It is also necessary to know what happens to the insecticide so that a better judgment can be made as to the true residue. In some instances the insecticide is converted to more toxic materials, and these should be considered part of

the residue. Also, non-toxic compounds are produced which should not be considered as part of the insecticide residue.

Although the first chemicals found to be active systemically in animals were chlorinated hydrocarbons, we have not been able to recommend their use because of associated high residues. Emphasis, therefore, has shifted to the organophosphorus insecticides with which the residues have generally been much lower.

In the Entomology Research Division's studies with radiolabelled organophosphorus insecticides, the principal isotope used has been phosphorus-32, but some work has been done with carbon-14. Other workers have also used sulphur-35. The isotope is always incorporated as part of the molecular structure of the insecticide. Animals are most often treated with the labelled insecticide prepared either as a capsule or bolus, or as a spray. Occasionally the insecticide is injected into the muscle tissue of the animal.

Information as to what happens to the insecticide or the associated residues is obtained from studies on samples of blood, urine, faeces, hair, meat, and milk.

Samples of blood are taken to determine the total amount of radioactive material present when the peak concentration occurs, and whether the radiation is from the parent insecticide or from oxidation or degradation products. The products can be bioassayed to determine their toxicity to various insects. The peak concentration of radioactive material in the blood differs with the compound used and the mode of treatment. With Dimethoate (*O,O*-dimethyl *S*-[*N*-methylcarbamoylmethyl] phosphorodithioate), given orally to cattle, the peak builds up in 2 to 6 h [6] [9], whereas with Ronnel (*O,O*-dimethyl *O*-[2,4,5-trichlorophenyl] phosphorothioate) or Co-Ral (*O*-[3-chloro-4-methyl-umbelliferone] *O,O*-diethyl phosphorothioate) it takes 8 to 12 h [7] [8] [11] [13]. A peak concentration from dermal treatment builds up more slowly than that from oral treatment. For example, when Co-Ral is applied dermally, the peak may be as late as 120 h after treatment [10]. With intramuscular treatments the peak arises faster in the blood stream than with oral treatments. The peak with Dimethoate applied intramuscularly may take less than 1 h.

Not all the radioactive material in the blood stream is the parent insecticide. The blood can be extracted with a solvent such as chloroform, and a nearly quantitative removal of the insecticide and its closely related compounds results. When this procedure was carried out with Co-Ral, a maximum of only 0.035 ppm was found to be the insecticide compared to a total of 0.27 ppm of radioactivity [14]. Another example in which higher concentrations occur is with Dimethoate in which 4.5 ppm was either Dimethoate or its oxygen analogue, compared to 8.4 ppm of total radioactivity [9].

The three compounds, Ronnel, Dimethoate and Co-Ral, are thiophosphates, whereas Ruelene (4-*tert*-butyl-2-chlorophenyl methyl methylphosphoramidate) is a phosphate. In all the extracts of blood from thiophosphate-treated cattle, both the sulphur and the oxygen compounds are obtained. In most instances the oxygen compound is more toxic to insects than the thio compound. Sometimes the difference is only slight, as with Co-Ral, whereas at other times it is considerable, as is illustrated by Dimethoate, in which the oxygen analogue is 40 times as toxic to stable flies [15]. Whereas the percentage of the parent insecticide decreases in the blood with time, that of the oxygen analogue increases [8] [9] [14].

The major route of excretion of phosphorus insecticides is by way of the urine. As with the blood, the peak concentration and the rate of elimination differ with the insecticide and mode of treatment. The elimination of 74 to 86% of the radioactive material from cattle treated orally with Ronnel requires 3 d, whereas about 90% of the radioactive material from cattle treated similarly with Dimethoate is eliminated within 24 h [7] [9]. An even faster rate is obtained with intramuscular treatment, in which 90% is eliminated within 9 h [9].

Elimination when dermal treatments are used is very slow and, as expected, the total elimination is much smaller. Even after 1 week with Co-Ral dermally applied to a confined steer, only 3% of the dosage was recovered in the urine [9]. In an unconfined steer as much as 6% was found in the urine, an indication that approximately half the dosage was taken in orally from licking [14]. As examples of the difference in peak-concentration times for urine, we may cite Dimethoate with its peak at 3 to 6 h from oral treatment, and Ronnel with its peak at 18 to 32 h [9] [11].

Our knowledge of the metabolism of the insecticides comes principally from the chromatography of urine extracts. With Dimethoate and Ruelene, as many as 11 different radioactive compounds have been found [4] [5]. Many of these have been identified. By the use of a compound such as Dimethoate we are now able to put together a fairly complete metabolism picture. The principal product of the Dimethoate breakdown is the carboxy derivative which, in turn, is converted to the dithiophosphate, thence to the thiophosphate, and finally to the dimethyl phosphate. Also, we have evidence that some simple phosphoric acid may be produced. With Ruelene we suspect that the amount of phosphoric acid produced is considerable, because even with oral treatment of cattle only 49% of an applied dosage is recovered [4]. Phosphoric acid from a degraded insecticide is used by the animal as a nutrient in its normal metabolism. The parent insecticides are either not found in the urine, or their amounts are very small [4] [5] [9] [14].

Faecal elimination of the insecticide or its products usually accounts for very small quantities of radioactive material. With Ronnel and Dimethoate given orally, 2 to 7% is found in the faeces [6] [7] [9] [11] 13], whereas with Co-Ral given similarly the amount equals that (34%) found in the urine [3]. The peak concentration in the faeces takes place after that in the urine. For example, the peak concentration, of orally administered Dimethoate in the faeces occurs in 6 to 30 h [9].

Residue determinations have been made for several of the systemic insecticides in animals, from a few hours to a month after treatment. In addition, the amounts of the insecticides appearing in milk have been determined. The largest residues have been obtained after Ronnel treatment, in which as much as 7 ppm was found in fat 2 weeks after treatment [13]. However, the residues in muscle were small, less than 0.05 ppm Co-Ral, Dimethoate, and Ruelene residues of 0.1 ppm were found in the subcutaneous fat of cattle 2 weeks after treatment [9] [12] [14]. Extrapolation of data showed that none of the residues persisted as long as 60 d.

The amount of the residue was always small when compared to the total radioactivity found in the tissue. For example, the residue for Ronnel was 0.05 ppm in liver, but the total radioactivity ranged from 9 to 12 ppm. Residues in milk are carefully observed in the United States; regulations forbid them. No systemic insecticide has passed the non-residue test. Residues in milk have ranged from 32 ppm for Ronnel at 8 h after treatment to a low of 0.1 ppm for Co-Ral [10] [11].

Further use of radioactive tracers

Radioactive tracers have been widely used in studies relating to insect control or insect repellents. It would be impossible to cover all that work within the text of this paper. However, some interesting results obtained at the Orlando laboratory are included since two of the authors have been directly involved.

Studies have been initiated on the toxicological action of insecticides on mosquito larvae. Prior to the use of radiolabelled insecticides, the chemical in a mosquito larva was too small to measure by chemical analyses. Radiolabelled insecticides have permitted precise

studies on the uptake of insecticide by mosquito larvae which can be related to mortality. Since the inception of this programme, toxicological studies of the action of DDT, Parathion, Co-Ral, Dimethoate, and Bayer 22408 (*O,O*-diethyl *O*-naphthalimide phosphorothioate) have been conducted with three species of mosquito larvae, *Anopheles quadrimaculatus* (Say), *Aedes aegypti* (L.), and *Aedes taeniorhynchus* (Weid.) [17] [19] [20].

Research is directed to showing the interrelationship between concentration of toxicant in test containers, dosages absorbed by the larvae, mortalities produced, and the length of exposure. One very interesting point has been demonstrated in these studies, and is illustrated below by a comparison of LC_{50} and LD_{50} values obtained in tests with fourth-instar *quadrimaculatus* larvae.

TABLE I

COMPARISON OF LC_{50} AND LD_{50} VALUES OBTAINED IN TESTS WITH FOURTH-INSTAR LARVAE

Insecticide	LC_{50} ppm	LD_{50} µg/larva	Insecticide found in larvae (%)
Dimethoate	2.6	0.004	0.02
Co-Ral	.04	.004	1.0
Bayer 22408	.009	.002	2.2
Parathion	.006	.0004	0.7
DDT	.006	.006	10.0

There is no actual correlation between the concentration required to kill and the dosage found in larvae. In fact, Dimethoate required almost 450 times the concentration to kill 50% of the larvae as did DDT and yet the dosage of Dimethoate found in larvae at the 50% level was only two-thirds that of DDT. Dimethoate is slightly more toxic to larvae than DDT, but some unknown factor is limiting the uptake of this chemical. This is illustrated above by the percentage of Dimethoate found in larvae.

Another interesting discovery pertaining to mosquito larval tests in the laboratory was made possible by the availability of C^{14}-labelled DDT. BOWMAN *et al.* [2] showed that DDT codistilled with water vapour from aqueous suspensions in which mosquito larvae were exposed. At low concentrations (of the order of 0.010 ppm) the loss of DDT from test jars was 50 to 60% in 24 h and 95% in 72 h. Furthermore, they showed that DDT was heterogeneously dispersed at these low concentrations. It has been shown [18] [21] that these two factors influenced the mortality of *quadrimaculatus* larvae and accounted for variations in larval mortality under different test conditions.

An important part of the research programme at Orlando, Florida, is the development of insect repellents. Research includes many phases of activities, such as screening new compounds, field testing of possible new repellents, and fundamental studies concerned with factors affecting repellency. All these studies involve several species of insects. The use of radioactive tracers has increased our ability to study the behaviour of insects in relation to the action of repellents. BAR-ZEEV and SCHMIDT [1] used P^{32}-labelled phosphoric acid as a tracer to study the behaviour of *Aedes aegypti* (L.) when repelled by diethyltoluamide. They concluded that most of the repellency was owing to vapour action, but that at a low concentration contact chemoreceptors on the labella may have been involved. SCHMIDT *et al.* [16] did a very interesting study on the fate of C^{14}-labelled diethyltoluamide when applied

to guinea-pigs. When 6.97 to 7.11 mg/in^2 was applied to the skin, 0.96 to 0.98 mg/in^2 was lost by evaporation and 1.32 to 3.40 mg/in^2 was absorbed in 6 h, at which time the remaining repellent was removed. The radioactivity in the urine reached a peak within 12 h of application. Over 80% of the absorbed dosage was excreted in the urine within 24 h and 93% within 8 d. During the 8 d, 0.75% of the absorbed dosage was excreted in the faeces. Very small amounts of radioactivity were found in the blood, skin, and hair.

From these examples it is clear that radioactive tracers will continue to play an increasingly important role in many different fields of insecticide research, and the development of insect repellents.

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DISCUSSION

M. S. QURAISHI (Pakistan): May I ask Dr. Weidhaas whether the portal of entry of Dimethoate and DDT in mosquito larvae was ascertained? Was it entirely oral or was there surface entry through the body wall as well? Since DDT is one of the most insoluble organic compounds known, is it possible that small particles of DDT admitted with water are retained inside the alimentary canal, thus indicating higher absorption?

D. E. WEIDHAAS: The site of entry is still in doubt, although it is probable that entry occurs both orally and through the body wall. The point that the oral dose accounts for the high absorption is an interesting one. However, this would be part of the toxicological action.

P. J. DEORAS (India): It seems to me that, when used on *Anopheles*, DDT is as good in powder form as in suspension, since the powder particles can be taken up equally readily. I should therefore like to know whether you used powder formulations of DDT as well as suspensions, and what differences were observed between suspension and powder.

D. E. WEIDHAAS: We have not used powder on the surface which, I presume, is what you are referring to. In all our studies DDT was dissolved in acetone and added by pipette to water, thus involving the use of DDT in what should, I think, be termed an aqueous acetone suspension.

J. TREHERNE (United Kingdom): It would be interesting to know whether you were able to demonstrate any accumulation of insecticide within the skin. I am asking this question because I discovered, some five years ago, that there was a diffusion barrier to water-soluble substances situated beneath the stratum corneum in mammalian skin, which also acted as a reservoir of diffusable molecules. It always seemed to me that this might be particularly important in studies of this kind. Such a diffusion barrier would act as a reservoir and allow a very slow efflux of insecticide from the skin.

D. E. WEIDHAAS: Yes, that is a very interesting point, but I'm afraid we have done no studies in this connection.

W. KLOFT (Federal Republic of Germany): I should be interested in hearing something about your measuring techniques for mosquito larvae. Did you extract the absorbed amounts of labelled insecticide, measure the larvae in total, or incinerate them?

D. E. WEIDHAAS: I should stress again that at the moment we are interested in gross differences in uptake, and so did not need to be too precise in our method of determining the amount in larvae. Our method is as follows: we remove fourth-instar larvae from exposure, wash them three times in an appropriate solvent, homogenize them in acetone, plate the ground homogenate on a steel planchet, dry it, and count with a proportional counter. We use this two-way standard with a known amount.

M. S. QURAISHI: As my colleague Dr. Banks points out, why could we not dissolve the larvae in dilute acid and count them in a liquid counter? Or is there some difficulty in the way of this?

D. E. WEIDHAAS: We have not tried it, but I should say it is possible. There is one difficulty, though: if I recall the figures correctly, the quantity we are dealing with is something like five thousandths of a microgram per larva, and although we generally work with groups of 25 larvae, I wonder whether one could operate in the liquid phase with so little material?

F. P. W. WINTERINGHAM (United Kingdom): I should like to comment on the last question, because I think it is of fundamental importance to all this work. There is one very important reason why a liquid β-counter—I take it that this is what you had in mind—could not be used for assaying C^{14}-labelled DDT in suspension, and that is that the maximum energy of the C^{14}-beta particle is insufficiently high to penetrate the glass walls of the conventional GM liquid tube. Thus, it is improbable that C^{14}-labelled DDT could be detected. P^{32}-labelled organophosphorus compounds could, however, be assayed in this way because of the high energy of the P^{32}-beta particle.

D. E. WEIDHAAS: Yes, that is a very good point. Actually, four of our materials were P^{32}-labelled and one was C^{14}-labelled.

SECTION 3

STUDIES ON INSECTICIDE RESISTANCE

RADIOISOTOPE TECHNIQUES AND RECENT RESEARCH ON METABOLISM OF INSECTICIDES IN INSECTS

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Abstract — Résumé — Аннотация — Resumen

Radioisotope techniques and recent research on metabolism of insecticides in insects. Insecticide metabolism, which represents only a part of the complex interaction between insect and chemical, reflects the defensive mechanisms of the organism and sometimes yields clues as to the mode of intoxication. An understanding of these processes can aid in the search for more specific control agents and for solutions to the enormous problem of insect resistance to insecticides. Radiotracer techniques combined with micromethods of chemical separation, such as paper chromatography, are ideally suited for elucidating the fate of insecticides applied to insects. This contribution will briefly review techniques useful for investigations of radio-labelled insecticides and insects, and their application to the study of insect mechanisms of resistance. Data are presented on the quantitative fate and metabolism of P^{32}-labelled Dipterex, *O,O*-dimethyl 1-hydroxy-2,2,2-trichloroethyl phosphonate, in normal and Dipterex-resistant houseflies. The resistant fly strain was able to detoxify the insecticide and excrete the water-soluble metabolites at a more rapid rate than the normal flies. Metabolites were identified by paper chromatography, and no qualitative differences were found between strains.

Recherches nouvelles sur le métabolisme des insecticides chez les insectes, au moyen des radio-indicateurs. Le métabolisme des insecticides, qui ne constitue qu'une partie des interactions complexes entre insectes et produits chimiques, s'explique par les mécanismes de défense de l'organisme et donne parfois des indications sur le mode d'intoxication. Si l'on comprend ces processus, on pourra plus facilement trouver de meilleurs agents destructeurs et résoudre l'immense problème de la résistance des insectes aux insecticides. L'emploi des radioindicateurs, combiné avec des méthodes microanalytiques de séparation chimique telles que la chromatographie sur papier, est un moyen idéal pour suivre les insecticides dans l'organisme des insectes. Le mémoire donne un bref aperçu des techniques qui peuvent être utiles dans les recherches sur les interactions entre insectes et insecticides marqués, et de leurs applications à l'étude des mécanismes de résistance chez les insectes. L'auteur fournit des données quantitatives sur le cycle du métabolisme du Dipterex (phosphonate d'*O,O*-diméthyl-hydroxy-1-trichloro-2,2,2-éthyle) marqué au phosphore-32 chez la mouche commune normale et la mouche commune résistante au Dipterex. La mouche résistante rend l'insecticide non toxique et excrète les métabolites solubles dans l'eau, à un rythme plus rapide que la mouche normale. L'auteur a identifié les métabolites par chromatographie sur papier; à cet égard, il n'a trouvé aucune différence qualitative entre les deux espèces de mouches.

Радиоизотопные методы и последние исследования в области метаболизма инсектицидов в насекомых. Инсектицидный метаболизм, который является лишь частью сложной проблемы взаимодействия между насекомым и химикалием, отражает защитный механизм организма и иногда дает ключ к пониманию действия отравления. Понимание этих процессов может помочь в поисках более конкретных контрольных агентов и растворов для решения крупной проблемы сопротивляемости насекомых воздействию инсектицидов. Методы радиоактивного мечения в комбинации с микрометодами химического разделения, такими как бумажная хроматография, идеально пригодны для выяснения судьбы инсектицидов, применяемых к насекомым.

В этом докладе будут кратко рассмотрены методы, полезные для исследования меченных радиоактивными изотопами инсектицидов и насекомых, и их применение для исследования механизма сопротивляемости насекомого. Приводятся данные о количественной судьбе

и метаболизме меченного фосфором-32 диптерекса, *O,O*-диметил 1-окиси-2,2,2-трислорэтил фосфата у обычной и устойчивой против диптерекса комнатной мухи. Штамм стойкой мухи смог обезвредить инсектицид и выделить растворимые в воде метаболиты более быстро, чем нормальные мухи. Метаболиты опознавались с помощью бумажной хроматографии. и не было обнаружено никаких количественных различий между штаммами.

Recientes investigaciones radioisotópicas sobre el metabolismo de los insecticidas en los insectos. El metabolismo de los insecticidas, que sólo constituye un aspecto de las complejas interacciones entre las sustancias químicas y el insecto, refleja los mecanismos de defensa del organismo y a veces proporciona indicios sobre el proceso de intoxicación. El conocimiento de estos procesos puede facilitar el descubrimiento de agentes insecticidas más específicos y la solución del enorme problema que plantea la resistencia de los insectos a dichos agentes. La utilización de los indicadores radiactivos, combinada con los métodos de separación química en microescala, tales como la cromatografía sobre papel, es un medio ideal para dilucidar el comportamiento de los insecticidas en los insectos.

En la memoria se examinan brevemente los procedimientos adecuados para estudiar los insecticidas e insectos marcados con sustancias radiactivas, y su aplicación al examen de los mecanismos de resistencia en los insectos. Se exponen datos cuantitativos referentes al comportamiento y metabolismo del Dipterex (fosfonato de *O,O*-dimetil-1-hidroxi-2,2,2-tricloroetilo) marcado con ^{32}P, tanto en moscas comunes normales como en las resistentes a esa sustancia. Estas últimas son capaces de eliminar la toxicidad del insecticida y de excretar los metabolitos hidrosolubles a un ritmo más rápido que las moscas normales. Los metabolitos se identificaron por cromatografía sobre papel y no se observaron diferencias cualitativas entre ambas cepas de moscas.

I. Introduction

Radioisotope tracer techniques have played an important role in elucidating interactions between living organisms and organic pesticides. Like other research tools, they cannot singly solve a complex problem but they do play a unique role when co-ordinated with other methods such as chromatography, biological and enzymatic assay, spectroscopy, and chemical analysis.

The worker investigating the fate of an insecticide applied to an insect needs an analytical method of a sensitivity beyond most conventional chemical techniques. First, the quantity of insecticide that can be applied may be only a fraction of a microgram for a housefly to several micrograms for larger insects, depending on the toxicity of the compound to the particular species and the degree of biological response necessary for the experiment. Secondly, dilution occurs as the chemical is absorbed, translocated and excreted by the organism, and its structure altered by metabolic processes.

Radioactive tracers are well suited for this work because they can be quantitatively assayed with an accuracy and sensitivity unrivaled by most other methods. Paper chromatographic methods of fractionation combined with radiometric analysis provide a means of characterizing the labelled metabolites, deducing metabolic routes, and determining the magnitude of the metabolic changes involved.

This paper briefly discusses certain radioactive tracer techniques and their application to the study of insecticide metabolism by normal and resistant insects. Several recent reviews of insect metabolism of insecticides are available [1—3] and no extensive discussion of this topic is, therefore, attempted. Procedures of radiotracing and their application to biological research may be found in several references [4—6].

II. Radio-labelled Compounds

The initial problem in a metabolism study is the synthesis of the radio-labelled insecticide. Basic considerations must be the isotope and its position in the molecule, a convenient

synthetic route, specific activity, and radiochemical purity. The isotopes phosphorus-32, carbon-14, and sulphur-35 are most widely used to label insecticides. Chlorine-36 and tritium (H^3) may also be suitable. The synthesis of a number of radiolabelled insecticides has been reported and reviewed [7], and a partial list is found in Table I.

TABLE I

REFERENCES FOR THE SYNTHESIS OF SOME RADIO-LABELLED ORGANIC INSECTICIDES AND RELATED COMPOUNDS

Common name	Chemical name and isotope	Reference
Chlorinated Hydrocarbon		
Endrin	1, 2, 3, 4, 10, 10-hexachloro-6, 7-epoxy-1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-1,4-*endo*, *endo*-5,8-dimethanonaphthalene-6,7-C^{14}	[8]
DDT	2, 2-*bis*-(*p*-chlorophenyl)-1, 1, 1-trichloro-2-C^{14}-ethane	[9] [10]
	2, 2-*bis*-(*p*-chlorophenyl-4, 4′-C^{14})-1, 1, 1-tricholoroethane	[11]
Thiodan	6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3-oxide-5a, 9a-C^{14}_2	[12]
Organophosphorus		
Co-Ral	*O*,*O*-diethyl *O*-3-chloro-4-methylumbelliferone-P^{32}-phosphorothioate	[13]
Demeton	*O*, *O*-diethyl *S*(and *O*)-2-(ethylthio)ethyl-P^{32}-phosphorothioate	[14]
DDVP	*O*, *O*-dimethyl 2, 2-dichlorovinyl-P^{32}-phosphate	[15] [16]
Diazinon	*O*, *O*-diethyl *O*-(2-*iso*propyl-6-methyl-4-pyrimidinyl)-P^{32}-phosphorothioate	[17]
Dimethoate	*O*, *O*-dimethyl *S*-(*N*-methylcarbamoylmethyl)-P^{32}-phosphorodithioate	[18]
Dipterex	*O*, *O*-dimethyl 1-hydroxy-2, 2,2-trichloroethyl-P^{32}-phosphonate	[15] [16]
Malathion	*O*,*O*-dimethyl *S*-(1, 2-dicarbethoxyethyl)-P^{32}-phosphorodithioate	[19] [20]
Parathion	*O*, *O*-diethyl *O*-(*p*-nitrophenyl)-P^{32}-phosphoro-S^{35}-thioate	[21]
	O, *O*-diethyl *O*-(*p*-nitrophenyl) phosphoro-S^{35}-thioate	[22]
Ronnel	*O*, *O*-dimethyl *O*-(2, 4, 5-trichlorophenyl)-P^{32}-phosphorothioate	[23]
Carbamate		
Sevin	1-naphthyl-1-C^{14}-*N*-methyl carbamate	[24]
Pyrethroid		
Allethrin	*d*, *l*-allelrethonyl-*d*, *l*-*cis*, *trans*-2-C^{14}-chrysanthemate	[25]
	d, *l*-3-allyl-2-C^{14}-methyl-4-oxo-2-cyclopenten-1-C^{14}-yl chrysanthemate	[26]
Synergist		
Piperonyl Butoxide	α-[2-(2-butoxyethoxy)ethoxy]-4, 5-methylenedioxy-2-propyltoluene-1-C^{14}-methylene	[27]
Repellent		
Deet	*N*, *N*-diethyl-*m*-toluamide (C^{14}-carboxy)	[28]

III. Quantitative tracing

Perhaps the most rapid and direct method of assaying phosphorus-32 in biological material is solid-sample counting. Aliquots of tissue homogenates or fluids are transferred to sample pans, dried, and counted. Lens paper disks attached to the pans facilitate spreading which gives a more uniform area and thickness. Volatility of radioactive insecticides in the sample can be decreased by adding a few milligrams of a polyethylene glycol in acetone to reduce losses and counter contamination [29]. Self-absorption, which is a major problem with soft β-emitters, is of no consequence with P^{32}, unless unusually thick samples are prepared.

Samples for soft β-analysis must be prepared so as to avoid loss of radioactivity from self-absorption or to allow an accurate correction to be made. Tissue extracts can often be prepared as "weightless" samples if the residue remaining after evaporation of the solvent is sufficiently small. When this is not practical, the material is usually oxidized and the carbon-14 or sulphur-35 assayed as barium precipitates of uniform area and thickness [30] [31]. Chlorine-36 in insect tissue has been analysed as mercurous chloride precipitates after wet oxidation [32]. HENDLER [33] has described a simple technique for correcting C^{14}-measurements for loss of radiation due to self-absorption in samples of variable weight. A correction factor F for a particular sample weight is equal to the ratio of the observed specific activity to the specific activity at a reference weight. When correction factors are plotted against sample weights, one obtains a straight line which can be used to find F for any sample weight for correction to the standard condition.

Counter efficiency is an important consideration in selecting radioassay equipment for insect studies. With increased efficiency, smaller quantities of the isotope or labelled compound can be detected, lower specific activities can be handled, or compounds labelled with short half-life isotopes, such as P^{32}, have a longer period of effective use. Gas-flow counters, windowless or with mylar-plastic windows, have been found suitable for most biological work. Liquid scintillation counting [34], in which the radioactive material and scintillation phosphors are combined in solution or suspension, offers the advantages of high counting-efficiency and relatively little self-absorption of radiation. HERBERG [35] has described methods for C^{14}- and tritium-analysis in blood and whole tissues of mammals and there is little doubt that these techniques could be adapted for insect fractions.

IV. Identification of radio-labelled compounds

Minute quantities of the labelled insecticide or metabolites in biological fractions can be rapidly separated and analysed by paper chromatography. Non-labelled authentic standards are added before developing the chromatogram and their positions on the paper, as indicated by a chemical test, are compared with the radioactive peaks of the labelled unknowns. One-dimensional ascending chromatography is most commonly employed in insecticide metabolism studies, and many techniques and solvent systems have been reported in the literature. MITCHELL [36] has described this method in detail in relation to separation of insecticides. It is desirable, whenever possible, to use more than one chromatographic system to establish the identity of a compound. A 'normal phase' system, in which the mobility of the organosoluble insecticide is greatest and the water soluble metabolites are least, is usually complemented by a method which reverses the order of migration.

Extraction, clean-up, and concentration of the radioactive material usually precede paper chromatographic analysis. Organosoluble insecticides or metabolites can be separated from ionic metabolites by extracting tissue homogenates with organic solvents such as chloroform and hexane. Lipids that interfere with chromatography must then be eliminated from the

solvent by clean-up prodecures. Many organic insecticides can be quantitatively separated from lipids by extraction from hexane with acetonitrile [37] or by low-temperature extraction of tissue with acetone [38]. MENN *et al.* [39] have purified insect-tissue extracts by spotting the crude material on filter paper wicks pointed at one end. The insecticides are then eluted from the interfering lipids by upward migration of acetonitrile. As the soluble substances collect at the tip of the paper, they are transferred by contact to the origin of a paper strip for chromatography. GREGORY [40] previously used this technique to transfer a compound from a developed chromatogram to filter paper to rechromatograph by another system. Continuous transfer of solvent from the paper wick to the chromatogram was possible by regulating the spot size with a warm air stream.

Radiometric assay of the labelled compounds on the chromatogram may be accomplished in several ways. The chromatograms can be cut into sections and each section assayed with a counter, or the spots eluted from the paper and prepared as samples to be counted. The whole chromatogram may be scanned with a strip counter, rate meter, and synchronous chart recorder. The areas under each peak, as measured with a planimeter, will give a quantitative estimation of the radioactivity in each spot. Sensitivity can be increased by windowless counting or 4-π geometry [6], and several chromatogram scanners are available commercially. Soft β-emitters present the greatest problem, which has been approached by immersing the paper sections of the chromatogram in a phosphor solution for assay in a liquid scintillation counter [41].

V. Radiotracers and insect resistance to insecticides

Radiotracer techniques are ideally suited for comparing the fate of an insecticide in susceptible and resistant insects. Uptake and absorption rates, distribution and storage, qualitative and quantitative differences in metabolism, and the ultimate elimination of the

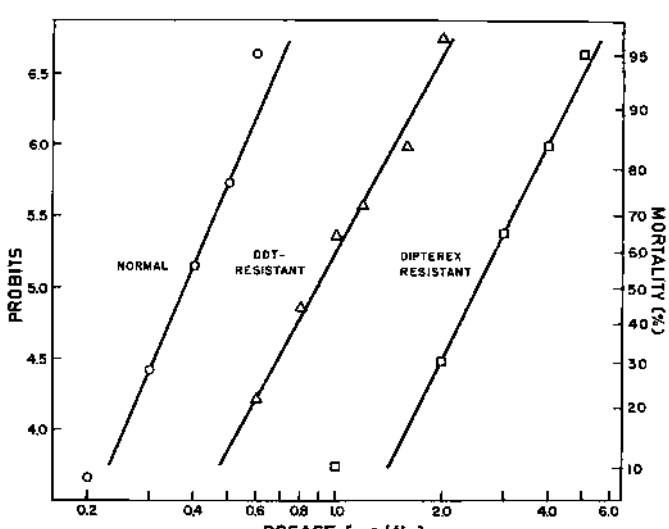

Fig. 1

The toxicity of topically applied Dipterex to normal, DDT-resistant, and F_4 Dipterex-resistant houseflies

parent compound or its metabolites from the insect are important aspects of such a study.

BRADBURY *et al.* [42—44], in a series of detailed papers, have demonstrated that BHC-resistant houseflies absorb less C^{14}-γ-BHC vapour and convert it to water-soluble metabolites at a faster rate than do susceptible flies. By fractionation and isotopic dilution methods, certain γ-BHC metabolites were isolated and identified.

Experiments comparing the fate of the S^{35}-labelled sulphur analogue of Dieldrin in Dieldrin-resistant and susceptible houseflies indicate a different mechanism of resistance [45]. There appeared to be no evidence of gross differences in cuticular penetration, metabolism, or excretion of *S*-Dieldrin in the two strains. Susceptible flies, prostrate at higher dosages, ceased to excrete but metabolism of the absorbed Dieldrin continued for several hours. The defensive mechanism operating in the resistant strain remained unexplained.

The fate of P^{32}-labelled Dipterex (*O,O*-dimethyl 1-hydroxy-2,2,2-trichloroethyl phosphonate) in normal and resistant houseflies has been compared by HOPKINS *et al.* [46]. The resistant strain was selected by topical application of Dipterex to adult females of a DDT-resistant colony already tolerant to the phosphonate (Fig. 1). The P^{32}-labelled Dipterex was synthesized by Fred Acree, Jr. of the US Department of Agriculture, and had a specific activity of 1×10^4 cpm/μg. Four-day-old female houseflies were treated and held in stationary positions throughout the post-treatment interval for quantitative collection of excreta as previously reported [47]. At three-hour intervals following treatment, batches of 25 flies were taken for analysis and rinsed with acetone to remove any remaining external insecticide. Homogenates were made of the rinsed flies for total radioactivity determinations and then extracted with five equal volumes of chloroform to recover any internal Dipterex. Radioassay and paper-chromatography techniques have been described elsewhere [29].

TABLE II

THE FATE OF P^{32}-LABELLED DIPTEREX APPLIED TOPICALLY TO NORMAL AND DIPTEREX-RESISTANT HOUSEFLIES

Results, the mean of two replicates of 25 flies each, expressed as percentage of total dose (0.3 μg/fly)

	Hours following treatment							
	3		6		9		12	
	Normal	Resistant	Normal	Resistant	Normal	Resistant	Normal	Resistant
External rinse	21.0	24.9	12.9	10.9	11.8	10.8	8.9	9.0
Internal								
Total	46.7	40.9	39.1	26.3	38.0	26.4	37.0	18.1
$CHCl_3$ soluble	24.6	15.4	13.9	9.5	11.8	8.1	10.4	5.8
Metabolized*	47.3	62.4	64.5	63.9	68.9	69.3	71.9	68.0
Excreted	13.5	20.7	26.1	36.2	32.8	38.5	31.0	51.9
Absorbed (Internal + excreted)	60.2	61.6	65.2	62.5	70.8	64.9	68.0	70.0
Total recovery	81.2	86.5	78.1	73.4	82.6	75.7	76.9	79.0

* Percentage of the total internal fraction.

The results (Table II) indicate no difference in the rate or quantity of Dipterex absorbed by the two strains over a 12-h period. Most of the cuticular penetration occurred in the first three hours after treatment with about 10% of the total dose absorbed thereafter. The flies

also contained the largest amounts of internal radioactivity at three hours, confirming rapid penetration of the insecticide.

The resistant flies consistently had less internal radio-labelled compounds than the normal strain, and this may be correlated with the larger excreted amounts of radioactive material. The total amount of chloroform-soluble radioactivity, identified as Dipterex by paper chromatography, was also less in the resistant strain. However, except for the three-hour analysis, about the same percentage of the internal fraction had been metabolized to water-soluble compounds in both strains. Approximately 20% of the total applied dose was unaccounted for at all intervals, apparently lost by evaporation from the cuticle or by regurgitation. Approximately 4% of the radioactivity in the chloroform extracts of 12-h normal flies had an R_f value corresponding to DDVP. Only trace amounts were present in the resistant strain extract.

Paper chromatography of the radio-labelled compounds excreted by the normal and resistant flies demonstrated no qualitative or gross quantitative differences between strains (Fig. 2). Very little Dipterex was excreted intact, with most of the radioactivity corresponding to dimethyl and monomethyl phosphate. These two metabolites were also identified in the urine from mice orally treated with Dipterex. The major metabolite excreted by mice was unidentified, and appears to be identical with that eliminated by cows [29] (Fig. 3).

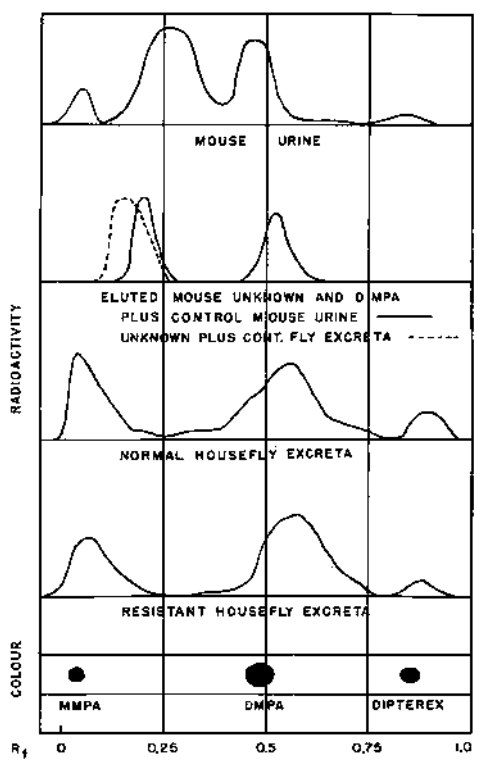

Fig. 2

Paper chromatography of Dipterex metabolites excreted by mice, Dipterex-resistant houseflies, and normal houseflies. (Mobile phase. 7.5 pt isopropanol, 2.5 pt NH_4OH). DMPA = dimethy phosphoric acid, MMPA = monomethyl phosphoric acid

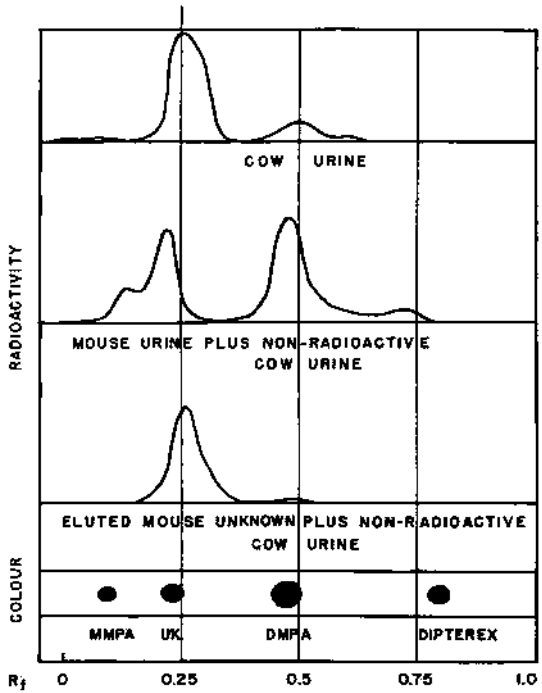

Fig. 3

Paper chromatography of Dipterex metabolites excreted by mice and cows. (Mobile phase: 7.5 pt ethanol, 2.5 pt 0.2% conc. HCl). DMPA - dimethyl phosphoric acid, MMPA - monomethyl phosphoric acid, UK - unknown metabolite

From these results it was concluded that the houseflies resistant to Dipterex were able to detoxify the insecticide and excrete the metabolites at a faster rate than the normal strain. The latter consistently had larger amounts of the intact insecticide present internally. DDVP, which is thought to be responsible for the *in vivo* toxic action of Dipterex [48], may be a transient intermediate between the parent compound and the hydrolysis products found in the excreta.

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DISCUSSION

THE CHAIRMAN (J. de Wilde, Netherlands): Contrary to my initial idea, I feel that metabolism and resistance must here be considered in association with one another, so that the discussion we are about to hold on Dr. Hopkins' most interesting paper should deal with both.

J. E. CASIDA (United States of America): Do you think that metabolism has anything to do with Dipterex resistance?

T. L. HOPKINS: The ability of the resistant strain apparently to metabolize more of the insecticide? I think it probably contributes to resistance, but I would not consider it as the entire cause.

J. E. CASIDA: We shall soon be publishing a paper on the metabolic fate of P^{32}-labelled Diazinon, methyl Parathion and Malathion in one susceptible and three organo-phosphate strains of housefly. In none of the characteristics—oxygen analogue, hydrolysis products, rate of penetration, or rate of hold-up in the cuticle—could we detect a difference in metabolism rate between the susceptible and the resistant. Do you believe that with Dipterex and your strains of housefly that detoxication of the organophosphate was a major factor in resistance?

What do you think the unknown from Dipterex can be?

T. L. HOPKINS: To your first question I can answer 'yes', but to the second, 'unfortunately we do not know'. However, we have some clues which I intend to follow up, time permitting.

J. E. CASIDA: Would these clues be consistent with a monodemethylated DDVP, which, on the basis of some experiments of ours, definitely forms under analogous conditions?

T. L. HOPKINS: This may be so; our work suggests that this unknown compound breaks down to form dimethyl phosphate, with the result that a monomethyl derivative would be eliminated. Although it runs with the R_f of dimethyl phosphate, there is always the possibility that this system does not separate more than one metabolite. Usually the monomethylated derivatives separate quite distinctly from the dimethyl derivatives.

J. E. CASIDA: Another check would be to test whether the metabolite is susceptible to bromination.

T. L. HOPKINS: Yes.

D. E. WEIDHAAS (United States of America): I am also curious about the question of metabolism and resistance. We did one test at Orlando—and only one, so the possibility of an artifact must be borne in mind—with some Malathion-resistant and susceptible houseflies. We found that, when we treated both resistant and susceptible flies topically with the same dose, effectively a 24-h LC_{90}, there was a gradual inhibition of cholinesterase activity, percentage-wise, in the susceptible individuals, and a percentage inhibition, followed by recovery, in the resistant ones. What was of the greatest interest to me, however, was that at about two hours there was a greater percentage inhibition of cholinesterase in the resistant than in the susceptible individuals. This seemed an anomaly to me, unless one assumed, for example, a conversion to more toxic metabolites followed by degradation. Have participants any views on this?

T. L. HOPKINS: As I recall, Chadwick did some experiments in this connection some years ago, and I do not believe he found any difference in inhibition of cholinesterase in susceptible and DFP-resistant houseflies.

J. E. CASIDA: If I may take my comments a little further, we made comparable studies to the ones you mentioned, using resistant strains of varying resistance and about 20 different phosphates. The inhibition occurs in the susceptible and not in the resistant strains, if you work specifically with thoracic ganglion, which is what we have been doing. The amount of available organophosphate for inhibition in a homogenate is greater in the susceptible than in the resistant. This result is comparable to that obtained by Van Asperen and Oppenoorth, in Utrecht, but it does depend on the definition given to "available". This is not only localized but it is bound; I think that that is all I should like to say at present on this aspect of the matter.

THE CHAIRMAN: I think this is a very good point, and here of course, the substrate protection technique might be strongly recommended. I should like to ask Mr. Hopkins if he

ever tried incubating different parts of insects in DDVP and endeavouring to obtain the metabolism products demonstrated in total flies.

T. L. HOPKINS: No, we have not tried this. We did start some *in vitro* work with Dipterex in mice, and we found out that even in our enzyme-inactivated liver slices we were getting DDVP* from a spontaneous rearrangement of Dipterex. That, actually, was as far as we went.

J. E. CASIDA: There is just one more remark I should like to make. We have worked with the enzymes in houseflies that break down phosphates. One can purify the enzyme that breaks down DDVP, and we have in fact purified it about a hundredfold. There is no difference in the activity of this enzyme in resistant and susceptible flies, and the same holds good for the enzyme that breaks down para-oxon. There are at least three different housefly enzymes that break down phosphates, and some were surprisingly active when properly supplied with co-factors. But supplying the co-factors was the difficulty. They are apparently lipoproteins—at least we have not been able to dissociate the enzymes from the lipid fraction.

* *O,O*-dimethyl 2,2-dichlorovinyl phosphate.

RADIOACTIVE TRACER TECHNIQUES IN INSECT BIOCHEMISTRY

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Abstract — Résumé — Аннотация — Resumen

Radioactive tracer techniques in insect biochemistry. Radioactive tracer techniques combined with microfractionation techniques such as paper chromatography have provided powerful tools for studying the biochemical problems of insect control by chemicals.

An important aspect of this type of work is the separation, assay and identification of labelled compounds recovered in trace amounts from insect tissues. Automatic radio-chromatographic techniques are available and identification may be established by co-chromatography with authentic compounds, and by studying the action of chemical reagents and specific enzymes on the labelled fraction.

A serious threat to progress in the chemical control of agricultural pests is the increasing numbers of insects which display such high levels of resistance to the established insecticides that their use has had to be abandoned. Labelled insecticides have been extensively used for comparing their absorption, metabolism and excretion by normal and insecticide-resistant insects. The use of labelled Dieldrin and its chemical relatives in this way has provided essential data on the mechanisms of resistance. The evidence to date strongly suggests that resistance to Dieldrin in diptera is not due to detoxication or failure of the insecticide to penetrate the insect cuticle.

By labelling pools of related metabolites *in vivo* the effects of insecticides upon the insect may be studied. Labelled pools are formed *in vivo* by feeding or injecting insects with suitable labelled compounds.

Comparison of the labelled pools formed when acetate-2-C^{14} and P^{32}-labelled PO_4^{3-} were injected into adult insects provided valuable data on the biochemistry of insect nerve and muscle. For example, the significance of *l*-α-glycerophosphate in the carbohydrate metabolism of the flight muscle has been demonstrated, and studies made of the kinetics of acetylcholine metabolism in the nervous tissue of the intact insect.

Pools of metabolites labelled with C^{14} or P^{32} have been used for studying the mode of action of Dieldrin and other insecticides in the insect. Thus, the organophosphorus insecticides appear to slow the rate of acetylcholine synthesis *in vivo* although they are without effect on the corresponding enzyme systems *in vitro*.

Caution must be exercised in interpreting tracer experiments applied to the problems of insect resistance. For example, a difference between the metabolism or excretion of a labelled insecticide by susceptible and resistant insects may not explain resistance but simply be a consequence of susceptibility.

Emploi des indicateurs radioactifs dans la biochimie des insectes. Les méthodes utilisant les indicateurs radioactifs, combinées aux techniques de microfractionnement telles que la chromatographie sur papier, sont des outils puissants pour l'étude des problèmes de biochimie que pose la lutte contre les insectes au moyen de produits chimiques.

Un aspect important des travaux qu'exige cette étude est la séparation, l'analyse et l'identification des composés marqués récupérés sous forme de traces dans les tissus des insectes. On peut recourir, à cette fin, aux techniques de radiochromatographie automatique; l'identification peut se faire par co-chromatographie à l'aide de composés authentiques, ainsi que par l'étude de l'action de réactifs chimiques et de certains enzymes sur la fraction marquée.

L'essor de la médecine tropicale est sérieusement menacé par l'apparition d'un nombre croissant d'insectes porteurs de maladies, qui présentent une telle résistance aux insecticides classiques que

l'emploi de ceux-ci a dû être abandonné. On a largement utilisé des insecticides marqués pour comparer leur absorption, leur métabolisme et leur élimination chez les insectes normaux et chez les sujets rebelles aux insecticides. Ainsi, l'emploi de la dieldrine marquée et de certains produits chimiques apparentés a permis de réunir des renseignements d'importance fondamentale sur les mécanismes de cette résistance. D'après les données dont on dipose à l'heure actuelle, on a tout lieu d'admettre que la résistance opposée par les diptères à la dieldrine n'est pas due à la désintoxication ou à l'incapacité de l'insecticide de pénétrer à travers les téguments de l'insecte.

On peut étudier les effets des insecticides sur l'insecte en marquant *in vivo* des pools de métabolites apparentés. Pour marquer ces pools *in vivo*, on administre aux insectes, par nutrition ou injection, des composés marqués appropriés.

La comparaison entre les pools marqués obtenus par injection d'acétate 2-^{14}C et de PO_4^{3-} marqué au phosphore-32 à des insectes adultes, a fourni des données précieuses sur la biochimie des nerfs et des muscles de l'insecte. Il a été démontré, par exemple, que le *l*-α-glycérophosphate joue un rôle important dans le métabolisme de l'hydrate de carbone du muscle de l'aile; des études ont été faites sur la cinétique du métabolisme de l'acétylcholine dans le tissu nerveux de l'insecte intact.

Des pools de métabolites marqués au carbone-14 ou au phosphore-32 ont été utilisés pour l'étude du mode d'action de la dieldrine et d'autres insecticides chez l'insecte. Il semble ainsi qu'*in vivo* les insecticides à base de substance organique et de phosphore ralentissent le rythme de la synthèse de l'acétylcholine, bien que *in vitro* ils n'aient aucun effet sur les systèmes enzymatiques correspondants.

Les résultats des expériences faites au moyen d'indicateurs en vue de résoudre les problèmes de la résistance des insectes doivent être interprétés avec prudence. Par exemple, une différence constatée entre insectes sensibles et insectes résistants dans le métabolisme ou l'élimination d'un insecticide marqué ne prouve pas forcément la résistance; elle peut être simplement due à la sensibilité.

Методы радиоактивного мечения в биохимии насекомых. Методы радиоактивного мечения вместе с методами микрофракционирования, таким как хроматография на бумаге, представляют собой мощные средства для изучения биохимических проблем уничтожения насекомых с помощью химикалиев.

Важным аспектом этого вида работы является разделение, количественный анализ и опознавание меченых соединений, обнаруженных в меченых веществах в тканях насекомых. Имеется автоматическая радио-хроматографическая техника и опознавание можно проводить с помощью сохроматографии с аутентичными соединениями и путем изучения действия химических реагентов и специфических энзимов на меченую фракцию.

Серьезной угрозой успеху борьбы с сельскохозяйственными вредителями при помощи химикалиев являются возрастающие количества насекомых, которые проявляют такие высокие степени сопротивляемости установленным инсектицидам, что от их использования вынуждены были отказаться. Меченые инсектициды широко использовались для сравнения их поглощения, метаболизма и удаления нормальными насекомыми и насекомыми, стойкими к инсектицидам. Использование меченого диелдрина и родственных ему химикалиев в этом отношении предоставило существенные данные о механизме сопротивляемости. Имеющиеся в настоящее время данные с большой определенностью говорят о том, что сопротивляемость диелдрину у диптера не является результатом детоксикации или неспособности инсектицида проникнуть через внешний покров насекомого.

Воздействие инсектицидов на насекомое может быть изучено путем использования мечения метаболических узлов *in vivo*. Меченые узлы создаются *in vivo* путем кормления насекомых или введения в них соответствующих меченых соединений.

Сравнение меченых узлов, созданных при инъекции (2-C^{14}) ацетата и (P^{32}) FO_4^{3-} взрослым насекомым, предоставило ценные данные по биохимии нервной и мускульной тканей насекомого. Например, демонстрировалось значение *l*-α-глицерофосфата в метаболизме углеводов летательной мышцы и проведены исследования кинетики метаболизма ацетилхолина в нервной ткани неповрежденного насекомого.

Метаболические узлы, меченные C^{14} или P^{32}, использовались для исследования того, как действуют диелдрин и другие инсектициды на насекомое. Так, органическо-фосфорные

инсектициды, видимо, замедляют скорость синтеза ацетилсолина *in vivo*, хотя они не действуют на соответствующие системы энзимов *in vitro*.

Следует соблюдать осторожность при интерпретации экспериментов с радиоактивными индикаторами, связанных с проблемами сопротивляемости насекомых. Например, разница между метаболизмом или удалением меченого инсектицида чувствительными и стойкими насекомыми может быть объяснена не только сопротивляемостью, она может оказаться следствием чувствительности.

Empleo de los indicadores radiactivos en la bioquímica de los insectos. Las técnicas de empleo de los indicadores radiactivos, combinadas con las de microfraccionamiento, como la cromatografía sobre papel, proporcionan poderosos instrumentos para el estudio de los problemas bioquímicos que plantea la lucha contra los insectos mediante productos químicos.

Un aspecto importante de los trabajos que este estudio requiere lo constituye la separación, análisis e identificación de los vestigios de compuestos marcados que se extraen de los tejidos de insectos. A tal efecto, es posible recurrir a las técnicas de radiocromatografía automática y la identificación puede hacerse por co-cromatografía con ayuda de compuestos auténticos, así como estudiando la acción de reactivos químicos y de ciertas enzimas sobre la fracción marcada.

El progreso de la medicina tropical se ve seriamente amenazado por la proliferación de un número creciente de insectos cuya resistencia a los insecticidas habituales es tan elevada que el uso de éstos ha debido abandonarse. Los insecticidas marcados se han utilizado ampliamente para comparar su absorción, metabolismo y excreción en los insectos normales y en los resistentes a los insecticidas. El uso del dieldrín marcado y de algunos productos químicos emparentados ha permitido reunir valiosos datos sobre el mecanismo de la resistencia. Las pruebas disponibles en la actualidad indican claramente que la resistencia que los dípteros oponen al dieldrín no se debe a una desintoxicación o a la incapacidad del insecticida de penetrar a través de los tegumentos del insecto.

Los efectos de los insecticidas sobre los insectos pueden estudiarse marcando *in vivo* pools de metabolitos afines. Los pools marcados se obtienen *in vivo* suministrando por vía bucal o por inyección sustancias convenientemente marcadas a los insectos.

La comparación de los pools marcados que se forman inyectando a los insectos adultos acetato [2-^{14}C] y PO_4^{3-} marcado con ^{32}P proporciona interesantes datos acerca de la bioquímica de los nervios y músculos de los insectos. Por ejemplo, se ha demostrado que el *l*-α-glicerofosfato desempeña un importante papel en el metabolismo de los hidratos de carbono en el músculo del ala, y se ha estudiado la cinética del metabolismo de la acetilcolina en el tejido nervioso del insecto intacto.

Se han utilizado pools de metabolitos marcados con ^{14}C o con ^{32}P para estudiar el modo de acción del dieldrín y de otros compuestos sobre los insectos. Así, los insecticidas orgánicos fosforados parecen disminuir la velocidad de síntesis de la acetilcolina *in vivo*, aunque *in vitro* no ejercen efecto sobre el sistema enzimático correspondiente.

Los resultados de los experimentos de marcación realizados con el objeto de resolver los problemas que plantea la resistencia de los insectos se han de interpretar con cautela. Por ejemplo, la observación de diferencias en el metabolismo o en la eliminación de un insecticida marcado no prueba necesariamente la existencia de una resistencia; la diferencia puede ser sencillamente consecuencia de cierta sensibilidad.

Introduction

Since 1945 the progress of biochemical research has been greatly influenced by the impact of two techniques: (a) the techniques of chromatography, electrophoresis, etc., by which mixtures may be resolved on the microgram scale, and (b) radioactive tracer techniques by which suitably labelled substances may be detected and determined with an unrivalled sensitivity and specificity. It is the purpose of this contribution to illustrate how the combination of micro-fractionation and radiochemical techniques may be effectively applied to the study of insect biochemistry. Within the space available it will not be possible to do

more than explain some basic principles and techniques. Some examples of their application in the author's own laboratories will be offered by way of illustration and for later discussion.

Two kinds of application of the combined techniques may be distinguished. In the first, the experimental insects are exposed to a labelled insecticide. The radioactive material may be later extracted from the insect tissues, and unchanged insecticide and its metabolites separated and assayed radiometrically. In this way the effect of the insect upon the insecticide may be studied.

In the second type of application, it is the insect or, more specifically, a pool of biochemically related metabolites of the insect which is labelled. This is accomplished by injecting or feeding an insect with a suitably labelled substrate so that metabolites derived from the substrate through the normal biochemistry of the insect also become labelled. When the mixture of labelled metabolites so formed is extracted and resolved, the radioactivities of the separated fractions indicate their relative concentrations or, at least, the relative rates at which they become labelled *in vivo*. By conducting such "labelled-pool" experiments with normal and with poisoned insects the effects of an insecticide upon the insect may be studied [1].

Preparation of labelled insecticides

The most important method of preparing labelled insecticides is by chemical synthesis from an intermediate suitable for radioactivation in the pile or particle accelerator. Many compounds, including insecticides, are prepared routinely in this way and are available commercially through National Atomic Energy Agencies. Usefully detailed descriptions of many labelled insecticide syntheses have also been published [2] [3].

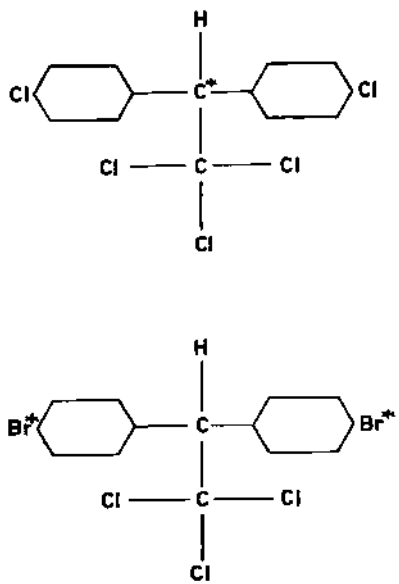

Fig. 1
C^{14}-DDT and Br^{82}-analogue

Figs. 1 and 2 show types of labelling of some important chlorohydrocarbon insecticides. PEARCE and JENSEN [4] successfully labelled DDT with C^{14} in the tertiary position, as shown in Fig. 1. The difficult synthesis of C^{14}-Endrin (Fig. 2) was first accomplished by the author's

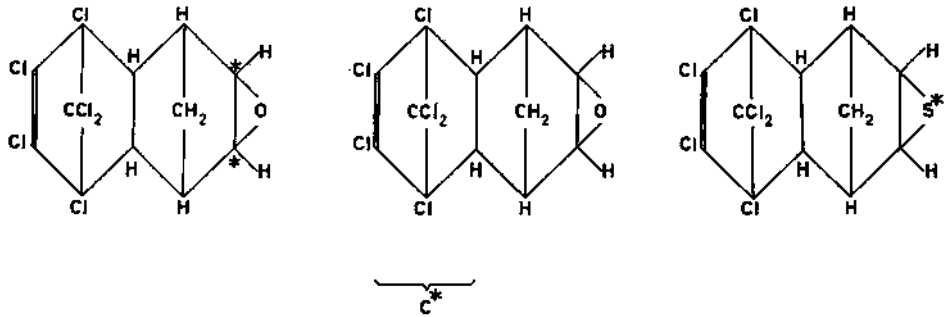

Fig. 2
C^{14}-Endrin, C^{14}-Dieldrin and S^{35}-analogue of Dieldrin

colleague Dr. Brooks [5]. The C^{14}-labelled Dieldrin was later made available by the Radiochemical Centre of the United Kingdom. When the labelling of the insecticide itself is not feasible, it may be of great value to use a labelled analogue. For example, the Br^{82}-analogue of DDT shown in Fig. 1 has been effectively used by my colleagues and me in studies of insect resistance [6] [7] and of food contamination [8]. The bromine analogue very closely resembles DDT in its insecticidal, chemical and physical properties, but it has the advantage of being easily labelled with the 35-h Br^{82} at very high specific radioactivities. The S^{35}-analogue of Dieldrin [9] was similarly used in insect resistance studies pending the much more difficult synthesis of the C^{14}-labelled insecticides. Fig. 3 shows doubly labelled Parathion [10]. Since P^{32} and S^{35} are readily differentiated radiometrically, the fates of different portions of the molecule may be studied simultaneously.

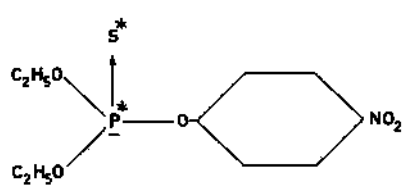

Fig. 3
P^{32}-S^{35} doubly labelled Parathion

For the isotopic synthesis of labelled insecticides of botanical origin such as the Pyrethroids, Rotenone, Nicotine etc., biosynthetic techniques may be used. For example, PELLEGRINI, MILLER and SHARPLESS [13] successfully prepared uniformly C^{14}-labelled Pyrethrins in this way (Fig. 5). Pyrethrum plants (*Chrysanthemum cinerariaefolium*) were grown in an atmosphere containing $C^{14}O_2$, and the labelled insecticides finally extracted from the radioactive flowers and purified. By the ingenious method of feeding tobacco plants with *dl*-ornithine-2-C^{14} LEETE [14] was able to recover specifically labelled Nicotine as shown in Fig. 6.

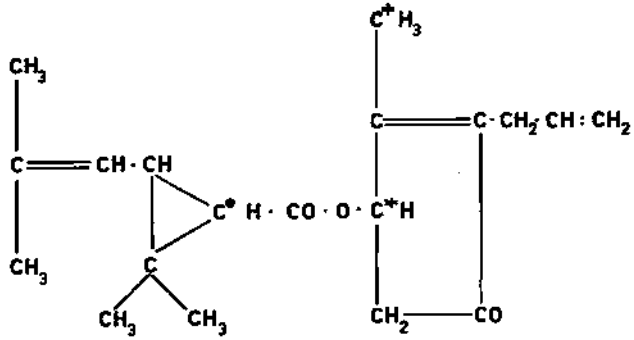

Fig. 4
Specifically labelled C^{14}-Allethrin

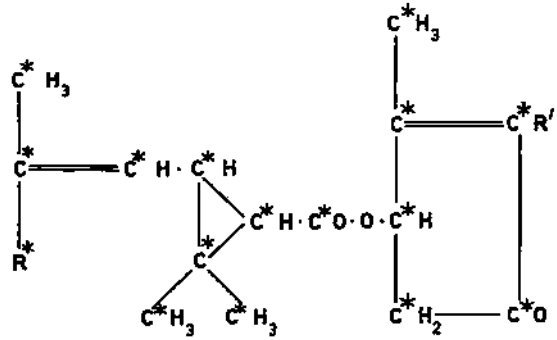

Fig. 5
Uniformly C^{14}-labelled Pyrethroids (by biosynthesis)

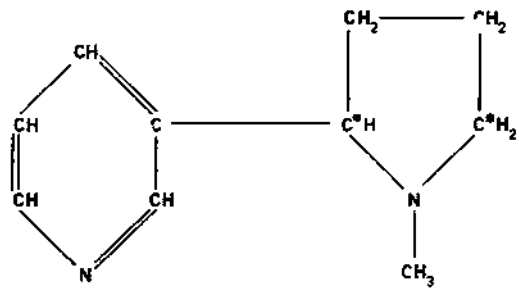

Fig. 6
Specifically C^{14}-labelled Nicotine (by biosynthesis)

Finally, labelling with tritium may be accomplished by the novel techniques of tritium recoil labelling [15] or by activated tritium exchange [16]. In the former technique a mixture

of the H-containing compound and a lithium salt is irradiated in the pile. Fast tritons produced by the Li^6 (n, α)H^3 reaction break chemical bonds, and exchange with hydrogen. In the Wilzbach technique the compound to be labelled is exposed to tritium gas, the necessary activation-energy arising from the β-decay of the tritium itself. Dr. K. G. Das (personal communication) has successfully labelled both DDT and Dieldrin (Fig. 7) by tritium exchange. The disadvantage of these techniques lies in the radiation decomposition of the target compounds and the necessity of rigorous radiochemical purification of the required product. In concluding this section, it may be said that it is most unlikely that a particular synthetic or naturally derived insecticide cannot be labelled by one or more of the techniques of chemical synthesis, biosynthesis or isotope exchange.

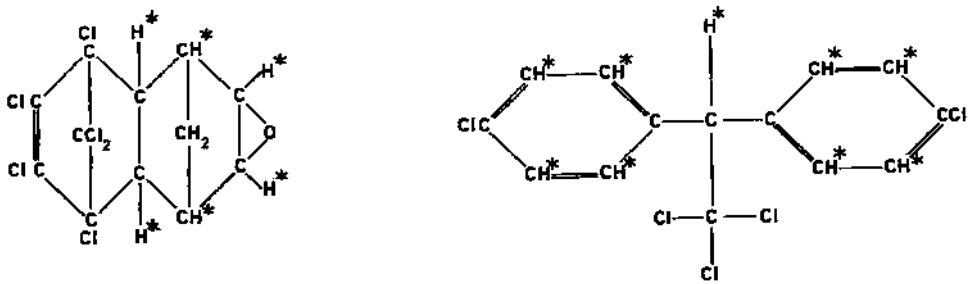

Fig. 7
Semi-uniformly H^3-labelled DDT and Dieldrin

Formation of labelled pools *in vivo*

The formation of labelled pools by insects *in vivo* is readily accomplished by injecting them or feeding them with a suitable substrate. Thus, the pool of soluble phosphorylated intermediates concerned in glycolysis and muscle function is readily labelled in adult house-

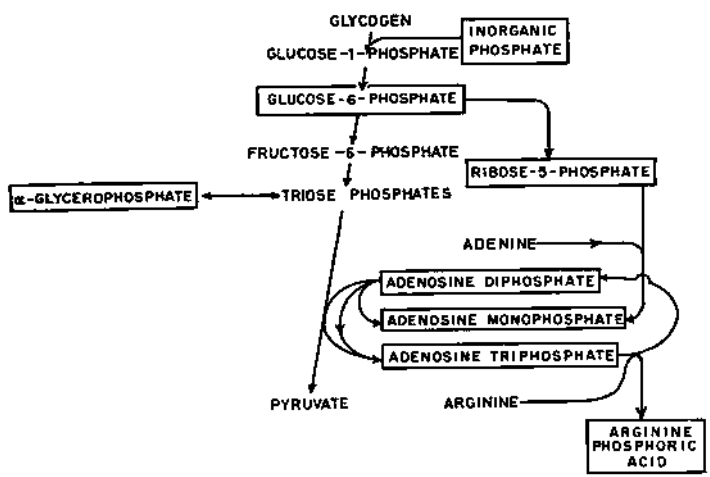

Fig. 8
Likely metabolic pathways by which the phosphorus pool of insect muscle could become labelled with P^{32}. Boxed compounds are those found to accumulate in the muscle of the adult housefly

flies by providing them with aqueous sucrose containing carrier-free P^{32}-labelled PO_4^{3-}. Effective isotopic equilibrium was achieved within 48 h so that the radioactivities of the various fractions were a measure of their relative tissue concentrations [17]. The likely metabolic pathways by which the intermediates of flight muscle become labelled are shown in Fig. 8. Boxed compounds are those which tend to accumulate in measurable concentrations and which have been identified. The related pools which become labelled following the injection of acetate-2-C^{14} into adult houseflies are shown in Fig. 9. The work of HILCHEY, COTTY, HENRY and their colleagues shows how organic sulphur pools may be labelled by feeding insects with S^{35}-labelled SO_4^{2-} or S^{35}-labelled amino acids. [18—21].

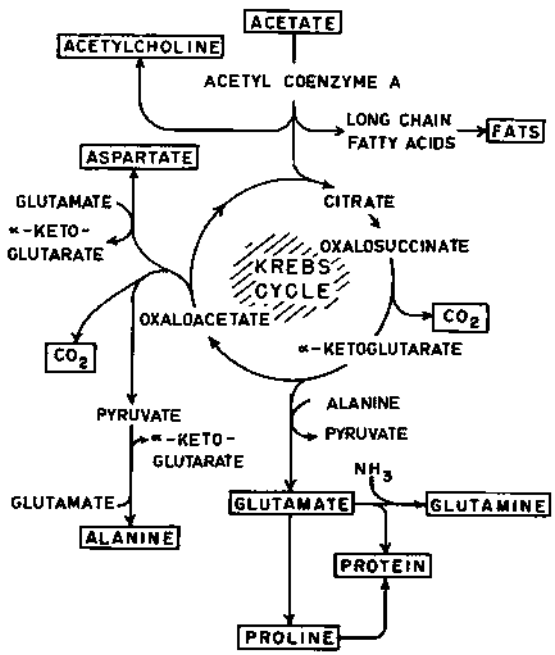

Fig. 9

Likely metabolic pathways by which the C^{14} of injected acetate-2-C^{14} could become incorporated into the free amino acid pool, etc., of the adult housefly. Boxed compounds are those found to accumulate in the thoracic and head tissues

Extraction, fractionation and radiometric assay of labelled substances in biological tissues

Whether labelled insecticides or labelled pools are being studied, the stage will be reached when the labelled compounds present in the insect must be extracted, separated and assayed radiometrically. In the case of labelled pool studies, especially, it will be important to extract the tissues under conditions which preclude further enzymic or chemical changes. Otherwise, the final analysis will not reflect the biochemistry of the tissues which obtained *in vivo*. For this purpose the tissues may be rapidly homogenized at low temperatures (e.g., at —10°C in acid-alcohol) or extracted at normal temperatures after first inactivating enzymes by rapid heating (e.g. immersion in boiling water for 1 min or more). Almost

invariably the necessary fractionation may be accomplished by some form of paper-, column- or gas-chromatography. Automatic radiometric scanning of paper chromatograms

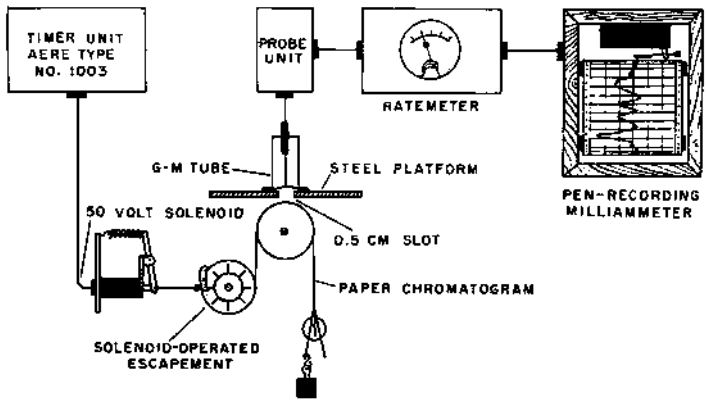

Fig. 10

Assembly for automatically scanning unidimensional paper chromatograms with provision for independent speeds of recorder paper and paper chromatogram. The 50-V solenoid is taken from register of time unit; the solenoid-operated escapement advances the paper chromatogram in 0.5-cm steps

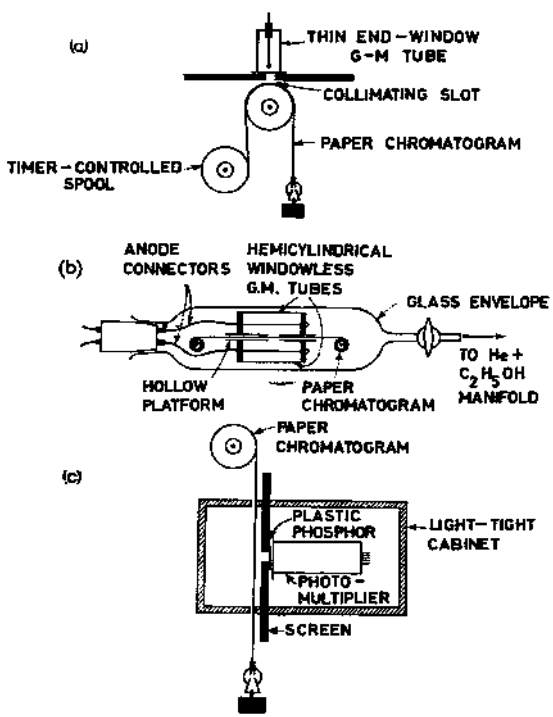

Fig. 11

Detectors for scanning paper chromatograms

or monitoring of column effluents is generally employed for comparing accurately and quantitatively the radioactivities of the separated fractions [22] [23]. A simple assembly for automatically scanning unidimensional paper chromatograms is shown in Fig. 10. Typical detector arrangements are shown in Fig. 11. Absorption of the β-particles

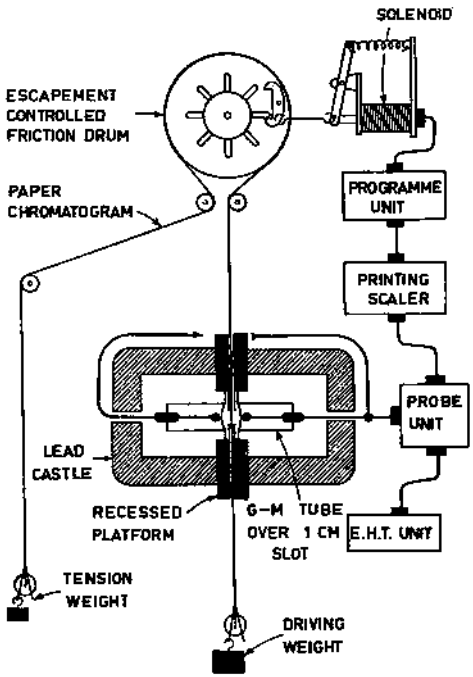

Fig. 12

Diagrammatic illustration of 4-π automatic-scanning assembly for paper chromatograms

by the end-window itself is a major source of loss in sensitivity of the G-M tube to soft beta emitters like C^{14} and S^{35}, as illustrated in Fig. 11a. Mounting paper chromatograms within the sensitive volume itself of a pair of G-M tubes, as shown in Fig. 11b, eliminates this loss, and the detector becomes usefully sensitive even to H^3-labelled compounds separated on paper. In the arrangement shown in Fig. 11c the paper chromatogram is exposed to a thin disc of plastic phosphor in optical contact with a photomultiplier window.

Fig. 12 shows an arrangement in which the total count of each exposed section of paper chromatogram is recorded automatically by a printing scaler. A pair of selected thin end-window G-M tubes of low background is mounted in 4-π geometry.

Fig. 13a shows an arrangement for continuously monitoring the liquid effluent from a chromatographic column. Liquid flow counters of the type shown in Fig. 13b possess glass walls of thickness 30 mg/cm^2, and are therefore insensitive to soft β-emitters like C^{14} and S^{35}. In the counter shown in Fig. 13c the thin layer of liquid sample (to minimize self-absorption) is separated from the sensitive volume of a G-M tube by a 2.5 mg/cm^2 mica-window which will pass a useful proportion of soft β-particles [24].

POPJAK *et al.* [25] have developed an ingenious arrangement for monitoring the gaseous effluent in gas chromatography. Gaseous effluent enters the diamond-shaped tube at I

(Fig. 14) which contains a solution of phosphor (e.g. diphenyl-oxazole in toluene). Radioactive constituents dissolve in the phosphor which tends to circulate in front of the photomultiplier on the right. The inert entraining gas escapes at E. The entire assembly is illustrated in Fig. 15. The three-channel recorder simultaneously records the response of a gas-density balance and radioactivity at two different recorder sensitivites.

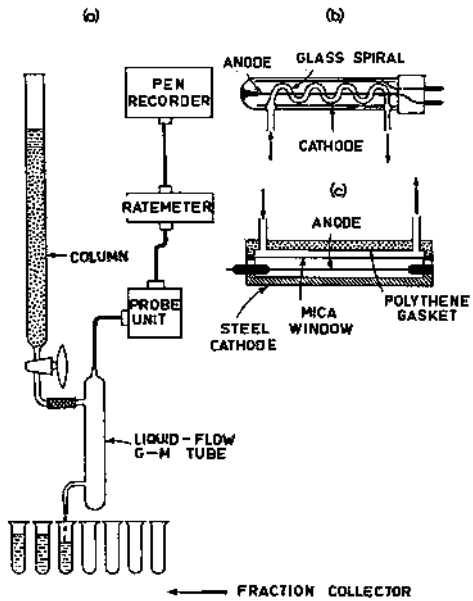

Fig. 13

Radiometric assay of liquid effluent from chromatographic column: (a) automatic assembly; (b) flow-type counter; (c) specially designed flow-counter for soft β-emitters

Identification of labelled compounds in amounts below the limits of chemical detection

The exhaustive fractionation of labelled compounds provides information on their number and the radioisotopic distribution between them. Chemical identities may also be required, however, and these may have to be established with amounts of material below the limits of conventional chemical tests. Almost invariably the problem is not so much a matter of identification *ab initio*, but of establishing which of some likely compounds is represented by the radioactive fraction.

In the very important co-chromatographic technique an authentic compound is added as "carrier" to the labelled mixture before fractionation, and in sufficient amount to permit its detection by a physical or chemical test. After extended fractionation, the carrier is located by the test and the labelled compound is located radiometrically. If the carrier and a labelled compound of the original mixture be identical there will be exact coincidence between their boundaries either in terms of serial fraction numbers, positions and shapes (e.g. as determined autoradiographically) on a paper chromatogram.

A simple technique is illustrated in Fig. 16 for reconcentrating a labelled fraction, together with unlabelled carrier, if required, of a paper chromatogram so that it may be subjected to further chromatography in a different solvent system [26]. That section of paper carrying the labelled fraction under examination is cut from the parent chromatogram

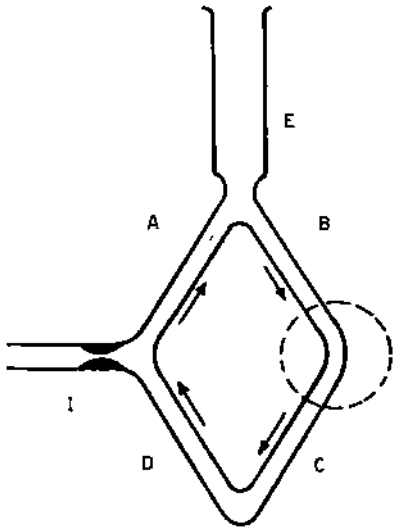

Fig. 14
Schematic presentation of principle used in monitoring the gaseous effluent in gas chromatography (from Popjak *et al.* [25])

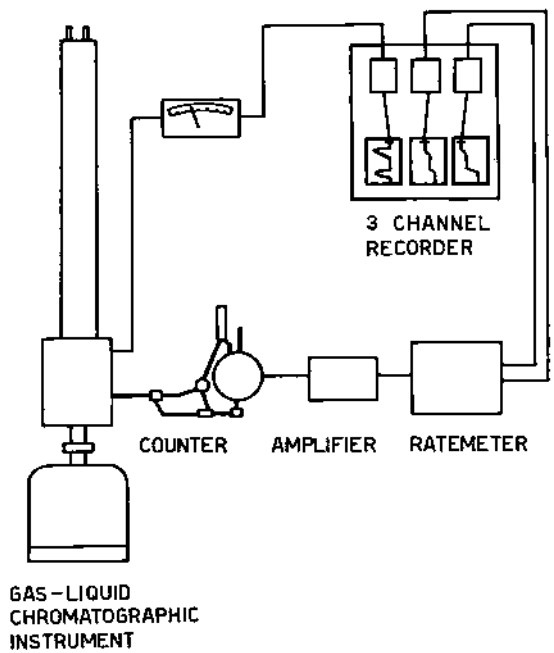

Fig. 15
Complete counting and recording arrangement for monitoring the gaseous effluent in gas chromatography (from Popjak *et al.* [25])

and attached to a fresh strip of paper at A. A suitable volatile solvent is introduced at B and this rises up the section, passes between the ground glass surfaces of the joint and emerges at R. The solvent is no longer in an atmosphere saturated with its own vapour, and therefore evaporates. The net result is that the labelled fraction and carrier are reconcentrated in a narrow band just above R. The paper strip may now be removed and chromatography repeated in a new solvent system. This process may be repeated indefinitely, subject to the stability of the labelled compounds present. If these conditions of, in effect, multidimensional chromatography, are applied in the presence of detectable carrier, the coincidence tests of identity can be made to be as exacting as one pleases. There must, of course, be no possibility of radioisotope exchange between carrier and labelled substance unless they are chemically identical.

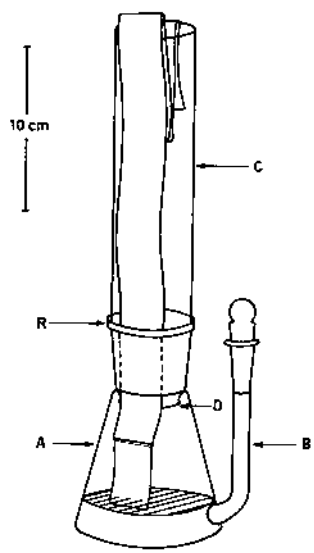

Fig. 16
Simple all-glass unit for eluting and reconcentrating labelled fractions separated on paper chromatograms

A surprising amount may be learned by studying the behaviour of detectably radioactive material under different conditions of fractionation, chemical, enzymic treatment etc. For example, P^{32}-labelled *l*-α-glycerophosphate was identified on paper chromatograms because it was oxidized in the presence of a purified *l*-α-glycerophosphate dehydrogenae resulting in the formation of acid-labile P^{32}-labelled PO_4^{3-} which could readily be identified by paper chromatography [27].

Labelled insecticides in the study of the mechanisms of insect resistance

There are numerous examples in which labelled insecticides have been used for studying their metabolism in insects, plants, and mammals and details may be sought through recent reviews [2] [28—31].

Since the author and his colleagues were perhaps among the first to use labelled insecticides for studying the mechanisms of resistance and synergism, an early finding is shown in Fig. 17 [7]. The radiochromatograms indicated a high level of detoxication in the resistant insect as a result of the enzymic conversion of the absorbed insecticide to its non-insecticidal ethylene derivative. It was also shown that in the presence of piperonyl cyclonene which appeared to enhance the effectiveness of DDT against the resistant insect, the metabolism of DDT was much reduced. This confirmed the earlier observation of PERRY and HOSKINS [32] that the action of piperonyl cyclonene was to inhibit DDT detoxication. Recent similar studies of the fate of the labelled Dieldrin-type insecticides in susceptible and resistant houseflies and mosquitoes strongly suggested that resistance to Dieldrin in these insects was not due to enhanced detoxication or excretion, or failure of the insecticide to penetrate the cuticle of the resistant insect [9] [33—35].

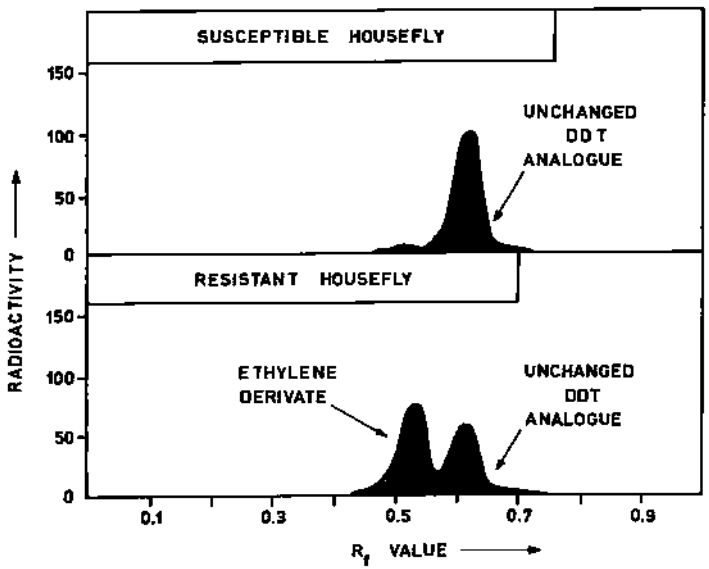

Fig. 17
Chromatograms of radioactive insecticide metabolites recovered from houseflies

P^{32}- and C^{14}-labelled pools in comparative biochemical and toxicological studies

The radiochromatogram shown in Fig. 18 was obtained by feeding the adult housefly *Musca domestica* with carrier-free P^{32}-labelled PO_4^{3-}, later extracting the thoracic tissues (mainly flight muscle) and carrying out a radiochromatographic analysis. This radiochromatogram was automatically plotted by the scanning device shown in Fig. 10. The conditions of the experiment were such that the distribution of total soluble P^{32}-activity represented the relative concentrations of soluble phosphorus compounds in the insect tissues. By repeating such experiments with normal and with poisoned insects it has been possible to study effects on carbohydrate metabolism of intact insects.

It was found, for example, that under conditions of anoxia there was an accumulation of *l*-α-glycerophosphate (fraction IV of Fig. 18) in the thoracic tissues of the adult housefly

[27]. Of considerable interest in this connexion have been the observations that insect flight muscle contains remarkably high and low concentrations respectively of α-glycerophosphate dehydrogase and lactic acid dehydrogenase [36] [37]. Now it is well established that for glycolysis to proceed in vertebrate muscle under the temporary conditions of oxygen lack (such as sudden exercise), reduced coenzyme I ($DPNH_2$) formed glycolytically

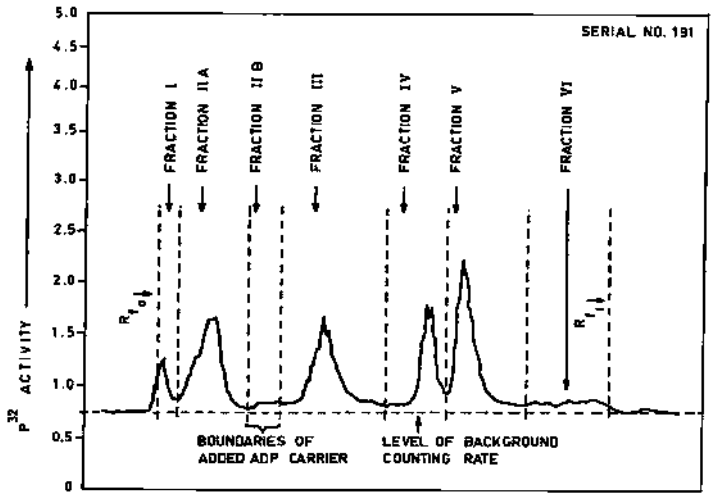

Fig. 18

Facsimile of automatically plotted radiochromatogram of P^{32}-labelled total soluble phosphorus pool recovered from insect flight muscle. Fraction I, phosphoprotein; fraction IIA, ATP; fraction IIB, ADP; fraction III, arginine phosphoric acid + glucose-6-phosphate + AMP; fraction IV, α-glycerophosphate; fraction V, inorganic phosphate

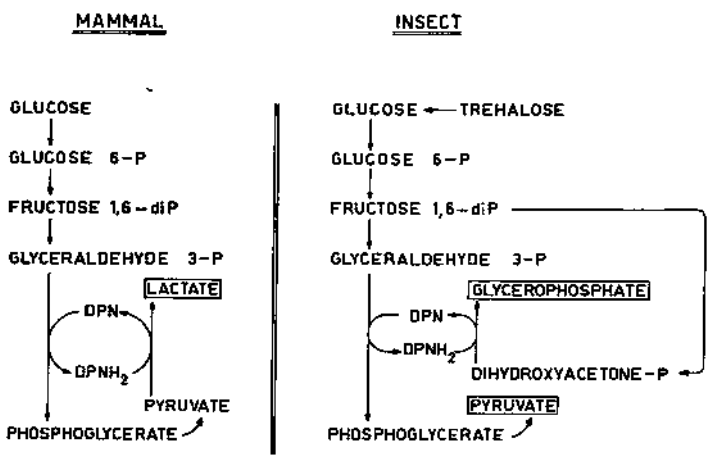

Fig. 19

Comparison between glycolytic mechanisms in mammals and insects

must be reoxidized anaerobically, and this occurs by the action of lactic acid dehydrogenase. Lactic acid thus tends to accumulate. If, on the other hand, α-glycerophosphate dehydrogenase replaced lactic acid dehydrogenase as in insect flight muscle, then an accumulation of α-glycerophosphate would be expected instead of lactic acid. Exactly this was demonstrated in the housefly by the labelled pool technique (see above). The glycolytic mechanisms concerned are compared in Fig. 19. KUBISTA [38] first demonstrated such an accumulation of α-glycerophosphate in the thoracic muscle of the cockroach, *Periplaneta americana*.

It has already been mentioned that recent studies suggest that insect resistance to Dieldrin is not due to lack of penetration of the insecticide through the cuticle of the resistant insect, nor to enzymic detoxication, excretion, etc. Instead, resistance may be due to some fundamental insensitivity at the normal site of action. The question then arises: What is this site of action in biochemical or physiological terms? Labelled pool techniques are being currently used for studying this.

When an active housefly is brought to rest by the minimal use of *cyclo*propane anaesthesia the thoracic α-glycerophosphate rapidly rises to its "resting level" [27]. However, at quite an early stage of Dieldrin poisoning, anaesthesia fails to restore the α-glycerophosphate to its resting level. This suggested that Dieldrin was possibly interfering with the regulation of carbohydrate metabolism at the α-glycerophosphate level [39].

There is evidence that Dieldrin unstabilizes the central nervous system of insects. This could be explained if Dieldrin affected acetylcholine metabolism *in vivo* under conditions of steady state concentrations, i.e. under conditions in which there would be no demonstrable change in the chemical content of acetylcholine in the nerve. This possibility has been studied by measuring the rate of acetate-2-C^{14} incorporation into tissue acetylcholine *in vivo*. When acetate-2-C^{14} is injected into adult houseflies the ketogenic amino acids, glutamate, glutamine, proline, aspartate etc. rapidly become radioactive in both head (rich in central nervous tissue) and thorax (rich in muscle). Since these amino acids may be derived by transamination with ketoacids of the tricarboxylic acid cycle (Fig. 9) this was strong evidence of the operation of the cycle in insect muscle and nerve [40] [41]. These amino acids similarly became radioactive following the injection of C^{14}-glucose which is not surprising since both glucose and acetate share the central intermediate of acetyl coenzyme A [42]. More recently, TREHERNE [43] has applied similar techniques and the same argument for the operation of the tricarboxylic acid cycle in the central nervous tissue of the cockroach *Periplaneta americana*. Returning to the adult housefly it has been found that, following the injection of acetate-2-C^{14}, besides free amino acids there is also a rapid incorporation of labelled acetate into the acetate moiety of acetylcholine in the nervous tissue. The labelled acetylcholine so formed *in vivo* may be separated and assayed radio-paper-chromatographically as shown in Fig. 20. The major labelled fractions separated in the formic acid — acetone solvent consist of the amino acids glutamine, glutamate, proline, etc. The fast-running fraction may be further resolved, as shown, in a formic acid propanol solvent, the impure C^{14}-acetylcholine being present in the slower running fraction at R_F 0.46 [44]. Finally, on running the crude acetylcholine fraction in the neutral aqueous-propanol solvent, the pure C^{14}-acetylcholine separates at about R_F 0.24. It is appropriate here only briefly to mention some of the preliminary findings of this investigation. Continuous *cyclo*propane anaesthesia lowered the rate of formation of C^{14}-acetylcholine and also the overall metabolism of acetate. Perhaps this is the first demonstration of a direct relationship between acetylcholine turnover and nervous activity *in vivo*. Anticholinesterase appeared to "uncouple" acetylcholine synthesis in the sense that the overall metabolism of acetate and respiration were increased, while the formation of C^{14}-acetylcholine was considerably reduced. There was no evidence of Dieldrin effects.

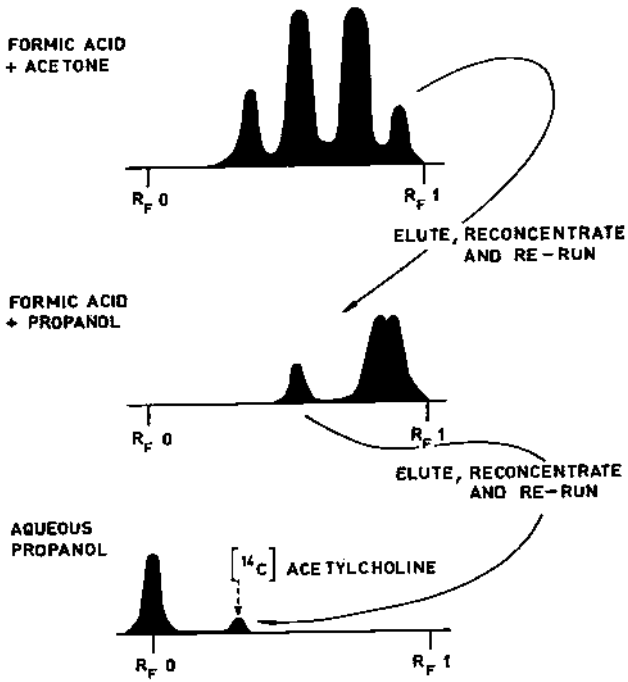

Fig. 20
Radiochromatographic assay of C^{14}-labelled acetylcholine extracted from insect tissue

Concluding comments

Like all other techniques, the use of combined radioactive tracer and microfractionation techniques is not without its pitfalls and limitations. For example, an unlabelled carrier and a labelled but chemically different compound may sometimes show such similar properties during chromatography as quite wrongly to suggest chemical identity. Exact co-chromatographic coincidence should be demonstrated in at least three different solvent systems before identity can be assumed. In the labelled pool technique the distribution of radioactivity between the different fractions may provide information on their *relative* concentrations or turnover rates, but can provide no information on the absolute weights of metabolites present.

Limitations notwithstanding, however, there are, indeed, exciting potentialities — such as the use of labelled cytidine pools for studying nucleic acid metabolism in living cells [45].

ACKNOWLEDGEMENTS

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DISCUSSION

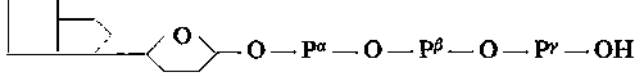
THE CHAIRMAN (J. de Wilde, Netherlands): I feel sure that the wealth of information Dr. Winteringham has given us will provide an excellent basis for a highly interesting discussion. After all, the labelled pool technique provides a key to many otherwise insoluble problems. For example, there is the work being done on the determination of essential amino acids in phytophagous insects. Some work is being done in the physiology laboratories in Utrecht, on nutrition. As you know, nutrition is very difficult to study in phytophagous insects because they will eat only living leaves. Now, after injecting C^{14}-labelled acetate into the pupa, we can see, on analysis of the amino acids in the adult, that two or three separate groups are present: the unlabelled amino acids which apparently are essential, and the labelled which are non-essential. Definite progress is being made in this field which is only one of the many avenues opened up by this method.

A. R. GOPAL-AYENGAR (India): I should like firstly, to express my great admiration for the extraordinary lucidity with which the whole subject has been presented to us. My only comment is that, despite the labelled pool technique and the variety of other techniques that are being brought into action to solve the resistance problem, we are still without a final solution of the problem of resistance and susceptibility, and I was wondering whether Dr. Winteringham could indicate the lines along which, having come so far, we should now proceed.

F. P. W. WINTERINGHAM: Well, the position, as I see it, is this: the first major case of resistance to be studied biochemically was that of DDT and I think it is clear that resistance to DDT is associated with quite a spectacularly high level of DDT metabolism. This led many of us to believe that the same mechanisms might account for the resistance to other insecticides, such as the cyclodiene derivatives like Dieldrin, and BHC and all the OP compounds. But I think it is only fair to say that the problem is not proving as simple as that. In the case of cyclodiene-derivative insecticides, like Dieldrin, we have no evidence to date, as far as I know, that resistance is due to metabolism, excretion or failure of the insecticide to penetrate the cuticle. And we therefore have to face up to the fact that resistance may involve, as I said earlier, some fundamental insensitivity at the site of action. Thus, if we are really going to understand the mechanisms of resistance, we must know more about the action of the insecticide itself. With the organophosphorus compounds, I think there is perhaps some indication that metabolism may indeed be an important factor — but even here, as some of the data of cross-resistance strongly suggest, it is unlikely to be the only factor. But I think van Asperen's work especially has indicated the possible role of phosphatases in detoxication.

T. L. HOPKINS (United States of America): In the labelled pool technique, do the labelled compounds achieve uniform labelling and is the same relative percentage of molecules of each compound labelled?

F. P. W. WINTERINGHAM: Uniformity of labelling can often be established. For example, adenosine triphosphate (ATP) has the skeleton structure



which, on acid hydrolysis yields adenine, ribose-5-phosphate from P^{α} and $2 \times PO_4^{3-}$ from P^{γ}.

Therefore if the ATP were uniformly labelled, the inorganic phosphate should be twice as radioactive as the ribose phosphate. By applying this test to the labelled ATP formed in insects *in vivo* it was found that ATP was uniformly labelled.

One other method which has been successfully applied is the following: a paper chromatogram containing P^{32}-labelled metabolites is allowed to decay for 2 or 3 months so that the original radioactivity has largely disappeared. The paper chromatogram is then reactivated in the reactor. All the chemical phosphorus originally present, not necessarily that which had been labelled, becomes reactivated according to the P^{31} (n, γ) P^{32} reaction so that the new radioactivity in the paper chromatogram is a measure of the total chemical phosphorus on the chromatogram. In our experiments, the peaks of radioactivity produced were roughly the same as the relative proportions on our original paper chromatograms. This proves that the original P^{31}:P^{32} ratio, i.e. the specific radioactivity, is the same for the various labelled fractions. However, I think it is only fair to say to Dr. Hopkins that there are cases which are more difficult to solve.

J. TREHERNE (United Kingdom): I was wondering if Dr. Winteringham would agree with me that, despite the very precise nature of the radioisotope technique, the method as applied to insect physiology and biochemistry still remains largely semi-qualitative. Frequently, radioisotope experiments yield information which would allow quantitative transfer constants, permeability constants and turnover rates to be calculated. Unfortunately, these data are often not utilized in these ways and, instead of being used for obtaining physical constants, remain in an "undigested" state which does not permit full light to be thrown on the nature of many biochemical and physiological processes.

F. P. W. WINTERINGHAM: I think the important thing to remember is that the tracer only measures accurately the turnover, or the chemical or physical distribution of the *radioactive isotope*. This by no means necessarily measures either net chemical synthesis or turnover of a particular compound. In many studies two factors are required: the radioisotope activity, and the chemical concentration. I agree that, ideally, there are many simple applications in which one can reasonably assume chemical concentrations to remain constant, so that the changes in radioactivity do really mean changes in turnover rate.

P. B. CORNWELL (United Kingdom): In an attempt to bring the two aspects of the Symposium together—radiotracer techniques and radiation damage in insects—I would like to ask Dr. Winteringham if he considers that radiotracer techniques may be of value in evaluating what physiological changes occur in radiation susceptibility studies?

F. P. W. WINTERINGHAM: Yes, I think they can in the sense that the labelled pool technique may well demonstrate accumulations or depletions of essential metabolites which might be missed by conventional, pharmocological or chemical analysis. One advantage of the labelled pool technique, provided it is used intelligently, is that it brings about changes in various intermediates, even before they may have been identified chemically: it draws attention to changes and phenomena which may be worth studying. The other possible advantage is that the technique may be considered as representing a radiation source in itself. In short, an experiment may be carried out as we in fact have done, at different levels of radioactivity. If there is a correlation of some change in the level of radioactivity, then this is an illustration of radiation damage in biochemical terms.

G. G. SENGUPTA (India): Is it possible to isolate the component parts and by-products of different metabolic cycles involved in insect tissue?

F. P. W. WINTERINGHAM: Yes, I think it is. By methods such as gas chromatography and electrophoresis, one can separate probably most of the more important metabolites involved in lipid, carbohydrate or amino acid metabolism. The important point in labelled pool work is to ensure that the methods of extraction, chromatography etc. do not themselves affect the proportion of metabolites obtaining in the original tissues.

However, I should perhaps mention here one important limitation of the tracer technique, namely, that we have not got suitable radioactive isotopes of all the natural elements — nitrogen is perhaps the most regrettable case. We are unable to follow the metabolism of nitrogen *per se* in insects, although we can, by fractionation techniques, separate most of the important nitrogen compounds, whether they are amino acids, purine bases, and so on

A. R. GOPAL-AYENGAR: Since, as appears, the OP substances work by affecting the nervous. system, is there any actual evidence of alteration in the structure of the nervous system under the action of insecticides?

F. P. W. WINTERINGHAM: I am probably treading on rather dangerous ground here, but I do not think there is any direct evidence of changes in nervous structure in histological terms. Histological studies have been made of nervous structure at late stages of poisoning, but I am rather doubtful whether these changes are the direct result of insecticidal action. I think they are possibly rather irrelevant consequences of nervous degeneration with the onset of death. It has been suggested that changes in structure might be determined histologically even at early stages of poisoning, but I am frankly not one of the supporters of this idea. I think there are better methods of approaching the problem. On the other hand, there are other grounds for believing that such changes do occur, and I think one of the most important hypotheses advanced on these lines has been put forward by Mullins at Purdue University. A particular lipo-protein structure of the nerve cell was postulated and it was shown or, rather, argued quite cogently that the dimensions of the BHC and the DDT molecule are such that they might distort this particular structure and so greatly affect the permeability of the nerve cell wall—and I speak loosely—to such important ions as potassium and sodium. But this is only a hypothesis, and as far as I know there is no experimental evidence of such a distortion.

P. J. DEORAS (India): When radioisotopes are used in insecticides, is there any demonstrable radiation effect on the histology of the insect—as distinct from the biochemically detectable effect?

F. P. W. WINTERINGHAM: There are two possible effects we have to take into account. One is the effect of the mass of the isotope, and this does suggest a possible limitation in the use of something like tritium as a tracer, which has a mass number three times that of the natural isotope, hydrogen. It has been amply demonstrated that turnover rates under non-equilibrium conditions are very different for tritium-labelled compounds and for natural hydrogen compounds. So the distribution studies carried out with tritium under non-equilibrium conditions might be misleading. On the other hand, Glascock at Reading has made a study of the significance of using tritium, and has concluded that under equilibrium conditions the effect could probably be negelected for most purposes. For other isotopes, where the mass number ratio is very much less—such as C^{14}:C^{12}—the effect can be neglected for most purposes, I think, nor is there evidence of biological fractionation. If there were, we should, of course, have a very nice method of separating and concentrating isotopes.

The second possibility is of radiation damage, and this is a very real factor to be taken into account. Using the labelled pool technique one can very easily feed or inject into an

insect such a high level of radioactivity as to affect its well-being. However, this can at least always be checked by doing one experiment at a very low level of radioactivity—compatible with detection and measurement, of course—and one at a very high level. I am unaware of demonstrable histological effects as a result of the self-absorbed radiation normally associated with tracer experiments.

SECTION 4

INSECT PHYSIOLOGY AND BIOCHEMISTRY

RADIOISOTOPES AND THE INSECT CENTRAL NERVOUS SYSTEM

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Abstract — Résumé — Аннотация — Resumen

Radioisotopes and the insect central nervous system. The use of radioisotopes has facilitated research at Cambridge on the physiology of the insect central nervous system. In particular, this work has been concerned with the influx of various radioactive compounds through the continuous cellular and fibrous membrane which envelopes the nervous system. It is currently believed that this structure, the perilemma, functions as a diffusion barrier restricting the entry of such substances as sodium and potassium ions and acetylcholine into the underlying nervous tissue. On the other hand, this structure must also, as Wigglesworth has pointed out, be specialized to allow adequate exchanges of nutritive substances between the central nervous system and the haemolymph. This important role has been little studied, and an attempt has been made to understand these processes by studying the uptake of C^{14}-labelled molecules in the abdominal nerve cord of the cockroach. At the same time, the opportunity has been taken to learn something of the biochemical events in insect nervous tissue by following the metabolism of some labelled compounds in the nerve cord of this insect. It is anticipated that this information may help to throw some light on the entry and subsequent behaviour of insecticide molecules in the insect central nervous system. These findings are discussed in relation to current concepts of the physiology of the vertebrate nervous system.

Les radioisotopes et le système nerveux central de l'insecte. L'emploi des radioisotopes a facilité les recherches qui ont été faites à Cambridge sur la physiologie du système nerveux central de l'insecte. Les travaux ont surtout porté sur la pénétration de divers composés radioactifs à travers la membrane cellulaire et fibreuse continue qui enveloppe le système nerveux. On estime généralement que cette membrane forme une barrière qui s'oppose à la pénétration de substances telles que les ions sodium et potassium ou acétylcholine dans les tissus nerveux sous-jacents. D'autre part, comme l'a souligné Wigglesworth, elle doit aussi avoir des fonctions spécifiques permettant les échanges nutritifs nécessaires entre le système nerveux central et l'hémolymphe. Ce rôle important a été peu étudié, mais on s'est efforcé d'expliquer les processus mentionnés en examinant chez la blatte l'absorption de molécules marquées au carbone-14 par le cordon nerveux abdominal. D'autre part, on a saisi cette occasion pour essayer de se faire une idée des phénomènes biochimiques qui s'opèrent dans le tissu nerveux de l'insecte, en observant le métabolisme de certains composés marqués dans son cordon nerveux. On espère que ces renseignements permettront de mieux comprendre le processus de pénétration et le comportement subséquent des molécules d'insecticide dans le système nerveux central de l'insecte. Les résultats obtenus sont examinés à la lumière des conceptions courantes de la physiologie du système nerveux des vertébrés.

Радиоизотопы и центральная нервная система насекомых. Использование радиоизотопов облегчило проведение в Кембридже исследований физиологии центральной нервной системы насекомых. Эти исследования в особенности касались потока различных радиоактивных соединений, проходящего через сплошную клетчатую и волокнистую мембрану, которая окружает нервную систему. В настоящее время считается, что эта структура — перилемма — выполняет роль диффузионного барьера, препятствующего проникновению таких веществ, как натриевые и калиевые ионы и ацетилхолин в расположенную под ней нервную ткань. С другой стороны, эта структура, как указывал г-н Уигглезворт, должна также быть приспособлена для обеспечения соответствующего обмена питательными веществами между центральной нервной системой и гемолимфой. Эта важная роль мало исследовалась и была сделана попытка познать эти процессы путем изучения усвоения меченных углеродом-14

молекул в брюшном нервном сплетении таракана. В то же время была использована возможность познать некоторые стороны биохимических явлений в нервной ткани насекомых путем наблюдения за метаболизмом некоторых меченых соединений в нервном корде этого насекомого. Ожидается, что эти сведения, возможно, прольют некоторый свет на проникновение и последующее поведение молекул инсектицидов в центральной нервной системе насекомых. Результаты этих исследований освещаются в связи с современными понятиями по физиологии позвоночной нервной системы.

Los radioisótopos y el sistema nervioso central de los insectos. Las investigaciones sobre fisiología del sistema nervioso central de los insectos que se vienen realizando en Cambridge se han visto facilitadas por el empleo de los radioisótopos. El trabajo descrito en la presente memoria se refiere en particular a la penetración de varios compuestos radiactivos a través de la membrana fibrosa y celular continua que envuelve al sistema nervioso. Se suele admitir que tal estructura, llamada perilema, actúa como una barrera de difusión que se opone a la penetración de sustancias como los iones sodio y potasio y la acetilcolina en el tejido nervioso subyacente. Por otra parte, como ha indicado Wigglesworth, esta estructura también debe estar diferenciada para que se puedan verificar los adecuados intercambios de sustancias nutritivas entre el sistema nervioso central y la hemolinfa. Como esta importante función ha sido objeto de pocos estudios, el autor ha intentado llegar a comprender dichos procesos de intercambio investigando la absorción de moléculas marcadas con ^{14}C en el cordón nervioso abdominal de la cucaracha. También ha aprovechado la oportunidad para adquirir algunos conocimientos acerca de los procesos bioquímicos en el tejido nervioso del insecto; a tal efecto, estudió el metabolismo de ciertos compuestos marcados en el cordón nervioso de la cucaracha. Se estima que la información reunida puede contribuir a explicar la penetración y el comportamiento ulterior de las moléculas de insecticida en el sistema nervioso central del insecto. Los hechos observados se examinan en el contexto de los conocimientos actuales acerca de la fisiología del sistema nervioso de los vertebrados.

Research on the physiology and biochemistry of the insect central nervous system has lagged far behind comparable studies on vertebrate nervous systems. Undoubtedly much of our ignorance concerning the metabolism of insect nervous tissue can be attributed to the difficulties associated with research on small organisms in which, even in the larger species, the whole nervous system rarely exceeds a few milligrams in weight. In this respect the use of radioisotopes represents an extremely valuable technique, facilitating the study of processes lying far beyond the limits of conventional chemical methods.

During the past few years at Cambridge, radioisotopes have been extensively used in research on some aspects of the physiology of the insect central nervous system. In particular this work has been concerned with the factors involved in the permeability of the continuous cellular and fibrous membrane which envelopes the nervous system. This membrane, the perilemma of SCHARRER (1939), had been found to serve a protective function apparently limiting the entry of sodium and potassium ions and acetylcholine molecules into the underlying nervous tissue (HOYLE, 1953; TWAROG and ROEDER, 1956). The presence of this relatively impermeable sheath made it difficult, however, to understand how the necessary exchanges of nutritive and excretory substances could take place between the haemolymph and the nervous system (WIGGLESWORTH, 1960).

In an attempt to throw some light on the processes involved in the permeability of the nerve sheath, the uptake and metabolism of some C^{14}-labelled sugars by the abdominal nerve cord of *Periplaneta americana* were studied (TREHERNE, 1960). In these experiments the increase in radioactivity within the abdominal nerve cord was measured following the injection of a small amount of C^{14}-labelled glucose into the haemolymph. As was to be expected from a previous investigation on the locust (TREHERNE, 1958) the injected glucose was fairly rapidly converted to the disaccharide trehalose which accumulated in the

haemolymph, only very small amounts of glucose remaining in equilibrium with the trehalose molecules. The level of trehalose in the haemolymph of these cockroaches was approximately 1.39 g/100 ml, while the concentration of glucose averaged 0.039 g/100 ml.

It was found that immediately following injection of the C^{14}-glucose there was an extremely rapid rise in radioactivity in the abdominal nerve cord relative to the haemolymph. This increase in activity within the nervous system occurred before an appreciable amount of the glucose had been converted to trehalose, and thus represented the movement of a relatively few glucose molecules of high specific activity into the nerve cord. Following this, there was a drop in the radioactivity of the nervous tissue resulting from the efflux of C^{14}-glucose as the specific activity of this compound in the haemolymph declined due to the incorporation of glucose molecules as trehalose. This initial rise and fall in activity due to the movements of the glucose molecules can therefore be regarded as an experimental artifact. The true entry of radioactive sugars from the haemolymph was thus represented by the subsequent increase in activity within the nerve cord in which the C^{14} originated as trehalose in equilibrium with the small amount of glucose.

The total influx of C^{14} originating as trehalose and glucose in the haemolymph was calculated to be equivalent to the movement of approx. 65.4 mM C^{14}-glucose units/litre nerve cord water per hour. From experiments on isolated preparations in which only the monosaccharide molecules were labelled with C^{14}, the massive concentrations of trehalose being non-radioactive, it was found that the influx of the radioactivity originating as glucose was roughly equivalent to 4.8 mM/l nerve cord water/h. Apparently then, only about 7% of the C^{14} passed into the nerve cord in the form of glucose, the greater part of the sugar

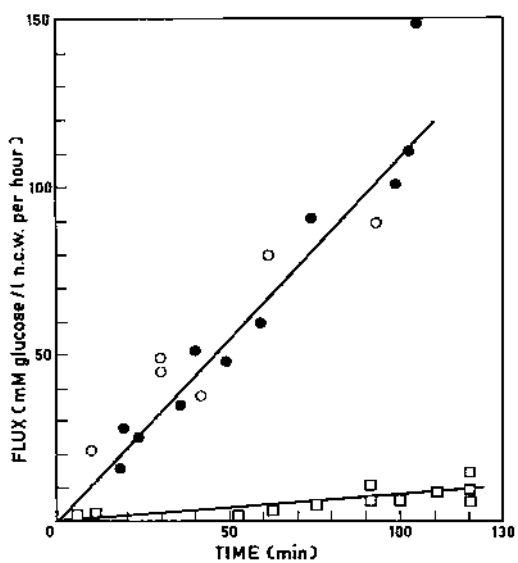

Fig. 1

The calculated influxes of radioactivity in the abdominal nerve cord of *Periplaneta americana*
● C^{14} originating as trehalose and glucose in the haemolymph of intact insects;
○ C^{14} originating as trehalose and glucose entering connectives isolated in radioactive haemolymph;
□ For isolated connectives in saline in which only the glucose (2.19 mM/l) was labelled with C^{14} the trehalose being non-radioactive.

The fluxes are expressed as mM C^{14}-labelled glucose units/l nerve cord water

movements being accounted for by the influx of the relatively large trehalose molecules. These results mean, in effect, that one molecule of glucose passed into the abdominal nerve cord for approximately every seven molecules of trehalose. The disaccharide molecules were, however, about seventeen times more concentrated than those of glucose, which implies that the individual monosaccharide molecules were in fact passing into the nerve cord at about 2.5 times the rate of those of trehalose.

In addition to the experiments on the movement of sugar molecules through the perilemma, some observations were also made on the subsequent metabolism of these compounds within the central nervous system. This metabolism was followed by separating the extracts of radioactive nerve cords on paper chromatograms developed in a variety of different solvent systems. It was found that the extracted C^{14} could be separated into at least seven peaks of radioactivity (Fig. 2). Several of these zones were associated with ninhydrin-positive spots on the chromatograms and were subsequently identified as aspartic acid, glutamic acid, glutamine and alanine. Appreciable amounts of glucose and trehalose were also detected, in addition to substantial amounts of a substance occurring at the base line of the chromatograms; this was found to be alcohol-insoluble, water-soluble, and to yield only glucose on acid hydrolysis. This latter substance was presumed to be the glycogen demonstrated by WIGGLESWORTH (1960) to occur in the nerve cord of this insect.

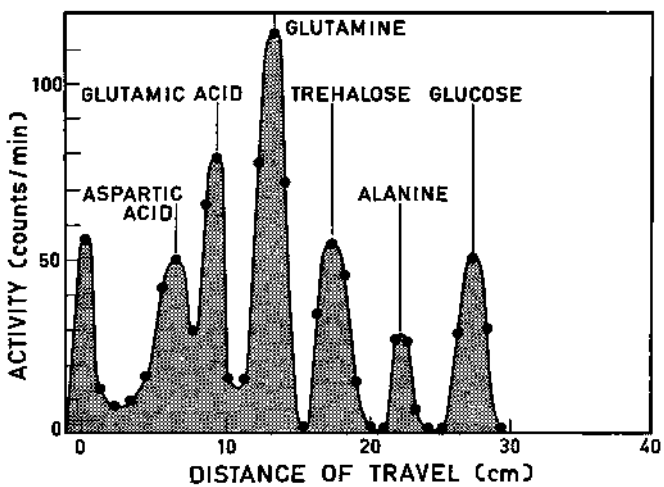

Fig. 2

The distribution on paper chromatograms of radioactivity extracted from cockroach abdominal nerve cords 3 h after injection of C^{14}-labelled glucose into the haemolymph. The chromatogram was developed with propanol/ethyl acetate/water

As has already been pointed out (TREHERNE, 1960), the prompt appearance of the C^{14} supplied as trehalose and glucose in the carbon skeletons of aspartic acid, glutamic acid, and alanine represents circumstantial evidence for the presence of the Krebs tricarboxylic acid cycle in the central nervous system of *Periplaneta*. HESLOP and RAY (1958), using the labelled-pool technique of WINTERINGHAM (1956), have identified glucose-6-phosphate and phosphoglyceric acid in the cockroach nerve cord, so that it is possible that the carbohydrate metabolism in this system could be linked to the tricarboxylic acid cycle by the conventional glycolytic pathway.

The fact that about half the administered C^{14} was found to be incorporated as glutamic acid and glutamine suggests that, as in the nervous tissue of mammals (*cf.* McILWAIN, 1959), this very reactive amino acid occupies a central position in the metabolism of the central nervous system of this insect. The linkage with glutamine is of special importance, for this substance must function as an important reservoir of amino-nitrogen in the abdominal nerve cord of *Periplaneta*.

Returning to the central theme of these studies, the permeability of the perilemma, it was rather unexpected that such a postulated diffusion barrier should allow relatively rapid influxes of C^{14}-labelled sugars to occur. In an attempt to throw some further light on the properties of the perilemma an investigation was initiated on the exchanges of sodium and potassium ions between the haemolymph and the abdominal nerve cord of *Periplaneta* (TREHERNE, 1961). In this study the penetration of K^{42} and Na^{24} into the nerve cord was measured, following the injection of small amounts of these ions into the haemolymph.

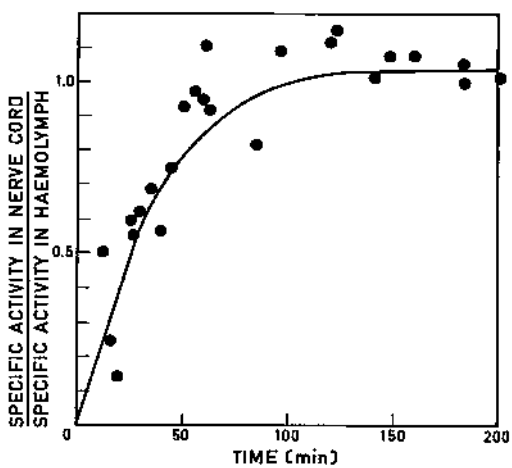

Fig. 3

The penetration of K^{42} into the abdominal nerve cord of the *Periplaneta americana*

The rate of entry of K^{42} ions into the abdominal nerve cord is illustrated in Fig. 3, which shows the change in the specific activity of the potassium in the nerve cord relative to that in the haemolymph. It will be seen that the exchange of these ions was rapid and complete. There was certainly no evidence of appreciable amounts of very slowly exchanging or 'bound' potassium such as was postulated to occur in nerve axons by ROTHENBERG (1950). The influx of the potassium was calculated to be equivalent to 312 mM/l nerve cord water/h (Fig. 4), which was very approximately calculated to represent an influx per unit surface area of nerve cord of 13.5×10^{-12} M/cm^2 s. Similar studies using Na^{24} showed that the calculated influx from the haemolymph was approx. 320 mM/l nerve cord water/h which was roughly equivalent to 13.9×10^{-12} M/cm^2 s.

The results showing these relatively rapid fluxes of sodium and potassium ions between the haemolymph and the nerve cord were rather unexpected, in view of the supposition that the perilemma functioned as a diffusion barrier. Indeed, the calculated influxes for potassium ions even approached the values which have been measured for isolated individual cephalopod

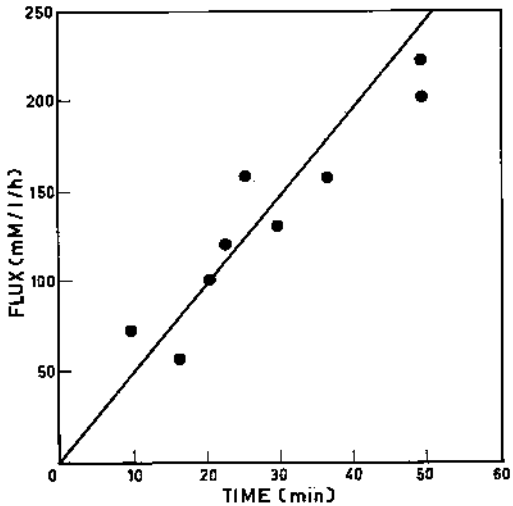

Fig. 4

The calculated influx of potassium ions into the abdominal nerve cord, expressed as mM/l nerve cord water, derived from the data in Fig. 3

axons (KEYNES, 1951). The possibility exists that these results could be explained in terms of an exchange diffusion mechanism of the type postulated by USSING (1949) by which unlabelled ions on one side of a membrane are exchanged on a 1:1 basis with the labelled ions on the other side. In such a system the perilemma might still be impermeable in the sense that no net ionic movements could occur. However, the work of TOBIAS (1948) has clearly shown that the total potassium content of the cockroach nerve cord showed a fairly rapid increase, following a rise in the haemolymph potassium induced by the ingestion of 1.4 *N* KCl. It must be concluded then that an impermeability of this sort does not play an appreciable part in this system. The measured fluxes of the Na^{24} and K^{42} clearly suggest that a dynamic rather than a static equilibrium must exist between the haemolymph and the central nervous system, and that the concept of the perilemma as a passive diffusion barrier must be abandoned as far as these substances are concerned. In such a dynamic system the accessibility of nutritive substances to the underlying tissues of the central nervous system becomes explicable. The relatively rapid influxes of trehalose and glucose molecules described earlier, for example, fit in very well with this concept.

These conclusions about the nature of the permeability processes associated with the perilemma have some important implications in relation to insect toxicology. The possible importance of the nerve sheath in protecting the insect nervous system from insecticide molecules in the haemolymph has already been emphasized by WIGGLESWORTH (1956). It is conceivable, therefore, that this structure might be effective in contributing to some of the dramatic insecticide resistances which have been acquired by certain species. On the basis of an impermeable nerve sheath it might have been imagined that such resistance could have been achieved merely by a rigid exclusion of the poison molecules by such a static system. The demonstration, however, of the apparent permeability of this structure, which might be associated with a dynamic ionic regulation, seems to exclude the possibility of a simple

resistance mechanism of this kind. It seems more likely, in the light of this evidence, that any exclusion of insecticide molecules from the nerve cells of the central nervous system must be the result of some 'active' processes. The fact that in *Rhodnius* the cellular layer of the nerve sheath, the perineurium, contains an abundance of mitochondria and a high content of succinoxidase and non-specific esterase (WIGGLESWORTH, 1958) might suggest that some localized detoxication processes could occur in this layer. Alternatively, some mechanism involving an outward secretion of particular toxic molecules might very tentatively be postulated, for in at least one biological system, the mammalian kidney tubule, it has been shown that secretion of a variety of non-physiological molecules can occur (*cf.* SMITH, 1951).

It is hoped that this brief account of several recent research topics will illustrate some of the ways in which the use of radioisotopes has affected our conception of certain aspects of the physiology of the insect central nervous system. Whatever the final solutions to the many problems involved in this subject, it seems fairly certain that techniques involving the use of radioisotopes will be among the most valuable tools available to future investigators.

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DISCUSSION

T. L. HOPKINS (United States of America): Do you think that trehalose undergoes metabolism and resynthesis in crossing the perilemma, or has trehalose been demonstrated in the nerve cord?

J. E. TREHERNE: Trehalose molecules entering the central nervous system of the insect are very rapidly converted to a variety of amino compounds and to glycogen. It is not possible to say where this metabolism occurs.

P. PÉLEGRIN (France): Could the movement of sodium or potassium across the perilemma be connected with hormonal action? The work done by Morel and Maetz of the Commissariat à l'Energie Atomique at Saclay suggests that this may be the case.

J. E. TREHERNE: I have not yet tried any experiments on the possible effects of hormones on sodium or potassium movements in this system. Preliminary studies show, however, that sodium efflux from the whole central nervous system may be an 'active' process, in that it can be reduced by the addition of metabolic poisons.

W. KLOFT (Federal Republic of Germany): Have you done any experiments with nerve tissue in various phases of function? I think your techniques will be a very useful tool for explaning nerve functions.

J. E. TREHERNE: I am, of course, working with the membrane surrounding the whole central nervous system. I doubt that, with this rather complex system it would be very easy to interpret results on the effects of different levels of electrical stimulation at this stage. These experiments are not directly comparable with those on single giant axons, on which most of this type of work has been done. It may well be worth while to consider such studies, once the present work has reached a more advanced stage.

D. F. HEATH (United Kingdom): As is well known, ionic organophosphorus compounds and some other pharmacological agents are very much less active against insects than their non-ionic, lipid-soluble analogues. If the whole neural sheath is freely permeable to ionic compounds and large water-soluble, oil-insoluble molecules, their effects are hard to explain. It is, however, only necessary to postulate that the synaptic gaps are protected against such compounds. Is there anything in your work inconsistent with such an assumption?

J. E. TREHERNE: No, there is not, and I think your suggestion extremely valuable. This is the sort of approach which we may have to adopt for this problem.

A STUDY OF THE PHOSPHOLIPIDS OF DIELDRIN-RESISTANT AND SUSCEPTIBLE HOUSEFLIES, WITH PARTICULAR REFERENCE TO THOSE OF THE THORACIC GANGLION

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Abstract — Résumé — Аннотация — Resumen

A study of the phospholipids of Dieldrin-resistant and susceptible houseflies, with particular reference to those of the thoracic ganglion. A study of the phospholipids of the housefly has been made because of the possibility that some alteration in membrane structure or active transport is implicated in the mechanism of Dieldrin-resistance in insects. The phospholipids of a Dieldrin-resistant strain of housefly and of a susceptible strain from which it has been derived have been labelled by feeding the flies on P^{32}-labelled orthophosphate solution for 24 h, and then allowing them to metabolize the adsorbed P^{32}-phosphate for periods up to 336 h. Thoracic ganglia have been removed and the phospholipids separated by chromatography on silicic acid impregnated paper strips. Four major phospholipid fractions can be separated which appear to be the same as those obtained from extracts of whole flies. In the case of whole fly extracts the identity of the fractions is as follows: fraction II phosphatidyl inositol, fraction III lysocephalin, fraction IV a mixture of phosphatidyl choline and an unidentified phospholipid and fraction V phosphatidyl ethanolamine and phosphatidyl serine. Fraction I consists of non-phospholipid phosphorus-containing compounds. Maximum labelling of the fractions was obtained after feeding the flies (on glucose and water) for one week after removal from the P^{32}-phosphate. The distribution of the P^{32}-activity in the separated fractions from the ganglia of the resistant and susceptible fly showed no significant differences at the time of maximum labelling. There was, however, some evidence for a slower turnover of P^{32}-activity into the two major phospholipid fractions (IV and V) from the resistant fly. This was made more obvious when the flies were fed on milk in addition to glucose and water. Preliminary studies on turnover for much shorter periods have been carried out on phospholipid extracts from whole flies injected with P^{32}-labelled orthophosphate solution. These have shown a more rapid turnover of P^{32}-activity into the phosphatidyl inositol fraction (II) in the case of the resistant flies, although in these short-term experiments there seems to be no difference in turnover into fractions IV and V from the two strains. The possible significance of the findings is discussed.

Etude sur les phospholipides des mouches communes sensibles et résistantes à la dieldrine, notamment sur ceux du ganglion thoracique. On a étudié les phospholipides de la mouche commune, car il est possible qu'une altération de la structure des membranes ou du processus de transport actif joue un rôle dans le mécanisme de la résistance des insectes à l'action de la dieldrine. Chez une souche résistante à la dieldrine et chez la souche- mère sensible, on a marqué les phospholipides en nourrissant les mouches pendant 24 h avec une solution d'orthophosphate marqué au phosphore-32 et en laissant le métabolisme du phosphate absorbé s'opérer pendant un laps de temps allant jusqu'à 336 h. Après extirpation des ganglions thoraciques, on a séparé les phospholipides par chromatographie sur bandes de papier imprégnées d'acide silicique. On a pu ainsi isoler quatre fractions principales de phospholipides qui semblent être identiques à celles que l'on obtient d'extraits de mouches entières. Dans le cas d'extraits de mouches entières, les fractions sont les suivantes: fraction II, phosphatidylinositol; fraction III, lysocéphaline; fraction IV, mélange de phosphatidylcholine et d'un phospholipide non identifié; fraction V, phosphatidyléthanolamine et phosphatidyl-sérine. La fraction I consiste en composés phosphorés autres que des phospholipides. Les fractions marquées au maximum ont été obtenues lorsque les mouches avaient été nourries au glucose et à l'eau pendant une semaine après suspension de l'alimentation au phosphate marqué au phosphore-32. Au moment du marquage maximum, la distribution du ^{32}P dans les fractions extraites des ganglions de mouches résistantes et

de mouches sensibles n'a pas accusé de différences notables entre les deux souches. Certains indices semblaient cependant montrer que chez la souche résistante l'incorporation du ^{32}P aux deux fractions principales de phospholipides (IV et V) s'opérait plus lentement. Ce phénomène est apparu avec plus d'évidence lorsque, au glucose et à l'eau qui constituaient la nourriture des mouches, on a ajouté du lait. On a fait des études préliminaires sur l'incorporation du ^{32}P pendant des périodes beaucoup plus courtes, en utilisant des extraits de phospholipides provenant de mouches entières auxquelles avait été injectée une solution d'orthophosphate marqué au ^{32}P. Ces études ont montré que l'incorporation du ^{32}P à la fraction II (phosphatidyl-inositol) était plus rapide chez les mouches résistantes, mais elles n'ont révélé aucune différence entre les deux souches quant à l'incorporation de cet élément aux fractions IV et V. Les auteurs du mémoire examinent la signification possible des résultats obtenus.

Исследование комнатных мух, устойчивых и восприимчивых по отношению к фосфолипидам диелдрина и особенно мух с грудным ганглием. Исследование фосфолипидов комнатной мухи было проведено ввиду существования возможности того, что механизм сопротивляемости диелдрину у насекомых включает некоторые изменения в структуре мембраны или активной транспортировке. Фосфолипиды устойчивой по отношению к диелдрину разновидности комнатной мухи, а также ее восприимчивой разновидности, из которой была получена устойчивая разновидность, были мечены путем кормления их в течение 24 часов меченным фосфором-32 ортофосфатным раствором, после чего мухи имели возможность метаболизировать поглощенный фосфат фосфора-32 в течение периода продолжительностью до 336 часов. Был удален грудной ганглий, а фосфолипиды были разделены методом хроматографии на полоски бумаги, пропитанные кремниевой кислотой. Можно выделить четыре основные фосфолипидные фракции, которые, по-видимому, не отличаются от фракций, полученных из экстрактов целых мух. При использовании экстрактов целых мух состав фракции является следующим: фракция II — фосфатидил инозита, фракция III — лизоцепалин, фракция IV — смесь фосфатидила холина и неустановленного фосфолипида и фракция V — фосфатидил этаноламина и фосфатидил серина. Фракция I состоит из соединений, не содержащих фосфолипидного фосфора. Максимальный уровень мечения фракций был получен после того, как вслед за удалением из фосфата фосфора-32 мухи кормились (глюкозой и водой) в течение одной недели. Распределение активности фосфора-32 в разделенных фракциях из ганглия устойчивой и восприимчивой мухи не выявило каких-либо значительных различий во время максимального уровня мечения. Однако некоторые данные свидетельствуют о том, что у устойчивой мухи происходил более медленный переход активности фосфора-32 в две основные фосфолипидные фракции (IV и V). Это стало еще более очевидным, когда мух стали кормить молоком в дополнение к глюкозе и воде. Предварительные исследования по значительно более кратковременному переходу были проведены на фосфолипидных экстрактах из целых мух, в которые был введен ортофосфатный раствор, меченный фосфором-32. В них происходил более быстрый переход активности фосфора-32 во фракцию (II) фосфатидила инозита при использовании устойчивых мух, хотя при этих кратковременных опытах, по-видимому, не наблюдалось различия в ее переходе из обеих разновидностей во фракции IV и V. В работе освещается возможное значение полученных результатов.

Estudio de los fosfolípidos del ganglio torácico de moscas comunes, sensibles y resistentes al dieldrin. Los autores han procedido al estudio de los fosfolípidos de la mosca común por considerar que la resistencia de los insectos al dieldrin puede tal vez estar relacionada con alguna alteración de la estructura de las membranas o del transporte activo. Los fosfolípidos de una cepa de mosca común, resistente al dieldrin, así como los de la cepa madre, sensible, fueron marcados alimentando los insectos durante 24 h con una solución de ortofosfato que contenía ^{32}P y dejándolos metabolizar el fosfato absorbido por períodos de hasta 336 h. Los fosfolípidos de los ganglios torácicos extirpados se separaron por cromatografía sobre tiras de papel impregnado de ácido silícico. Pudieron aislarse cuatro fracciones principales de fosfolípidos que, al parecer, son idénticas a las obtenibles de los extractos de moscas enteras, en cuyo caso las fracciones son las siguientes: fracción II, fosfatidil-inositol; fracción III, lisocefalina; fracción IV, mezcla de fosfatidil-colina y un fosfolípido no identificado, y fracción V, fosfatidil-etanolamina y fosfatidil-serina. La fracción I consiste en compuestos fosforados distintos de los fosfolípidos. El grado de marcación más elevado se alcanzó al alimentar las moscas con glucosa y agua una semana después de suspender el suministro de ^{32}P. En el momento de máxima marcación, la distribución del ^{32}P en las fracciones extraídas de los ganglios de moscas resistentes y sensibles no reveló diferencias significativas. Sin embargo, ciertos indicios sugieren que la in-

corporación del ^{32}P se verifica con más lentitud en la cepa resistente. Este fenómeno se hizo más manifiesto cuando se añadió leche a la glucosa y al agua que constituía la dieta de los insectos. Los autores han efectuado estudios preliminares sobre la incorporación del ^{32}P durante períodos mucho más breves utilizando extractos de fosfolípidos de moscas enteras a las que se había inyectado solución de ortofosfato marcado con ^{32}P. Estos estudios han demostrado que la incorporación del ^{32}P en la fracción II (fosfatidil-inositol) es más rápida en el caso de las moscas resistentes, aunque los experimentos a corto plazo no habían revelado diferencia alguna entre las dos cepas en lo que se refiere a la incorporación del radionúclido en las fracciones IV y V. Los autores analizan el posible significado de estas observaciones.

I. Introduction

No satisfactory explanation for the development of resistance by insects to the action of Dieldrin and its related group of insecticides has so far been advanced. No similar mechanism to the enzymic detoxication of DDT has been found in the case of Dieldrin. Work at this laboratory using C^{14}-labelled Isodrin, Endrin, Aldrin and Dieldrin [1], the S^{35}-labelled sulphur analogue of Dieldrin [2] and C^{14}-labelled γ-benzene hexachloride (γ-BHC) [3] on a strain of Dieldrin-resistant flies, also cross-resistant to γ-BHC, and the susceptible strain from which it was derived has shown that there are no differences in the rates of penetration, excretion or metabolism of the insecticides by the two strains. In fact the adults of the resistant strain, bred with Dieldrin in the larval medium, contain on emergence as much as 8μg of Dieldrin/fly [3]. This internal Dieldrin is only slowly lost by excretion over the adult life time of the fly. These facts suggest that the 'site of action' of Dieldrin has been modified in the resistant fly, making it less sensitive to the action of the insecticide. Such modification might lie in the cell wall, mitochondrial structure etc., resulting in an alteration of transport across the membrane. Phospholipids are important constituents of membrane structures and have recently been implicated in active transport of sodium ions [4], secretory activity of hormones [5], and the transport of γ-amino-butyric acid [6]. Very little work has been done on the phospholipids of insects. Reports have appeared on the phospholipids of bee brain [7], *Drosophila* [8] and the blowfly [9]; considerable quantities of phospholipids have also been reported in blowfly sarcosomes [10]. A private communication from Dr. S. C. Chang of the Department of Entomology, University of Illinois indicates that he has also made a study of the phospholipids of the housefly and his identification of the phospholipid fractions appear to be similar to those presented in this paper.

II. Experimental

The following strains of housefly (*Musca domestica*) have been studied:

S-strain

Susceptible strain reared at this laboratory, without any previous contact with an insecticide.

MVS-strain

Obtained from Dr. J. R. Busvine and originally collected from Omdurman (Sudan). In spite of a history of Dieldrin-resistance and of being more resistant than the S-strain it was used in most of the experiments as the susceptible strain, for comparison with the more resistant strains derived from it.

R-strain

Obtained from the MVS-strain by applying Dieldrin pressure. Stock maintained under continuous pressure by incorporating 150 ppm of Dieldrin in the larval medium. This strain showed a high level of resistance to both Dieldrin and γ-BHC.

RND-STRAIN

Obtained from the R-strain by omitting Dieldrin pressure. Showed a slight drop in resistance on initial removal of pressure but no subsequent fall on continued breeding in the absence of Dieldrin.

For experiments in which the phospholipids of the thoracic ganglia were studied, groups of 30 two-day-old female flies were fed on a glucose solution containing 0.5 mc of P^{32}-labelled 'carrier-free' orthophosphate (obtained from the Radiochemical Centre, Amersham) in aerated glass chambers at a temperature of 26—27°C [11]. After 24 h the flies were lightly anaesthetized with *cyclo*propane and transferred to muslin-topped, 7-lb jam-jars where they were fed on glucose and water or on milk, glucose and water. During milk feeding the milk-swab was changed daily. At various intervals after removal from the active phosphate, samples of flies were taken, lightly anaesthetized with *cyclo*propane and mounted on their backs on microscope slides with a spot of paraffin wax. The flies were allowed to recover from the anaesthetic, and the thoracic ganglia were dissected out as rapidly as possible under a binocular. Each dissected ganglion was immediately placed 10 cm from the end of a silicic-acid-impregnated strip of Whatman No. 1 filter paper, moistened with a drop of 3:1 ethanol: ether mixture and lightly crushed with a glass rod. The strips were developed with *di-iso*butyl ketone: acetic acid: water (40:30:7 v/v) [12] by ascending chromatography at 25°C for 72 h. The strips were air-dried, and the P^{32}-activity detected in a 4π scanning device [13] by which both sides of 1-cm sections of the strip were exposed to Geiger-Müller tubes and the counts recorded on a printing scaler. After correction for background, paralysis and decay, a direct measure of the distribution of the P^{32}-activity along the strip was obtained.

In the experiments in which whole flies were studied, flies were injected with 1 µl of P^{32}-labelled 'carrier-free' orthophosphate solution neutralized with sodium bicarbonate, left for periods up to 6 h after the injection, and the water soluble phosphorus fraction and phospholipid fraction extracted [14]. Chemical estimations of phosphorus were made by digestion in perchloric acid, the phosphate being determined by a modification of the Fiske-Subbarrow method [15] and also by neutron activation analysis of the chromatograms run on the phospholipid extracts.

III. Results and discussion

The radioactivity separated by the chromatographic procedure described above could be divided into five major fractions. Although these have not been identified positively in the case of the chromatograms run on the ganglia, their position on the chromatogram, their relative size and their speed of turnover make it likely that they are identical with the phospholipid fractions obtained from the whole fly, which have been identified by a variety of hydrolytic, chemical and chromatographic procedures. Fraction I which remains at the point of application consists of non-lipid phosphorus-containing compounds. By analogy with the fractions obtained from the whole fly, fraction II is phosphatidyl inositol, fraction III lysocephalin, fraction IV a mixture of phosphatidyl choline and an unidentified phospholipid which appears to be a sphingomyelin-like substance but with ethanolamine replacing the choline, and fraction V a mixture of phosphatidyl ethanolamine and phosphatidyl serine. Table I gives the distribution of P^{32}-activity in these fractions at different times after removal from the source of P^{32}-phosphate, the flies being fed on glucose and water only. These figures indicate that maximum labelling is reached in all fractions within one week, and that there is then no significant difference between the RND- and MVS-ganglia in the distribution of activity in the various fractions. Using the distribution of activity at the

time of maximum labelling as a measure of the chemical amounts of phosphorus in the fractions it is possible to calculate the ratio of the specific activity of the phospholipid fraction to the specific activity of the non-phospholipid fraction. These values are shown in Table II and suggest a slower rate of incorporation of the P^{32} into the phospholipids of the ganglion of the RND-fly. Both fractions IV and V show this effect.

TABLE I

DISTRIBUTION OF P^{32}-ACTIVITY IN PHOSPHOLIPID FRACTIONS SEPARATED FROM GANGLIA OF RND- AND MVS-HOUSEFLIES

Time after removal from P^{32}-phosphate (h)	Strain of fly	Total radioactivity on chromatogram (%)		Radioactivity of phospholipid fractions (%)			
		Fraction		Fraction			
		I	II to V	II	III	IV	V
0	MVS	67.3	32.7	10.1	1.3	40.2	48.4
	RND	69.4	30.6	9.6	2.5	38.6	49.3
24	MVS	56.1	43.9	6.9	1.8	38.7	52.6
	RND	58.9	41.1	7.2	1.7	40.2	50.9
48	MVS	53.6	46.4	5.6	1.7	34.6	58.1
	RND	52.8	47.2	5.1	1.5	32.7	60.7
168	MVS	48.0	52.0	4.4	1.1	29.5	65.0
	RND	47.3	52.7	4.5	1.0	29.0	65.5
336	MVS	48.7	51.3	4.4	1.2	28.5	65.9
	RND	48.2	51.8	3.9	1.2	28.1	66.8

TABLE II

RELATIVE SPECIFIC ACTIVITIES OF THE PHOSPHOLIPID FRACTIONS TO THE NON-LIPID PHOSPHORUS FRACTION FOUND IN GANGLIA OF RESISTANT AND SUSCEPTIBLE FLIES AFTER FEEDING FOR 24 h ON P^{32}-ORTHOPHOSPHATE AND, SUBSEQUENTLY, ON GLUCOSE AND WATER

Time after removal from active phosphate (h)	Total phospholipid		Fraction IV		Fraction V	
	MVS	RND	MVS	RND	MVS	RND
0	.461	.400	.658	.593	.343	.329
24	.752	.687	1.040	1.000	.614	.539
48	.832	.828	1.000	.983	.759	.767
168	1.039	1.049	1.090	1.096	1.045	1.052
336	.994	1.000	1.015	1.018	1.010	1.020

This slower rate of turnover was shown more clearly when the flies were fed on milk in addition to glucose and water. In this case the specific activity of the non-phospholipid fraction fell rapidly, the 24-h and 336-h values being 50% and 10% respectively of the zero time value. When values for the ratio of the specific activity of the phospholipid fraction to that of the non-phospholipid fraction were calculated they again suggested a difference

between the strains. In this case a slower loss of P^{32}-activity from the phospholipids of the RND-strain is apparent, which is shown by both fractions IV and V (Table III).

TABLE III

RELATIVE SPECIFIC ACTIVITIES OF THE PHOSPHOLIPID FRACTION TO THE NON-LIPID PHOSPHORUS FRACTION FOUND IN GANGLIA OF RESISTANT AND SUSCEPTIBLE HOUSEFLIES AFTER FEEDING ON P^{32}-ORTHOPHOSPHATE FOR 24 h AND, SUBSEQUENTLY, ON MILK, GLUCOSE AND WATER

Time after removal from active phosphate (h)	Total phospholipid fraction		Fraction IV		Fraction V	
	MVS	RND	MVS	RND	MVS	RND
0	.461	.400 ($P<0.05$)*	.630	.539	.363	.322
24	.796	.833 ($P<0.6$)	.948	.982	.733	.770
48	.968	1.093 ($P<0.2$)	1.330	1.590	.793	.878
168	1.201	1.333 ($P<0.1$)	1.570	1.620	1.073	1.135
336	1.428	1.684 ($P<0.001$)	1.840	2.030	1.340	1.640

* Figures in paranthesis indicate the levels of probability P of the significance of the differences between the MVS and RND values, as estimated by the t test.

By the nature of the experimentation used comparisons could only be made between the phospholipid fractions of the ganglion with slow turnover. No information on the relative turnover rates of the phosphatidyl inositol fraction (II) could be obtained, as it had reached its maximum activity within the 24-h labelling period. It was also a very small fraction making accurate assay difficult. However, some preliminary short-term turnover studies have been made in which P^{32}-orthophosphate solution was injected into flies, and the activity incorporated into the phospholipids of the whole fly measured up to 6 h after injection. In these experiments no difference was found in the relative rates of incorporation of P^{32} into the phosphatidyl choline and ethanolamine fractions of the resistant and susceptible flies. However, a more rapid incorporation into the phosphatidyl inositol and the unidentified fraction, possibly the ethanolamine analogue of sphingomyelin was found in the case of the resistant fly. The ratio of the specific activity, determined by direct estimation, of the phosphatidyl inositol fraction to that of the water-soluble fraction 6 h after injection was found to increase with increasing resistance of the strains (Table IV). It is hoped to extend the turnover studies on the ganglia to cover shorter periods of time.

TABLE IV

RATIO OF SPECIFIC ACTIVITY OF PHOSPHATIDYL INOSITOL FRACTION TO SPECIFIC ACTIVITY OF WATER-SOLUBLE PHOSPHORUS COMPOUNDS 6 h AFTER INJECTION OF P^{32}-ORTHOPHOSPHATE

Strain of fly	LD_{50} µg γ-BHC/fly	sp. activity of phosphatidyl inositol / sp. activity of water-soluble phosphorus compounds
S	.031	.223
MVS	.114	.252
RND	1.73	.270
R	13.0	.293

While it is premature to suggest that these small differences in phospholipid turnover are directly connected with the mechanism of Dieldrin-resistance it is interesting to observe that they are consistent with the concept of some change in membrane permeability. The routes for the enzymic syntheses of the phospholipids as found by various workers [16—19] using a range of vertebrate preparations is summarized in Fig. 1: If the turnover of

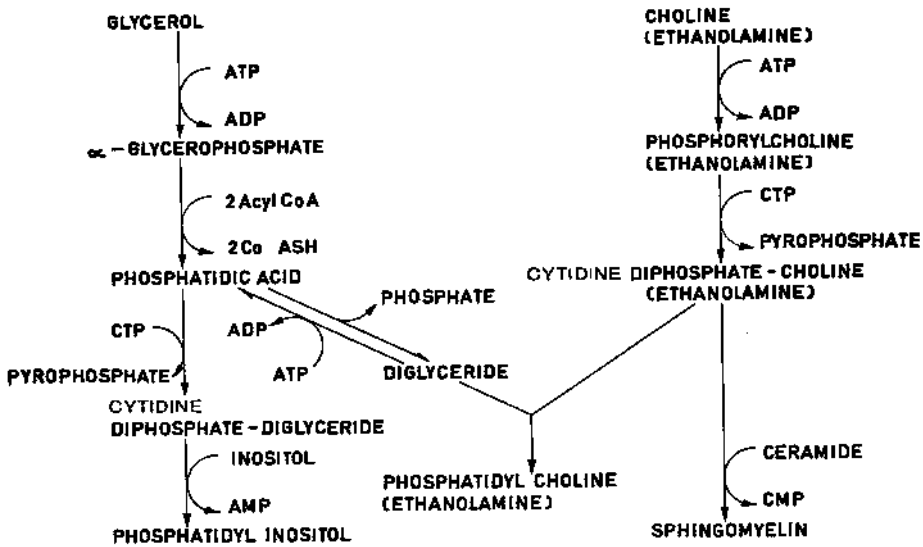

Fig. 1
The routes for the enzymic syntheses of the phospholipids using a range of vertebrate preparations

phosphatidyl inositol is more rapid in the resistant fly, it could mean that the formation of cytidine-diphosphate-diglyceride is favoured, together with a more rapid turnover of phosphatidic acid and a reduction in diglyceride concentration. This, in turn, could favour the formation of sphingomyelin or its ethanolamine analogue at the expense of phosphatidyl-choline or ethanolamine formation. Such an explanation would fit all the observations made to date, with the possible exception of the failure to find any difference in the rates of labelling of the phosphatidyl choline and ethanolamine fractions during the short-term turnover experiments. It seems likely, however, that this latter difference would only become apparent in the long-term turnover studies because of the much larger amounts in which these fractions occur, as compared with the phosphatidyl inositol-fraction. The turnover of phosphatidic acid has been associated with active sodium transport [4], so that the above observations could reflect a modification of some membrane structure in the resistant fly.

ACKNOWLEDGEMENT

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DISCUSSION

THE CHAIRMAN (F. P. W. Winteringham, United Kingdom): After this penetrating exposé, it is encouraging to know that at last one, or possibly two, workers are coming to grips with the problems of lipid metabolism in insects. Some of our most important insecticides such as DDT, Dieldrin and BHC are, after all, highly oil-soluble compounds, and it would be in the lipid phases of the living tissue that we would expect them to accumulate. And yet we know far less about the metabolism of lipids than about almost any other branch of insect metabolism.

J. E. TREHERNE (United Kingdom): It would be interesting to know how much of the phospholipid content is situated in the cells associated with the nerve sheath, and how much in the glial cells and the axons. Would you have enough specific activity to de-sheath the thoracic ganglion and determine the phospholipid content of the cellular perineurium? This would, I imagine, require a high specific activity of P^{32}.

R. G. BRIDGES: The level of activity may be high enough for the de-sheathed ganglion to be examined. We realize, however, that the approach at the moment is rather a gross one.

J. E. TREHERNE: You mentioned an association of phospholipids with sodium movements. Do you imagine that the effect of these lipids is upon passive sodium influx or upon some sort of sodium pump?

R. G. BRIDGES: No. Increased turnover of phosphatidic acid has been associated by Hokin and Hokin with *active* sodium transport. Mullins has suggested that γ-BHC blocks a lipoprotein membrane. It seems possible to extend his idea to the cyclodiene insecticides and to consider that a slight modification of the membrane structure could cause resistance to the insecticide. Such a modification might then affect the passive Na influx, and in order to maintain the normal ionic levels within the nerve the active transport would have to be modified. I think that the differences in phospholipid turnover reported here may

reflect such a modification in the active transport system in the resistant fly, as a result of some membrane modification.

THE CHAIRMAN: I wonder whether Mr. Bridges would like to comment on the possible significance of the ethanolamine analogue rather than the choline analogue in the insect.

R. G. BRIDGES: It does indeed seem rather strange that we have in the insect a much larger amount of the phosphatidyl ethanolamine than has been reported in any mammalian tissue, and of note also is the interesting possibility that we have the ethanolamine rather than the choline analogue of sphingomyelin. But I should not like to comment on its significance.

STUDIES ON THE PERSISTENCE, DECAY AND DISTRIBUTION OF RADIOPHOSPHORUS IN GRASSHOPPERS AND THE MADEIRA COCKROACH

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Abstract — Résumé — Аннотация — Resumen

Studies on the persistence, decay and distribution of radiophosphorus in grasshoppers and the Madeira cockroach. Three species of grasshoppers and the Madeira cockroach were labelled with P^{32} for persistence, decay and distribution studies.

The persistence of P^{32} in the grasshoppers and the Madeira roach was detected in measurable quantities beyond 30 days, whilst the biological half-life was found to range from 6.8 to 7 d in grasshoppers and 8.5 to 10 d in the Madeira roach.

Radiophosphorus, when introduced into test insects in reasonable quantities, showed no toxic or detrimental effects on any stage of development of the insects.

P^{32} was found in the cast skin and the oöthecae of the Madeira roach. Very small amounts of radioactivity were found in the egg capsules of the grasshoppers but not in their exuviae.

The distribution of P^{32} in both insects (grasshoppers and the roach) was very similar after 48 h. The distribution study was also checked by radioautograph techniques. The radioautograph plates revealed that the concentration of the isotope was greatest in the region of the hind gut and the metathoracic legs.

Etudes sur la persistance, la désintégration et la distribution du radiophosphore chez la sauterelle et la blatte de Madère. En vue d'études sur la persistance, la désintégration et la distribution du radiophosphore, l'auteur a marqué au ^{32}P trois espèces de sauterelles ainsi que des blattes de Madère.

Il a pu constater la persistance du ^{32}P en quantités mesurables chez les sauterelles et la blatte de Madère après 30 jours, et il a déterminé que la période biologique se situe entre 6,8 et 7 jours chez les sauterelles et entre 8,5 et 10 jours chez la blatte de Madère.

Le radiophosphore introduit en quantités appropriées dans des insectes de laboratoire n'a d'effets toxiques (ou nocifs) à aucun stade du développement des insectes.

L'auteur a trouvé du ^{32}P dans les dépouilles et les oothèques des blattes de Madère, et de très faibles quantités de radioactivité dans les capsules des œufs des sauterelles, mais aucune trace dans leur dépouille.

Chez les deux insectes (sauterelle et blatte), il a observé une distribution du ^{32}P tout à fait analogue après 48 h. L'étude de la distribution a été aussi vérifiée par des méthodes d'autoradiographies. Les autoradiographies ont révélé que la concentration de l'isotope était la plus forte dans la partie postérieure du tube digestif et dans les pattes du métathorax.

Исследования устойчивости распада и распределения радиоактивного изотопа фосфора в саранче и в мадейрском таракане. Три вида саранчи и мадейрского таракана были мечены фосфором-32 с целью проведения исследований устойчивости распада и распределения этого изотопа.

Как было установлено, устойчивость фосфора-32 в саранче и мадейрском таракане в измеримых количествах превышала 30 дней; одновременно было обнаружено, что биологический полупериод жизни у саранчи равен 6,8—7 дням и у мадейрского таракана — 8,5—10 дням.

Введенный в подопытные насекомые в разумных количествах радиоактивный изотоп фосфора не обнаружил токсических (или вредных) свойств ни на какой стадии развития насекомых.

Фосфор-32 был обнаружен в сброшенном панцире и скорлупе яиц мадейрских тараканов. Очень небольшие количества радиоактивности были обнаружены в скорлупе яиц саранчи; в их сброшенных покровах радиоактивности обнаружено не было.

Распределение фосфора-32 в обоих насекомых (саранче и таракане) по истечении 48 часов являлось довольно аналогичным. Распределение фосфора-32 было также зарегистрировано при помощи метода радиоавтографии. Радиоавтографические пластины позволили обнаружить, что концентрация изотопа достигла наибольшей величины в районе задней кишки и метагрудных лап.

Estudios sobre la persistencia, desintegración y distribución del fósforo radiactivo en los saltamontes y en la cucaracha de Madeira. Tres especies de saltamontes y cucarachas de Madeira se han marcado con ^{32}P a fin de estudiar la persistencia, desintegración y distribución de este radioisótopo.

En los saltamontes y en la cucaracha de Madeira, el ^{32}P persiste en cantidades mensurables después de más de 30 d; el período medio biológico oscila entre 6,8 y 7 d en los saltamontes y entre 8,5 y 10 d en la cucaracha de Madeira.

La introducción de cantidades moderadas de fósforo radiactivo en los insectos experimentales no produce efectos perjudiciales (tóxicos o de otra índole) en ninguna fase de desarrollo de los insectos.

Se ha encontrado ^{32}P en los caparazones desprendidos y en las ootecas de las cucarachas de Madeira. La radiactividad hallada en las cápsulas de los huevos de saltamontes es muy reducida, en tanto que es nula en los tegumentos desprendidos.

La distribución del ^{32}P en ambos insectos (saltamontes y cucarachas) es muy parecida al cabo de 48 h. El estudio de la distribución se ha completado por determinaciones autorradiográficas. Las placas obtenidas demuestran que la concentración del isótopo es más elevada en la parte inferior del intestino y en las patas metatorácicas.

Introduction

Among the various nutrients of insect food, phosphorus occupies an important place particularly due to its vital role in the process of metabolism. Because of its other advantages, radioactive phosphorus was chosen to label orthopterous insects such as grasshoppers and the Madeira cockroach for determining the persistence and mode of biological decay of the radioelement in the insect body.

The techniques followed for labelling insects and measuring their radioactivity *in vivo* were the same as described previously (HUQUE and MYSER, 1959).

Persistence of P^{32} in grasshoppers

Three species of grasshoppers, *Melanoplus differentialis*, *Melanoplus femur-rubrum* and *Dichomorpha viridis*, were labelled with P^{32} through bean plants. The radioactivity was measured *in vivo* for a period of 30 d and the radioactive content of the grasshopper was found to decrease rapidly during the initial days following treatment (Table I). After 20 d, the rate of biological decay declined, the residual activity remaining measurable with a Geiger counter. The physical law of decay is not the only factor which determines the level of radioactivity within the animal body, since radioactive material may further be lost through egestion, salivation and excretion. When the composite net-counts (the mean values of corrected mean counts for a set of similar experiments) for grasshoppers of the same size, age and sex were plotted against time on semilog graph paper, the maxima formed a straight line. This graph, when compared with one showing natural physical-decay alone, shows clearly that excretion contributes to a relatively more rapid loss of radioactivity. The biological half-life of P^{32} in the three species of grasshoppers as determined from the graph varied from 5.6 to 7 d.

TABLE I

RATE OF P^{32} DECAY FROM ADULT GRASSHOPPERS FED ON RADIOACTIVE* BEAN PLANTS

Days after treatment	1	3	5	7	10	19	22	25	28	30	Physical half-life of isotope (d)	Biological half-life of isotope (d)
Experimental insect	Activity**											
Melanoplus differentialis	6799	4572	3926	3023	1954	600	370	260	170	135	14.3	5.6
Melanoplus femur-rubrum	4190	3159	2576	1954	1807	479	350	240	160	125	14.3	7.0
Dichomorpha viridis	3241	2195	2027	1574	926	370	262	185	140	100	14.3	7.0

* Specific activity 85.14 cpm/mg of leaf ** Activity, expressed as net counts/min, from *in vivo* measurements

TABLE II

RATE OF P^{32} DECAY IN THE MADEIRA COCKROACH, *LEUCOPHAEA MADEIRA* (F)

Days after treatment	1	3	5	7	9	11	13	15	19	21	25	28	30	Physical half-life of isotope (d)	Biological half-life of isotope (d)
Stage tested and method of tagging	Activity*														
Standard	6565	5895	5421	5103	4546	4141	3831	3402	2948	2762	2188	1914	1785	16	
Adult male injected**	3803	2907	2800	2418	2223	1842	1553	1332	988	845	600	696	410	14.3	10
Adult male fed***	8838	7182	6051	5516	4201	3874	2905	2429	1718	1558	976	729	612	14.3	8.5
Instar 4th nymph injected**	3730	3362	2337	2200	1995	1650	1400	1203	880	740	571	—	360	14.3	9.4
Instar 4th nymph fed***	4426	3565	2646	2500	2100	1700	1400	1200	800	660	451	—	340	14.3	8.4

* Activity, expressed as net counts/min, from *in vivo* measurements ** 0.3 μc solution injected *** Fed dog biscuit containing 0.5 μc/g

TABLE III

DISTRIBUTION OF P^{32} IN THE GRASSHOPPER AND THE MADEIRA COCKROACH

	Grasshoppers fed on bean plant registering 85.14 cpm/mg and subsequently sacrificed for assay						Madeira cockroach fed on dog biscuit containing 1 μc P^{32}/g and subsequently sacrificed for assay					
Body portion	48 h			72 h			48 h			72 h		
	wt.	cpm	cpm/mg	wt.	cpm	cpm/mg	wt.	cmp	cpm/mg	wt.	cpm	cpm/mg
Head	50	154	3.0	51	153	3.0	54	59	1.0	50	46	0.9
Thorax	110	225	2.0	108	169	1.6	398	1194	3.0	401	862	2.1
Abdomen	130	2356	18.1	128	1650	12.6	1080	6048	5.6	851	4039	4.7
Legs	90	233	2.5	87	210	2.4	181	366	2.0	180	341	1.8
Wings	15	56	3.7	15	51	3.4	75	166	2.2	70	141	2.0
Intact insect	395	3178	7.8	394	2335	5.9	789	8022	4.4	1558	5315	3.4

Weight is expressed in milligrams and counts are quoted per minute (cpm) and also per milligrams of body weight (cpm/mg).

Following ingestion of radioactive leaves, the tested species of grasshoppers showed an average initial activity of 6799, 4190 and 3241 net counts/min, respectively. After a period of 30 d these net counts fell to 135, 125 and 100 counts/min. Without loss of material through excretion from the animal body, the count rate would decrease in exact accordance with the physical decay of P^{32}.

Persistence of P^{32} in the roaches

In another experiment, P^{32}-labelled adult and fourth-instar nymphs of the Madeira roach, *Leucophaea madeira*, retained an appreciable amount of radioactivity beyond 30 d. The mode of decay of P^{32} followed almost the same pattern as with the grasshoppers. The biological half-life of P^{32} in the fed roaches was found to be about 8.4 d and in the injected roaches about 10 d (Table II).

Distribution of P^{32} in the grasshopper

The distribution of P^{32} in the different grasshoppers was determined after feeding the insects on "hot" plants, and sacrificing them at intervals of 48 and 72 h. The former allowed sufficient time for the radioisotope to become distributed in the tissues. The insects were then killed and weighed, and the radioactivity in the whole insect determined. Such parts of the body as the head and antennae, the thorax, abdomen, wings and legs were separated carefully, and their weight (in mg) noted. Each part was then placed in an aluminium planchet, and its radioactivity determined. The specific activity, i.e. the count rate per minute per milligram of body weight for each treatment, was recorded. The same procedure was adopted for assaying the radioactivity in the different parts of the grasshopper body after 72 h. Except for a greater concentration in the abdominal region, P^{32} appeared to be fairly uniformly distributed. After 48 h, the specific activities of the head, thorax, abdomen, legs and wings of the different grasshopper species were found to be 3.0, 2.0, 18.1, 2.5 and 3.7 cpm/mg respectively (Table III). Percentage losses of specific activity during the following 24 h from the thorax, abdomen, legs and wings were found to be 20%, 30%, 4% and 8%, respectively; no loss was observed from the head portion.

Distribution of P^{32} in the roach

In the case of Madeira roaches fed on dog biscuit impregnated with a few microcuries of P^{32}, and dissected after 48 h, the head, thorax, abdomen, legs and wings were assayed for radioactivity (Table III). They all showed depletion of specific activity in the following 24 h. Percentage losses of specific activity from the head, thorax, abdomen, legs, and wings amounted to 10%, 30%, 16%, 10%, 9% and 22%, respectively. From these observations it seems likely that P^{32} enters the metabolic pathways and follows the same pattern in both these orthopterous insects, and hence follows a similar mode of decay.

P^{32} distribution study by radioautography

Attempts were made to check the distribution of the radioelement in the different grasshoppers and in the Madeira cockroach. The radioautographic plates gave a general idea of distribution. Both in the grasshoppers and the roach, P^{32} was found in all body parts, but the concentration was greater in the region of the hind gut and the metathoracic legs.

P^{32} in exuviae

The cast skins of radioactive grasshoppers and Madeira roach nymphs were assayed for radioactivity; no trace of P^{32} was found in either case. These results compare favourably with the findings of FULLER *et. al.* (1954) on *Comnula pellucida.* Contrary to these observations, KETTLEWELL (1955) reported that the exuviae of desert locust nymphs did contain a very small amount of P^{32}.

P^{32} in egg capsules

Egg capsules of tagged grasshoppers and roaches were also examined for radioactivity. It was found that the oöthecae of the roaches were free from radioactivity, but the individual egg capsules of the grasshoppers registered 15—20 counts/min above the background. This observation is in contrast to that of BABER *et. al.* (1956), who reported that the oöthecae of radioactive German roaches were very flaccid and contained no eggs, but registered counts of several hundred per minute.

Mortality due to P^{32}

KETTLEWELL (1955) while working with the desert locust nymphs reported increased mortality among the tagged hoppers of *Schistocerca gregaria,* and attributed it to P^{32}. In the present study, several hundred adults and nymphs of grasshoppers and Madeira roaches were tagged; P^{32} did not appear to affect the life-span of the test insects.

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DISCUSSION

THE CHAIRMAN (J. Treherne, United Kingdom): I wonder if I might ask a question before we start the general discussion? Have you considered the possibility of studying the distribution of the P^{32} in the body of the insect at successive intervals, starting after a very short time, in order to build up a dynamic picture of its accumulation?

H. HUQUE: I did not work precisely along those lines because the purpose of my experiments was not so much to make a physiological observation as to see how long the tag persisted, with a view to undertaking ecological studies such as work on migration and dispersal.

W. KLOFT (Federal Republic of Germany): You give the biological half-life of P^{32} in grasshoppers as being 6.8 to 7 d and as 8.5 to 10 d in the Madeira roach. How did you measure your insects *in vivo,* at what intervals were the measurements made, and how many measuring points were taken?

H. HUQUE: There is very little published data on techniques for *in vivo* measurements on radioactive insects. However, for the present studies, a special device was developed to hold the radioactive insects under the end-window of the GM tube. Pillboxes of diam. 1.5 in and 0.5 in deep were chosen to confine the radioactive insects separately. The bottom of

each pillbox was fitted with a foam-rubber pad to prevent the insect from moving about and, also, to hold it snugly against the nylon cap in the top of the box. A comparative study was then made to ascertain the efficacy of *in vivo* measurement of intact insects in pillboxes as against measurement of nitric-acid digested insect residues in aluminium planchets. The differences in specific activity varied from 0 to 6%. Measuring the activity of insects in pillboxes was, therefore, an accurate and effective technique for *in vivo* measurements.

Each measurement was continued for long enough to avoid a standard error of more than $\pm$ 5%.

W. KLOFT: The biological half-life values for P^{32} seem to me to be somewhat long, and, as I intend to show in the following paper, there exists a great source of error through the possibility of cuticular excretion of P^{32} with a subsequent apparent increase in the counting rate. In my experiments with ants, termites, bees and aphids I always found a very short biological half-life—only about 30 h in the case of aphids, for example.

H. HUQUE: Nevertheless, my findings are in very good agreement with those of BABER *et al.* in 1956, who found the biological half-life of P^{32} in the adult German cockroach to be 9 d. RADELEFF *et. al.* in 1952 reported that the biological half-life of P^{32} in the screwworm fly was approximately 7 d, but indicated that there was considerable variation.

W. KLOFT: I have measured the biological half-life of P^{32} in *Calliphora*, and have evidence to show that, because of the source of error to which I have referred, the biological half-life can appear longer than the physical—which is, of course, impossible. I think we should be sceptical of all biological half-life values for P^{32} given in the literature, because most of the authors appear unaware of the continuous cuticular excretion.

H. HUQUE: For mosquitoes the half-life has been calculated, according to the literature, to be something like one to two days and considerable variations have been recorded for other insects.

D. W. JENKINS (United States of America): The work that Dr. Huque has reported is extremely valuable in ecological studies, and I was very interested to hear it and to compare it with that of others.

Dr. Huque mentioned Baber's work. Baber's work in determining biological half-life in the German cockroach was based on injection of P^{32} in the adults, and he found that the half-life in the male was 9 d and in the female 14 d. Some excellent work has been done in the USSR, showing that the biological half-life in the same roach was 3 d. Here, the P^{32} was fed internally. It is extremely important to determine these half-lives; for example, Dr. Hassett and I found that with zirconium-95—we were trying to obtain a good γ-emitter—the biological half-life was only 1 d, while the physical half-life is 65 d.

Perhaps the physiologists could help us on the following difficult subject: in determining a half-life, it is necessary to have some sort of standardization of the amount of radioisotope given to the insect. You could give 1 mc to a roach and it would probably get rid of most of it within a day. On the other hand, you could also give 0.5 µc, and the whole amount might be retained and physiologically taken up. I think I have accumulated all the available data on biological half-life, and I am quite sceptical as to what it means until we get some firm basis for the initial dose. I should like to hear Dr. Winteringham's views on this.

F. P. W. WINTERINGHAM (United Kingdom): This is indeed a problem of interest to ecologists, physiologists and biochemists alike, and I agree entirely with Dr. Jenkins on the rather urgent need for some clarification of our terms in this matter.

Firstly, we must be careful to distinguish between biological and radioactive half-life. I suggest that biological half-life should relate only to the chemical element, all measurements by tracer being automatically corrected for radioactive decay. Otherwise we shall find ourselves in the position of reporting different biological half-lives of a particular element—say sodium—merely because different workers have used different isotopes, e.g. Na^{22} and Na^{24}. For ecological studies we are of course concerned with *effective measurable radioactivity*. This will indeed be a function of biological and radioactive half-life.

I would dispute Dr. Jenkins' suggestion that biological half-life would be influenced by radioactive dose in terms of microcuries. I am sure he will agree that biological half-life will be a function of chemical dose rather than radioactive dose. For example, if one provided, say, 0.5 μc of P^{32} in the form of 10 mg of inorganic phosphate, I think this would be lost rather rapidly by the cockroach. If, on the other hand, one provided 10 mc of P^{32} as 10^{-2} μg of inorganic phosphate, it would be retained a long time. Finally, I would mention that biological half-life will depend on the conditions of the experiment. Thus, the persistence of P^{32} in an insect will be greatly influenced by whether or not phosphorus is present in the diet. If one is feeding the insect on its normal phosphate-containing diet the radioactive phosphate tends to be excreted, because it is almost flushed out of the system, so to speak, by the continual provision of dietary phosphate. If, on the other hand, the adult housefly is deprived of phosphate (after giving it radioactive phosphate), it will retain a very high percentage for a very long time, and the biological half-life thereby becomes very much greater.

All these things should, I think, be borne in mind, and I shall be interested to hear further comment on this very important matter.

J. HALBERSTADT (IAEA, Scientific Secretary): Perhaps it would be useful to repeat some of the experiments with P^{33}, which is available now, and make a comparison with P^{32}. Perhaps somebody could comment on this.

F. P. W. WINTERINGHAM: Many biologists probably do not realize it, but every time one uses P^{32}, one almost certainly uses P^{33} as well. Fortunately, it does not detect very easily, having a much slower β-emission than P^{32}. But Dr. Halberstadt's suggestion is an excellent one, and I think it would illustrate the point I just made. That is, if one measured the biological half-life by P^{32} and P^{33} respectively, we should—provided we use the correct interpretation of biological half-life—find no difference in the result. We should, of course, find a difference in practice, because P^{33} has a much longer radioactive half-life and the insect would therefore appear to retain its radioactivity over a much longer period.

THE CHAIRMAN: May I ask another question? What advantage did you gain from feeding the phosphorus *via* the leaf as distinct from injecting it? I should have thought injection would have been more economical and would probably have yielded higher specific activity, in addition to which you would have been able to control the dose more accurately.

H. HUQUE: As I mentioned before, the purpose of tagging the insects was to see how long the tag persisted. This being so, the feed method, by which you can label many insects and select for your work those having absorbed the most radioactivity, seemed to me to be more convenient than the rather tedious and cumbersome injection method.

D. F. HEATH (United Kingdom): I should like to extend Dr. Winteringham's statement a little. Provided that the tracer used is in a chemical form which is also found *in vivo*, and the chemical dose is small compared with the natural pool, the half-life should be independent of dose. For example, a few micrograms of P^{32}-phosphate in a large insect soon become part of the much greater natural phosphate pool, and the excretion of P^{32} is completely

controlled by the loss of natural P. At sufficiently low chemical doses, therefore, the half-life should be independent of dose and strictly reproducible.

H. HUQUE: I quite agree both with you and with Dr. Winteringham.

W. KLOFT: I should also like to add to Dr. Winteringham's statement. As I indicate in my second paper, we have shown in aphids that the biological half-life of P^{32} follows two different rates. First, there is excretion of unbound inorganic phosphate, giving a short half-life; then, as there is subsequently only excretion of metabolic phosphate, we have a longer half-life in the second phase. Once labelled, the aphids were exposed to conditions of continued uptake of unlabelled phosphorus, as I shall report later.

TECHNICAL PROBLEMS OF RADIOISOTOPE MEASUREMENT IN INSECT METABOLISM

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Abstract — Résumé — Аннотация — Resumen

Technical problems of radioisotope measurement in insect metabolism. In studying metabolism-reactions or the transfer of substances in insects by radiation measured from the outside, one must take into account many factors which influence the result. Of special importance are the absorption and the back-scattering in the relatively thin and also inhomogeneous tissue-layers of the insects. A direct method for determination of the absorption coefficient for β-radiation of various types of tissue will be specified. In addition to this it is necessary to take off the exterior tissue layers which have to be examined. The change of intensity of radiation that is caused by this procedure can then be examined. By an indirect method, the former rate of impulses is re-established by interposition of aluminium foils of known thickness in mg/cm². The data of the suitable absorption filters for substitution will be transferred to the tissues. After detection of the absorption coefficient for the tissues (especially the cuticula) of the used insect species and stages, it is possible to determine a correction factor for calculating the real activity from the measured rate.In a similar way the back-scattering, especially at the cuticula, is taken into account for quantitative measurements. Other problems of measurement, as, for example, the apparent increase of activity by cuticular excretion over the whole surface are discussed. This cuticular excretion results in a higher counting rate because part of the radioactivity is on the surface so that radiation from it is not reduced by absorption.

Problèmes techniques que pose la mesure des radioisotopes dans l'étude du métabolisme des insectes. Lorsque l'on étudie les réactions métaboliques ou le transport de substances chez les insectes au moyen de rayonnements mesurés de l'extérieur, il faut tenir compte de nombreux facteurs qui ont une influence sur le résultat. L'absorption et la rétrodiffusion dans les couches tissulaires relativement minces et non homogènes des insectes revêtent une importance particulière. L'auteur expose une méthode permettant de déterminer directement le coefficient d'absorption du rayonnement bêta pour divers tissus. Pour cela, il faut enlever les couches de tissu extérieures que l'on veut examiner. On peut alors étudier la variation d'intensité du rayonnement qui en résulte. La fréquence des impulsions première est rétablie par une méthode indirecte qui consiste à interposer des feuilles d'aluminium dont l'épaisseur exprimée en mg/cm² est connue. Les données relatives aux filtres d'absorption voulus sont appliquées aux tissus. Après avoir déterminé le coefficient d'absorption des tissus (notamment de la cuticule) pour les espèces et phases de développement considérées, on peut établir un facteur de correction pour déduire l'activité réelle à partir du taux mesuré. En procédant de même, on tient compte de la rétrodiffusion, notamment dans la cuticule, pour les mesures quantitatives. L'auteur examine ensuite d'autres problèmes de mesure tels que l'accroissement apparent de la radioactivité dû à des excrétions cuticulaires sur toute la surface. Ces excrétions entraînent une augmentation du taux de comptage, car une partie de l'activité se trouve alors sur la surface et le rayonnement qu'elle émet n'est donc pas réduit par absorption.

Технические проблемы измерения радиоизотопов при изучении метаболизма насекомых. При изучении реакций метаболизма или перемещения веществ у насекомых при помощи радиации, измеряемой извне, следует учитывать многие факторы, которые оказывают влияние на результаты исследований. Особое значение имеют поглощение и обратное рассеяние в сравнительно тонких, а также неоднородных слоях ткани насекомых. В докладе охарактеризован непосредственный метод определения коэффициента поглощения бета-радиации различными видами ткани. Для проведения исследований необходимо удалить внешние слои ткани. Затем можно исследовать изменение интенсивности радиации, вызываемое этой процедурой. При помощи косвенного метода восстанавливается прежняя скорость

импульсов путем введения алюминиевой фольги известной толщины в мг/см². Данные о подходящих поглощающих фильтрах для замены будут использованы на тканях. После регистрации коэффициента поглощения для тканей (особенно кутикулы) использованных видов насекомых и стадий возможно установить поправочный коэффициент для расчета действительной активности, исходя из измеренной скорости. Аналогичным образом принимается во внимание обратное рассеяние, особенно в кутикуле, для количественных измерений. В докладе освещаются другие проблемы измерения, как, например, очевидное возрастание активности путем кутикулярных выделений на всей поверхности. Это кутикулярное выделение вызывает более высокую скорость счета, так как часть радиоактивности расположена на поверхности, ввиду чего вызываемая ею радиация не уменьшается ввиду поглощения.

Problemas técnicos que plantea la determinación cuantitativa de los radioisótopos en el metabolismo de los insectos. Cuando el transporte de sustancias o las reacciones metabólicas se estudian en los insectos por medición externa de radiaciones, han de tenerse en cuenta numerosos factores que influyen en los resultados. Revisten particular importancia la absorción y la retrodispersión en las capas tisulares de los insectos, relativamente delgadas y carentes de homogeneidad. Para determinar directamente el coeficiente de absorción de las radiaciones beta en diversos tipos de tejidos, el autor describe un procedimiento con arreglo al cual se han de quitar las capas exteriores del tejido que se desea examinar. Pueden estudiarse entonces las variaciones resultantes en la intensidad de la radiación. El índice de recuento primitivo se restablece con arreglo a un método indirecto, que consiste en interponer láminas de aluminio de espesor conocido (expresado en mg/cm²). Los datos relativos a los filtros de absorción adecuados se aplican a los tejidos. Después de haber determinado los coeficientes de absorción correspondientes a cada tejido (sobre todo de la cutícula) para las especies de insectos y las fases de desarrollo que interesen, es posible hallar un factor de corrección que permite calcular la actividad real con los datos obtenidos por medición directa. De modo parecido se tiene en cuenta, para las determinaciones cuantitativas, la retrodispersión, sobre todo en la cutícula. El autor examina luego otros problemas de medición como, por ejemplo, el incremento aparente de actividad debido a la excreción cuticular, en toda la superficie, que origina un aumento del índice de recuento, ya que parte de la radiactividad se halla entonces en la superficie y no sufre merma por absorción.

Introduction

Metabolic processes and the transfer of substances within living insects are being studied by means of radioactively labelled compounds. Repeated measurement of the radiation from an intact, live test-insect for determining the sequence of such phenomena must take into account a series of factors which affect the readings. Along with such physical constants as the energy of the radiation and the half-life, technical characteristics of measuring apparatus such as the geometrical arrangement between the radioactive test insect and the GM tube, and even the absorption by air and the effect of particles scattered by the wall of the chamber must be taken into account. These factors and the characteristic properties of the GM tube may, however, easily be maintained constant. Measurements of β-particles are, on the other hand, greatly affected by the absorption and back-scattering effects of body tissue. If, in addition, the tracer used also participates in metabolic processes, the possibility of a further, rather important, source of error arises, namely that of cuticular excretion of the tracer. Measurements on P^{32}-labelled insects are reported here with a view to studying such possible errors.

I. Direct and indirect means of measuring the quantity of β-particle absorption

Absorption must be taken into account even when using a relatively hard β-source such as P^{32} in small insects. HUOT and VERLY [1] have measured the relationship between the

radiation rates from live and then incinerated larvae of *Tenebrio molitor* L., and developed a correction factor which is a function of the weight. There remains, however, a total correction factor which combines body absorption, the back-scattering in the animal and even the particle-scatter in the measuring cell designed for holding the live insect. One of my own methods is of interest if the absorption of single layers of tissue, especially of the cuticle, is to be measured. Absorption constants are to be found in the literature, but it is almost impossible to interpolate the values from medical tables since insect tissue layers are very thin and furthermore non-homogeneous.

Direct measurement of absorption by the insect body

This work was carried out on ants that had been fed on a P^{32}-labelled aqueous solution of honey. If the insects are killed within 30 min of feeding, the total activity remains in the proventriculus [2—8] which neither digests nor absorbs the labelled food. The source of radiation is therefore localized in a spherical form in the abdomen (gaster) of the insect without contaminating the tissues. The freshly killed insect is then fixed on a slide using an adhesive, and is positioned directly under the centre of the GM tube. After measuring the background of the counter, the activity of the freshly killed insect is measured over a sufficiently long interval in order to reduce the statistical error. The dorsal cuticle is then carefully removed with fine dissecting instruments. A further measurement is then taken, which shows an increased impulse-rate due to the absence of cuticular absorption (Table I). These measurements give I_{cu} and I_o, the impulse rates with and without cuticula, respectively.

Table I

EXAMPLES OF MEASUREMENTS TO DETERMINE THE ABSORPTION OF THE TERGAL CUTICULA OF AN INSECT

Test insect no.	Measurements on the test-insect before substitution				After substituting Al-Filters of thickness d			
	I_o	I_{cu}	d_{cu}	$\mu = \frac{\log (I_o/I_{cu})}{d}$	Filter no.	mg/cm²	d_{Al}	I_{Al}
1	10509	9928	0.020 mm	1.23 mm^{-1}	F_2	3.4	0.0126 mm	10105
					F_3	6.2	0.023 mm	10023
					F_4	10.7	0.0396 mm	9758
2	6357	5903	0.020 mm	1.47 mm^{-1}	F_2	3.4	0.0126 mm	6098
					F_3	6.2	0.023 mm	5996
					F_4	10.7	0.0396 mm	5834

μ is the calculated absorption-coefficient for the cuticula,
I_o = impulse rate after dissection,
I_{cu} = impulse rate with cuticle,
I_{Al} = impulse rate after substituting Al-Filter (given in counts/min),
d_{cu} = cuticle thickness.

From these data absorption may be expressed as a percentage of I_o. More useful, however, is a calculation of the absorption-coefficient μ (consisting of a real absorption value and a scatter value), given below by the exponential equation for β-absorption.

$$I_{cu} = I_o \times e^{-\mu d} \qquad \text{or} \qquad \mu = [\log (I_o/I_{cu})] / d$$

where d = thickness of the absorber, in this case the dorsal cuticula. It was determined microscopically by means of median sections through entire ants (*Formica nigricans* Em.), the average value being 0.020 mm. The calculated values of μ entered in Table I apply to the cuticle and hypodermis. The latter always remains attached to the cuticle in the dissection and its value was always considered in conjunction with the cuticle. It must be stressed that the μ values thus determined are dependent on the degree of activity and the particular measuring system used, and must therefore be determined for any one equipment set up as well as for each insect species.

Indirect method of measuring the absorption of the insect body (substituting Al-Filters for the excised tissue layers)

In the direct absorption measurements mentioned above, the absorption coefficient was calculated for the dissected cuticula layer. In order to do this the layer had to be measured microscopically. The absorption value of the excised tissue may also be easily determined indirectly by substituting aluminium filters of known weight per unit-area (mg/cm^2).

For this purpose a set of 30 calibrated aluminium filters (Filter set A, manufacturer: Frieseke & Hoepfner, Erlangen) were used, the weight per unit-area ranging from 1.3 to 65.8 mg/cm^2 (corresponding to 0.0048—0.243 mm in thickness). These filters were fixed in the path of the rays, 8 mm above the test insect, and the impulse rate was measured after each filter substitution. The purpose of the measurements was to find a filter which would reduce the impulse rate I_o to the original value I_{cu}. The weight per unit-area in mg/cm^2 of the corresponding filter might then be considered equal to that of the cuticle. Similarly the next tissue layer, the fatty tissue lying between the digestive tract and the dorsal cuticle, was removed and its weight per unit-area determined by substituting aluminium filters. This value was a little above 3.4 mg/cm^2. The thickness of the layer was determined microscopically; an average value of 0.030 mm for worker ants (*Formica nigricans* Em.) was obtained. A summation of the values for individual layers gives the total absorption by the layers (between the digestive tract and the outer surface) in mg/cm^2. If about 8 mg/cm^2 (between filters F_3 and F_4) is taken to be the weight per unit-area of the cuticule including the hypodermis, and 4 mg/cm^2 as the weight of the fat bodies between the cuticula and proventriculus, then the total amounts to approximately 12 mg/cm^2.

Discussion of the results

The absorption values of the excised tissues are expressed in terms of weight per unit-area (mg/cm^2). This is the quantity usually reported in handbooks; knowing the specific gravity, it is possible to calculate those layer thicknesses which completely absorb β-radiation of a certain energy (maximum range R) or reduce it to half the original energy (half-thickness $R/2$). R has been reported to be about 740 mg/cm^2 for P^{32}. This value was checked by the author in a control experiment which used aluminium as absorber. $R/2$ is about 110 mg/cm^2. If the weight per unit-area of the absorbing tissue layer is known, the important half-thickness may easily be determined. Three separate steps may now be taken:

(1) The weight per unit-area of the cuticule based on microscopic measurements of its thickness (0.020 mm) may be determined, and combined with the specific weight reported in the literature (max. 1.3). From these data a weight per unit-area of 2.6 mg/cm^2 was deduced for the dorsal cuticula of the ant. Referred to 110 mg/cm^2, based on aluminium, the calculated half-thickness was 0.84 mm.

(2) The weight per unit-area may be determined according to the substitution method. A value of about 8 mg/cm^2 was thus obtained. Using the known layer thickness of 0.020 mm

and $R/2 = 110$ mg/cm², a value of only about 0.275 mm was calculated for chitinous insect cuticula.

(3) Once the absorption coefficient, μ, has been determined for the excised tissue, the layer thickness which lowers the radiation to the half-value level, $I_o/2$, may be calculated from the equation

$$\mu = [\log (I_o/I_{cu})]/d.$$

Hence, for the material under consideration, the value corresponding to the half-thickness ($d_{\frac{1}{2}}$) is given by

$$d_{\frac{1}{2}} = (\log 2)/\mu = 0.245 \text{ mm}.$$

The substitution method, in close agreement with direct measurement, shows a greater reduction in radiation intensity in the cuticula than would be expected from calculation. This discrepancy may be due to the biconvex form of the dorsal cuticula which may introduce a higher back-scattering effect, or even to the difference in material, aluminium being used as filter. The effective layer thickness may also be greater than the value measured microscopically since many rays pass through at an angle to the perpendicular. Cuticle thicknesses of 0.2—0.8 mm are found in large, well armoured sclerotized forms, especially beetles. In such cases a reduction of I_{cu} to $I_o/2$ might be expected.
The real activity I_o in the insect may easily be calculated from the reduction coefficient μ and the microscopically measured thickness d, of the cuticula. The following equation holds true:

$$\log I_o = d \times \mu + \log I_{cu}.$$

Thus, correct values for $\log I_o$ may be obtained without any dissection by only adding the value $d \times \mu$ (Table I) to the externally measured impulse rate.

II. The back-scattering effect of the insect cuticula

The scattering of β-particles is a very complex process and plays an important part in absorption by the body. The back-scattering at 180° of particles emitted downward from the source is of particular interest, since they will cause an increase in pulse rate. It is known that scattering increases as the square of the atomic number of the scattering material [9]. When a β-source is mounted on a solid backing material (such as slides) an increase in the counting rate, due to the back-scattered particles entering the tube, has to be taken into account, the so-called back-scattering effect. This factor can be maintained constant, in so far as it is dependent on the substrate, by always using the same material. If a very thin membrane is used, this factor can be discounted in practice. The quantity of back-scattering within the insect itself, however, is of great interest. It occurs almost throughout the tissues but should be relatively greater in the cuticle. It is in this portion, the cuticle, that the value of the back-scattering effect may be determined very easily.

Freshly killed beetles, (*Tenebrio molitor* L.), received an enclosed P^{32}-source in the digestive tract and were mounted on a perforated metal slide so that their upper and lower surfaces remain exposed. Back-scattering due to the substrate was thus avoided. The impulse rate without the back-scattering caused by the elytra, I_o, can be determined by measuring the beetle with spread wing covers (elytra)* (Fig. 1); when closed the impulse rate I_{cu} is obtained.

* The membranous hind-wings were removed to simplify matters.

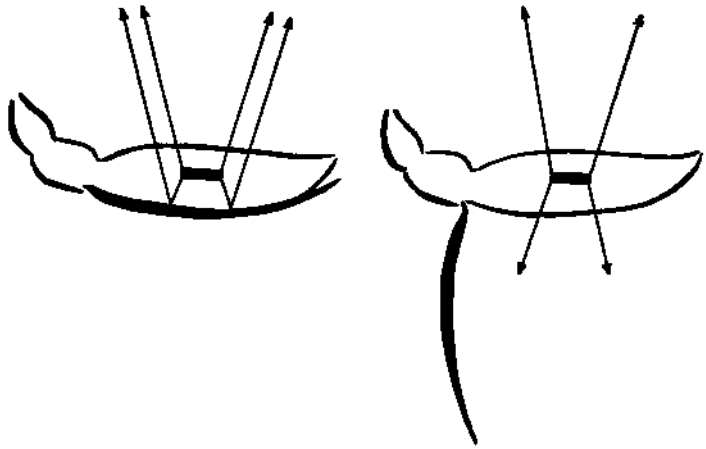

Fig. 1
Arrangement by which the back-scattering effect of the elytra of *Tenebrio molitor* may be measured

Measured values for two beetles are given in Table II. They clearly show that the back-scattering within and from the cuticula of the elytra increases the impulse rate by about 6—7%. The increase from I_o to I_{cu} which corresponds to the effect of the elytra may be determined by interposing the appropriate aluminium filters. These experiments will, however, not be discussed here. The true impulse-rate is decreased by self-absorption and increased by the back-scattering demonstrated above. The absolute and relative magnitude of these factors differ from case to case.

TABLE II

RESULTS OF MEASUREMENTS TO DETERMINE THE BACK-SCATTERING EFFECT IN IMAGINES OF *TENEBRIO*

Insect	I_o (counts/min)	I_{cu} (counts/min)	back-scattering effect
beetle no. 1	15706 ± 125	16620 ± 129	5.82%
beetle no. 2	21508 ± 147	23035 ± 152	7.09%

I_o = counting rate without elytra,
I_{cu} = impulse rate with elytra; higher due to the back-scattering.

III. The cuticular excretion of labelled phosphorus and its technical significance to measurements

If an insect is fed with labelled phosphorus, the latter may possibly be excreted from the cuticular surfaces as shown in some work on ants in these laboratories [10]. Such excretion can present a significant source of error if attempts are made to determine the biological half-life of P^{32} from repeated measurements made on an intact insect. A study of this sort using adult ♂♂ + ♀♀ of *Calliphora erythrocephala* Meig. showed an effective decrease in

radioactivity which was comparable to an effective half-life, T_{eff}, of between 12.3 and 17.0 d. The averages calculated from 14 test insects showed $T_{eff} = 14.42$ d, whereas the physical half-life T_{phys}, is only 14.3 d. On theoretical grounds T_{eff} can be equal to T_{phys} but only on the total absence of excretion; T_{eff} can never exceed T_{phys}. This is shown by the following relationship:

$$T_{biol} = \infty; \frac{1}{T_{biol}} = \frac{1}{\infty} = 0;$$

$$\frac{1}{T_{eff}} = \frac{1}{T_{phys}} + \frac{1}{T_{biol}}; \frac{1}{T_{eff}} = \frac{1}{T_{phys}} + 0;$$

$$T_{eff} \equiv T_{phys}.$$

T_{eff} cannot possibly equal T_{phys} in the case of labelled phosphorus, since the phosphorus enters into the metabolic processes and is also excreted.* If, therefore, the measured decrease is less than the physical decay (Fig. 2.), some error must be present which prevents a direct comparison of the various measurements. It has been shown that the adult *Calliphora* excretes phosphorus through the cuticle. This excretion may be removed by external washing, as confirmed by the measurements. The activity of surface phosphorus is then no longer weakened by absorption in the body, and the counting rate increases. Thus, despite excretion, a higher impulse-rate is recorded. This rate is not comparable to the rate determined some days previously for the same specimen.

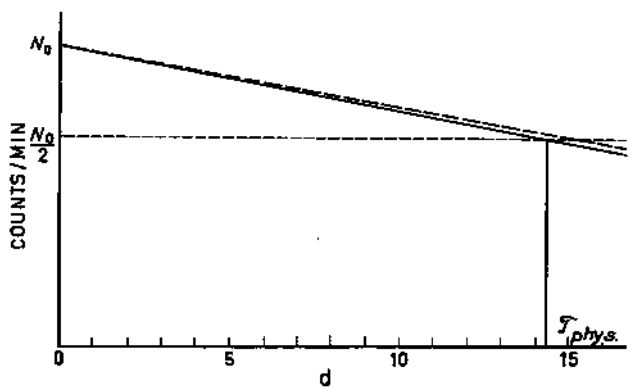

Fig. 2

Physical (———) and effectively measured (------) decay of P^{32} in imagines of *Calliphora erythrocephala* Meig. The erroneous result is due to the cuticular excretion of the tracer
N_0 = log of initial measured pulse-rate

A grossly erroneous value such as cited for *Calliphora* may be recognized easily but it is the smaller errors which are more dangerous. If $T_{eff} < T_{phys}$, one may easily be tempted to consider these values as being correct even if T_{eff} is too large.

In very small insects with a thin cuticle—in aphids [11] the cuticle was found to be only 0.0042 mm thick—this error may be neglected. It is a prerequisite, however, that the

* In the case of injections of colloidal Au^{198}, no excretion occurs and the relationship $T_{eff} = T_{phys}$ may apply.

first measurement in half-life determinations should be taken only after the tracer has become well distributed throughout the organism.

Summary

The use of β-emitting isotopes as tracers in insects is accompanied by certain complications when measurements are made on intact insects. Technical and mathematical ways of determining the value of the absorption and back-scattering have been described, and mention is made of the problem of cuticular excretion of P^{32}, with subsequent changes in counting rates. All such complications are greatly reduced if γ-sources are used as tracers, and the measurements made with scintillation counters. If the γ- and β-radiations from the same insect are measured in close succession in the various experiments described, the size of the effects may be easily determined for the particular isotope used. Work of this nature is now in progress.

ACKNOWLEDGEMENTS

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DISCUSSION

D. W. JENKINS (United States of America): Perhaps our use of the term "biological half-life" is not very suitable in connection with ecological studies, where we need a standard of measurement of the radioactivity present. Could Dr. Kloft firstly define his term "effective half-life", and secondly, indicate his views on its value for ecological studies, where we need a measure of the amount retained in the insect and of that given off?

W. KLOFT: In order to explain what I mean by effective half-life, I must have recourse to a diagram which will demonstrate the inter-relationships between physical, effective and biological half-lives.

The measured initial pulse-rate of the insect is entered logarithmically as N_0. In half-life measurements, the point in time at which half the original activity is still present ($N_0/2$) is the one that interests us. With P^{32}, the physical decay curve cuts the $N_0/2$ level after a lapse of 14.3 d, and by dropping a perpendicular we obtain the physical half-life, T_{phys}. What we are measuring, however, is the effective decay: the decay as actually measured. This is

conditioned by the physical decay *and*, simultaneously, the excretion of the tracer, hence by biological processes. Where the effective decay curve cuts $N_0/2$ the effective half-life, T_{eff}, is found by dropping a perpendicular. From the relation

$$\frac{1}{T_{eff}} = \frac{1}{T_{phys}} + \frac{1}{T_{biol}}$$

we can easily derive the biological half-life T_{biol}, since we have measured T_{eff} and can obtain T_{phys} from tables (when we do not wish to determine it ourselves). After T_{biol} has thus been determined, the value obtained is plotted on our semi-logarithmic diagram, and we can then draw in the biological decay curve. In my next paper, on excretion in aphids, written in collaboration with P. Ehrhardt, I illustrate these relationships more clearly with reference to a test insect.

D. F. HEATH (United Kingdom): The true half-life of P^{32} and the half-life calculated from experiments in which there is cuticular excretion are shown as 14.3 and 14.8 d, respectively. What is the statistical significance of this small difference? I am very surprised that you are able to determine a difference of 3% in a half-life.

W. KLOFT: My rate is the average of 14 to 20 samples measured *in vitro*. In fact, the effective half-life values ranged from 12.3 to 17 d. Individual values were thus genuinely significant with respect to the physical half-life of 14.3 d.

V. K. SASTRY (India): On the last slide that you showed us, the physical half-life and the average effective half-life are almost the same. Can we take it that, in general, the average effective half-life and the physical half-life are almost the same for practical purposes?

W. KLOFT: No, because the effective half-life as recorded was affected by an error of measurement caused by cuticular excretion of P^{32}. The effective half-life is actually substantially less than the physical. It is always necessary to know the error involved in T_{eff} since it is with the aid of the effective half-life that biological half-life is to be calculated.

T. L. HOPKINS (United States of America): Do you know what the nature of cuticular excretion is in *Calliphora*, and have you checked the possibility of cuticular contamination from external sources such as excreted and regurgitated radioactive materials?

W. KLOFT: I think that there is excretion both of inorganic substances and of phospholipids. We are now using chemical methods to separate the various components of the cuticular phosphate excretion, but we do not yet know the precise chemical composition. With regard to the possibility of external contamination, this can be excluded, I think: on the one hand the insects were confined separately, and on the other they were moved to fresh containers at hourly intervals.

F. P. W. WINTERINGHAM (United Kingdom): I should just like to make a very brief comment. I feel that in studies of biological half-life it is most important that all measurements of radioactivity be made under carefully standardized geometrical conditions. I think we should be cautious in attempting to make some arbitrary correction on the basis of the geometry of the insect and of the physical density of the tissues. In your derivation of the equation, I was not quite clear, in fact, whether you were taking into account a point source of radiation at the centre of the insect, or if you were considering a uniformly distributed source. It is most important to discriminate between the two.

W. KLOFT: I quite agree. In our measurements we worked with ants, the great advantage of this being that the radioactive solution fed to the insects is retained in the gaster for a

considerable time, and we found that we had, in fact, a point source of radiation which remained without any absorption for more than half an hour.

A. R. GOPAL-AYENGAR (India): It would be of interest to know the radiation dose received by the insect, and I wonder whether Dr. Kloft has calculated it in rads?

W. KLOFT: As a matter of fact I am working on such calculations at the moment. Until they are completed I can give you the dose in r only.

SECTION 5

STUDIES ON FEEDING BEHAVIOUR

SOME RECENT STUDIES, INVOLVING THE USE OF RADIOISOTOPES, OF THE FEEDING BEHAVIOUR OF TWO PHYTOPHAGOUS INSECTS

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Abstract — Résumé — Аннотация — Resumen

Some recent studies, involving the use of radioisotopes, of the feeding behaviour of two phytophagous insects. Three examples from the author's researches on insect pests illustrate the solution, with radioisotopes, of problems which could not readily be solved in other ways.

The feeding and excretion rates of *Aphis fabae* Scop. were estimated by allowing the insects to feed on bean leaves made radioactive with P^{32}, and then relating the radioactivity of the aphids to that of the leaves. The results are presented and the method criticized.

By allowing groups of *A. fabae* to feed on bean plants made radioactive with P^{32}, the hypothesis that ant-attendance significantly increases the aphid's feeding and excretion rates was confirmed.

The movements and feeding behaviour in the field of adult Senn bugs (*Eurygaster integriceps*, Put.) were studied in Iran by tagging individual insects with small pieces of Ta^{182} so that they could be detected from a distance with a suitable instrument. The results are described.

Etudes récentes au moyen de radioisotopes sur les habitudes alimentaires de deux espèces d'insectes phytophages. Trois exemples tirés des travaux de recherche que l'auteur a effectués sur les insectes nuisibles montrent comment les radioisotopes ont permis de résoudre certains problèmes dont la solution aurait été difficile par d'autres moyens.

L'auteur a déterminé les taux d'alimentation et d'excrétion chez l'*Aphis fabae* Scop. en nourrissant les insectes de feuilles de haricots rendues radioactives à l'aide de phosphore-32 et en comparant ensuite la radioactivité des pucerons à celle des feuilles. Il expose les résultats obtenus et soumet la méthode employée à une étude critique.

En élevant des *Aphis fabae* sur des plants de haricots rendus radioactifs au moyen de phosphore-32, on a pu confirmer l'hypothèse selon laquelle l'intervention des fourmis augmente d'une manière significative les taux d'alimentation et d'excrétion des pucerons.

On a étudié, en Iran, dans le milieu naturel, les mouvements et les habitudes alimentaires de l'*Eurygaster integriceps*, Put. adulte, en marquant certains spécimens à l'aide de petits morceaux de tantale-182, de manière à pouvoir les détecter à distance au moyen d'un appareil approprié. L'auteur expose les résultats obtenus.

Некоторые последние исследования, поведения при поглощении пищи двух видов питающихся растениями насекомых с использованием радиоизотопов. Три примера из исследований, проведенных автором на насекомых-вредителях, иллюстрируют решение с помощью радиоизотопов проблем, которые нельзя было легко решить другими методами.

Скорости поглощения пищи и выделения у *Aphis fabae* Scop. определялись путем кормления насекомых листьями бобов, содержащими радиоактивный фосфор-32, с сопоставлением в дальнейшем радиоактивности тли и листьев. В докладе сообщаются результаты исследований и разбирается метод исследований.

Путем кормления групп *A. fabae* бобовыми растениями, содержащими радиоактивный фосфор-32, была подтверждена гипотеза, что присутствие муравьев значительно увеличивает скорость поглощения пищи и выделения у тли.

В Иране проводились исследования движений и поведения при поглощении пищи в поле у взрослого Senn (*Eurygaster integrieps*, Put.) путем мечения отдельных насекомых небольшими порциями тантала-182 с тем, чтобы их можно было регистрировать на расстоянии при помощи соответствующего прибора. В докладе описываются результаты исследований.

Recientes estudios, efectuados con radioisótopos, sobre los hábitos alimentarios de dos especies de insectos fitófagos. Tres ejemplos de las investigaciones realizadas por el autor sobre los insectos dañinos revelan de qué manera los isótopos permiten estudiar con éxito algunos problemas que no podrían resolverse fácilmente por otros procedimientos.

El autor ha calculado los índices de alimentación y de excreción del *Aphis fabae* (Scop.), nutriendo los insectos con hojas de haba radiactivadas con ^{32}P, y comparando luego la actividad de los áfidos con la de las hojas. Expone los resultados obtenidos y comenta el método aplicado.

Criando los *Aphis fabae* en plantas de haba radiactivadas con ^{32}P, se ha confirmado la hipótesis de que la intervención de las hormigas aumenta notablemente el índice de alimentación y de excreción en los áfidos.

En el Irán se han estudiado los desplazamientos y los hábitos alimentarios del *Eurygaster integriceps* (Put.) adulto marcando algunos insectos con pequeñas partículas de ^{182}Ta, a fin de poder detectarlos desde cierta distancia con ayuda de un instrumento apropiado. El autor expone los resultados obtenidos.

Each year man suffers enormous losses of his crops from the direct and indirect damage caused by insects. A rational basis for control of an insect pest must be based on thorough research into all aspects of its life. Radioisotopes are but one of the many new tools available to the research worker and they can sometimes be used to solve a problem which cannot otherwise be solved readily or even at all. In illustration of this, I have selected three examples from my own researches on insect pests in which radioisotopes were used to solve particular problems.

In temperate climates, *Aphis fabae* Scop. is a serious pest of beans and sugar beet, which it damages by feeding in large numbers on the phloem sap. Estimates of the amount of sap ingested by aphids are difficult to obtain and attempts to measure it have, therefore, been few. One way of estimating the uptake of sap is to allow the insects to feed on leaves made radioactive with P^{32} and then to relate the radioactivity of the aphids to that of the leaves.

The feeding and excretion rates of nymphs of *A. fabae*, feeding on bean leaves, were studied in this way for feeding periods of 1 h to 24 h [2]. The maximum rate of feeding, which was reached after 12 to 16 h of feeding, was estimated at 0.2 mg of sap/h, an uptake of 59% of the average body weight of the insect per hour. A bean plant infested with several thousand aphids would thus lose a large quantity of vital phloem sap in a short time.

For aphids, the method suffers from the defect that the radioactivity of the ingested sap is unknown, but is assumed to be equal to that of a sample of the leaf tissue. The criticism would not apply to those insects which ingest solid material from the leaf.

Aphis fabae is sometimes attended by ants for the honeydew it excretes, just as many Coccids in the tropics are attended by ants.

For a long time, it was thought that attendant ants stimulate the feeding and excretion of the aphid but there was no experimental proof [4]. As part of a study of the complex, plant—aphid—ant—aphid—predator, it was essential to know whether ant-attendance causes the aphid to feed and excrete more.

Groups of nymphs of *A. fabae* were allowed to feed on leaves of bean plants made radioactive with P^{32} in water culture, so that the aphids took up the isotope and excreted it in their honeydew. The radioactivity of the honeydew taken from them by attendant *Lasius niger* L. was then compared with that of the honeydew excreted concurrently by unattended aphids on separate plants.

By increasing their uptake of sap, the ant-attended aphids produced twice as much radioactivity in their excreta as did the ant-free aphids [1].

Ant-attendance therefore increases the rates of feeding and excretion of this aphid and consequently increases the damage to the plant. The results are also of interest in that

they show that the aphid is able to control its rates of feeding and excretion, which are not dependent solely on pressures within the plant as was sometimes previously thought.

The Soun (or Senn) bug, *Eurygaster integriceps* Put., is a serious pest of wheat in the Middle East and countries bordering the Caspian Sea. Related species of pentatomidae occur in North Africa and other countries of the Mediterranean area and cause considerable damage to crops there.

Existing descriptions of the insect's behaviour are sometimes conflicting and are rarely supported by evidence. A thorough understanding of its behaviour is essential to a proper basis for control measures and for assessing the advantages and disadvantages of various methods of sampling.

The problem of locating individual insects in the field, so as to observe their behaviour, is easily solved by tagging them with small pieces of radioactive tantalum, Ta^{182} or some other radioisotope with similar properties, so that they can be detected from a distance with a suitable portable instrument.

In Iran last summer, the behaviour of young adult Senn, tagged with labels of Ta^{182}, was studied for several days [3]. The insects were located and observed at half-hourly intervals throughout the day from sunrise to sunset during the intensive feeding period and again, later, when they had finished feeding and were about to migrate to the mountains for hibernation.

The insects restricted their movements to a very small area of the wheat crop. Where the wheat was thickly planted, the leaves and stems still green and the grains still soft, the bugs fed frequently and were nearly always found on the wheat ears and rarely in the soil. In a part of the crop where the wheat was sparsely sown, the air hotter and drier, and the plants dry with ripe grains, the insects spent much more time in cracks in the soil, where, presumably, it was not so hot and dry and, therefore, they did not feed as often as those in the green part of the crop. In both parts of the crop, feeding occurred chiefly in daytime. It increased in frequency from sunrise to a maximum at 8 a.m., declined to a minimum at midday and rose to another peak in the afternoon. Feeding almost ceased at sunset.

At the end of the feeding period, just before the flight to the mountains, the insects spent most of the time in cracks in the soil and were rarely seen feeding.

By this simple method the movements and behaviour of insects under field conditions can be observed. Effects of radiation on the insects, of which so little is known, would probably be negligible for periods of a few days and the method deserves exploitation.

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DISCUSSION

THE CHAIRMAN (J. E. Treherne, United Kingdom): In the aphid experiment you were presumably not able to correlate the concentration of P^{32} in the honeydew directly with the phloem sap, since the concentration of this fluid cannot be estimated. Were you confident, therefore, of the statistical significance of your results which correlate the honeydew concentration with the gross concentration in the bulk of the leaf tissue?

C. J. BANKS: I emphasized that this method of estimating the sap-uptake of *Aphis fabae* is unsatisfactory because we do not know the radioactivity of the sap which the

aphid actually ingests. The results obtained are, however, consistent with those obtained by others, in particular with those of Mittler who used the cut-stylet technique to obtain phloem sap samples when working with the large willow aphid, *Tuberolachnus salignus*.

THE CHAIRMAN: Have you tried using Mittler's cut-stylet technique yourself to obtain the true sap concentration? Is this possible with *Aphis fabae?*

C. J. BANKS: I have, in fact, tried the technique with *Aphis fabae* but without success because the insect is too small.

W. KLOFT (Federal Republic of Germany): I should like to make a few supplementary remarks to Dr. Banks' paper, which will at the same time have a bearing on the matter raised by Dr. Treherne.

Dr. Banks himself pointed out that the specific activity of the phloem sap is not known, but that it is assumed to be identical with that of the leaf tissue—from which he calculates the quantity of nutrient ingested. I believe, however, that this is not a valid procedure for, according to the plant physiologist H. ZIEGLER [*Planta* (Berlin) **47**, (1954) 477—494] the carbohydrates transported in the phloem sap are not phosphorylated. The phosphate content of the phloem sap is therefore not equimolecular with that of the sugars, and the phosphorus content of the sieve tube sap is much lower than in the surrounding tissue. I therefore believe that Dr. Banks' estimates of food ingestion by his experimental insects are probably too high.

I should also like to point out, in connection with the food ingestion curve, that the curve was obtained from a group experiment and thus only represents an average. The ingestion rate of an individual insect is much faster and appears to follow an exponential curve with a sharp initial rise. As Dr. Banks himself points out, his curve allows for the fact that the externally visible act of insertion of the stylet by the insect cannot be assumed simultaneous with the actual start of feeding. However, as my collaborators H. Kunkel and P. Ehrhardt have shown in their tracer experiments, there are varying intervals between the actual penetration and the time when actually 100% of the insects begin to feed. In one species, although the time-lag is 8—10 h at 24°C, certain individuals may nevertheless start feeding 6—8 min after insertion (W. KLOFT, *Z. angew. Entomol.* **45** (1960) 337—381) and 5—10% of the experimental insects already show considerable radioactivity 10 min after insertion. This activity can derive only from ingestion of nutrient from the phloem sap. (W. KLOFT and H. KUNKEL, Proc. XI International Congress of Entomology, Vienna 1960, in press).

In conclusion, I would therefore emphasize that this kind of experiment should, as far as possible, be carried out with individuals.

C. J. BANKS: I recognize that Dr. Kloft has carried further the work which Nixon and myself did using groups of aphids only; however, regarding his criticisms as to what is actually taken in by the aphids, I think that I have already dealt with the point myself earlier.

D. W. JENKINS (United States of America): You mentioned that pieces of tantalum-182 were stuck to the insects. How was this done, and did the pieces remain on the insects for long periods? In other words, would the tantalum technique be comparable to that used by Dr. Andreev, by which he was able to follow the *Eurygaster* right through the winter? I should also like to ask whether you did any further experiments on hibernation? Dr. Andreev has shown that in autumn the *Eurygaster* migrate distances of up to some 12 km for hibernation purposes, and that in summer there is very little migration.

C. J. BANKS: In answer to the first part of the question, we obtained a piece of tantalum strip, irradiated at Harwell. From this strip small chips of dimensions 0.05 × 0.16 × 0.46 mm

with an average weight of 0.06 mg—the weight of an average bug being approx. 11 mg—were cut with a knife kept for the purpose, picked up with the point of a needle covered with gum and attached to the insect's back with a spot of coloured paint. As you saw on the slide, the insect had two red spots on it; one of those spots was in fact cellulose paint which acted as an adhesive for the piece of tantalum and also served to identify the insect in the field. I don't think the tantalum would stay on very long because the paint tends to chip off, and I think you would have to find something more reliable as an adhesive. For the experiment here described, however, it certainly stayed on long enough. When, on another occasion, I used a similar method for labelling coccinellid larvae, I did in fact use an ordinary, water-soluble glue; this would not be suitable here because of the effects of damp.

With regard to the second part of your question, we did this work in Iran for a few days last summer; I have not studied any of the insect's overwintering habits.

P. B. CORNWELL (United Kingdom): I think perhaps one should stress the importance in virus-transmission studies of an understanding of feeding by Homoptera. Radiotracer techniques can make a valuable contribution in this field, and Dr. Banks' graph of uptake of labelled phosphorus from the plant with time is of particular interest in this connection. In virus-transmission studies in the laboratory it is necessary to distinguish between the "settling period", before insertion of the stylets, and the true feeding period with uptake of plant sap.

The subsequent feeding-behaviour of aphids which have been subjected to starvation periods also seems to me to be quite important in virus-transmission studies, and I should like to ask Dr. Banks whether he has carried out any studies which involved starvation of aphids before their being placed on labelled plants.

C. J. BANKS: No, I have not done any such work myself, but I understand some of my colleagues in the Plant Pathology Department at Rothamsted have done so.

W. KLOFT: We have carried out the kind of study to which Dr. Cornwell refers at our laboratories, and I show the results in the next paper.

C. KRISHNAMOORTHY (India): Dr. Banks told us that there are two peaks in the feeding rates of *Eurygaster*, one at 8 a.m. and the other at about 4 p.m. Have these peaks been correlated with temperature?

C. J. BANKS: My graphs showed that air temperatures and relative humidities were recorded hourly but, as I stated, the feeding activity of the insects could not be correlated with these records. Further research is required to explain the two peaks of feeding activity.

STUDIES ON THE ASSIMILATION AND EXCRETION OF LABELLED PHOSPHATE IN APHIDS

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Abstract — Résumé — Аннотация — Resumen

Studies on the assimilation and excretion of labelled phosphate in aphids. Aphids are plant-sucking insects with a relatively high metabolism and therefore very suitable for studies of the incorporation and excretion of labelled substances. A particular advantage is the uptake, nearly free of contamination, of radioactive compounds from labelled plants through the stinging and sucking mouth-parts. Measurements show that the insects take up food only some time after stinging, at a moment which corresponds with reaching the phloem. The activity then suddenly increases to an approximate maximum, which is reached after the intestinal tract has become full of labelled phloem-sap. Subsequently, however, the activity increases slowly because of resorption from the midgut. In the meantime, incorporation (assimilation) and excretion of radioactive material begins in different ways. Its incorporation into the saliva and its subsequent re-injection into the plant are of special interest. The interval between the uptake of the tracer from a plant to its re-excretion with the saliva is 5 h at a temperature of 22—24° C. This period, as well as the exact course of excretion of saliva during sucking and the distribution of saliva in the plant itself, have been analysed in detail because of their great importance for the physiology of nutrition and for the phytopathological effects of aphids. Furthermore, these tracer experiments indicate how the problem of virus transmission (especially of persistent ones) by plant-sucking insects may be attacked. Further excretion of the radioisotope takes place in the honeydew as well as through the bearing of larvae. On hatching, these larvae have only a low activity but this increases after longer periods of resorption in the maternal organism. On account of continuing ovoviviparie and by the use of P^{32} it is possible to obtain a record of the phosphate metabolism of the ovary in the live aphid. By measuring the different kinds of excretion as well as the remaining activity, the biological half-life and the amount of tracer actually assimilated may be determined. Any circulating radioactivity may be detected by measuring the haemolymph. Constant temperature must be ensured since the processes are highly sensitive to changes in temperature.

Expériences sur l'assimilation et l'excrétion du phosphate marqué chez les aphidiens. Les aphidiens sont des insectes phytophthires qui ont un métabolisme relativement élevé; ils se prêtent donc fort bien à l'étude de l'incorporation et de l'excrétion de substances marquées. Ils présentent l'avantage particulier d'absorber presque sans contamination les composés radioactifs des plantes marquées par leurs organes buccaux de pénétration et de succion. Les mesures montrent que les insectes ne pompent la nourriture que quelque temps après avoir piqué, au moment où le bec atteint le liber. L'activité augmente alors brusquement pour se rapprocher du maximum, qui est atteint une fois que l'appareil intestinal est rempli de la sève marquée du liber. Puis l'activité augmente lentement, du fait de la résorption par l'intestin moyen. Dans l'intervalle, l'incorporation (assimilation) et l'excrétion des radioisotopes commencent de différentes manières. L'incorporation à la salive et la réinjecton consécutive dans la plante sont particulièrement intéressantes. A une température de 22 à 24°C, cinq heures s'écoulent depuis le moment où l'insecte absorbe l'indicateur contenu dans la plante jusqu'au moment où il l'excrète avec la salive. Cette période, ainsi que le mode exact d'excrétion de la salive pendant la succion et la distribution de la salive dans la plante sucée par les insectes, ont été analysés avec précision, en raison de leur grande importance pour l'étude de la physiologie de la nutrition des aphidiens et des effets phytopathologiques dont ces insectes sont cause. En outre, ces expériences au moyen des indicateurs servent de modèles pour l'étude du problème de la transmission des virus, notamment des virus persistants, par les phytophthires. L'élimination des radioisotopes se produit aussi par l'excrétion du miellat, ainsi que par la naissance de jeunes larves vivantes. L'activité des premières de ces larves à leur sortie de l'œuf est faible; elle s'accroît après des périodes prolongées de résorption dans l'organisme maternel. Si l'on utilise du phosphore-32, l'ovoviviparité continue

permet d'enregistrer le métabolisme du phosphate dans l'ovaire de l'aphidien vivant. En mesurant tous ces modes d'excrétion, ainsi que la radioactivité résiduelle, on peut déterminer la période biologique et la quantité d'indicateurs effectivement assimilée. On déterminera la partie circulante en mesurant séparément l'hémolymphe. Ces opérations exigent une température constante, car les variations de température ont une grande influence sur les divers processus.

Эксперименты по усвоению и выделению меченых фосфатов в тле. Тля — насекомое, питающееся соками растений и обладающее сравнительно высоким метаболизмом. В связи с этим она удобна для изучения усвоения и выделения меченых веществ. Особым преимуществом является усвоение, почти без заражения, радиоактивных соединений из меченых растений при помощи надкусывающих и сосущих частей рта. Измерения показывают, что насекомые принимают пищу лишь некоторое время спустя после укуса, а именно в момент, когда их жало доходит до флоэмы (phloem). Затем активность резко увеличивается приблизительно до максимума, что достигается после заполнения кишечного тракта меченым соком флоэмы. Однако после этого активность возрастает медленно в результате рассасывания из среднего брюшка. В то же время начинается усвоение и выделение радиоизотопов различными путями. Особый интерес представляет их усвоение в слюну и последующая реинъекция (re-injection) в растение. Промежуток времени между засасыванием индикатора из растения и до обратного выделения его со слюной составляет 5 часов при температуре 22—24° С. Этот промежуток времени, а также точный ход выделения слюны во время высасывания и распространение слюны в самом растении были точно изучены ввиду их большого значения для физиологии питания и фитопатологического воздействия тли. Далее, эти эксперименты с индикаторами указывают каким образом можно подходить к изучению проблемы перенесения вирусов (особенно стойких вирусов) питающимися соками растений насекомыми. Последующее выделение радиоизотопов происходит в виде сходного с медом вещества и в появляющихся личинках. С самого начала эти личинки обладают лишь малой активностью, которая возрастает после более продолжительных периодов рассасывания в материнском организме. Вследствие постоянного яйцеживорождения и путем использования фосфора-32 можно точно проследить за метаболизмом фосфата яичника живой тли. Измерением выделений всех этих видов, а также остаточной активности можно определить биологический период полувыведения радиоактивных веществ из организма и количество действительно ассимилированных индикаторов. Участвующая в циркуляционном процессе радиоактивность может быть обнаружена измерением гемолимфы (haemolymph). Следует соблюдать постоянную температуру, так как эти процессы чрезвычайно чувствительны к изменениям температуры

Estudios sobre la asimilación y excreción de fosfatos marcados en los áfidos. Los áfidos son insectos fitoftirios, de metabolismo relativamente intenso y apropiados, por tanto, para estudiar la incorporación y excreción de sustancias marcadas. En particular, presentan la ventaja de absorber, casi sin contaminación, los compuestos radiactivos de las plantas marcadas, por medio de sus órganos bucales de penetración y succión. Las mediciones demuestran que los insectos no ingieren alimento sino hasta algún tiempo después de efectuada la picadura, en el momento de entrar en contacto con el floema. En ese instante, la actividad experimenta un aumento súbito y llega casi al máximo, que se alcanza cuando el insecto ha llenado su tubo digestivo con savia marcada del floema. Seguidamente, la actividad aumenta lentamente a causa de la resorción en el mesogastrio. Entretanto, la incorporación (asimilación) y excreción de las sustancias radiactivas comienza de diferentes maneras. La incorporación en la saliva de los insectos y su ulterior reinyección en las plantas es particularmente interesante. A una temperatura de 22 a 24°C, el intervalo que media entre el momento en que el insecto ingiere el indicador contenido en la planta y el instante en que lo excreta con la saliva es de 5 horas. Por su gran importancia para el estudio de la fisiología de la nutrición de los áfidos y de los efectos fitopatológicos causados por esos insectos, se ha estudiado minuciosamente dicho intervalo, así como el proceso exacto de excreción de la saliva durante la succión y la distribución de la misma en la planta chupada por los insectos. Además, estos experimentos con indicadores radiactivos revelan cómo puede abordarse el problema de la transmisión de virus (sobre todo de virus persistentes) por los fitoftirios. Los radioisótopos también se excretan con la ligamaza y con las larvas vivas recién

nacidas de los insectos. Durante la incubación éstas presentan una actividad reducida, la cual aumenta después de prolongados periodos de resorción en el organismo materno. Debido a la ovoviviparidad continua y mediante el uso del ^{32}P, se puede registrar el metabolismo de los fosfatos en el ovario del áfido vivo. Midiendo las distintas excreciones y la actividad remanente, se puede determinar el período biológico y la cantidad de indicador realmente asimilada. La cantidad de indicador circulante puede averiguarse midiendo la actividad de la hemolinfa. Estas operaciones deben llevarse a cabo a temperatura constante, dado que los distintos procesos son muy sensibles a las variaciones térmicas.

Introduction

Radioactive isotopes are being used more and more in work on insect metabolism. Because of their contamination-free intake of tracer from radioactively labelled plants insects which suck the sap of such plants make especially good experimental animals. In those species which suck from the phloem there is not only a relatively rapid intake and conversion, but also an easily followed periodic excretion in the form of honeydew and saliva, and production of young larvae. All these processes may be easily followed by serial measurements.

A variety of authors have already worked on insects which suck plant sap; they used radioactive isotopes mainly for studying the virus-vectoring properties of the insects tested. HAMILTON [1] worked with marked polonium as early as 1935, but it appears that this radioactive element plays no active part in the normal physiology of insects. The development of artificial radioactive isotopes, especially the production of the physiologically important P^{32}, represents a great advance in this field. Studies on intake and excretion have been performed on the jassid *Orosius argentatus* Evans, [2], and on the aphids *Myzus persicae* and *Brevicoryne brassicae* [3]. WATSON and NIXON [4] working on *Myzus persicae*, and BANKS and NIXON [5] on *Aphis fabae* pursued similar studies. All the authors mentioned, however, worked with groups of experimental animals. In such group-studies, the individual variability in the time-lag between the insect's beginning to pierce and actually to feed on the phloem [6] [7] introduces errors. To avoid these, individuals were used in our studies on the duration of salivary injection by *Myzus ascalonicus* [8] [9] and the time which elapses between uptake of P^{32} and its re-injection with the saliva [10]. HENNIG [11] also sought to determine the moment at which food uptake begins in *Aphis fabae* Scop.; he worked with individual specimens.

Methods

The use of a sufficiently large experimental animal was essential to our study. The aphid, *Megoura viciae* Buckt., which sucks the phloem of *Vicia faba* met our specifications. The apterous imagines, which weigh 2.5 to 5 mg, lend themselves well to individual experiments since they may easily be marked by coloured dots, and can be caged separately. Furthermore, a single drop of honeydew is sufficiently large to be easily visible and measured accurately. The activation was carried out on sprouts of approx. 10 cm of *Vicia faba*, raised on phosphate-free Knop solution to which labelled phosphate of a specific activity of about 0.05 mc/ml had been added. Experience has shown that it is necessary to work with intact plants, since *M. viciae* is very sensitive and seems to require an undamaged phloem system for continuous feeding and normal honeydew excretion.

Using special techniques, it was possible to obtain the honeydew drops of an individual aphid on a transparent foil; by cutting out the section its activity could easily be measured. Individual aphids were caged for measuring in such a way that movement was only possible along the central axis. The aphids being nearly oval, the geometry of the measuring system

was not affected by their limited movement (Fig. 1). All measurements were taken with the same GM tube, using a constant potential difference and the same geometrical arrangement

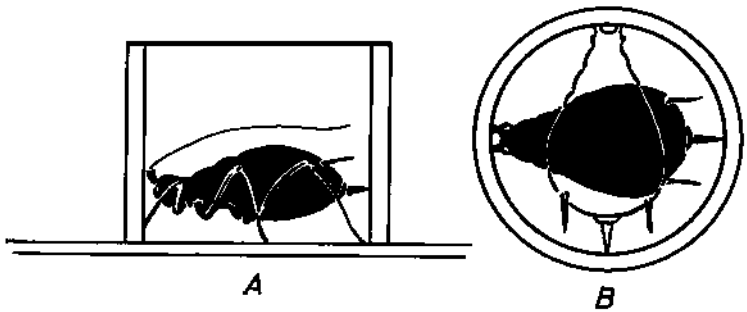

Fig. 1
Aphid cage for measuring purposes, consisting of a ring of glass, covered by a thin membrane
A profile view
B view from above

throughout. An electronic scaler produced by Frieseke & Hoepfner, Erlangen, was used in conjunction with an end-window counting-tube having a window thickness of 1.21—1.30 mg/cm^2. Live aphids were counted for 5 min; the honeydew for 10 min. The tests were carried out at temperatures ranging from 20° to 22°C.

Results

1. Ingestion of the tracer by the aphids

The beginning of penetration can be accurately determined from the behaviour of the aphid [12] [13]. It should be noted that the moment of puncture does not mark the beginning of ingestion, which in this case can only take place after 5 min at the earliest, i.e. when the phloem has been reached [7]. The specimens chosen began to ingest almost immediately on penetrating the phloem (following the initial 5 min mentioned above); this was evident from the rapid increase in their activity. After about 24 h there appears to be no further increase in activity even if feeding continues. A phosphate saturation has apparently been reached. Uptake and excretion seem to have reached equilibrium.

2. The start of tracer excretion

According to studies by Ehrhardt [7], the P^{32} is already absorbed in the alimentary tract within 50 to 60 min after the phloem has been reached, as can be determined from the haemolymph. Absorption is therefore extraordinarily rapid and the tracer, along with the circulating haemolymph, is distributed just as quickly throughout the organism.

While more tracer is being taken up with the food substances, excretion starts as follows:

1. Via *the honeydew*

Drops of honeydew produced by aphids on activated plants were under continuous observation. Activity in this excretion was not detected earlier than 2½ h after the start of

penetration. The insects produced an average of about 10 drops in a 24-h period*. The total activity of the specimen at the time of producing the first honeydew is about 10% of the maximal activity that can be reached in the span of 24 hours. This value, presumably, indicates that the digestive tract had become filled but it must be remembered that resorption had already set in after 1 h. The results also reveal the speed of food transport in the digestive tract. The activity of the honeydew rises subsequently, and reaches a maximum value (Fig. 2) after 20 h. This reflects the time-lapse of 20 to 24 h that the aphids need in order to reach maximum activity.

2. Via *deposition of young larvae*

The newly born young larvae, deposited at the rate of about 10 per day were also measured for radioactivity, in order of their appearance, with the counter used for the mother. At first, the larvae are not radioactive; the first radioactive larvae appear only 6 h after feeding on the activated plant. Larvae deposited from 6 to 24 h after the mother has begun to feed show an increase in activity. After about 20 h there is no further increase and saturation of the organism, specifically of the ovaries, seems to have been reached (Fig. 2).

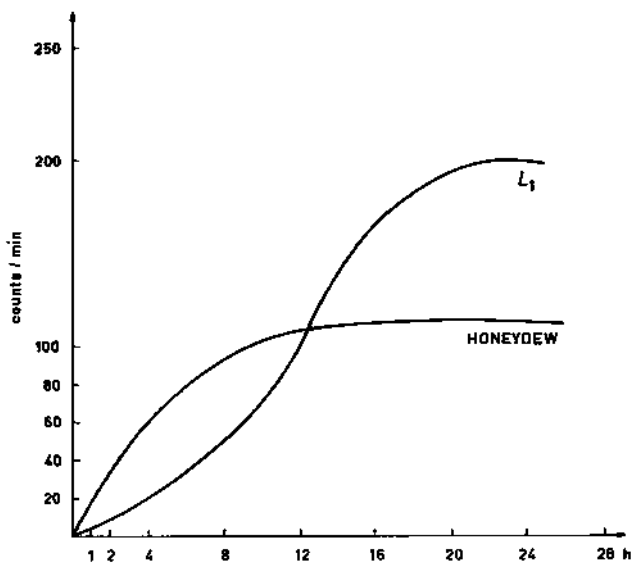

Fig. 2
Counting rate of the honeydew and the young larvae in relationship to the time of the first detectable appearance of excreted radioactivity

3. Via *the saliva*

According to the studies by KLOFT and KUNKEL [10] on *Myzus ascalonicus* and by EHRHARDT [7] on *M. viciae*, P^{32} is excreted in the saliva at the earliest 5 hours after the start of feeding. In order to make such measurements, the insects must be transferred to a non-radioactive test plant after a relatively short feeding time.

* This number should not be considered the natural rate of excretion of this species since the normal feeding process was interrupted once a day in order to measure activity.

3. Further course of tracer excretion, following maximum saturation and aphid-transfer to non-active plants

It is interesting to follow the course of P^{32}-excretion in the form of honeydew and young larvae over several days once the insect, now saturated with radioactive phosphate, has been transferred to an unlabelled plant from which there can be no replenishing of the tracer. The total activity of the aphid and that of every drop of honeydew and of every young larva at birth was measured at regular intervals of 24 h.

The impulse rate of the drops falls off very rapidly at first but more slowly later. After 24 to 30 h the impulse rate has dropped to about 5% of its initial value, and remains at this level over a period of 5 d. This value can be traced back to excretion from the haemolymph into the alimentary tract. On the 7th day after transfer to a non-radioactive plant, it is practically impossible to detect any radioactivity in the honeydew.

In comparison with the honeydew activity, that of the larvae decreases much more slowly. Even from the 3rd to the 6th day these values are several times higher than the impulse rate from the honeydew. The activity of the young larvae is still clearly measurable above the background-level even 7 days after removing them from the labelled plants.

A continuous determination of the activity lost from the specimen with the saliva was not possible by the methods used. The quantity of P^{32} which had been deposited in the plant could only be determined at the end of the experiment, by incinerating the plant and testing the ash. Even after 4 days of feeding, this value was found to be below 0.5% of the initial maximal activity of the whole insect, and may therefore be neglected in the total calculation. A value of similar magnitude was reported by Day and Irzykiewicz [3] in the percentages quoted for salivary secretions from *Myzus persicae* and *Brevicoryne brassicae*.

4. The course of phosphorus-labelled metabolism

The quantity of a material excreted may only be considered a measure of its metabolism in the organism if the substance normally participates in metabolism and if its habitual supply from the outside is not interrupted. Both conditions are met in this case, since phosphorus plays an important role in normal metabolism and the experimental animals were allowed to continue feeding on unlabelled host plants. The effective decrease in the radioactivity remaining in the animal has been represented by means of a curve obtained from periodic measurements. It is the sum of the natural radioactive decay of P^{32} (physical half-life $T_{phys} = 344$ h) and the decrease, from excretion of the tracer through the various channels mentioned above. The effective decrease in impulse rate follows an exponential law, as does the physical and the biological decrease. The biological decay depends on excretory losses, and may be computed from the other two values. The curves of the effective as well as the biological decrease may be characterized by a half-life value, the effective half-life being represented by T_{eff}, and the biological by T_{biol}. The following relationship holds for these functions:

$$\frac{1}{T_{eff}} = \frac{1}{T_{phys}} + \frac{1}{T_{biol}}.$$

Since T_{phys} for P^{32} is known to be 344 h, and T_{eff} may be determined graphically from the experimental data, the biological half-life T_{biol} may easily be computed. In order to determine the effective half-life, the data from periodic measurements on 21 individual specimens were averaged. In *Megoura viciae*, the effective half-life varies between 27 and 45 h, with an average of 32—33 h. The biological half-life varies correspondingly, ranging from 29.3 to 51.7 h, with an average of 36.5 h. These relations are illustrated semi-logarithmically

for an apterous imago; they are similar, in principle, for other imagines (Fig. 3). An attempt was made to determine the effective half-life separately, by mathematical means only, and the corresponding biological half-life value for P^{32} by means of the two main factors, i.e. the excretion of phosphorus in honeydew and the loss of phosphorus due to deposition in the larvae. In the case of the imago represented in Fig. 3, $T_{eff} = 36$ h for the first 5 days after incorporation has begun, whereas $T_{biol} = 40.2$ h. If the honeydew excretion were to be exluded, T_{biol} would be increased to approx. 44 h. If, on the other hand, the excretion with young larvae were not to be counted, T_{biol} would be even longer, about 65 h.* The excretion of P^{32} by means of young larvae is, therefore, of great significance to viviparous imagines. Within 4—5 days after transferring them from radioisotope-labelled to unlabelled plants, the rate of P^{32}-elimination changes in the imagines as well as in the larvae. This is shown in Fig. 4 for a larva.

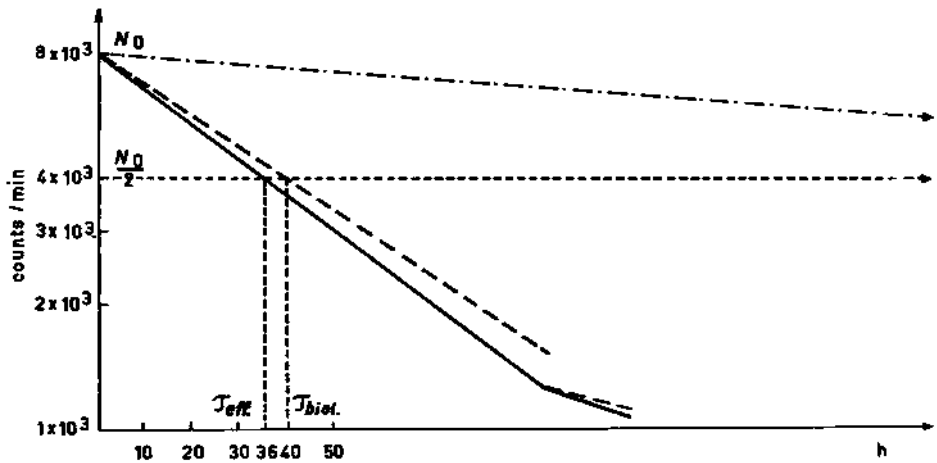

Fig. 3

Relationship between effective, physical and biological decay in an imago of *Megoura viciae*
The half-lives, T_{eff} and T_{biol}, were found graphically; N_0 = initial activity of the aphid
——— effective decay, — · — · — physical decay, — — — calculated biological decay

It should not be concluded that the biological half-life of P^{32} is longer in larvae because they do not possess the ability to excrete it by means of giving birth to young larvae. Measurements indicate that in the first phase, the three days after the tracer has begun to be incorporated, the larvae show an effective decrease similar to that in imagines (apterous virgins). The example in Fig. 4 gives a T_{eff} of 28 h and a T_{biol} of 30.4 h for a 3rd instar larva in this first phase. Beginning with the 4th day after the start of the experiment there is, as already mentioned for imagines, a change in the curve which clearly shows the excretion to be slower. T_{eff} is then 57 h and T_{biol} is computed to be 68.3 h. This change in the biological half-life value is believed to be traceable to the fact that during the first phase the digestive tract evacuates not only the quantity of the phosphorus that would normally be

* These calculated values should only be considered as approximate, since the absorption factors and the distribution of the phosphorus differ between imagines, larvae and honeydew drops. It is not yet known how these differences affect the results. The possibility of such errors may be avoided by using a γ-source as tracer.

excreted, but all the excess phosphorus present in the tract. In the correspondingly longer second phase, on the other hand, only the phosphorus recently freed in the metabolic processes is being excreted. The higher value for T_{biol} during the second phase therefore corresponds to the natural metabolic processes and is of greater biological interest than the first.

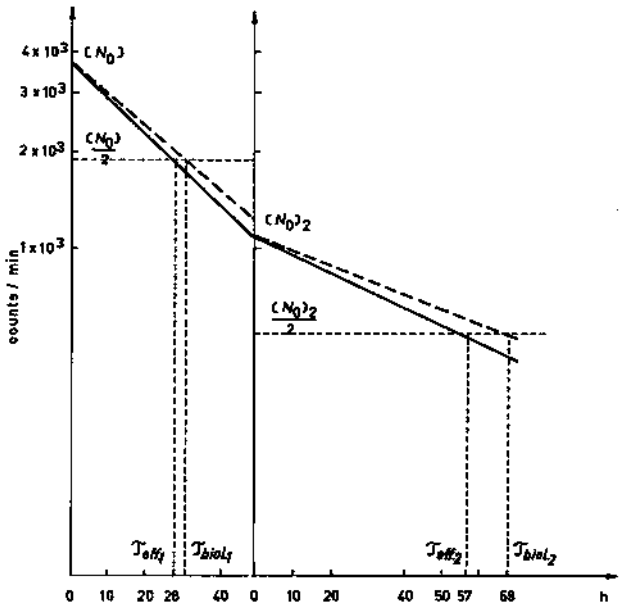

Fig. 4
Effective and biological half-lives in a larva of *M. viciae*
$(N_0)_1$ = initial activity for the first phase
$(N_0)_2$ = initial activity for the second phase

The results give a first indication of the distribution of phosphate in the organism studied. It is not yet possible, however, to say whether the phosphate is present in an organically bound or free form. Biochemical studies are thus needed to elucidate the phosphate metabolism. Experiments along such lines are being pursued in these laboratories.

ACKNOWLEDGEMENTS

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DISCUSSION

J. DE WILDE (Netherlands): Could Dr. Kloft tell us whether he has observed permeation of P^{32} through the salivary sheath which develops as soon as the aphid penetrates into the epidermal cells, and whether he has obtained any evidence as to which components of the saliva can pass the salivary sheath?

W. KLOFT: In some as yet unpublished studies, my collaborator Marek has shown with the aid of the phase contrast microscope and autoradiographs that P^{32}—in which precise form we do not yet know—passes from the salivary sheath into epidermal cells of allium and even penetrates into neighbouring cells.

R. G. BRIDGES (United Kingdom): Have you any idea of the phospholipid content of your aphids? The results which we have obtained from houseflies suggest that maximum labelling of the phospholipid fraction does not occur until some 5—7 d have elapsed. I was surprised to hear that you have obtained maximum labelling within 24 h.

Secondly, do you think that your differences in rates of excretion are due to the fact that in the early stage the rate of loss of P^{32} is governed by loss of activity from the water-soluble phosphorus compound, but in the later stage by loss of activity from the phospholipid fraction, which would be turning over more slowly?

W. KLOFT: We have not yet investigated the phospholipid content of our aphids. Although, as you say, the labelling of phospholipids requires some time, nevertheless the insects showed their maximum activity approx. 24 h after P^{32} had begun to be taken up. I think that the phospholipid content is not so great that labelling it should constitute a substantial proportion of the total labelled phosphate taken up.

Regarding your second point, we do in fact consider that in the first stage it is mainly the unbound (and water-soluble) phosphate which is excreted. Your suggestion that excretion proceeds more slowly in the second phase strikes me as very interesting and worth further investigation.

G. G. SENGUPTA (India): By allowing the labelled aphids to feed on an unlabelled plant leaf, is it possible to study the disturbance in plant metabolism which results in leaf-curl?

W. KLOFT: I have shown in some other work that disturbances in growth, respiration, photosynthesis etc. of leaves upon which the aphids have fed are closely connected with saliva injection. The various phases of injection could be more closely identified with the tracer method. Free amino acids have been identified as phytopathological agents in the saliva (see KLOFT, *Z. angew. Entomol.* **45** (1960) 337—81 and **46** (1960) 42—70).

P. B. CORNWELL (United Kingdom): One of the important problems in the control of Homoptera is, of course, their relationship with ants. I think that we should recognize that radiotracer techniques can give us a very much better understanding of the relationship in

question, making it possible to assess the relative importance of various ant species in sustaining host populations on plants.

Turning to another point, we are conscious, with Homoptera, and particularly aphids, of a tendency for the population to concentrate on a particular part of the plant. Is there any evidence from tracer studies that this concentration occurs where a labelled insect may have fed on an unlabelled plant?

W. KLOFT: I do not think that any tracer studies have yet been carried out on this subject, and it is something that might profitably be taken up.

PART II

RADIATION STUDIES

SECTION 1

DIRECT EFFECTS OF RADIATION

EFFECT OF RADIATION ON MEXICAN FRUIT-FLY EGGS AND LARVAE IN GRAPEFRUIT

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Abstract — Résumé — Аннотация — Resumen

Effect of radiation on Mexican fruit-fly eggs and larvae in grapefruit. A limited study has been made of the effect of gamma-radiation from cobalt-60 on the eggs and larvae of the Mexican fruit-fly. Various doses from 5000 to 90 000 rad were given to fruits one and twelve days after infestation to explore the effects of irradiation on the egg and first-instar larval stage. Fruits containing fully grown larvae and larvae at intermediate stages were also irradiated with similar doses. Frequent examination and dissection of the fruits were used to determine larva mortality, larval damage to fruits, presence of pupae and presence of adult flies.

Dissected fruits given radiation doses of 5000 rad or more during the egg or first-instar larval stage showed no presence of insects and no trace of insect damage. Fruit infested with adult larvae produced numerous pupal recoveries but no adult flies emerged from pupae recovered from fruits receiving a radiation dose of 5000 rad or more. It is concluded that a gamma-radiation dose of 5000 rad (and possibly less, based on limited tests of 2000 rad) will break the life cycle of the Mexican fruit-fly although the larval stage may survive appreciably greater dosages.

A design for a railway mobile gamma-irradiator for treating infested fruit is proposed.

Effets des rayonnements sur les œufs et larves de la mouche à fruit mexicaine dans le pamplemousse. Les auteurs ont étudié l'action des rayons gamma du cobalt-60 sur les œufs et larves de la mouche à fruit mexicaine. Ils ont soumis des fruits à différentes doses de rayonnements, allant de 5000 à 90 000 rads, 1 et 12 jours après leur infestation, pour étudier les effets de l'irradiation sur l'œuf et sur le premier stade de l'état larvaire. Des fruits contenant des larves entièrement développées et des larves à des états intermédiaires ont également été soumis à des doses de même ordre. On a pratiqué des examens fréquents et la dissection des fruits pour déterminer la mortalité des larves, les dommages causés aux fruits par les larves, la présence de pupes et celle de mouches adultes.

A la dissection de fruits qui avaient reçu des doses de 5000 rads alors qu'ils contenaient des œufs ou des larves encore incomplètes, on n'a constaté ni présence d'insectes, ni traces de dommages causés par les insectes. Dans des fruits infestés de larves adultes, il y a formation de nombreuses pupes, mais il n'est pas sorti de mouches adultes des pupes recueillies dans les fruits qui avaient reçu une dose de rayonnements de 5000 rads ou davantage. On en conclut qu'une dose de rayons gamma de 5000 rads (peut-être même moins, à en juger par quelques essais effectués avec des doses de 2000 rads) arrête le processus de formation de la mouche à fruit mexicaine, bien que les larves puissent survivre à des doses sensiblement plus fortes.

Le mémoire propose un modèle de wagon-irradiateur gamma destiné au traitement des fruits infestés.

Действие радиации на яйца и личинки мексиканской фруктовой мухи в грейпфруте. Были проведены ограниченные исследования действия гамма-радиации кобальта-60 на яйца и личинки мексиканской фруктовой мухи. Плоды были облучены различными дозами от 5000 до 90000 рад через один и 12 дней после их заражения для исследования действия облучения на яйцо и первую скрытую стадию развития личинки. Аналогичными дозами были облучены плоды, содержащие полностью развившиеся личинки и личинки, находившиеся на промежуточных стадиях развития. Частое исследование и рассечение плодов использовалось для определения процента погибших личинок, ущерба, нанесенного личинками плодам, наличия куколок и взрослых мух.

В рассеченных плодах, получивших дозы облучения в 5000 и более рад при наличии в них яиц или личинок в первой скрытой стадии развития, не оказалось насекомых, а также следов

нанесенного ими ущерба. Из плодов, зараженных взрослыми личинками, было получено много куколок, однако куколки, изъятые из плодов, которые были облучены дозами в 5000 и более рад, не произвели взрослых мух. Вывод состоит в том, что гамма-облучение в 5000 рад (а возможно и меньшая доза, о чем делается вывод на основании ограниченных опытов с использованием облучения в 2000 рад) нарушает жизненный цикл мексиканской фруктовой мухи, хотя ее личинка может переживать и более значительные дозы облучений.

Предлагается схема передвижного гамма-излучателя для использования на железных дорогах с целью обработки зараженных плодов.

Efectos de las radiaciones sobre los huevos y larvas de la mosca mejicana de la fruta en las toronjas. Los autores han estudiado el efecto de las radiaciones gamma del cobalto-60 sobre los huevos y larvas de la mosca mejicana de la fruta. Irradiaron toronjas 1 y 12 d después de la infestación con dosis comprendidas entre 5000 y 90000 rad con el propósito de explorar los efectos de la irradiación sobre los huevos y las larvas en la primera etapa de su desarrollo. También irradiaron con dosis del mismo orden de magnitud frutas que contenían larvas en estados intermedios, así como completamente desarrolladas. Mediante el examen frecuente y la disección de las toronjas, observaron la mortalidad de las larvas, los daños causados por éstas y la presencia de ninfas y de moscas adultas.

Al disecar frutas que habían recibido dosis de 5000 rad o más cuando contenían huevos o larvas en la primera etapa de su desarrollo, no se observó la presencia de insectos ni vestigios de daños. En las frutas infestadas por larvas adultas, se formaron ninfas en cantidad elevada, pero no salieron moscas adultas de las ninfas procedentes de las toronjas irradiadas con dosis de 5000 rad como mínimo. Se deduce que una dosis de 5000 rad (y quizá inferior, a juzgar por los resultados de algunos ensayos realizados con dosis de 2000 rad) interrumpe el ciclo vital de la mosca mejicana de la fruta, aunque las larvas pueden sobrevivir a dosis considerablemente más elevadas.

En la memoria se propone un modelo de irradiador gamma montado sobre vagón de ferrocarril, destinado al tratamiento de frutas infestadas.

I. The problem of Mexican and Mediterranean fruit-flies in the United States of America

The Mexican fruit-fly (*Anastrepha ludens*) is indigeneous to Mexico and southern Texas near the USA-Mexican border. This fly infests a variety of soft fruits but is particularly troublesome in citrus fruits which are a valuable farm crop in southern Texas. Fig. 1 shows an enlarged photograph of a male Mexican fruit-fly on a grapefruit. A variety of control techniques are used; spraying the fruit-producing area with chemical insecticides destroys insects in the mature fly stage. However, it is not possible to eradicate or completely control the fly in southern Texas by this process because the adult flies migrate into Texas from Mexico. A large co-operative effort by both countries might bring this fly under better control.

A long application of low-temperature steam is used on citrus fruits grown in infested areas and intended for shipment to other areas. This steaming process destroys the eggs which are deposited on the skins of the fruit by the adult flies.

If infested fruits are not treated by a suitable process to destroy the eggs, the eggs hatch in about one to two weeks to produce larvae which feed on the fruit. The larvae bore exit holes in the infested fruit, permitting the entrance of other organisms such as molds and yeasts which produce secondary damage. When mature, the larvae emerge from the exit holes. In the case of fruit in the field, the larvae drop to the ground and enter the pupal stage. Fig. 2 shows an infested fruit, with exit holes indentified by rings. Note the larva emerging from one of the exit holes.

Many other important fruit-growing areas in the United States such as southern California, are at present free from this infestation. Rigid quarantines and inspection procedures are used to prevent the spread of this pest. These quarantines may be raised only if the fruits are treated by a process such as vacuum fumigation, steaming, or possibly in the future by irradiation

so as to assure complete destruction or biological sterilization of all eggs and larvae in fruit from infested areas. Vacuum fumigation is an expensive batch process. Low-temperature steaming requires considerable time if the minimum temperature is used to kill the eggs and larvae. Use of higher temperatures shortens the time but results in excessive damage to the fruit and appreciable loss in ascorbic acid (Vitamin C). An irradiation treatment can be a

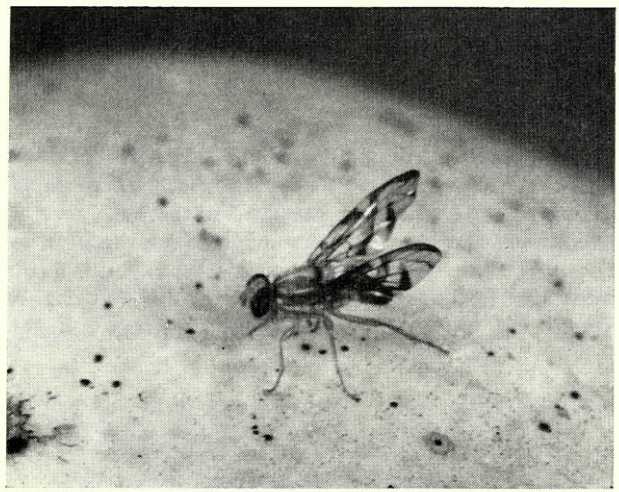

Fig. 1
Enlarged photograph of male Mexican fruit-fly (*Anastropha ludens*) on grapefruit

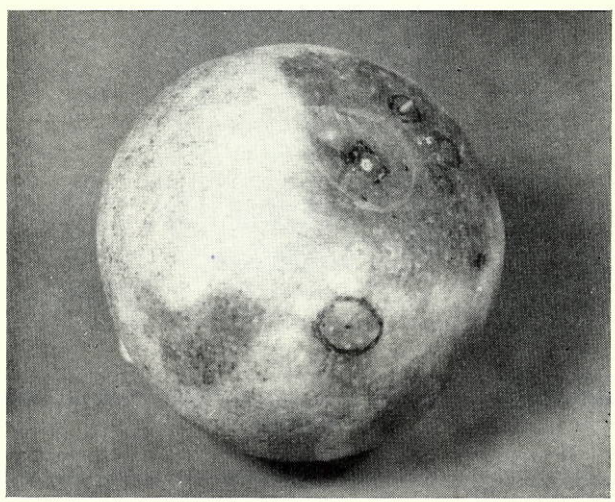

Fig. 2
Larva of Mexican fruit-fly emerging from upper exit-hole (holes are ringed) in infested grapefruit

rapid continuous process which may offer many advantages over vacuum fumigation or steam treatment. For this reason, limited tests were conducted at the University of Michigan on the effects of gamma-radiation on the eggs and larvae of this fly, using grapefruit as the host.

A very similar pest, the Mediterranean fruit-fly (*Ceratitis capitata*), has recently infested the citrus fruit-growing areas in Florida. Vigorous, extensive and expensive control measures using fumigation, aerial spraying, insect traps, quarantines, road blocks, and confiscation of fruit finally brought this infestation under control. If irradiation techniques had been available this task might have been easier and less expensive.

II. Description of experimental radiation source

The gamma-radiation source used in these studies consisted of 100 rods of metallic radiocobalt, each $\frac{1}{4}$ inch in diam. and 10 inches long, jacketed in aluminium to prevent corrosion [1] [2]. These rods were neutron-irradiated in the NRX reactor at Chalk River, Ontario. The 100 rods were arrayed in an aluminium rack to form a hollow cylinder, as shown in Fig. 3. The central axial space receives the highest radiation flux (about 200000 r/h at the time these tests were made) and will accommodate a commercial No. 10 can (diam. $6\frac{3}{16}$ in and length 7 in). The source is used in a "radiation cave" as illustrated in Fig. 4.

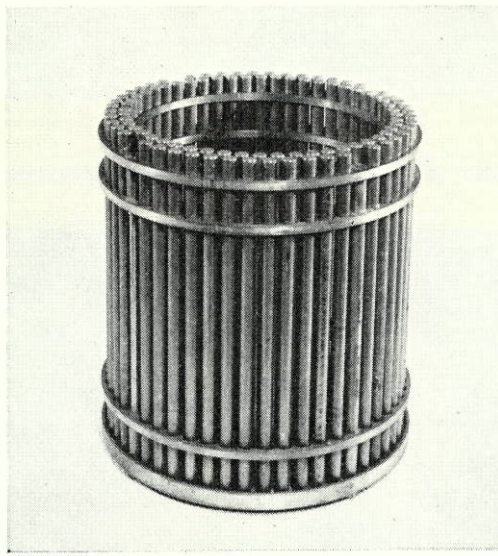

Fig. 3
Photograph of model of 3000-c cobalt-60 source used in Fission Products Laboratory, University of Michigan

When the source is not in use, it is stored under 10 ft of water. Fig. 5 shows the rods in the storage position under water, as photographed by the light of the Cerenkov radiation. The water shielding enables the operator to enter the cave for the purpose of arranging experimental apparatus or placing samples around the source position without experiencing

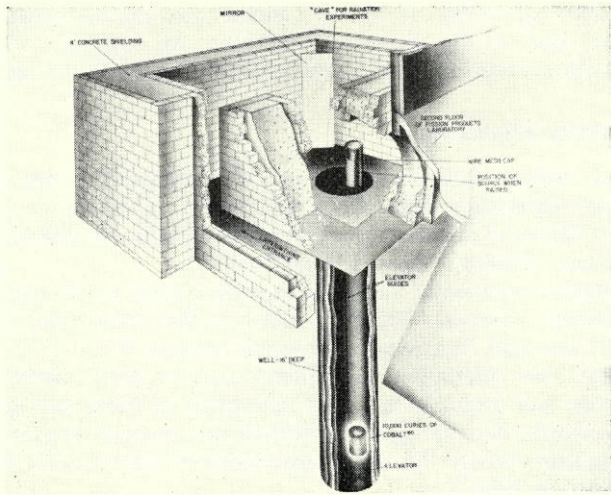

Fig. 4
Radiation "cave" showing labyrinthine passageway, water storage well, shielding and viewing mirror

significant exposure to radiation. Only when the operator has left the cave, and closed and bolted the entrance door, can the source be raised into its operating position in the cave. The source is raised by a hand-operated winch located outside the cave in the laboratory. When the source is in the operating position, a mechanical interlock prevents the entrance door from being opened.

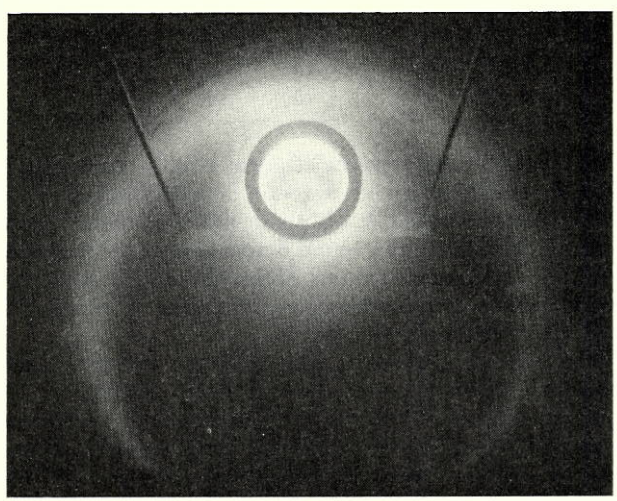

Fig. 5
Gamma-radiation source under water taken by the light of the Cerenkov radiation

A concrete barrier-wall in the cave forms a simple labyrinthine passage that permits the use of an ordinary door at the entrance to the cave. An opening in this door and two 6-ft-high mirrors allow observation of experiments in progress in the radiation cave.

III. Study of irradiated infested fruits

Texas grapefruit infested with the eggs of the Mexican fruit-fly were obtained from the US Department of Agriculture. These fruits were stored on beds of sand in special insect cages to develop a colony of adult fruit-flies. Fresh grapefruit were placed in cages with the adult flies to produce infested fruit used in the tests.

The effect of gamma-radiation at the egg and the first-instar larval stage was investigated, using gamma-radiation treatments between 1 and 12 d after infestation. At the temperature at which the fruit was kept, egg eclosion occurred between 6 and 12 d after oviposition. Within the 12 days after infestation, the gamma-radiation acted mainly at an advanced embryonic-egg stage and probably upon few specimens of first-instar larvae. Beyond the 12-d period, the majority of viable eggs were eclosed and some larvae were further than their first instar. The dosages tested at this developmental period were 2, 5, 10, 20, 30 and 90 krad. The results are listed in Table I.

TABLE I

DATA ON GAMMA-IRRADIATED GRAPEFRUIT INFESTED WITH THE MEXICAN FRUIT FLY (AT EGG AND FIRST-INSTAR LARVAL STAGE)

Radiation dosage (krad)	Number of fruit	Fruit with insect damage	Pupal recoveries	Flies emerging
0 (control)	52	27	151	73
2	10	1	0	0
5	13	0	0	0
10	32	0	0	0
20	13	0	0	0
30	33	0	0	0
90	13	0	0	0

Insect damage or infestation and presence of pupae and fly emergence were evaluated by frequent observations and dissection of the fruit in checking larval damage or larval mortality in fruit that did not exhibit exit holes or did not present natural recoveries. As shown in Table I, the 5, 10, 20, 30 and 90-krad dosage-groups did not show the presence of insects nor any trace of insect damage in any of the samples dissected. In comparison, the control-groups related to these dosages showed an infestation of almost 52%, an abundance of pupal recoveries, and a 48.3% fly emergence.

The experiments at this developmental stage involved 166 grapefruit, all exposed to similar conditions of infestation. Only 52 fruit, randomly separated as control, were not irradiated. They proved to be the only group with infested fruit on which the normal insect cycle was completed with subsequent fruit damage. From the 114 fruit that were irradiated at various dosages, no further insect development nor damage were found, with the exception of one fruit at the 2-krad dose, which exhibited damage and two dead larvae inside. Fig. 6 shows a half-section of an irradiated fruit on the left and a half-section of a control-fruit on the right. Note the secondary damage in the control-fruit.

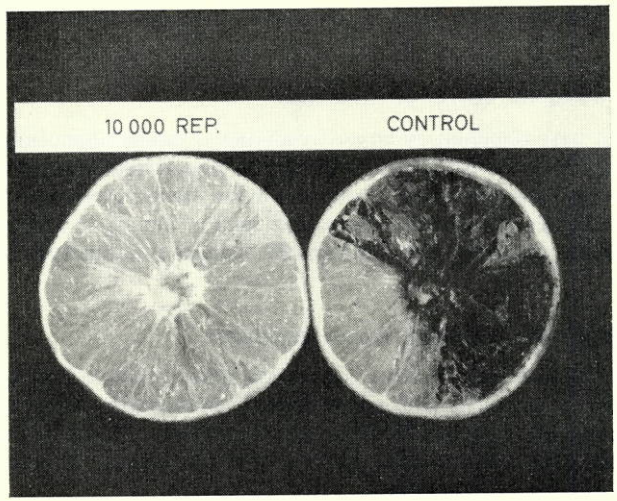

Fig. 6
Half-section of irradiated infested fruit (on left) and a half-section of a non-irradiated infested control-fruit (on right). Note secondary damage in control-fruit

The effect of gamma-irradiation of full-grown larvae was also investigated. It was possible in this case to deliver radiation with a certain degree of accuracy within the developmental period and to apply radiation to fruit known to be infested. Infestation of fruit was carried out as in the developmental stages. In this set of experiments, radiation was applied at the time when exit holes were present and only to fruit showing these exit holes.

The fruit with exit holes were allocated randomly between the control and dosage groups. The doses tested were slightly different from those of the previous experiments. It was expected that a more differentiated dose would provide a better basis for evaluating results. Doses of 5, 15, 45 and 70 krad were used; results are listed in Table II.

TABLE II

DATA ON GAMMA-IRRADIATED GRAPEFRUIT INFESTED WITH THE MEXICAN FRUIT FLY (AT MATURE LARVAL STAGE)

Radiation dosage (krad)	Number of fruit	Fruit with insect damage	Pupal recoveries	Flies emerging
0 (control)	19	19	277	174
5	10	10	82	0
15	10	10	53	0
45	10	10	27	0
70	6	6	12	0

Table II shows that many pupae recovered when the mature larvae were irradiated, whereas no pupae survived when the irradiation was performed in the egg or first-instar larval stage, as is shown in Table I. However, none of the pupae from irradiated fruit

developed flies, whereas about 60% of the pupae from the control fruit produced flies (*cf.* Table II).

IV. Conclusions about the effects of irradiation

Additional data were obtained on larvae irradiated at intermediate stages and on studies of dissected fruit. The conclusion was made that a gamma-radiation dose of 5 krad (and possibly less, based on limited tests at 2 krad) will break the life-cycle of the Mexican fruit-fly, although the larval stage may survive appreciably greater dosages. Taste-panel tests showed no changes in flavour, nor any other undesirable changes in grapefruit irradiated at up to 30000 rad.

V. Conceptual design for a railway mobile irradiator

The use of a mobile unit can be more economical than a built-in irradiation facility when the material to be irradiated is seasonal in nature. Rather than remaining idle for many months between seasons, a mobile facility might be moved to Texas or Florida to irradiate fruit; to Idaho or Maine to irradiate potatoes; to Washington, Oregon or Michigan to irradiate apples; to Kansas or Minnesota to irradiate grain.

A conceptual design is presented for a railway mobile gamma-radiation-facility that could be moved to various citrus-shipping areas in Florida and Texas to sterilize fruit shipped from infested areas [3]. The proposed railway gamma-irradiation-facility is shown in section in Fig. 7. The estimated capacity of this facility is 10 t/h with a 10000-rad dose. This production is accomplished through efficient absorption of gamma-photons, and use of a simple, flexible conveying-system. The cost was minimized by using hot-rolled mild-steel mill-plate, 2-in thick, for shielding. Shielding thickness is varied from 4 to 16 in by varying the number of plates with the radiation field. The steel shield is used to carry the car-load, eliminating the structural undercarriage in the car. Double trucks are attached directly to the steel shield which acts as the car frame. The total car weight is estimated to be 173 t. Radiation sources are kept in two water-cooled lead containers weighing about 4 t each and located on either side of the car. A gear box on the conveyor-drive permits variation of conveyor speed to allow delivery of dosages from a few thousand rad to several million rad.

To operate the facility, the sources of radiation must first be installed. The shipping containers are water-cooled and are supplied with a reserve tank of make-up water to replace that lost by evaporation during shipment to the mobile facility. The shipping containers "U" indicated in Fig. 7 are loaded into shield "Q". A handwheel and crank are connected to provide the mechanism to raise the source doors and to move the source elements out of the shipping container.

When the car is on location, the source doors are raised and sources "S" withdrawn from lead containers "V", making the facility operative. Fruit or other material to be irradiated is brought in *via* conveyor "A" from an adjacent warehouse, processing plant or grain elevator and is fed into buckets at point "B". The conveyor in the car carries the material from point "B" past and over shield "C", under shield "D" over shield "E" into radiation zone "F". After travelling the length of the upper pass "F", a turn is made about sprocket "G" and a lower pass "H" is made directly over sources "S". A turn is made around sprocket "I" and two identical passes "J" and "L" are made below the source passing around sprocket "K". Thus the material is first irradiated from the underside of the buckets in two passes and then from the top side of the buckets in two identical passes to equalize the dosage on top and bottom of the conveyor buckets. The material leaves the radiation zone, passing under shield "E", over shield "M" and under shield "C", travelling up incline

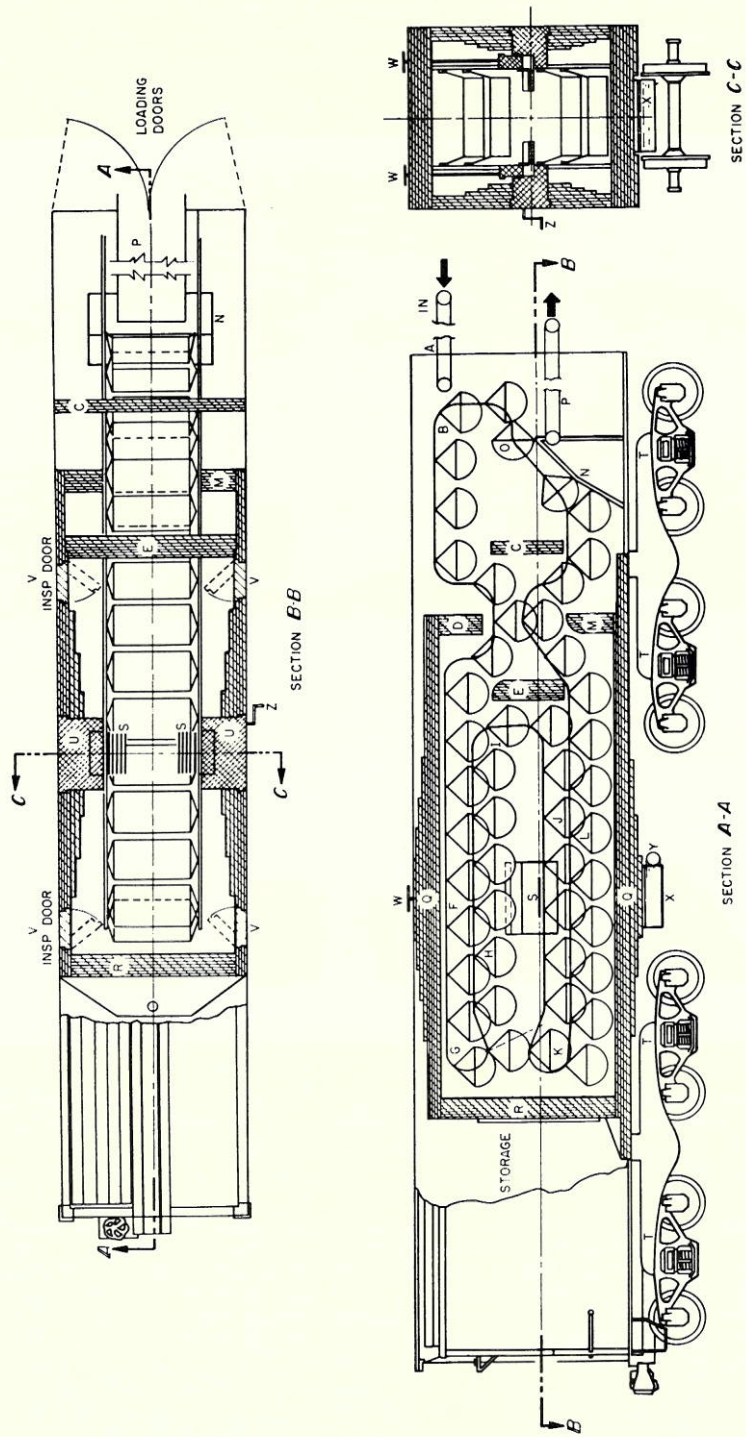

Fig. 7
Sectional views of proposed railway mobile irradiator [3]

"N" where a guide-cam tilts the bucket and empties it at point "O". Conveyor "P" returns the irradiated material to the warehouse or processing plant.

A model of the proposed car has been built as shown, by courtesy of American Motors Inc., in Fig. 8.

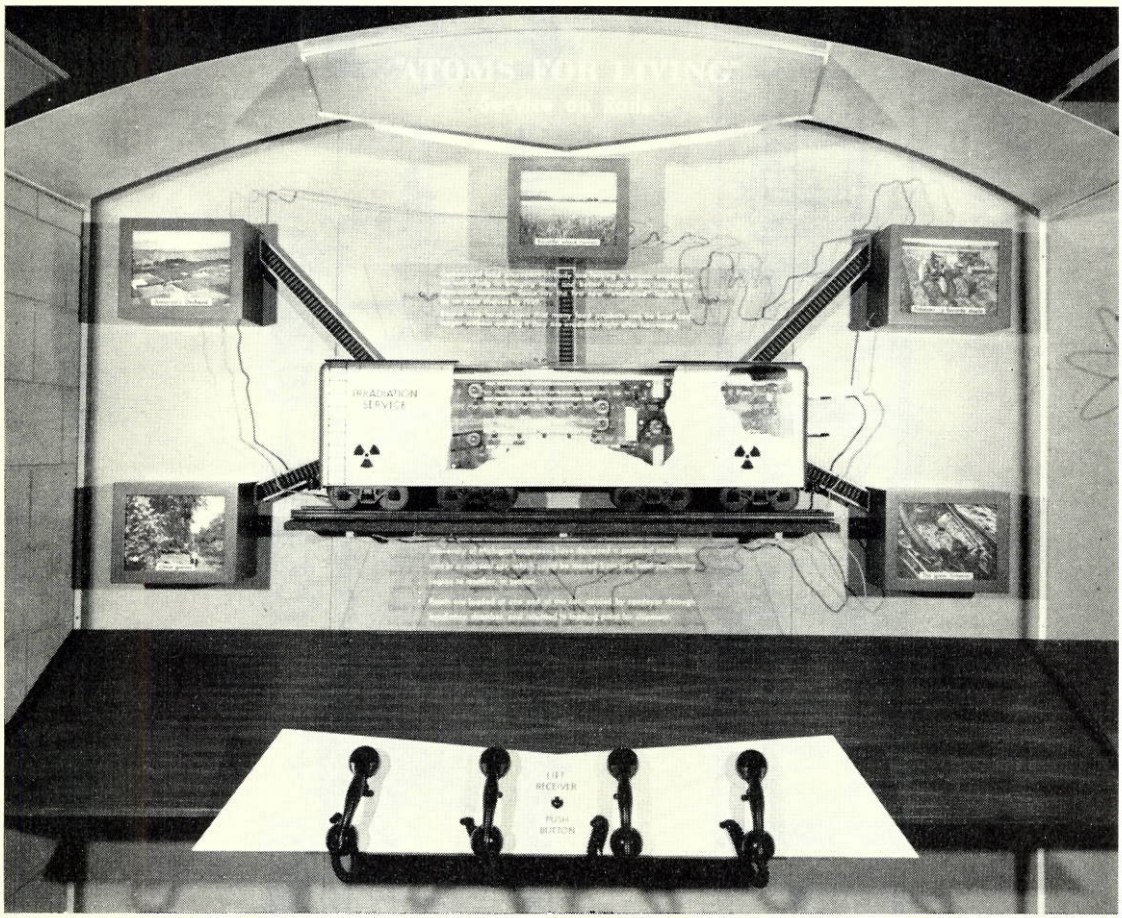

Fig. 8

Model of mobile irradiation-facility on rails—a railway car which would be moved from one food-producing area to another for mass irradiation (Telephones give recorded explanation in English) (Courtesy American Motors Inc.)

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[2] BROWNELL, L. E. *et al.*, *Chem. Eng. Progr.* **49** (1953) 569; NEHEMIAS, J. V. *et al.*, *Am. J. Phys.* **22** (1954) 88; NEHEMIAS, J. V. *et al.*, *ibid.*, **22** (1954) 511.

[3] BROWNELL, L. E. *et al.*, "Design of a Railway Mobile Gamma Source for Industrial Irradiations", Progress Report No. 1943: 7-67-P, Eng. Res. Inst., The University of Michigan, Ann Arbor, Mich. (1956).

DISCUSSION

THE CHAIRMAN (S. V. Andreev, USSR): There are two or three questions which I should like to put to Dr. Brownell. First, I should like to know what kind of source, and of what strength, he would use in his proposed vehicle? Secondly, what does he estimate the treatment-capacity of the plant to be? And thirdly, did he observe any change in flavour of fruit after irradiation at 5000 rep?

L. E. BROWNELL: In answer to your first question, the source originally designed for this vehicle consisted of ten reactor fuel elements from the NTR reactor. Their strength varies with age. During recent studies, however, we have found that a slight amount of induced radioactivity is caused when fuel elements are used to irradiate fruit. At present we would therefore suggest cobalt as a source, and the amount of radiation required depends upon the capacity of the plant. Ten reactor fuel elements would have an activity of about two million curies.

With regard to your second question, capacity does, of course, depend a good deal on how efficiently the radiation is used, but to give you an idea, the installation might have a capacity of 10000 lb/h if we used a 3000-rad dose, for example.

Replying to your third query, there was no flavour-change in the citrus fruit up to doses of 25000 rad, at which level a very slight change was detected by some taste-panel observers. Most observers, however, detected no flavour-change until 50000 rad were reached.

A. R. GOPAL-AYENGAR (India): Would you not obtain a non-uniform dose using spent fuel rods as opposed to a cobalt gamma-source?

L. E. BROWNELL: That is quite correct; using spent fuel rods one would need a variable speed conveyor-system. Working with a total of ten fuel rods we suggested substituting two rods every month to maintain the activity, and varying the speed of the conveyer system according to the level of activity of the rods. In the case of cobalt, which has a much longer half-life, this problem would of course be much less serious.

K. K. NAIR (India): I should like to know whether the grapefruit you irradiated were fully ripe or in process of ripening. If the latter was the case, did you observe any change in ripening properties as between the control and the irradiated fruit?

L. E. BROWNELL: Well, the grapefruit we received from Texas was the infested fruit, but we raised our colony of insects and then bought fresh fruit on the open market. We did not know its age, but it was ripe, ready for eating. This fruit was placed in the insect cages to become infested.

V. K. SASTRY (India): Have any studies been made on carbohydrate change as a result of irradiation?

L. E. BROWNELL: Yes. The numerous studies that have been made over a number of years show that carbohydrates undergo breakage as a consequence of irradiation. Cellulose, for example, breaks down into dextrins and eventually glucose, and after vegetables have been subjected to large radiation doses an increase in sweetness as a result of this breakage has been observed. However, carbohydrates, when irradiated, usually do not acquire an objectionable flavour, and for this reason primarily carbohydrate fruit is quite free from irradiation flavour-changes, even after high doses.

G. KRISHNAMOORTHY (India): Is there any residual protective effect, so to speak?

L. E. BROWNELL: No, there is no residual protective effect, so that fruit that has been sterilized by irradiation must be protected against re-infestation. Our scheme was to irradiate

fruit ready for transport from a growing area to another area free from the pest, and thereby to prevent the spread of the pest. You see—and this is something the importance of which is not always appreciated—we can do something with radiation that we cannot do with insecticides, namely, guarantee 100% destruction of the pest, including eggs, larvae and pupae. Thus, provided we then store the sterilized product in insect-proof containers, protection against insect infestation lasts indefinitely. The difficulty is, of course, in storing many products, such as grain, in insect-proof containers, and where we do not do so the enormous initial benefit of 100% sterility is lost. The aim must therefore be to store the product in insect-proof containers in areas where re-infestation is possible.

P. B. CORNWELL (United Kingdom): There are a number of points I should like to ask Dr. Brownell. He mentioned that he was seeking, or has already obtained, approval for the use of doses of 5 Mrad in food. May I ask first of all whether this is in respect of a specific commodity or of a variety of commodities?

L. E. BROWNELL: The dose of 5 Mrad is that required to ensure protection of canned meats or any protein substance against botulinum. Actually, a safety factor is here included, the precise dose as determined being 4.6 Mrad. The 5-Mrad dose is thus equivalent to thermal sterilization of protein foods.

The United States Army has carried out extensive studies on irradiation of foodstuffs with the idea of improving the quality of food supplied to the armed forces in areas where it is difficult to obtain commodities locally. Since irradiation can sterilize without cooking, it is possible to prepare certain food items of better quality than when using the normal canning methods.

I should finally mention, of course, that for purposes of radio-pasteurization much lower doses could be used.

P. B. CORNWELL: If food thus irradiated is in fact going to be supplied to the armed forces, may we assume that the recipients will tolerate any unusual flavours produced by this high dose?

L. E. BROWNELL: We have worked for several years on this problem. Among our experiments was one carried out two months ago, when I ate a steak irradiated at 5 Mrad—and left none of it. We have solved some of the problems of flavour-change in certain fruits. It is true that the proteins are very susceptible to flavour-change, but we have found various means of meeting this difficulty. All the basic studies and practical experiments on one particular food-item have been completed, and the US Army Quartermaster Corps has requested the Surgeon General's Office and the Food and Drug Administration to grant approval for irradiation of this product. We believe that this approval will be granted, but there is delay due to the need for a re-interpretation of the present law, which lays down that no action may be taken which results in an increase in the radioactivity of any food. This is the reason why we stopped using fuel elements, and also why we do not look with favour upon the use of high-voltage electronic accelerators. Now although no one, even with the help of the most sensitive equipment available, has ever been able to measure any induced radioactivity caused by irradiation with cobalt-60, physical calculations showed that there is in fact some activity so induced. The amount is so small, however, that even if you ate irradiated foods all your life, your exposure might be increased by the insignificant extra amount involved in moving from say Chicago to Denver, i.e. to a higher altitude. As I was saying, therefore, we are awaiting an interpretation of the law to the effect that zero shall be deemed to be zero for practical purposes, and not theoretical absolute zero.

H. HUQUE (Pakistan): Did radiation have any effect on the vitamin content of the fruit?

L. E. BROWNELL: Vitamins are susceptible to radiation damage, ascorbic acid being particularly susceptible. In fruit it is, however, partly protected by other free radical acceptors. Thus, at a dose of 25000 rad there is no appreciable loss of vitamin C content. Vitamin E, tocopherol, is also susceptible to radiation damage, being easily oxidized, and we did an experiment at the University of Michigan to find out if vitamin E loss was being caused by irradiation. We fed a colony of animals for four generations with wheat which had been irradiated at 10000 rad for insect-control purposes. The animals' sole source of vitamin E was the whole wheat, and as you know, any shortage of vitamin E interferes with reproduction. However, we had normal reproduction and excellent growth, with the animals altogether very healthy.

P. B. CORNWELL: In the railway wagon irradiation-plant Dr. Brownell has described, is the fruit subjected to rolling or is it placed in some sort of container? If the former, is there likely to be a problem of damage, as does in fact occur when potatoes are rolled during irradiation?

L. E. BROWNELL: As I explain in detail in my second paper (*These proceedings*, p. 233), we have in many of our designs preferred to use the bucket-type conveyor which pivots under the force of gravity as it goes first over the source and then down and under it. By irradiating the product thus from both sides one obtains a more uniform dose-distribution, and obviates the need for any mechanical turning-over operation. The buckets would be loaded as shown in the railroad car by means of a rubber belt-conveyor. The fruit would not be rolled, but of course it would have to be fed into the buckets. With potatoes, which cannot be dropped more than six inches without damage, there might be a difficulty here, but with citrus fruit we think that there would be no damage.

A. R. GOPAL-AYENGAR: How economically feasible do you believe the use of insect-proof containers to prevent re-infestation to be? Perhaps you could elaborate on the techniques and methods you would employ?

L. E. BROWNELL: Actually this is also a thing I discuss in my second paper. I refer there to the use of both insect-proof elevators and storage bags. If we sterilize the contents of an insect-proof bag this product can be kept free from insect damage for an indefinite period, and a watch must be kept for chemical changes rather than for any others. The same results can be obtained by keeping irradiated grain in an insect-proof elevator. I hope that the elevators which I understand India is now building will not embody the errors of design all too prevalent in the United States. I have spent considerable time discussing with what I believe is the largest grain-handling company in the world—they handle $ 4000 million worth of grain annually—the feasibility of using radiation at their grain terminals. At present, up to $ 50000 a year are spent at a terminal on insecticides. Although in the North—where admittedly most of the grain storage is done —we are free from insect hazards during the winter months because of the low temperatures, in the South fumigation and turning may be necessary three times a year or more, with the annual cost of the insecticide alone ranging from a tenth of a cent to about one cent per bushel. Long-term grain storage, therefore, can become an expensive proposition. Hence, irradiation might well be cheaper for long-term storage purposes if we could treat the grain for a cost in the order of one to two cents per bushel. It would certainly be cheaper than refumigating every few months in warm climates.

P. B. CORNWELL: I wonder if Dr. Brownell is aware of the work going on in the Australian Atomic Energy Commission at the moment on the irradiation of citrus fruits to control dissemination of fruit-fly? In Australia they have come, tentatively, to the same conclusion—

that a dose of 5 Mrad is sufficient to inhibit the emergence of adults and that, in fact, this dose produces no adverse effect on the commodity. This is an application which was felt, as the result of a recent survey of insect problems in that country, to be one of the most promising, in that the present marketing arrangements, involving centralized handling for export, provide the ideal framework for irradiation treatment; a mobile radiation plant might also ease the strict quarantine regulations governing the distribution of citrus across interstate boundaries.

L. E. BROWNELL: I am very interested to hear of this development.

PRELIMINARY STUDIES ON THE EFFECTS OF GAMMA-RADIATION ON HOUSEFLY PUPAE WITH SPECIAL REFERENCE TO THE CRITICAL PERIODS IN RELATION TO THE MECHANISM OF EMERGENCE

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Abstract — Résumé — Аннотация — Resumen

Preliminary studies on the effects of gamma-radiation on housefly pupae with special reference to the critical periods in relation to the mechanism of emergence. Studies on the radiation sensitivity of housefly pupae in different stages of development have been carried out with special reference to the mechanism of emergence. The different dose levels employed were 500 r, 1000 r, 2000 r, 2500 r, 5000 r and 10000 r. The data obtained on percentage emergence in each group indicated that the early stages of development were most sensitive to radiation, since a dose of 2000 r and above applied to 5-h-old pupae resulted in no emergence at all, while the same doses applied to 30—80-h-old pupae did not appreciably affect the mechanism of emergence in these groups. Development in the 2—5-h-old irradiated pupae was found to be complete, but the flies failed to emerge. The significance of the findings is discussed.

Études préliminaires sur l'irradiation gamma de la pupe de la mouche commune, notamment pendant les périodes critiques, et de ses effets sur le processus d'éclosion. On a étudié la radiosensibilité de la pupe de la mouche commune au cours des différentes phases de son développement, notamment les effets des rayonnements sur le processus d'éclosion. Les doses d'irradiation étaient de 500 r, 1000 r, 2000 r, 2500 r, 5000 r et 10000 r. Les données recueillies sur le pourcentage d'éclosions dans les différents groupes montrent que la radiosensibilité est plus élevée au début de l'évolution puisque l'application de doses d'au moins 2000 r à des pupes âgées de 5 h a supprimé toute éclosion, alors que l'application des mêmes doses à des pupes âgées de 30 à 80 h n'a pas eu d'effet sensible sur le processus d'éclosion. On a constaté, en outre, que les pupes irradiées lorsqu'elles avaient 2 et 5 h parvenaient à leur plein développement, mais sans donner naissance à des mouches. L'auteur étudie la portée de ces constatations.

Предварительные исследования в области воздействия гамма-радиации на личинку комнатной мухи с особым уклоном на критические периоды, связанные с механизмом появления личинки. Проводились исследования в области чувствительности к радиоактивному облучению личинки комнатной мухи на разных стадиях развития с особым уклоном на механизм появления личинки. Применялись следующие различные дозы: 500, 1000, 2000, 2500, 5000 и 10000 рентген. Полученные данные о проценте выводящихся личинок в каждой группе показали, что ранние стадии развития наиболее чувствительны к радиации, так как доза в 2000 рентген и больше, примененная к личинкам в возрасте 5 часов, прервала дальнейшее развитие личинки, в то время как те же самые дозы, примененные к личинкам в возрасте от 30 до 80 часов не оказали заметного влияния на личинки в этих группах. Более того, наблюдалось также, что в случае с личинками в возрасте 2 часа и 5 часов развитие облученной личинки было полным, однако мухи не рождались. Рассматривается значение этих выводов.

Estudio preliminar de los efectos de las radiaciones gamma sobre las larvas de la mosca común, particularmente en los períodos críticos para la eclosión. Se ha estudiado la radiosensibilidad de las larvas de mosca común, en diversas etapas de su desarrollo, dedicándose particular atención al proceso de eclosión. Las dosis de radiación utilizadas fueron de 500, 1000, 2000, 2500, 5000 y

10000 r. Los datos relativos al tanto por ciento de eclosión en los diferentes grupos indican que los organismos presentan mayor radiosensibilidad en las primeras etapas de su desarrollo, puesto que la aplicación de una dosis del orden de 2000 r a larvas de 5 h impidió totalmente su eclosión, mientras que la irradiación de larvas de 30 a 80 h con dosis similares no influyó sensiblemente sobre el proceso de eclosión en estos grupos. Además, se observó que si bien el desarrollo de las larvas de 2 h y de 5 h irradiadas fue completo, las moscas no aparecieron. El autor analiza el significado de estos hechos.

I. Introduction

The effects of ionizing radiations on insects have been adequately reviewed by HILCHEY [1]. It is known that radiations can cause immediate death in a stadium or bring about noticeable deleterious changes in the later stages of development. It is not uncommon to find retardation of development as a consequence of these changes. An example of this effect is the delay of pupation in the irradiated larvae of *Drosophila melanogaster* [2—4]. BOURGIN *et al.* [4], by using a variety of experimental conditions, have attributed this delay of pupation to radiation damage to some target-organ in the anterior third of the body, most likely the ring gland. As an example of long-term effects of radiation may be mentioned the work of DAVIS *et al.* [5] on the pupae of the mosquito, *Anopheles quadrimaculatus*. The present work was undertaken with a view to studying the effects of different doses of gamma-radiation on housefly pupae at different stages of development, with particular reference to the mechanism of adult emergence.

II. Material and methods

Pupating third-instar larvae of the housefly, *Musca domestica* L., were removed from the larval containers and allowed to pupate in beakers containing moistened cotton. The age of the pupa was calculated from the time at which the puparium became pigmented. About 50 pupae from each of the 5, 10, 20, 30, 50, 60 and 80-hour-old groups were exposed to gamma-radiation from a cobalt-60 source. The doses employed were 500 r, 1000 r, 2000 r, 2500 r, 5000 r and 10000 r. The dose rate was approximately 280 r/min. After irradiation the pupae were transferred to suitable containers for emergence. Each experiment was repeated four times. The number of adults emerged in each case was recorded as percentage of the number emerged in the controls.

III. Results and discussion

The results obtained are presented in the following table:

TABLE I

ADULT EMERGENCE EXPRESSED AS PERCENTAGE OF CONTROLS

Age of the pupae at the time of irradiation (h)	Adult emergence (%) after given dose (r)					
	500	1000	2000	2500	5000	10000
5	95	84	0	0	0	0
10	95	89	0	0	0	0
20	95	84	0	0	0	0
30	98	93	81	94	68	72
50	100	99	100	97	99	93
60	100	87	98	93	79	84
80	100	89	100	95	84	84

It is apparent that doses of 500 r and 1000 r did not appreciably affect the emergence of adults in any of the age groups studied. Marked lethal effects were observed only in the 5 to 20-h-old pupae exposed to doses of 2000 r or more. Complete failure of emergence occurred in all these groups. These results are somewhat different from those obtained by DAVIS *et al.* [5] in their studies on mosquito (*A. quadrimaculatus*) pupae of various ages exposed to a dose of 8865 r of cobalt-60 gamma-rays. They stated that, although irradiation did not significantly reduce the number of adults emerged, mortality of the adults after three days was highest among the groups exposed as 1-h and 4-h-old pupae. The response of the 5 to 20-h-old housefly pupae to gamma-radiation was different in that there was total failure of emergence when exposed to a dose of 2000 r or more. When these pupae were dissected out from the puparium and examined, it was observed that development and differentiation had proceeded as in the controls. Figs. 1A, 1B, and 1C show pupae 20, 70 and 96 h old, respectively. Fig. 1D shows a pupa 96 h old which was exposed

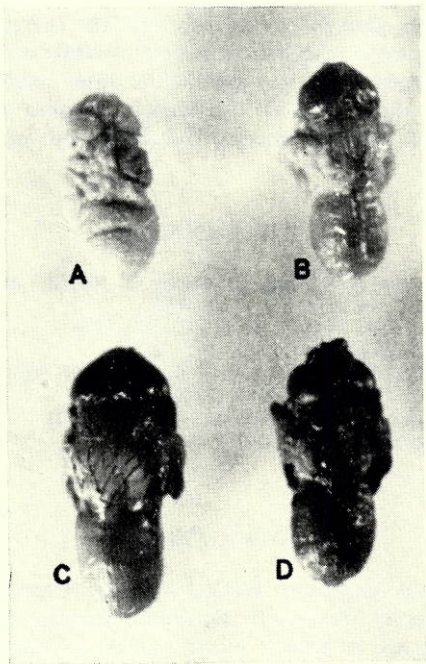

Fig. 1

Pupae of the housefly at various stages of development. A: 20-hour-old pupa; B: 70-hour-old pupa; C: 96-hour-old pupa; D: 96-hour-old pupa which was exposed to 2500 r when 5 h old

to 2500 r when 5 h old. Detailed examination of the external morphology of these specimens indicated that irradiation did not affect the eversion of the cephalic and thoracic imaginal discs. The wings and legs had undergone development to the same extent as controls.

The foregoing observations indicate that the imaginal discs which, by differentiation, give rise to the adult form, appear not to be susceptible to the levels of radiation used. Since unemerged pupae in irradiated and in control groups possessed identical morphological

features, the factors contributing to failure to emerge as a consequence of irradiation could not be ascertained from external morphology alone. Detailed investigations on the extent of anatomical and physiological damage in such groups are in progress.

Data on emergence obtained for the other age-groups show that there is a sudden drop in the radiosensitivity of pupae from the age of 30 h onwards. Since it is known [6] that susceptibility to gamma-radiation is inversely proportional to the degree of differentiation of the tissues, the higher percentage of emergence observed in these groups at all levels of radiation employed would imply that differentiation of tissues in the housefly pupae progresses rapidly from 20 to 30 h after pupation, at the end of which time most parts of the adult fly can be distinguished. Such a possibility had already been indicated [7].

IV. Summary

Studies on radiation sensitivity of housefly pupae of various ages exposed to different doses were carried out, with special reference to the mechanism of emergence. The data on adult emergence in the various groups indicated that the early stages of development (up to 20 h) were most sensitive to radiation. A dose of 2000 r applied to pupae 5 to 20 h old did not produce any emergence, whereas even higher doses applied to pupae 30 to 80 h old, had very little effect on the emergence mechanism. In spite of completed development, the flies of the first group failed to emerge. The significance of these findings is discussed briefly.

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DISCUSSION

P. B. CORNWELL (United Kingdom): While I was very interested to hear that Dr. Nair's results confirm those recently obtained by Donnelly, who also finds this marked transition in susceptibility with the age of blowfly pupae (*Lucilia sericata*), I feel that we should be a little careful here in the interpretation. I very much doubt whether the marked change in emergence is altogether connected with the degree of differentiation of the pupae, and one should bear in mind that there could be another, very simple, explanation. We know that when adult insects are irradiated there is a delay in response, a period of lethargy, and death may occur quite suddenly a few days after irradiation. Therefore, if we irradiate the pupal stage a sufficient number of days before emergence we might again expect delayed death, mortality occurring at just about the time of emergence. If we irradiate a considerable time before emergence, death will occur in the pupal stage. If we irradiate nearer the time of emergence, the adult will indeed emerge and death will supervene a few days afterwards. With irradiation we are also concerned here with the production of deformities; since, in fact, emergence depends on a certain amount of physical activity on the part of the

insect, emergence from the pupal case may be prevented either directly, by induced lethargy, or by deformity. Frequently, for example, we have observed the state of adult weevils half emerged from infected grain. In all, then, we shall expect a very marked transition in the pupal stage, not necessarily related to the degree of differentiation of the tissues but specifically related to the time when this irradiation is carried out in relation to the delayed-response period.

K. K. NAIR: Well, I still feel that this transition may be related to the degree of differentiation, and this has been confirmed by Wilson in his studies on rat embryos. When irradiating the embryos within the uterus itself he observed that the early stages of development were the more sensitive, the later stages being insensitive to both levels of radiation employed in his test. I would admit, of course, that this is only a suggestion, and I do not profess to know the actual mechanism by which radio-susceptibility is influenced by the degree of differentiation.

P. B. CORNWELL: In the comments you have just made you were referring to radiation effects on an embryo, the susceptibility of which, I would agree, is markedly affected by age. I also do not deny that there are changes in susceptibility in the pupal stage. It is just that I feel one should also take into account the physical factor of delayed response in interpreting experimental data.

THE CHAIRMAN (S. V. Andreev, USSR): Have you ever observed in your experiments any cases of stimulation or other changes of development in insects in their various stages—eggs, larvae or pupae?

K. K. NAIR: No.

THE CHAIRMAN: Although Dr. Nair refers to his studies as being of a preliminary nature only, I consider that they already throw a very interesting light on some important uses of radiation; I hope that he will continue these investigations in view of their practical significance.

THE EFFECTS OF CONTINUOUS AND FRACTIONATED DOSES OF GAMMA-RADIATION ON THE SURVIVAL AND FERTILITY OF *SITOPHILUS GRANARIUS* (*CALANDRA GRANARIA* L.)*

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Abstract — Résumé — Аннотация — Resumen

The effects of continuous and fractionated doses of gamma-radiation on the survival and fertility of *Sitophilus granarius (Calandra granaria* L.). Calculations have shown that many megacuries of Co^{60} are required to disinfest grain at 16500 rep, the dose level evaluated for commercial treatment, at 200 t/h, the minimal handling rate demanded by the trade. Maximum efficiency in the use of the radiation plant might be obtained with sources of lower curie strength to ensure continuous operation if the full dose for sterilization could be given in a process of repeated passes. The question arises as to whether many sub-lethal and sub-sterilizing doses will provide the same measure of control as continuous treatment.

Accordingly, an investigation was made into the effect of dose fractionation on the radiation susceptibility of the grain weevil, *Sitophilus granarius* (*Calandra granaria* L.). Comparison of fractionated and continuous treatment showed differences in survival and fertility which may be attributed to "recovery" of somatic and reproductive cells during the intervals between treatment. Differences in survival were obtained in all developmental stages, being particularly marked in pupae; recovery was obtained with intervals of 10 min and longer, the process being governed by the number of fractions, fractionated dose and interval temperature. Recovery in reproductive capacity was obtained in irradiated eggs, larvae, pupae but not in adults. Whilst these effects are manifest at low doses, fractionated treatment does not adversely affect the degree of control achieved at the commercial dose-level.

Effets de doses continues et de doses fractionnées de rayons gamma sur la survie et la fertilité du *Sitophilus granarius (Calandra granaria* L.). Les calculs ont montré qu'il faut un bon nombre de mégacuries de cobalt-60 pour désinfecter des céréales par une irradiation de 16500 rep, dose estimée nécessaire pour le traitement industriel à raison de 200 t/h qui est la cadence minimum exigée. On pourrait utiliser les installations d'irradiation avec le maximum d'efficacité tout en employant des sources moins intenses, s'il était possible d'administrer la dose totale, nécessaire à la stérilisation, par fractions successives. La question est de savoir si des doses répétées inférieures à la dose létale ou stérilisante assurent le même degré de désinfection qu'une dose forte unique.

On a donc étudié l'effet du fractionnement de la dose sur la radiosensibilité du charançon du blé (*Sitophilus granarius* [*Calandra granaria* L.]). La comparaison entre les effets du traitement fractionné et ceux du traitement continu a révélé une différence dans la survie et la fécondité de l'insecte, qui pourrait être due à une «guérison» des cellules somatiques et germinales durant les intervalles qui séparent les irradiations. Des différences dans la survie ont été observées à toutes les phases du développement de l'insecte, mais elles étaient particulièrement marquées chez les pupes. La «guérison» s'opérait lorsque les intervalles étaient de 10 min ou plus, ce processus étant influencé par le nombre et l'intensité des doses partielles ainsi que la température au cours des intervalles. La faculté de reproduction est reparue lorsque l'irradiation était limitée aux œufs, larves et pupes, mais les adultes irradiés sont restés stériles. Ces effets se manifestent sous l'action de faibles doses, mais le traitement fractionné n'a pas d'incidences défavorables sur le degré de désinfection, aux doses employées pour le traitement industriel.

* Full details of the experimental work presented in this paper are given in a report of the U.K.A.E.A. Research Group, A.E.R.E. R. 3503.

Влияние непрерывных и разделенных доз гамма-облучения на выживание и плодовитость *Sitophilus granarius (Calandra granaria* L.*)* Расчеты показали, что для промышленного уничтожения вредителей в зерне требуется большое число мегакюри кобальта-60 при 16500 фэр, (оза для коммерческой обработки) на 200 т/ч, что является минимальной экономически Выгоаной скоростью об работки. Максимальная эффективность радиационной установки может быть достигнута при наличии источника с меньшей мощностью в кюри для обеспечения непрерывности операции, если можно обеспечить полную дозу стерилизации в процессе повторяющейся обработки. Возникает вопрос, не обеспечит ли большое количество сублетальных и субстерильных доз ту же степень контроля, что и непрерывная обработка.

В соответствии с этим было проведено исследование разделения дозы на восприимчивость зернового долгоносика *Sitophilus granarius* (*Calandra granaria* L.) к облучению. Сравнение разделенной и непрерывной обработок показало наличие различий в выживании и плодовитости, что может быть объяснено „восстановлением" соматических и воспроизводящих клеток во время интервалов в ходе обработки. Различия в выживании наблюдались на всех стадиях развития, особено на стадии куколки; восстановление происходило с интервалами в 10 минут и более, процесс определялся числом делений, размером разделенной дозы и температурой во время интервала. Восстановление воспроизводящей способности наблюдалось в облученных яйцах, личинках, куколках, но не у взрослых особей. Хотя эти влияния являются очевидными при низких дозах, разделенная обработка не оказывает вредного воздействия на степень контроля, достигнутую при облучении коммерческими дозами.

Efectos de dosis continuas y fraccionadas de rayos gamma sobre la supervivencia y fecundidad del *Sitophilus Granarius (Calandra Granaria* L.*)*. Los cálculos efectuados por el autor demuestran que se requiere una fuente de ^{60}Co de muchos megacuries para esterilizar cereales con 16500 rep, esto es, una dosis que se considera apropiada para el tratamiento comercial, a razón de 200 t/h, que es el ritmo mínimo exigido en el comercio. Si la dosis total esterilizante pudiera administrarse en una serie de exposiciones repetidas, el rendimiento máximo de la instalación de irradiación se alcanzaría utilizando fuentes de pocos curies que permitan llevar a cabo un tratamiento continuo. Se plantea la cuestión de saber si muchas dosis inferiores a la letal y a la esterilizante producirán los mismos efectos que un tratamiento continuo.

En consecuencia, se han estudiado los efectos del fraccionamiento de la dosis sobre la radiosensibilidad del gorgojo de los cereales *Sitophilus granarius* (*Calandra granaria* L.). La comparación entre la irradiación continua y el tratamiento con dosis fraccionadas revela diferencias en la supervivencia y fecundidad de los insectos; estas diferencias pueden atribuirse a la "recuperación" de las células somáticas y reproductivas durante los intervalos que median entre las irradiaciones. Las diferencias de supervivencia se observaron en todas las fases del desarrollo, siendo más acusadas en las ninfas; se registraron recuperaciones con intervalos de 10 min y más largos, siendo factores determinantes del proceso el número de fracciones, la dosis fraccionaria y la temperatura durante los intervalos. La recuperación de la capacidad de reproducción se observó en los huevos, larvas y ninfas irradiadas, pero no en los adultos. Si bien estos efectos se manifiestan para dosis bajas, el tratamiento con dosis fraccionadas no afecta desfavorablemente al grado de esterilización obtenido con dosis comerciales.

I. Introduction

Investigations into the possible use of ionizing radiations for the control of insects in stored foodstuffs have been concerned primarily with the radiation susceptibility of specific pests, *Lasioderma serricorne* [1] [2], *Tribolium castaneum* [3], *Tribolium confusum* [4—6], *Trogoderma granarium* [7] and *Trogoderma sternale* [8]. At this Establishment, small-scale tests have been carried out on seventeen species infesting cereal commodities [9]; a detailed study has been made of the radiation susceptibilities of the grain weevils, *Sitophilus granarius* (*Calandra granaria*) and *S. oryzae* (*C. oryzae*) [10], with an appraisal of the fundamental and applied problems of disinfesting grain by gamma-irradiation [11]. Many megacuries of Co^{60} would be required to treat grain at 200 t/h (the minimum handling-rate

demanded by the trade) at the dose level of 16500 rep for effective sterilization [10]. Smaller sources could, however, be employed if treatment were carried out by a series of repeated passes involving the circulation of grain from one storage bin to another. The present studies were carried out to compare the biological efficiency of continuous and fractionated treatments on different stages of *S. granarius*; the size of the fractional dose, number of fractions, interval time and interval temperature were also investigated.

II. Methods

(a) Insect culture

A strain of *S. granarius* from the Pest Infestation Laboratory, A.R.C., was reared and maintained on Manitoba wheat at 26°C and 76% relative humidity. Immature stages of required age were obtained by placing adults to oviposit for 24 h followed by incubation. The rate of development of *S. granarius* under these culture-conditions has been given in detail [10]. Adults of known age were obtained by daily removing the newly emerged insects from stock cultures, and retaining them on uninfested wheat until required for irradiation.

(b) Irradiation technique

Irradiations were carried out at dose rates varying from 5000—10000 r/h at 25° to 35°C. Doses measured in rep (roentgen-equivalent-physical, corresponding to an energy deposition of 93 erg/g of tissue) were delivered with an accuracy of $\pm$ 3%. Samples of 40 adults were irradiated on 100 grains of wheat; samples of immature stages consisted of 100 grains of infested culture medium. Manitoba wheat may be treated with doses up to 50000 rad without adversely affecting weevil development [12]. Samples were maintained at 26°C during the intervals between fractionated irradiations unless otherwise stated.

(c) Experimental procedure

Five samples of adults were used in each treatment. After irradiation each sample was incubated on 100 g of grain and examined periodically for survival. The surviving insects were transferred to fresh grain 28 and 56 d after irradiation, and discarded at 60 d.

Ten samples of eggs, larvae and pupae were used in each treatment. After irradiation, pairs of samples were combined and retained on 200 grains of uninfested wheat. Adults were counted 28 d after the beginning of emergence, and were periodically transferred to 100 g of grain throughout the emergence period. They were subcultured at 34 d and discarded at 62 d. Counts of progeny were made 7 and 9 weeks after initial inoculation.

(d) Handling of data

Three criteria were used to assess differences in the radiation susceptibility of developmental stages:

(1) Numbers of adults emerging from the grain.

(2) Survival after emergence.

(3) Production of adult progeny.

Appropriate corrections for the controls [13] were made to the data for emergence and survival. Fertility was expressed as the average number of progeny per live parent for certain periods after emergence (for immature stages) or after irradiation (for adults). These were then expressed as a percentage of progeny production in controls. Numerical data were treated by probit analysis using reiteration on the Mercury computer, A.E.R.E., Harwell.

III. Experimental

The effect of four fractions at intervals of 24 h

In the first experiment, continuous and fractionated treatments were given, at a range of dose levels, to eggs (0—4 days old), larvae (7—11 days old), pupae (28—32 days old) (Table I) and adults (7—11 days old). Samples given interrupted treatment received the first fractional dose on the first day of the age range; those given continuous treatment contained equal proportions of aged insects spanning the range. Similarly, untreated controls were of two types comparable with the age composition of the two types of irradiated samples.

Table I

EMERGENCE FROM IRRADIATED EGGS, LARVAE AND PUPAE AS A PERCENTAGE OF CONTROL

Eggs			Larvae			Pupae		
Total Dose	Continuous	Fractionated	Total Dose	Continuous	Fractionated	Total Dose	Continuous	Fractionated
500	96	97	500	99	93	500	98	82
800	89		1423	98	86	1423	114	124
1000		85	2010	107	94	2010	108	102
1100	58		2500	96		2840	107	107
1423	35	70	2840	68	91	4012	102	103
2010	15	47	3000	68		4500	105	
2500		2	3350	30		4900	78	
2840	7	7	3600	18		5250	77	
3250	7	1	4012	1	98	5666	104	109
4012	0	0	4500		96	6250	80	
			4900		88	7000	85	111
			5250		67	8004	66	102
			5666	0	43	8500		130
			6200		17	9000		47
			8004	0	0	9750		53
			11310	0	0	10500		58
			20000	0	0	11310	95	63
						20000	59	102

Note: Values over 100% indicate that more adults emerged from irradiated samples than from controls — a stimulatory reaction which is marked on irradiation of pupae.

Regression equations (Table II) allow calculation of the dose levels which permit 50% emergence. With eggs there was no difference between continuous and fractionated treatments; the dose level allowing 50% emergence from irradiated larvae increased from 3117 to 5546 rep ($P<0.1\%$). The dose level for 50% emergence from irradiated pupae was outside the range investigated.

Further regression equations (Table III) relating dose and survival of the emerged adults 60 d after irradiation show that the dose level for 50% mortality is significantly increased by fractionated treatment in all stages, particularly for pupae and larvae (Figs. 1 and 2). From the practical viewpoint of insect control the dose levels for 99.9% mortality are of

TABLE II

RELATIONSHIPS BETWEEN EMERGENCE AND DOSE IN CONTINUOUS AND FRACTIONATED TREATMENTS

Age group	Treatment	Equation Y (Probit) =	Lethal Dose — 50% Emergence Log Dose (x) ± standard error	LD_{50} Dose (rep)	Probability of a difference between LD_{50}'s	Standard error of slope
Eggs	Continuous	$-4.542 \times + 19.090$	3.1022 ± 0.0184	1266	$P = 10 - 5\%$	± 0.3653
	Fractionated	$-5.612 \times + 22.935$	3.1958 ± 0.0422	1570	(Not significant)	± 1.1720
Larvae	Continuous	$-17.071 \times + 64.641$	3.4937 ± 0.0045	3117	$P < 0.1\%$	± 1.4150
	Fractionated	$-18.542 \times + 74.422$	3.7440 ± 0.0047	5546	(Highly significant)	± 1.7690

TABLE III

RELATIONSHIPS BETWEEN SURVIVAL AND DOSE IN CONTINUOUS AND FRACTIONATED TREATMENTS

Age Group	Days after Irradiation	Treatment	Equation Y (Probit) =	Lethal Dose — 50% Survival Log Dose (x) ± ± standard error	LD_{50} (rep)	Probability of a difference between LD_{50}'s	Increase on continuous treatment (%)	Standard error of slope	Probability of difference between slopes
Eggs	60	Continuous	$-5.506 \times + 21.905$	3.0703 ± 0.0120	1176	$P < 1\%$		± 0.3465	$P = 80 - 70\%$
		Fractionated	$-5.910 \times + 23.893$	3.1968 ± 0.0388	1573	(Significant)	34	± 1.2247	(Not significant)
Larvae	60	Continuous	$-19.629 \times + 73.333$	3.4812 ± 0.0056	3028	$P < 0.1\%$		± 2.2840	$P = 40 - 30\%$
		Fractionated	$-16.122 \times + 65.019$	3.7228 ± 0.0075	5282	(Highly significant)	74	± 2.2967	(Not significant)
Pupae	60	Continuous	$-11.965 \times + 49.111$	3.6867 ± 0.0115	4861	$P < 0.1\%$		± 1.9566	$P = 70 - 60\%$
		Fractionated	$-15.639 \times + 66.853$	3.9550 ± 0.0253	9016	(Highly significant)	85	± 7.2526	(Not significant)
Adults	60	Continuous	$-9.620 \times + 40.042$	3.6426 ± 0.0058	4391	$P < 0.1\%$		± 0.6681	$P = 10 - 5\%$
		Fractionated	$-7.602 \times + 33.992$	3.8137 ± 0.0109	6512	(Highly significant)	48	± 0.8023	(Not significant)

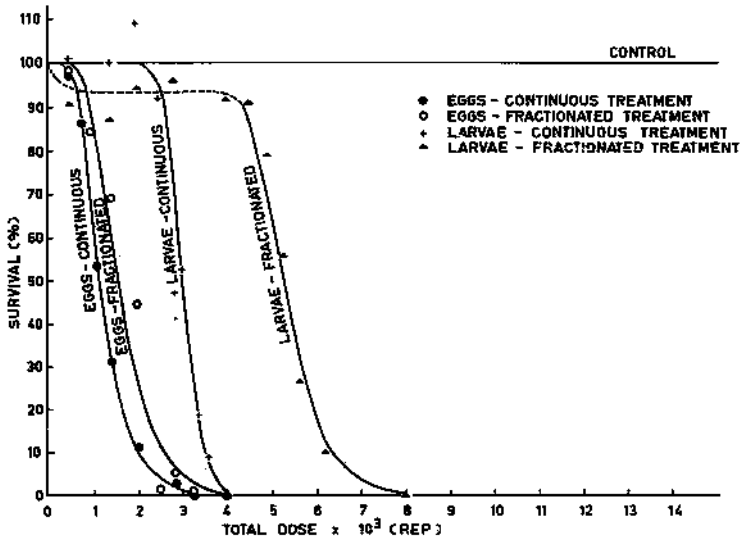

Fig. 1

Percentage survival 60 d after irradiation of eggs and larvae by continuous and fractionated treatments (calculated curves)

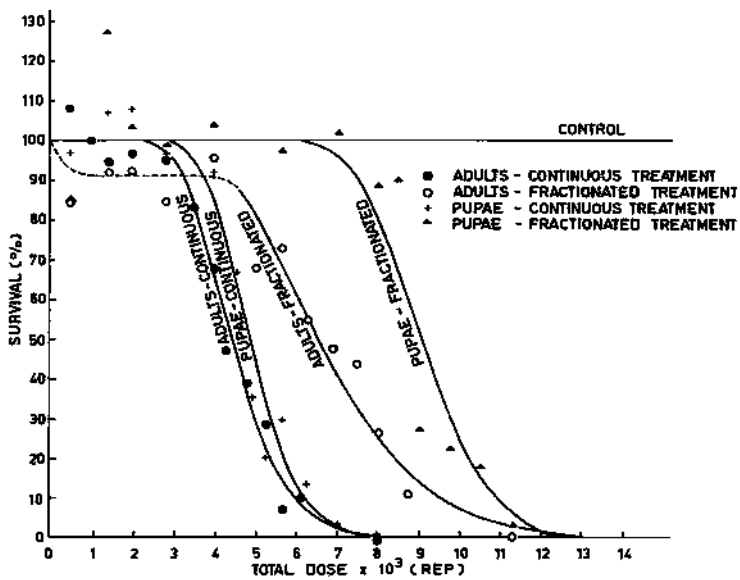

Fig. 2

Percentage survival 60 d after irradiation of pupae and adults by continuous and fractionated treatments (calculated curves)

greater significance than those for 50%. Using the equations shown in Table III the following values (Table IV) were derived for fractionated treatment:

TABLE IV

DOSE LEVELS FOR 99.9% MORTALITY

Stage	Log dose ± standard error	Dose (rep)
Eggs	3.7197 ± 0.1009	5240
Larvae	3.9145 ± 0.0291	8210
Pupae	4.1526 ± 0.0942	14210
Adults	4.2202 ± 0.0430	16610

The calculated doses for 50% reduction in fertility (Table VII) during the first month of oviposition are significantly increased by fractionated treatment with eggs, larvae and pupae but not adults. There is no foundation for the suggestion, based on preliminary work of JEFFERIES *et al.* [14], that fractionated treatment may lead to redevelopment of fertility in adults.

The calculated doses for 50% reduction in fertility during the second and third months' oviposition were as follows:

TABLE V

DOSE (rep) FOR 50% REDUCTION IN FERTILITY

Stage irradiated	Month of oviposition	Continuous treatment	Fractionated treatment
Egg	2nd	1251	1691
	3rd	1206	1242
Larva	2nd	2406	4394
	3rd	2339	4214
Pupa	2nd	1655	2791
	3rd	1355	2565
Adult	2nd	1495	1343
	3rd	1097	1366

Again, for the purpose of insect control the dose levels for 99.9% reduction in fertility are of significant interest. From the equations in Table VII the following values were obtained for fractionated treatment:

TABLE VI

DOSE LEVEL FOR FRACTIONATED TREATMENT (99.9% REDUCTION IN FERTILITY)

Stage	Log dose ± standard error	Dose (rep)
Eggs	3.6473 ± 0.1434	4440
Larvae	3.8982 ± 0.0123	7910
Pupae	3.9565 ± 0.0539	9050
Adults	4.2110 ± 0.1035	16260

TABLE VII

RELATIONSHIPS BETWEEN PROGENY PRODUCTION AND DOSE IN CONTINUOUS AND FRACTIONATED TREATMENT

Calculations based on numbers of progeny per parent and oviposition during the first month

Age group	Treatment	Equation Y (Probit) =	Dose for 50% reduction in progeny Log dose (x) ± standard error	Dose for for 50% reduction (rep)	Probability of difference between doses for 50% reduction	Increase on continuous treatment (%)	50% Dose as a percentage of $LD_{50/60}$	Standard error of slope
Eggs	Continuous	5.556 × — 11.856	3.0338 ± 0.0335	1081	$P < 2\%$		92	± 0.9740
	Fractionated	7.022 × — 17.521	3.2072 ± 0.0429	1612	(Significant)	49	102	± 2.2185
Larvae	Continuous	11.172 × — 32.670	3.3718 ± 0.0039	2354	$P < 0.1\%$		78	± 0.4374
	Fractionated	11.617 × — 37.195	3.6322 ± 0.0041	4287	(Highly significant)	82	72	± 0.6354
Pupae	Continuous	3.882 × — 7.357	3.1832 ± 0.0689	1525	$P < 1\%$		31	± 0.8754
	Fractionated	6.070 × — 15.926	3.4474 ± 0.0175	2802	(Significant)	84	31	± 0.6266
Adults	Continuous	3.764 × — 6.852	3.1488 ± 0.0280	1409	$P = 70 — 60\%$		32	± 0.3124
	Fractionated	2.844 × — 3.886	3.1245 ± 0.0497	1332	(Not significant)	— 5	20	± 0.3279

THE EFFECT OF VARYING THE INTERVAL TIME

Differences in the radiation susceptibility of weevils subject to continuous and interrupted treatments indicate the existence of a "recovery" process during the intervals between fractional doses. The second experiment was carried out to examine the interaction of this process with the interval time. To make this study as critical as possible, particularly for the purpose of investigating the effects of short intervals, doses were chosen which afforded 95% mortality with continuous treatment. These were, respectively, 2339 rep (4×585) for eggs, 3673 rep (4×918) for larvae, 6670 rep (4×1667) for pupae and 6510 rep (4×1627) for adults. Intervals of 10 min, 30 min, 1 h, 2 h, 4 h and 1 d were allowed between four fractions. A 2-d interval was also used for pupae and adults.

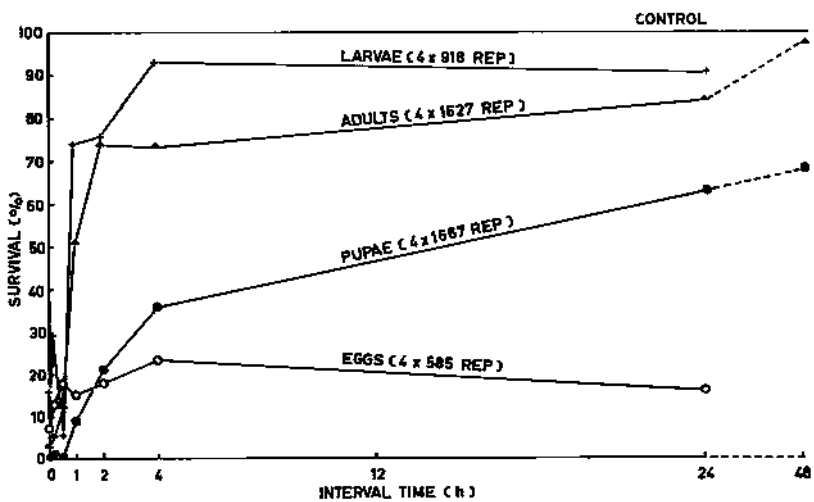

Fig. 3
Survival of developmental stages in relation to interval time in fractionated treatments

Note: The survival curve for pupae is much lower than would be expected at 6670 rep and may be accounted for by an error in the irradiation

The extent of recovery between treatments is reflected in the increase in survival and fertility with increasing interval. Recovery of somatic cells is rapid and almost complete in 4 h (Fig. 3). Increasing intervals produced only slight differences in the fertility of eggs, and no recovery of germ cells in irradiated adults (Table VIII). Larvae, however, showed a marked increase in fertility with intervals up to 2 h. The fertility of pupae was not tested.

THE EFFECT OF THE NUMBER OF FRACTIONS

Changes in susceptibility, afforded by varying the number of fractions, were tested with adults using two doses, 6510 and 7500 rep, divided into 2, 3, 4 and 5 fractions applied at 24-h intervals. Survival increased markedly with the number of fractions (Fig. 4); recovery was most marked during the first three intervals (up to about 70% survival), becoming progressively diminished with further subdivisions of dose.

TABLE VIII

THE EFFECT OF VARYING THE INTERVAL TIME ON FERTILITY OF EGGS, LARVAE AND ADULTS

Interval time	Progeny per parent as a percentage of control		
	Eggs (4×585r)	Larvae (4×918r)	Adults (4×1627r)
Continuous treatment	12.9	1.0	1.1
10 min	2.5	2.5	1.1
30 min	17.8	0.0	1.3
1 h	15.1	32.3	0.8
2 h	25.0	46.6	0.9
4 h	12.2	54.8	0.7
1 d	15.1	47.4	0.6
2 d	—	—	4.3

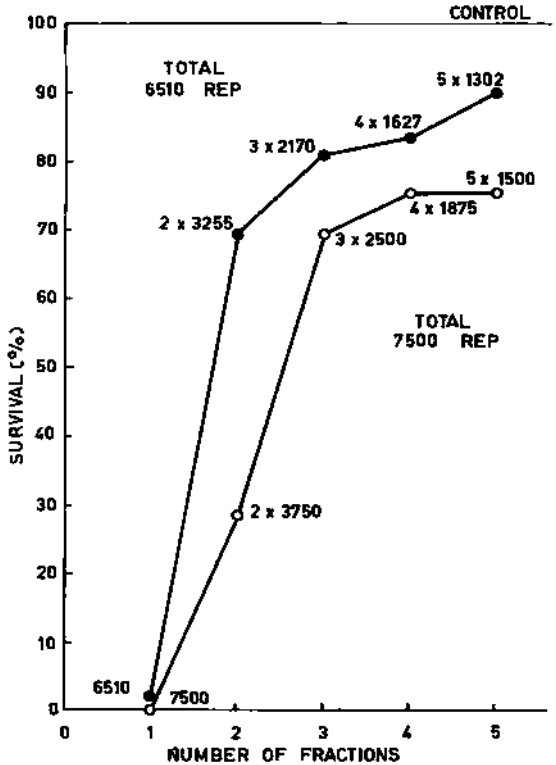

Fig. 4

Survival of adults 60 d after irradiation with fractional doses at 1-d intervals

THE EFFECT OF INTERVAL TEMPERATURE

It is recognized that within certain limits the rate of metabolism increases with temperature. To investigate the effect of temperature on the recovery process, adults were irradiated with 6510 rep by continuous treatment and with doses divided into 2 and 4 fractions, with 1-d intervals. Irradiations were carried out at 26°C; interval temperatures were 1°, 10°, 15°, 26° and 30°C, with all samples maintained at 26°C after the irradiation treatments had been completed.

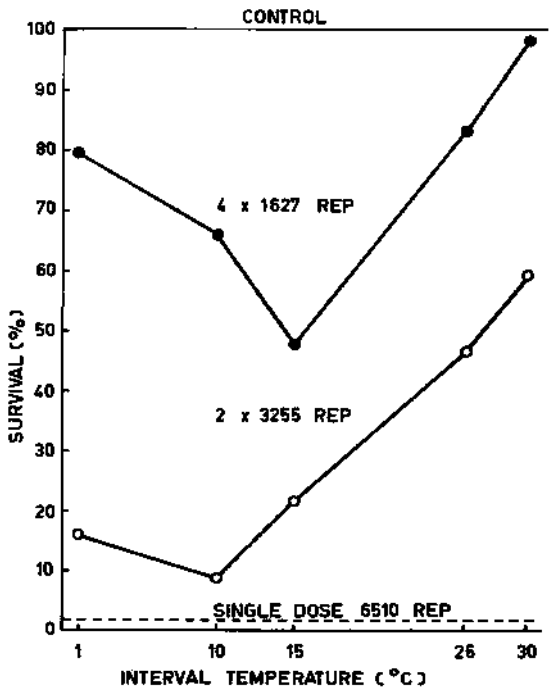

Fig. 5

Survival of adults 60 d after irradiation in relation to the interval temperature in fractionated treatments

At low temperatures, trends in survival (Fig. 5) were not consistent; with four fractions there was a decrease in survival with increasing temperature from 1° to 15°C, and a marked increase in survival from 15° to 30°. With two fractions, the initial decrease in survival was followed by an increase from 10° to 30°C. There was no significant difference in survival between controls held during the intervals at 1° and 30°C (2 fractions: $P = 40—30\%$; 4 fractions: $P > 90\%$) or at 1°C and 15°C (2 fractions: $P = 60—50\%$; 4 fractions: $P = 10—5\%$). As previous experiments had indicated no effect of fractionation on recovery of fertility in irradiated adults, progeny counts were confined to treatments shown in Table IX:

TABLE IX

PROGENY COUNTS FOR VARIOUS TREATMENTS

Treatment	Progeny/parent as % of control
6510 rep continuous treatment	1.1
4 × 1627 rep fractionated treatment with interval temperatures 15° C	0.9
26° C	0.6
30° C	1.0

Accordingly it may be concluded that the interval temperature has no effect on the susceptibility of the gonads.

THE EFFECT OF TEMPERATURE DURING IRRADIATION

As the rate of recovery of body cells varies with the interval temperature, irradiation over several hours at a low dose-rate and at high and low temperatures should show the presence of recovery during the irradiation period. Adults, 28—29 d old, were irradiated with 4500 rep at 450 rep/h. Fifteen samples of 50 insects were treated at both 7°C and 29°C. Controls were also retained at 7°C and 29°C during the 10-h irradiation period. Fertility was tested during an oviposition period of one month.

Percentage survival, compared with controls, 60 days after irradiation was $102 \pm 3\%$ and $78 \pm 3\%$ at 29°C and 7°C, respectively; the difference is highly significant ($P < 0.1\%$). Differences in fertility (2.1 ± 0.2 and 1.9 ± 0.1 progeny per parent) were not significant. There was no difference in survival ($P = 50$—40%) or fertility ($P = 20$—10%) between untreated controls retained at the two temperatures. Thus recovery of somatic cells also progresses during irradiation at low dose-rates, particularly at high temperatures.

THE EFFECT OF TWO FRACTIONS AND INCREASING INTERVALS

The final experiment was devoted to investigating the amount of recovery in a single interval of 4 h to 10 d. Adults, up to 11 days old, were treated as shown in Table X:

TABLE X

TREATMENTS ON ADULTS UP TO 11 DAYS OLD TO TEST AMOUNT OF RECOVERY

Dose (rep)	Time intervals
Fractionated treatments 2 × 4100 = 8200	4 h; 1, 1.5, 2, 3, 4, 5, 7, 10 d
2 × 3750 = 7500	4 h; 1, 1.5, 2, 3, 4, 5, 7, 10 d
2 × 3255 = 6510	10, 30 min; 1, 2, 4 h; 1, 1.5, 2, 3, 4, 5, 7, 10 d
2 × 3500 = 7000	1 d
2 × 3000 = 6000	1 d
2 × 2500 = 5000	1 d
Continuous treatments 8200, 7500, 6510, 4100, 3750, 3500, 3255, 3000 and 2500	—

The effect of the first three treatments on survival, 60 d after irradiation, is shown in Fig. 6. Survival gradually increased with interval time, except at 2 d when in all cases it was lower than at 1½ d. There was no further increase in survival above the level afforded by one fractional dose. Thus, insects surviving the first fractional dose, and given a sufficiently long interval, are able to withstand a further dose of the same magnitude with no further

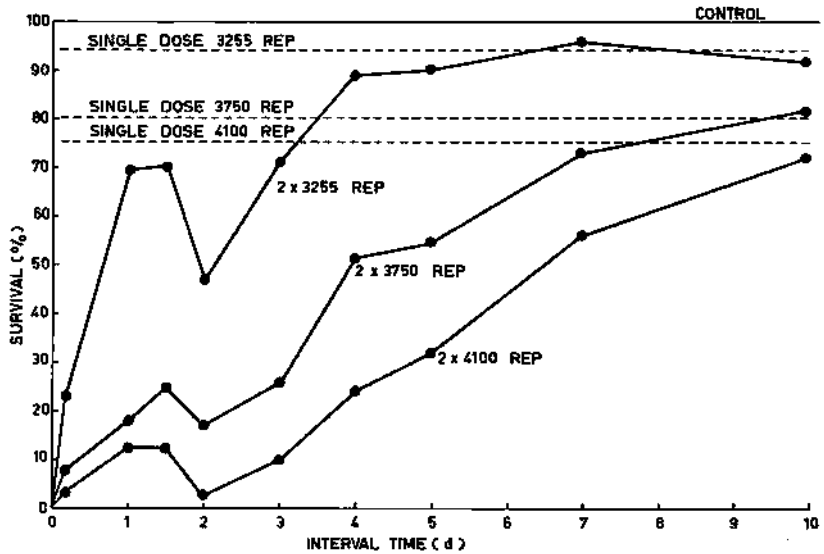

Fig. 6
Survival of adults given two fractional doses with increasing interval time. The survival level with one fractional dose is shown by a broken line

percentage mortality. Complete recovery from a dose of 3255 rep is obtained in about 6 d; larger doses require longer intervals. Survival after treatments with 2 × 3500, 2 × 3000 and 2 × 2500 rep separated by 1-d intervals, was 49, 74 and 91% compared with 89, 90 and 97% with single doses 3500, 3000 and 2500 rep, respectively. Survival after treatment with 2 × 3255 rep with intervals of 10 min, 30 min, 1 h and 2 h was 5, 6, 9 and 8%, respectively, compared with 0% after a continuous dose of 6510 rep. Thus an interval of only 10 min is sufficient to increase survival above that obtained with continuous treatment.

IV. Discussion

Few previous workers have used insects in studying the effects of small repeated *versus* large continuous doses of ionizing radiation. The life span of *T. confusum* has been extended with small daily doses of X-rays [4] [5] and gamma-rays [6]. The dose for sterility in *Trogoderma sternale* has been increased by fractionation [8] and greater emergence obtained from eggs of *Bombyx mori* [15]. Divided doses of X-rays given to *Habrobracon juglandis* provided only temporary sterility [16].

The experiments reported here have shown differences in the radiation susceptibility of developmental stages of *S. granarius*, when subjected to continuous and fractionated treatments, with regard to both survival and fertility. These differences must be attributed to

recovery of the somatic and reproductive cells. Recovery may be brought about by repair on the molecular level or replacement on the cellular level. Radiation may damage important macromolecules or disorganize the grouping of molecules in functional units [17]. In the intervals between damage, macromolecules may be repaired and the units reorganized, or new cells may take over the function of damaged cells if cell division is sufficiently rapid. Both repair and replacement would reduce the overall effect of radiation damage and it is probable that both mechanisms are in operation.

Repair and replacement probably continue throughout irradiation as well as between treatments. It is not the change in overall dose rate, however, which causes the observed variations in survival. With 2 × 3255 rep given to adults with one interval of 4 h (overall dose rate: 6510 rep in 4 h 39 min = 1400 rep/h) the corrected survival was 23%. On the other hand, 50% survived 4 × 1627 rep with 3 intervals of 1 h (overall dose rate: 6510 rep in 3 h 39 min = 1784 rep/h). The amount of recovery and the final survival are dependent on the length of the intervals, the number of fractions and the interval temperature.

Differences between continuous and fractionated doses are apparent at intervals of 10 min. Rate of recovery is highest during the first few hours after irradiation and decreases as the damage is reduced (Figs. 3 and 6). With low fractional doses (1627 rep in adults) and intervals of 24 h, survival is similar to that with intervals of 4 h or less. The level of survival afforded by a 2-d interval is not much higher than for 1 d (Fig. 3). With high fractional doses (3255 rep in adults) cell damage is too great to be completely recovered within 4 h, and the rate of recovery does not substantially decrease until 4 d after irradiation (Fig. 6). Increasing the interval between treatments produces no further recovery when survival has reached the level obtained with the fractional dose, viz. 3255, 3750 and 4100 rep. Thus, insects capable of surviving the first fractional dose and given long enough to recover completely would then be able to withstand a further dose of the same magnitude with no further (percentage) mortality. The three survival curves in Fig. 6 indicate that the higher the dose, the longer the time necessary for complete recovery. Approx. 6 d are needed to recover fully from 2355 rep, 9 d from 3750 rep and 11 d from 4100 rep. These values are plotted in Fig. 7, with the points derived by probit analysis of data obtained with two fractions and increasing intervals. Fig. 7 shows that insects capable of surviving a large dose would require extremely long periods for complete recovery before surviving a second, similar dose. The trend of the curve would indicate that complete recovery is impossible from a dose greater than 5000 rep, however long the interval between the two treatments. Fig. 7 also relates interval time and the fractional dose which provides the same amount of radiation damage as a continuous treatment (i.e. results in zero recovery). It would suggest that two doses of about 6200 rep separated by intervals up to 5 d allow no recovery, survival being equal to that obtained with a continuous dose of 12400 rep.

Increase in survival is expected with the subdivision of treatment into a greater number of fractions, retaining the same interval time, since this reduces the amount of damage to be recovered in each interval. The high rate of recovery exhibited between the first and second fractions is not, however, continued between the second and third, and is still lower between the third and fourth (Fig. 4). The additional treatments may be damaging the newly recovered tissue and impose greater demands on the recovery process which cannot be met.

The present work has also shown that the interval temperature is an important factor influencing recovery. LAMARQUE, [15], irradiating eggs of *Bombyx mori* with two doses, obtained recovery during an interval at 21°C; when, however, the eggs were kept 'in a refrigerator' the effects of the two fractions were strictly cumulative. COOK [18], on the other hand, found that eggs of *Ascaris equorum* exhibited greater recovery when retained at 5°C

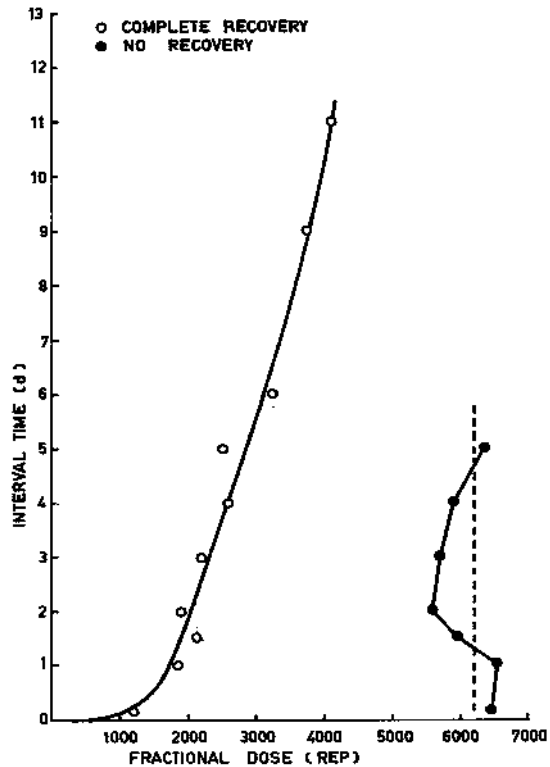

Fig. 7

The relationship between time for complete recovery and fractional dose. Also the interval time plotted against the fractional dose providing the same radiation damage as continuous treatment

after irradiation than at 25° C. The results for *S. granarius* differ from both these observations; recovery and survival increased with interval temperatures from 15° to 30°C and from 15°C to lower temperatures. The survival value at 1°C is also far greater than that obtained with continuous treatment (Fig. 5).

Differences were found between stages in their capacity for recovery from radiation damage (as measured by calculating the percentage increase on the $LD_{50/60}$ for continuous doses). The results shown in Table III indicate an increase in this capacity as the insect develops from egg to pupa with a decrease after the emergence of the adult. It is plausible to expect the capacity for recovery of insects passing from one developmental stage to the next during the interval between treatments to be similar to that of stages on either side of the moult. The capacity for recovering fertility also increases with development, but without recovery in the adult stage. In Table VII the dose for 50% reduction in progeny is calculated as a percentage of the $LD_{50/60}$. Similarity between the scores for continuous and fractionated treatments shows, except for adults, that recovery in the capacity for reproduction occurs to the same extent as recovery in somatic tissues. There was no indication of fertility being regained in later months by adults receiving fractionated doses as found in *Habrobracon juglandis* [16].

The recovery peak shown by adults at 36 h (Fig. 6) is similar to that obtained by other workers treating widely different material. Cell lines of ovarian tissue and female lung tissue of the Chinese hamster, *Cricetulus griseus*, have been irradiated [19] with 2 doses separated by intervals up to 25 h; the results were similar to those of the present work except that a peak was obtained with an interval of 2 h followed by a trough at 4 h. Pollen grains of *Tradescantia bracteata* treated with 2 doses separated by breaks of 2—18 h [20] resulted in fewer chromosome breaks as the interval increased to 4 h; at 12 h there was an increase in the number of breaks followed by a decrease again with longer intervals. Similar results are reported by HAQUE [21] with pollen grains of *Tradescantia paludosa* and by JAIN *et al.* [22] with barley root tips. LANE [20] has suggested that the drop in breakage frequency might be attributed to a reduction in sensitivity of the cell to subsequent damage — a radiation-induced protection which disappears with time. With *S. granarius* (Fig. 6) the three peaks certainly cannot be explained by fluctuations in radiosensitivity with age, since the susceptibility of adult weevils remains relatively constant over the first 10 d [10]. Adults receiving 4 fractional doses did not show this recovery peak with the trough at the 2-d interval, possibly because of the low fractional doses and the much-reduced recovery-rate observed with the increased number of fractions. There are, however, indications of a recovery peak and trough in survival at 1 h with eggs and 30 min with larvae and pupae (Fig.3).

CORNWELL *et al.* [10] have derived a dose of 16500 rep for the commercial disinfestation of grain, effective against all stages of *S. granarius*. Adults require the highest dose to kill and sterilize with both continuous and fractionated treatments; they are potentially the most dangerous stage in the event of inefficient control. Calculations from the equation in Table III show that less than 1 in 10000 adults can survive a continuous dose of 16500 rep. It has also been shown that when a dose is divided into 2 fractions, with fractional doses of 6200 rep and above, no recovery is apparent with intervals up to 5 d, survival being similar to that after continuous treatment. Moreover, survival is only increased to 1 in 1000 adults when a dose of 16500 rep is divided into 4 fractions with 1-d intervals at 26°C. With shorter intervals and lower temperatures, survival is less. Finally, with adults the level of sterility afforded by fractionated treatment is equal to that with continuous doses. It may therefore be concluded that any fractionation likely in practice would not reduce the efficiency of radiation disinfestation of grain. On the other hand, with low-dose applications to packaged cereal commodities, specifically to prevent the emergence of immature stages, fractionated treatment could seriously jeopardize the efficiency of control.

V. Summary

1. An investigation was made into the effect of dose fractionation on the radiation susceptibility of eggs, larvae, pupae and adults of *Sitophilus granarius* (*Calandra granaria* L.) to examine the biological efficiency of multiple pass systems for radiation disinfestation of grain.
2. Survival of all developmental stages was significantly increased by fractionation, particularly with larvae and pupae. The difference in survival afforded by continuous and fractionated treatments must be attributed to recovery during the intervals between damage.
3. Recovery was noted with intervals of 10 min and longer, the amount of recovery and final survival being governed by the number of fractions, the fractional dose, interval time and interval temperature, but not by the overall dose rate.

4. Rate of recovery was greatest in the first few hours after irradiation, and decreased with increasing number of fractions.
5. Irradiation is followed by a period of reduced sensitivity to subsequent treatment.
6. Recovery was obtained in the reproductive capacity of irradiated eggs, larvae and pupae but not adults. Adults subjected to fractionated treatment did not redevelop fertility in later months.
7. Pupae showed the highest capacity for recovery of somatic and reproductive cells. Adults require the highest dose to kill and sterilize by continuous and fractionated treatment and are potentially the most dangerous stage in the event of inefficient control.
8. Comparison of the effects on adult survival of continuous and fractionated treatments at 16500 rep would indicate no loss in the efficacy of radiation disinfestation of grain involving any system of multiple treatment likely to be met in practice. Fractionation could, however, lead to inadequate control in packaged cereal commodities given low doses to prevent adult emergence.

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DISCUSSION

J. A. QAYYUM (Pakistan): Did you record any differences in the behaviour of the two sexes with regard to recovery from irradiation in the adult stage?

P. B. CORNWELL (United Kingdom) who presented the paper: No. The experiments were carried out with unsexed samples and no attempt was made to assess the rate of recovery in males and females separately. The experiments were designed to examine the effects of dose fractionation should this type of treatment be applied in practice.

K. BORA (India): I must congratulate Dr. Jefferies on his very interesting paper. It is now fairly well established that the efficiency of a fractionated dose is less than that of a continuous dose. It depends, of course, on many factors. In view of the fact that radiosensitivity varies with the developmental stage, I would like to ask Dr. Cornwell whether he has some

information on the variation in radiosensitivity during the four days over which the fractionated doses were spread.

Secondly, Dr. Cornwell mentioned somatic and genetic effects. Could he tell us precisely what these effects were?

Thirdly, if a dose is fractionated, the material to be irradiated has to be brought near the source several times, depending on the number of fractions. Alternatively, multiple sources would be required. Would fractionation not, therefore, involve greater technical difficulties in handling the material?

P. B. CORNWELL: To answer your first question: Using continuous doses of radiation, we know a great deal about the radiation susceptibility of the grain weevil (*Sitophilus granarius*) from day to day throughout its life history. Only in eggs and pupae do we find marked changes in susceptibility within a developmental stage. Because treatments in the present experiments were spread over a number of days, those samples receiving continuous treatment were composed of equal proportions of aged insects covering the age range. The response obtained when comparing continuous and fractionated treatments is then a measure of the overall susceptibility of that sample.

As for your second question: In making the comparison between continuous and fractionated treatments an attempt has been made to compare the effects on somatic tissues and reproductive cells, the first illustrated by mortality and the second by the number of adult progeny produced.

With regard to your third question: Yes, dose fractionation does involve bringing the product to the source several times or using multiple sources, but this may in fact be desirable. Because grain is handled commercially at very fast handling rates, sources of many megacuries are required to give the effective sterilizing dose in one operation. The capital cost of these sources is prohibitive. It may be more desirable to use smaller sources, to give a substerilizing dose to grain entering storage and to complete the treatment by more leisurely, internal circulation at a later date. Similarly, in designing a radiation plant for the treatment of packaged products, in order to provide uniform dose distribution throughout the commodity, it may be necessary to convey the packages around a system of many source rods or a number of slab-type sources. In this type of facility the insect would receive an integrated dose of radiation of very low and very high intensities. There may well be conditions which simulate those of dose-fractionation.

D. E. WEIDHAAS (United States of America): Does "reduction in fertility" imply an effect directly on reproduction?

P. B. CORNWELL: The term implies an effect directly on reproduction, or more precisely to the lower numbers of adult progeny produced by irradiated parents compared with controls.

G. G. SENGUPTA (India): Have any observations been made on the sex ratio, in the course of experiments on the effects of continuous and fractionated doses of radiation?

P. B. CORNWELL: It has been assumed that the sex ratio of the test insects used in these experiments approximates to unity. The sex ratio of progeny produced after irradiation with continuous and fractionated doses has not been examined.

I have one comment to make. One use of fractionated treatments which has not been brought out in Dr. Jefferies' paper is possibly in the treatment of insects subsequently liberated in a programme of 'sterile-male release'. One of the major problems in the control of insects by this method is selection of the optimal pupal age which on irradiation provides

sterilized males with normal longevity, vigour, mating behaviour and mobility. When the doses for sterilization and mortality are very close it could be of considerable advantage to produce a differential effect on somatic and reproductive cells. In future control programmes on screwworm, for example, the release of adult males fully competitive with the wild population could result in considerable economy in manpower, and in the costs of rearing and aerial distribution.

THE USE OF RADIATION SOURCES FOR INSECT CONTROL

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Abstract — Résumé — Аннотация — Resumen

The use of radiation sources for insect control. The different radioisotopes and electrical machine sources of radiation are briefly discussed, but a greater emphasis is placed on radioisotopes because of their greater reliability and ease of operation. The numbers of curies of the most suitable isotopes for various applications are tabulated. The general principles of source design covering radiation flux intensity, optimum dose distribution and shielding requirements are outlined. Various procedures for the use of radiation in control of insect populations are reviewed, including the sterile-male release technique, direct irradiation of agricultural products, the combined use of insecticides with irradiation and the possible use of radiation as an insect repellant. Possible new applications are suggested, e.g., the combined use of insecticide and sterile-male release in a programme for the eradication of the malarial mosquito in the Indonesian islands.

A new method is put forward for storing grain for a long time without the need for periodic fumigation. This method involves the use of a special grain container and radiation processing. Designs for various irradiators and some of the problems encountered are reviewed.

Special reference is made to small semi-portable irradiators suitable for insect sterilization; to a flexible laboratory irradiation unit covering both research and field scale applications; and to some of the large production scale facilities which have been proposed for commercial use.

In view of the proven effect of radiation for insect control, it is urged that the health authorities give serious consideration to this technique for reducing losses in the world's food supply.

Emploi des sources de rayonnements dans la lutte contre les insectes. Le mémoire donne un bref aperçu des différentes sources de rayonnements (radioisotopes et appareils de grande énergie), en s'attachant surtout aux radioisotopes en raison de leurs plus grandes régularité de fonctionnement et facilité d'emploi. Il contient un tableau des nombres de curies des isotopes convenant le mieux aux diverses applications. Le mémoire présente les principales caractéristiques que doivent posséder les sources, en ce qui concerne notamment l'intensité du flux de rayonnement, la répartition de la dose optimum et le dispositif de protection nécessaire. Il étudie diverses méthodes d'emploi des rayonnements pour lutter contre les insectes, y compris le lâcher de mâles stériles, l'irradiation directe des produits agricoles, l'emploi combiné d'insecticides et de rayonnements et l'emploi éventuel de rayonnements pour chasser les insectes. Des nouvelles applications sont suggérées, par exemple l'emploi combiné d'insecticides et du lâcher de mâles stériles en vue d'une campagne de destruction du moustique de la malaria dans l'archipel de la Sonde.

Les auteurs exposent une nouvelle méthode de stockage prolongé de céréales ne nécessitant pas de fumigations périodiques. Cette méthode comporte l'emploi de silos spéciaux et le traitement par les rayonnements. Le mémoire présente différents modèles d'irradiateur et discute certains des problèmes à résoudre.

Le mémoire fait mention des petits irradiateurs semi-portatifs convenant pour la stérilisation des insectes; d'un appareil d'irradiation pour le laboratoire pouvant servir à la fois à la recherche et aux applications à la production d'un champ; enfin, de certaines des installations qui ont été proposées pour un traitement industriel.

Les rayonnements ayant des effets certains pour lutter contre les insectes, les auteurs insistent pour que les autorités sanitaires envisagent sérieusement leur emploi pour réduire les pertes que subissent les ressources alimentaires du monde.

Использование радиационных источников для борьбы с насекомыми. В докладе дается краткое описание источников радиации различных радиоизотопов и электрических машин, причем основное внимание уделяется радиоизотопам в связи с их большой надежностью и легкостью эксплуатации. В докладе приводится список количеств кюри наиболее подходящих для различного использования изотопов. В нем перечисляются общие принципы схемы источника радиации, включая напряжение радиационного потока, оптимальную дозу распределения и требования, предъявляемые к экранировке. В докладе дается обзор различных методов использования радиации для борьбы с популяциями насекомых, включая метод выпуска стерильных мужских особей, прямое облучение сельскохозяйственных продуктов, комбинированное использование инсектицидов и облучения, а также возможное использование радиации в качестве разрешающего средства для насекомых. В докладе предлагаются новые возможные методы использования изотопов, как, например, комбинированное применение инсектицидов и выпуск стерильных мужских особей по программе ликвидации малярийного москита на Индонезийских островах.

Предлагается новый метод хранения зерна в течение длительного времени без необходимости в периодическом окуривании. Этот метод предполагает использование специального хранилища для зерна и обработки облучением. В докладе дается обзор схем различных излучателей, а также возникших в связи с этим проблем.

Особо говорится о небольших полупортативных излучателях, годных для стерилизации насекомых; об универсальной лабораторной установке для облучения, пригодной для использования как в целях исследований, так и в полевых условиях; и о некоторых средствах крупномасштабного производства, которые были предложены для промышленности.

Принимая во внимание доказанное действие радиации для борьбы с насекомыми, в докладе настоятельно предлагается, чтобы органы здравоохранения обратили серьезное внимание на этот метод для сокращения потерь запасов продуктов, имеющихся в мире.

Empleo de fuentes de radiación en la lucha contra los insectos. En la memoria se describen sucintamente distintas fuentes de radiación (radioisótopos y aparatos eléctricos), pero se atribuye particular importancia a los radioisótopos, debido a su mayor regularidad de funcionamiento y facilidad de manejo. Se reproduce un cuadro en el que se indica el número de curies de los isótopos más adecuados para diversas aplicaciones. Se exponen los principios generales de diseño de fuentes, en lo que atañe a la intensidad del flujo de radiación, la distribución óptima de las dosis y las características del blindaje necesario. Se examinan diversos procedimientos para el empleo de las radiaciones en la lucha contra los insectos, comprendidos la técnica de la suelta de machos esterilizados, la irradiación directa de productos agrícolas, el empleo combinado de insecticidas e irradiación, y la posible utilización de las radiaciones para ahuyentar los insectos. Se sugieren nuevas aplicaciones posibles, tales como, por ejemplo, el empleo de insecticidas combinado con la suelta de machos esterilizados en un programa de erradicación del mosquito del paludismo en el archipiélago de la Sonda.

Los autores exponen un nuevo método para almacenar cereales durante largo tiempo sin necesidad de fumigaciones periódicas. Este método exige el empleo de silos especiales y un tratamiento por irradiación. Se examinan los diseños de diversos irradiadores y algunos de los problemas que su empleo plantea.

Se describen especialmente unos dispositivos semiportátiles adecuados para la esterilización de insectos; un aparato para la irradiación en laboratorio que puede aplicarse a la vez en la investigación y sobre el terreno; y algunas de las instalaciones que han sido proyectadas para uso comercial.

En vista de los efectos comprobados de las radiaciones en la lucha contra los insectos, se pide a las autoridades sanitarias que examinen seriamente su empleo a fin de reducir las pérdidas en las existencias mundiales de productos alimenticios.

Introduction

It has become apparent in recent years that insects can develop an immunity towards the conventional insecticides. This and the near availability of large sources of ionizing radiation have stimulated research into the possible use of radiation in insect control.

Both gamma-radiation and electron-beams have been used, and it has been shown [1] that the lethal and sterilizing effects are comparable in the two cases, provided adequate radiation penetration is assured.

Two radioisotopes have generally been considered for use as sources of gamma-radiation: caesium-137, which is a fission product, and cobalt-60 which is produced by exposing natural cobalt to the neutron flux in a reactor. The mixed isotopes of zirconium and niobium could be available in large quantities from the fission-product separation plants, and would be particularly suitable for seasonal applications. However, further technical development is required before their use can be considered.

Several types of generator for electron-beams are in use for research and industrial irradiation. These are used primarily as sources of electrons, and therefore suffer from limited penetration, and although conversion to highly penetrating gamma-radiation is possible, at the energies in use it is not very efficient.

All are highly developed complex pieces of equipment and, with suitably trained operators, can be relied upon for good service. However, as a research tool, cobalt-60 has been much the most widely used source of radiation.

Up to several hundred curies may readily be housed in a lead container which is convenient both for shipping and normal use. Calibration of the source in terms of air dose may be carried out at the time of the initial loading. Knowing the rate of decay of the isotope, the dose delivered subsequently may be calculated from the time of exposure.

For research purposes, a source of considerable flexibility is required. It should be possible to irradiate samples under any desired conditions of temperature, humidity and composition of atmosphere. For example, anoxia may exert an appreciable radiation protective effect. In addition, the rate at which the radiation dose is delivered should be controllable over a wide range, say 1000 to 1, and the total dose received by the sample must be known within close limits and be reproducible to within, say, $\pm 2\%$. In order to achieve this, it may be necessary to use small compact samples or a special spatial arrangement of source and sample.

Two procedures using radiation for the control of insect populations are generally distinguished. Firstly, direct control by irradiation of infested products, and secondly indirect control of a particular species by the release of sterilized adults of that species [2].

Direct control

Direct irradiation aims at complete and permanent reproductive sterilization and premature death of the insects. If the infestation is largely confined to eggs, then these should be prevented from further development, even into sterile adults, so that the problem of removing readily visible though dead insects from the product may be avoided.

The radiation sensitivities of many species of insect have now been studied (insects infesting grain [3], wheat flour and beans [4], other stored products [5] [1], mites [6] etc. [7]) and certain generalizations are possible. Doses of 0.5 Mrad will cause reproductive sterilization and death within 24 h. About 100000 rad will induce a radiation lethargy so that subsequent feeding damage is minimized, will cause reproductive sterilization and lead to death within one week.

Doses in the range 15—20 krad will cause reproductive sterilization and death within a few weeks. This generally is a sufficient measure of control and the only dose level which can be considered for economical application.

Sterile-male release technique

Radioisotopes and nuclear radiations can be employed in various ways in the efforts to control or eradicate insect populations. LINDQUIST [7] has reviewed the various entomological uses of radioisotopes. The most dramatically successful has been the sterile-male release technique. The success of this technique was first established in the classical experiment of eradication of the screwworm fly on the island of Curaçao [8].

The screwworm fly is a native pest in the southwestern United States where stockmen have spent millions of dollars annually for range riders to examine and treat infected herds. The fly did not exist in the southeastern United States until 1933 when it was introduced by infected animals brought from the Southwest. KNIPLING [8] estimated that this pest cost the livestock industry in Florida, Alabama, Georgia and the Carolinas at least $ 240000000 between 1933 and 1955. At the time of eradication in 1959 this cost had reached $ 20000000 p.a. [9].

Each female of the species lays about 200 eggs in cuts and other wounds in cattle or game. The eggs hatch to maggots (the larval stage) that destroy additional healthy tissue and produce an enlarged festering wound that attracts more flies. If the animal is not treated it will be killed by the insect.

In attacking the problem of screwworm eradication, it was found in laboratory tests that a dose of 3 krad caused complete sterility in male flies but left them with normal sexual behaviour [8]. Experimental mating of a number of flies in cages showed that the female mated only once and, if this mating was with a sterile male, the eggs laid were sterile. Also, the caged matings showed that the percentage of sterile eggs was almost directly proportional to the percentage of sterile males in the cage. This shows the possibility of decreasing the population of this insect if enough sterile males can be released in a given area. Table I shows the mathematical result of releasing, at four different periods, a number of sterile males equal to twice the original natural population of males. Theoretically such a procedure would eliminate the population [8].

To prove the method, it was necessary to attempt eradication in an isolated area of limited size. The island of Curaçao has an area of about 170 mile^2 and had a screwworm population, per square mile, as high as any area in Florida. A co-operative agreement was reached with the Netherlands Antilles Government to attempt eradication on this island.

The experiment began in March 1954. Flies were raised in a laboratory in Florida and, while in the pupal stage, were exposed to 5 to 7.5 krad of gamma-radiation. Sterile flies of both sexes emerged and were flown to Curaçao and released by air at a weekly rate of 400 sterile males/mile^2—three to four times the natural population of males. To test the results, goats were placed in 11 different animal pens located at various places on the island and were examined daily for screwworm eggs. The eggs found were allowed to mature in the laboratory to discover what proportion would produce larvae. The results of the experiment are summarized in Table II [8].

Stockmen on Curaçao reported no incidence of cases of screwworm in livestock after the ninth week and a check later showed no evidence of the pest on the island [8].

Assured by the success of the 1954 Curaçao experiment, a preliminary test was made in Florida in 1957; then a vigorous campaign for eradication in the southeastern United States was launched in 1958 [9] [10]. An unused aeroplane hangar was converted to a

TABLE I

THEORETICAL POPULATION-DECLINE WHEN STERILE MALES ARE ADDED TO A NATURAL POPULATION [8]

Natural population	Sterile males released	Ratio of sterile to fertile males	Population decline (theoretical max.)
1000000	2000000	2:1	333333
333000	2000000	6:1	46619
46619	2000000	42:1	1107
1107	2000000	18000:1	less than 1

TABLE II

SUMMARY OF CURAÇAO SCREWWORM EXPERIMENT [8]

Weeks	No. of egg masses		
	Fertile	Sterile	Egg masses sterile (%)
1	15	34	69
2	17	38	69
3	17	36	68
4	10	37	79
5	7	42	86
6	3	23	88
7	0	10	100
8	0	12	100
9	0	0	—

fly factory capable of producing 100 million sterile flies per week. The irradiators used consisted of six cobalt-60 units with only about 600 c of activity per unit. In 16 months 3000 million sterile flies were released and the project successfully completed with the screwworm eradicated from the southeastern United States. The total cost of the project was about $10 million—about half the previous annual figure for damage caused by this pest.

The sterile-male release technique as a means of control or eradication of other pests has been reviewed by BUSHLAND *et al.* [10] [11]. To be successful, the following requirements should be fulfilled [10]:

(1) A low natural population must exist or the population must be reduced by other means so that it is possible to release an excess of sterilized males.

(2) The insect must be easily reared in mass numbers in the laboratory.

(3) The mating behaviour of the males must not be adversely affected by sterilization and native females must be willing to accept sterilized males.

(4) Preferably the female should mate only once, but on theoretical grounds multiple matings should result in the production of infertile eggs in a ratio similar to that of released sterilized to native males.

(5) Methods of measuring the insect population per unit-area are needed so that the numbers required for release purposes can be accurately estimated.

(6) The area in which eradication is attempted should be isolated by quarantine or other measures against reinfestation.

In determining the feasibility of the sterile-male release technique for eradication of other insects, various problems must be considered, such as the effects of irradiation on the species, habits of the insect, population trends of the species, rate and extent of migration, etc. One such possible application will be briefly considered—the use of the sterile-male release technique in the eradication of malarial mosquitoes in the islands of Indonesia.

Consideration of the use of the sterile-male release technique in eradication of malarial mosquitoes in the islands of Indonesia

Lindquist, of the US Department of Agriculture, comments that "Malaria, carried by *Anopheles* mosquitoes, has been a scourge of mankind for thousands of years. This disease has been estimated to kill 2 million persons every year and to sicken approximately 100 times this number so that they are unable to perform effective work" [7]. The World Health Organization together with the International Cooperation Administration of USA and Federal governments are undertaking the eradication of malaria in many parts of the world. The plan involves the residual spraying of buildings and other retreats of the malaria mosquitoes. This type of approach destroys enough of the mosquitoes for transmission of the disease to be interrupted but will not lower the incidence of the mosquitoes greatly nor will it, of course, provide for eradication [12]. An extensive eradication programme of this nature is now under way in Indonesia.

Indonesia has numerous islands, about 3000, many of which would be ideal for use of the sterile-male release technique from the point of view of isolation as a means of prevention of reinfestation. Also, the islands vary greatly in size so that a complete eradication programme of a desired magnitude may be selected by using certain islands. This excellent opportunity of field-testing the sterile-male release technique for eradication of malarial mosquitoes ought certainly to be considered.

Insecticides may be very efficient in controlling insect populations when the population density is large. However, once the population is reduced by repeated use of insecticides the process becomes very inefficient. At low population densities, several pounds of insecticide may be required per insect killed when mass-spraying techniques are used. The efficiency of the sterile-male release technique, on the other hand, increases with decreasing population density until a limit is reached which is governed by the distance the insect will travel to mate. The fact that sterile insects can seek out native insects whereas insecticides cannot is a basic advantage of the sterile-male technique, once the population density has been reduced. This advantage permits complete eradication of the species which is otherwise very difficult with insecticides alone.

LINDQUIST states that "it might be feasible to eradicate one or more anopheline malaria transmitters (on some of the islands of Indonesia) by an all-out attack . . . first with chemicals and then use of the sterile-male technique" [12]. He also states that "first the insect should be decimated as much as possible by use of insecticide and then rely on the sterile-male technique, providing it works, to clean up the small remaining population". However, he cautions that many unknown factors are involved and the possibility of complete success of such an attack could not be predicted without further information. HARRIS, chief

malariologist of the malaria eradication programme in Indonesia has commented on this suggestion. "The new technique (sterile-male release) would be more economical in application but demands large expenditures in research and field trials before assurance of success. It is readily agreed that this peaceful use of atomic energy should not be eliminated from consideration" [13].

DAVIS *et al.* [14] have reported some exploratory studies on the use of the sterile-male technique on *Anopheles quadrimaculatus*, one of the malaria-carrying species of mosquitoes. They found that doses of 8800 r to 12900 r applied in the pupal or adult stage were required in order to cause complete sterility. Irradiated females mated to non-irradiated males produced no eggs, whereas non-irradiated females mated to irradiated males produced a normal number of eggs but none hatched. Irradiated males introduced into caged populations of normal males and females at 4:1:1 or less usually resulted in no reduction in the total number of viable eggs, but at ratios of 6:1:1 and 10:1:1 there was a reduction of about 80% [14].

DAVIS *et al.* concluded that "The demonstration that *A. quadrimaculatus* mosquitoes can be sterilized by gamma-irradiation in the pupal or adult stage, and that the release of adequate numbers of sterile males in a normal population results in a substantial reduction in the number of viable eggs produced, indicates sufficient promise to justify further exploration of the possibilities of the use of sterilized anophelines as a control method. The practicability of the method would depend on the species of mosquito, the development of the most efficient procedures for inducing maximum sterilizing effects, the size of the area to be treated, the abundance of the normal mosquitoes, the importance of obtaining complete eradication, which is difficult to obtain by other means, and the financial resources available. Although at first glance this appears to be an expensive method, it might be more economical in certain circumstances than the current methods" [14].

Biological sterilization of insects infesting foods and other agricultural products

Dosages of the same order of magnitude as used in the sterile-male release technique can be used to sterilize and thereby break the life-cycle of insects infesting foods and other agricultural products. This process may be used to prevent multiplication of insects in stored foods and other agricultural products or to remove quarantines on such items.

Throughout the world, insect infestation results in a tremendous loss of foods. The Food and Agricultural Organization of the United Nations estimated that the grain destroyed by insects annually would feed more than 100 million people. Stored grain, flour, meal and cereal products are particularly susceptible to insect attack. The loss of these products in the United States alone has been estimated to be $300000000 annually [15].

In the case of stored grains grown in areas having a grain surplus, the insects and their eggs are brought into storage elevators from the field. Once inside the elevator, unless destroyed by chemical fumigation, the insects will lay more eggs and these, plus those carried in with the grain will hatch, producing the larval stage. In this stage the insects consume and spoil great portions of cereal grains and beans stored in elevators. Some of the most destructive insects are the granary weevil (*Sitophilus granarius*), the rice weevil (*Sitophilus oryzae*), and the confused flour beetle (*Tribolium confusum*), although over 150 different varieties of insects have been reported to infest cereals and cereal products [16].

The insects, when in the adult or larval form, are easily killed by the use of chemical fumigants since their respiration carries the poison into their system. However, the unhatched eggs and the pupae carried in with the grain are the main problem. Insects in these stages respire slowly and are more resistant to the chemical poisons. As a result, they may hatch

inside the elevator after the chemical treatment is over, producing adult insects which multiply and feed on the grain.

Gamma-irradiation of grain being charged into elevators will sterilize these quiescent stages as well as the adult insects. The use of gamma-radiation to sterilize the eggs and adults of several types of insects has been studied in the research laboratories of the Curtiss-Wright Corporation. In wheat-irradiation, the varieties studied have included the flat grain beetle (*Leamophloeus pusillus*), the cadelle (*Tenebroides mauritanicus*), the sawtooth grain beetle (*Olyzacphilus surinameusis*), the granary weevil (*Sitophilus granarius*), and the Angoumois moth (*Sitotioga cerealella*). In addition, the Mediterranean flour moth (*Ephestia kühniella*) and the cigarette beetle (*Lasioderma serricorne*) have been studied in the irradiation of peanuts and tobacco, respectively. BAKER, TABOADA and WIANT reported [4] an extensive series of tests on the effects of ionizing radiation on insects which infest wheat flour and beans. At the University of Michigan it was found that a dose of only 5000 rad was sufficient to sterilize eggs of the Mexican fruit-fly (*Anastrepha ludens*) (*cf.* BROWNELL and YUDELOVITCH, *these proceedings*, p. 193).

From these and other studies, it is concluded that a radiation dose of 10000 rad will sterilize eggs of these insects and that the same dose will prevent the adults from reproducing.

Besides the ability of the radiation to destroy eggs, the treatment should eventually prove to be cheaper, safer and more reliable than chemical fumigation. Chemical processes involve the use of some poisonous compounds for the extermination of the insects. Such chemicals as phosphine, hydrogen cyanide, methyl bromide and carbon disulphide are poisonous to humans, and in the latter case also explosive. Their application and storage near a food-processing plant calls for stringent precautions. The chemicals must be such as not to persist in the grain to be consumed by humans. This requires, for some chemicals, that the grain be turned over and aired during storage which adds appreciably to the cost of chemical treatment. With irradiation, the grain requires no further handling after passing through the irradiator.

Quality of irradiated grain cereals and flour

A variety of tests were made at the Curtiss-Wright Corporation food-research laboratories on the baking quality of gamma-irradiated flour. Similar tests have been conducted at the Fission Products Laboratory of the University of Michigan [17]. It was found in both series of tests that there were no detectable effects of gamma irradiation until dosages of 50000 rad were reached, and even at this dosage the effects were minor. Thus, four or five successive irradiation treatments might be given over a long period of storage, if necessary, without causing damage. To substantiate these results, tests on the baking quality of flour made from stored irradiated grain would be desirable.

The use of gamma-radiation as a method of insect control must be approved by the Food and Drug Administration before the process can be used commercially in the United States of America. A study of the nutritional value of irradiated whole wheat has been made at the University of Michigan [18]. This study involved the use of four generations of experimental animals fed whole wheat given a dose of 10000 rad, as compared to four generations of control animals which were fed non-irradiated whole wheat. Irradiated and non-irradiated wheat, respectively, constituted 70% of the diet for both groups of animals. The sole source of Vitamin E (which controls reproduction) for the experimental animals was the irradiated and non-irradiated whole wheat, respectively. It was concluded that the irradiation of the wheat had no effect on the growth, reproduction and pathology of the animals used in this study [17]. Extensive studies on the wholesomeness of irradiated

foods have been conducted in the United States under the supervision of the Office of the Surgeon General of the Army. These studies are nearly completed and it is hoped that approval of the use of gamma-radiation as a method of food processing will soon be granted by the Food and Drug Administration of the United States.

Proposed use of a new type of grain container for long-term storage under minimum shelter and without the use of chemical insecticides

Irradiation can produce 100% insect sterilization permitting unlimited storage in so far as insect infestation is concerned, provided the storage is conducted in containers absolutely free of insect penetration. Otherwise, storage over long periods would necessitate additional irradiation of the grain.

India is constructing new grain-storage elevators for handling in bulk large quantities of imported grains. We would urge that these elevators be designed differently from existing grain elevators in the United States of America. Most of our elevators are constructed of reinforced concrete. After aging, cracks develop that provide retreats for insects. Also, existing designs contain many openings that enable new insects to enter from outside. The great value in radiation-processing of long-term storage without fumigation is lost in these elevators which were designed for use with fumigants. Full advantage can be taken of radiation processing when the new elevators are designed to be proof against insect reinfestation.

As an alternative, a new procedure is proposed which involves sealing the grain within specially prepared insect- and water-proof bags containing from 50 to 100 lb of grain apiece. The bags of grain would then be irradiated with doses sufficient to sterilize biologically the insects and the eggs contained in the grain. The bags of grain could then be stored indefinitely or transported great distances with no danger of spoilage. The bags need not be opened until the grain is ready to be used at mills or as food for humans or farm-animals.

To be suitable for use in the process described above, the bag should be made of material which is impermeable to water vapour and permeable to atmospheric gases. The impermeability to water vapour protects the grain from moisture, thus preventing swelling and spoilage. Permeability to atmospheric gases is beneficial since the grain during storage and shipment will give off carbon dioxide and other gases which must be able to pass through the bag to the atmosphere. Otherwise, the bag might rupture and the grain be lost.

One proposed bag-arrangement would utilize any ordinary bag used for grain treated with a suitable insecticide and provided with a polyethylene liner. The liner could be coated on the inside surface of the bag or be a separate bag within a bag. Polyethylene film might be used as the liner since it is heat-sealable, easily providing the necessary protection against the admittance of water and water vapours. However, any material having these properties of polyethylene would be suitable. Polymylar, for example, would have the additional advantage of forming a barrier to the transfer of odours and other undesirable external conditions.

The Indian scientists PINGALE [19] [20] and MAJUNDER *et al.* [21] have reported success in the use of lindane-impregnated jute bags for insect proofing. They report [3] that the quantity of lindane (19.2% gamma-BHC) required to impregnate bags is so small that there is no risk of contaminating foodstuffs stored in the bags. They also state that washing impregnated-bags with water did not remove the lindane.

We suggest that the storage-life of grain in such bags might be extended appreciably by use of a polyethylene liner and gamma-irradiation.

Once the grain has been placed in the bags and irradiated, it would not be necessary to store the bags within a grain elevator or the like. Rather, the bags could be arranged on the ground in the open air, with only some slight protection between the bags and the ground, and covered to prevent the accumulation of undue moisture.

It is conceivable that such a process as that described could solve some of the major problems involved in shipping bulk or bagged grain to India and to countries which have little or no grain-storage facilities. The grain stored in the insect- and water-proof plastic-lined bags might be left in the open in these countries with only a tarpaulin for cover, without adverse effect. To facilitate transport they might even be floated in groups on a raft along rivers and waterways.

The cost of such special bags would add a capital cost to grain storage. This could be minimized by using the bags again for, perhaps, 4 or 5 shipments and storings. Since grain stored in such containers would not need fumigation or storage in elevators for protection against weather, savings could be realized which could offset the cost of the new containers.

In general, the cost for the chemicals used for treating grain for insect control in the United States of America is between 0.1 cent and 0.6 cent per bushel; the lowest cost is for a mixture of carbon disulphide, carbon tetrachloride and sulphur dioxide, known as "Weevil-Cide", and higher costs for products such as methyl bromide, Dow fume, phosphine and others. In addition to the cost for chemicals, an additional cost of about 1 cent per bushel is involved for turning the grain. Some operators turn the grain, others do not. Some operators fumigate two or three times in one year and others only once in eighteen months. The differences in practice are influenced by the temperature and humidity in the storage bins, by the degree and type of initial insect infestation, and by the design of the storage elevator. Grain held at low temperature and low humidity is relatively free from insect attack and seldom requires turning. If "hot spots", with temperatures over 100°F develop, insect infestation or sprouting from excess humidity is indicated. Fumigation and turning of the grain is usually required under such conditions. In tropical climates stored-grain may require turning and fumigation every 2 to 4 months. Thus a storage of one year in southern States of the United States might involve a cost of several cents per bushel if frequent turning and fumigation were required. If we add to this cost the unit charge for capital invested in the grain elevator, we might be able to write off the cost of the new containers after about 2 or 3 years of service. The containers might well be used for a much longer time.

In another process, radiation might be used to sensitize insects to other methods of destruction. Pre-treatment of bacteria with a dose of radiation leaves the remaining living cells sensitive to heat so that the effective heating time for sterilization may be reduced several-fold. NAIR [22] of the Indian Atomic Energy Establishment, Bombay, reports a similar effect on insects, as a result of studies he made at the University of Michigan. Irradiation of dermestid larvae with doses up to 5000 rad did not break their life-cycle, nor did a heat treatment of 47°C for 1 h. However, larvae irradiated before heat treatment did not pupate, and the cycle was broken.

Although little is known about the possible synergistic effect of a radiation pre-treatment followed by use of chemicals, there is a strong possibility that a low dose of radiation would sensitize insects to chemical poisons.

The current techniques under consideration for radiation control of insects rely chiefly on means of sterilizing adults and killing eggs. Very little attention has been given to the possible exploitation of techniques involving radiation-induced changes in behaviour to rid stored-food of insect pests.

BAYLOR and SMITH [23] have shown that the water flea *Daphnia magna* wil dive from a position near the surface to the bottom of an aquarium when exposed to X-rays. Their explanation for this behaviour is that *Daphnia* perceives X-rays and reacts to them in this characteristic fashion. It is claimed, furthermore, that all animals perceive X-rays. Gamma-rays have evoked the same behaviour [23]. In higher animals X- and gamma-rays may well be only experienced as a vague disturbance in the normal field of vision whereas a more pronounced response may be expected in insects, since their normal phototropic responses are an all-or-nothing phenomenon which is only slightly moderated, i.e. they have little control over their responses. This is demonstrated by the suicidal behaviour of moths drawn to a flame. Other experiments using insects have confirmed the fact that exposure to ionizing radiations will cause atypical behaviour. This behaviour should be investigated with a view to its applicability to controlling insect populations.

Little evidence is available at present to indicate the nature of the response of insects to ionizing radiations. It is not known whether the observed behaviour is peculiar to each species, or if the stimulation by gamma- and X-rays occurs through the eyes or by acting directly on the central nervous system. Observations made in the Fission Products Laboratory at the University of Michigan range from data showing that rat fleas desert irradiated rats in which it was not possible to test for directional response, to specific directional reactions by irradiated grain beetles. In observations on the grain beetles the insects were mixed randomly into grain and irradiated at biological sterilizing dosages. After irradiation, the beetles had aggregated at the side of the container facing away from the source. These experiments were carried out under high radiation intensities. The behaviour at lower intensities has not been investigated. However, recent experiments performed at the school of air medicine at Randolph Field [24] show that the behaviour of mammals is altered by exposure to less than 50 r. Perhaps insects which respond almost totally (i.e. without control) to stimuli of high-intensity ionizing radiation may do the same at lower exposures.

A ring of radiation sources might serve as an insect repellent. However, little is known about such possibilities. Much research is needed to evaluate more fully the possible uses of radiation in insect control.

Available radiation sources

The simplest type of radiation source consists essentially of a cylindrical steel-drum filled with lead, except for a hollow central well. The cobalt-60 source material, generally as a number of individual pieces or slugs, is held in an annular container at the bottom of the well. The samples to be irradiated are placed in a cylindrical container attached to the bottom of a lead plug which slides in the central well. When an irradiation is to be carried out, this plug is raised by means of a light crane and swung to a point several feet away from one side of the source. The sample-can is attached to the plug and the whole is swung back over the central well in the source and lowered into the exposure position. After the necessary time interval, the sample is raised beyond the source and detached from the plug, which is then replaced.

With this type of source arrangement, considerable amounts of radiation stream upwards from the mouth of the well when the plug is removed. However, this presents no danger if the facility is maintained in a room separated from overlooking buildings, and is mounted on a pedestal so that the top of the lead container is approximately at shoulder height.

In this manner, with sources of up to 200 c, the radiation level at head height not closer than 5 ft to the source may be kept below the maximum permissible limits.

Using spiral tubes cast into the lead shielding, it is possible to circulate air or other gas through the irradiation chamber and so achieve a measure of atmosphere and temperature control during irradiation. The sample, size and dose rate are, however, limited by the design.

One example of this type of source is described in reference [25] and several models are available commercially. A modified design has a second plug fitted below the sample holder. As the double plug is raised, the lower one fills the void at the mouth of the well, thus preventing excessive radiation leakage. Models of this type, while more expensive, can be used in existing laboratories.

However, the maximum sample size in these irradiators is a cylinder of about 8 in diam. and 8 in high, and because of the closeness of the sources, even $\pm$ 10% variation in dose distribution can only be achieved over a portion of this volume. A much more adaptable source system may be used by exposing the source in a shielded room or cell. This must have walls and roof of adequate thickness to provide shielding down to maximum permissible levels for workers in the vicinity, and must be fitted with a door and sufficient interlocks to prevent the accidental exposure of personnel to radiation. Access may be by means of a shielded corridor or a heavy door.

The simplest source-system to use in such a cell is a rod normally kept in a lead cylinder but raised inside a fixed, vertical guide-tube for irradiation. The source-plug combination may be lifted manually and this operation linked so that exposure is not possible until the gate has been closed. Conversely, it is not possible to open the gate until the source has been lowered to its safe position.

Samples to be irradiated are mounted at a given distance from the source, and may be placed on small turn-tables to provide greater uniformity. With a single source, there must always be a compromise between uniformity and dose rate, since the most uniform radiation fields are to be found at the greatest distance from the source, where the dose rates are lowest.

A most flexible arrangement is provided by using several sources which can move along guide tubes from a safe, shielded position to a predetermined exposure-position. One method of carrying this out is to use the transport-cask to shield the sources in their safe positions and to attach sealed, flexible guide-tubes to openings in this cask (see Fig. 1). (Alternatively, rigid tubes bent to give a pre-set configuration may be used.) The sources are exposed by expelling them to the closed ends of the guide tubes and subsequently retracting them into the cask. Again, adequate controls and safety interlocks must be provided. In this manner, ten or more sources totalling up to 25000 c may be used in a facility which imposes no restriction on the size or condition of samples to be irradiated. Facilities of this kind are to be found in the leading radiation centres of the world. They are of the greatest value for research purposes and when loaded with sufficient cobalt-60, provide a straightforward transition to pilot scale operations.

Although the first commercial gamma-radiation processing-facility has only this year come into operation [26], many designs have been put forward. The earliest of these generally assumed spent fuel-elements to be the source of radiation, but would require very little modification to change to cobalt or cesium.

The design of a production-facility has to cope with two major groups of problems. Firstly, what shape and size of source arrangement should be used to provide the maximum of usable radiation-energy with a minimum absorbed in the source itself. Secondly, how may a conveyor system be arranged around the radioactive source so that maximum

absorption of radiation is achieved without over-exposure of parts of the product and without holding up an unduly large volume of product in the facility.

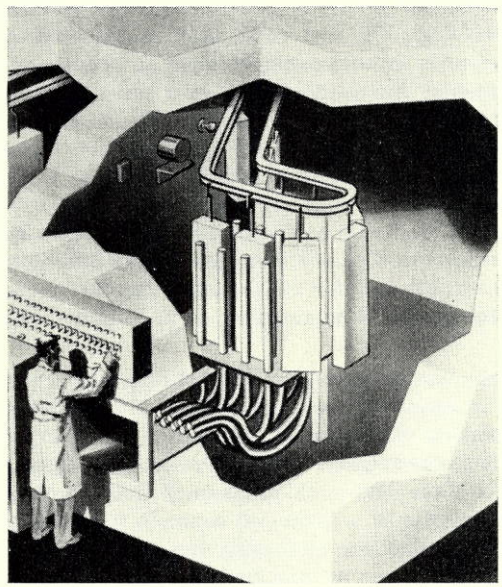


Fig. 1
Illustrates flexible guide tubes from Multi-Array Gamma-Irradiator mounted in wall of radiation cell

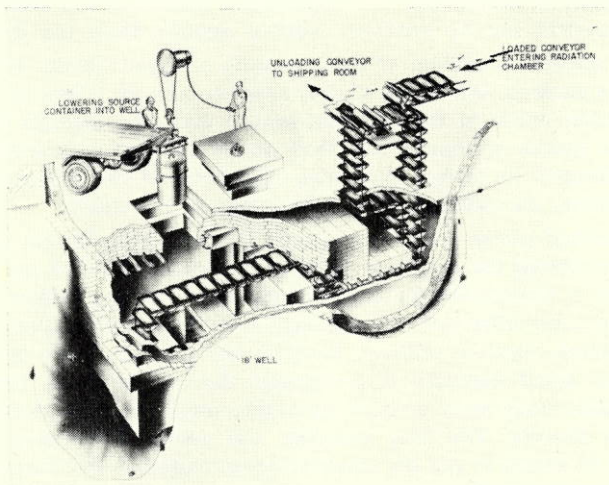


Fig. 2
Schematic arrangement of irradiator for bags of grain, situated in basement of existing mill

Fig. 2 illustrates the principles involved [27]. It shows a flour irradiator which might be situated in the basement of a large mill. The facility would become an integral part of the manufacturing operation and would be so located that the bagged flour could be conveyed to the entrance of the chamber, placed in a bucket conveyor and transported into, through and out of the radiation field. The bags could then be conveyed to a shipping or storage area. The bucket conveyor is shown passing through an opening in the floor to the access passageway of the chamber. Along this, it makes a series of 90° turns (incorporated to prevent the escape of radiation) and continues on into the radiation cell. Here it passes under, up and over the radiation source and leaves the cell *via* the access passage. For grain irradiation, it would be desirable to irradiate in bulk. Consequently, a design for a low-cost gravity-flow irradiator was developed by the Curtiss-Wright Corporation. In this design, a bulk-flowing food such as grain would pass by gravity through the irradiator at the top of a storage elevator. This eliminates the cost of additional conveyor-equipment. The use of a compact vertical duct for the radiation chamber greatly reduces the cost for shielding. Control of the dosage is obtained by an adjustable orifice at the bottom of the radiation chamber.

One of the chief design factors taken into consideration here is the method of positioning the radiation sources to maximize the absorption of the radiation with minimum overdosage. In an early analysis of the use of a cobalt-60 rod-source for food irradiation, it was shown that if a 3-lb commercial can of food was rotated adjacent to a single rod source, the dosage on the surface of the food would be about 10 times that received in the interior [28]. The inefficiency in this respect of a single-rod source is exceeded only by a point source. If multiple-rod sources are used, the uniformity can be increased greatly. For example, if 10 rods were placed around the can of food instead of rotating the food adjacent to a single rod, the activity in each rod might be reduced by a factor of 10 to maintain the same total activity, and the radiation flux between rods would be essentially uniform. The reduction of local overdosage approaches 10 for the example above, depending upon the size of the sample, the distance between sample and source, the energy of the gamma-source, and the absorption coefficient of the sample. Thus, by using a number of rod sources, an essentially uniform radiation-field can be obtained without penalty from the inverse-square law.

To utilize the maximum amount of the radiation, a single cylinder of food should not be irradiated by a circle of rods, since all the radiation outward is lost in the shield around the source and since much of the radiation inward may pass through the food into the shield. Instead, a matrix of sources and food should be used with a width the value of several half-thicknesses to permit maximum absorption of the gamma-radiation in the food. This minimizes the radiation lost at the edges to the shield.

The ideas described above were incorporated in the Curtiss-Wright design which was optimized by performing the necessary calculations on an IBM-704 machine. Fig. 3 is a sectional view of a Curtiss-Wright experimental-size, continuous-flow grain-irradiator. This design is for a portable unit utilizing lead shielding. If installation in a grain silo is desired, the shielding could be ordinary concrete and the size would be larger. Grain or other granular or liquid materials enter through the top labyrinth, flow downward by gravity past the distributed array of axial rod sources and leave through the lower labyrinth and flow-control throttle. The flow chamber, and source dimensions and geometrical arrangement can be varied to suit the material being irradiated and the isotope to be used. Essential specifications for the wheat configuration are shown in Fig. 3 and in Table III.

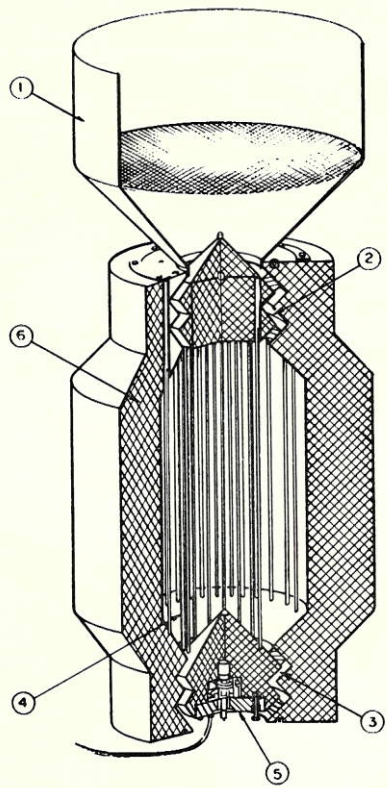
To explore the economic feasibility of the gravity-flow irradiator, a cost-breakdown and capacity-calculation can be made to provide an estimate of the cost per bushel of

TABLE III

DESIGN DATA FOR EXPERIMENTAL GRAIN IRRADIATION UNIT (MODEL GRP-4)

Capacity	120 bushels/h
Source	15000 c Co^{60}
Unit dimension	42 in diam. × 74 in high
Proposed trailer dimension	24½ ft long × 8 ft wide × 12½ ft high
Unit weight	28000 lb approx.
Lifting skid weight	2000 lb approx.
Trailer weight	9500 lb approx.
Tractor weight	13750 lb approx.
Gross weight	53250 lb approx.

Model GRP-4 grain unit
Capacity 120 bushels/h
Diam. 42 in
Cobalt-60 gamma-ray source
15000 c
Wt. 14 t



① Feed hopper and screen
② Entrance labyrinth
③ Exit labyrinth
④ Array of source tubes
⑤ Motorized throttle with position indicator
⑥ Lead shield

Fig. 3
Curtiss-Wright gravity-flow irradiator for wheat or granular material

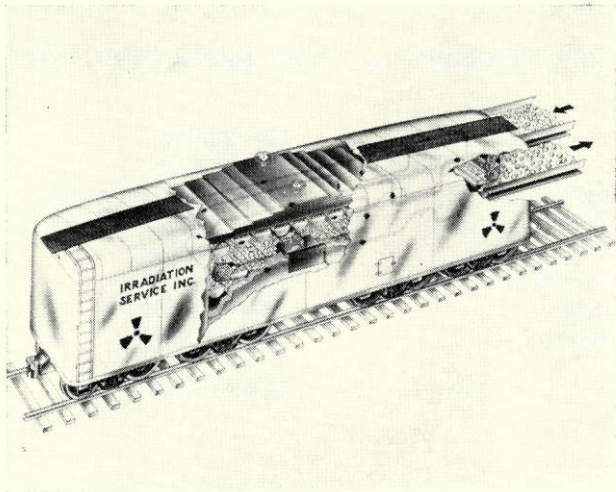


Fig. 4
Portable irradiator for potatoes, mounted on a rail car using lead shielding

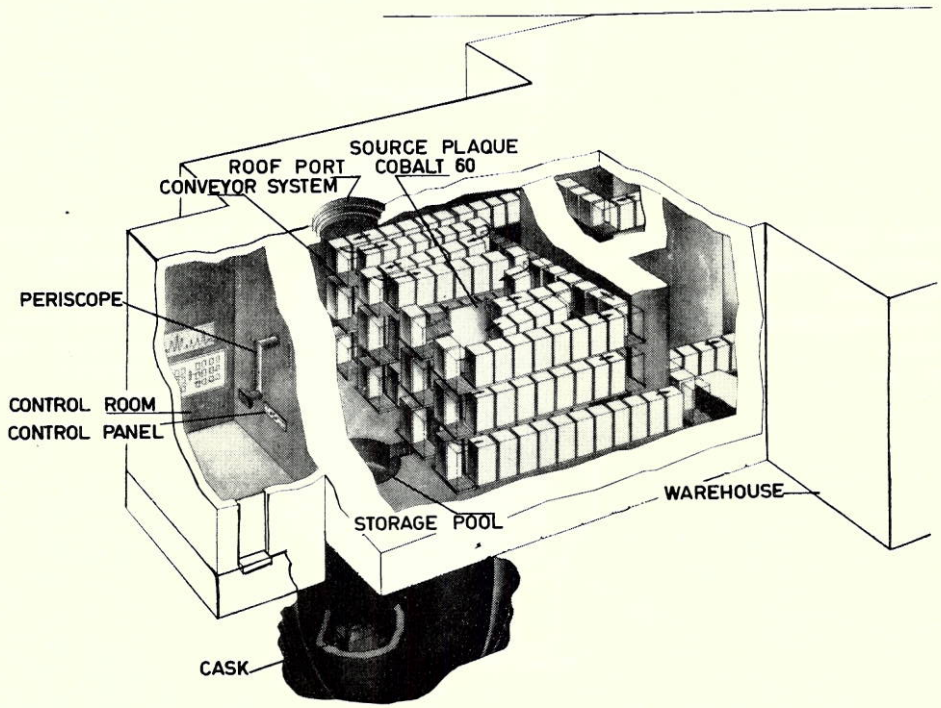


Fig. 5
Conveyor arrangement for package irradiation to provide maximum absorption of radiation

irradiating grain with a dose of 10000 rad. This estimate indicates the cost per bushel for disinfestation of wheat by irradiation to be in the same range as that for chemical disinfestation. The exact cost depends, of course, on the assumed price of the cobalt. Using a current price of $ 1.00/c, the cost is about 0.5 cent per bushel for 24 h/d operation.

An alternative is to use an irradiator consisting of a series of concentric cylinders, such as described by CORNWELL [2]. This facility consists of a source contained in a cylinder, diam. 8 in and length 10 ft, surrounded by a cylinder of diam. 2 ft. The annulus so formed has a volume of 28 ft^3 and with a flow rate of 5 ft/min would deliver 17 t/h of grain. By surrounding the source with two further cylinders to provide outer annuli with capacities of 56 and 84 ft^3 respectively, the flow rate could be increased to 25—30 t/h and the efficiency of the system to around 40%. This would require approximately 210000 c for a dose of 20000 rad.

Fig. 4 shows a mobile facility designed to irradiate bagged potatoes, which could also be used for the treatment of bags of flour or grain. In this application the irradiator is shown mounted on a rail car. It could equally well be fitted on-board ship.

Several facilities have been designed for the irradiation of packaged materials, e.g., dried fruit, which is liable to arrive after a long sea journey from producer to market, infested with larvae which have hatched from moth eggs laid in the fruit at the packing stations. Fig. 5 shows a concept of surrounding the source as completely as possible by packages so that they may absorb the maximum possible amount of radiation. The packages move along the conveyor system so that each package occupies, in turn, each position in the system and so all receive equal doses.

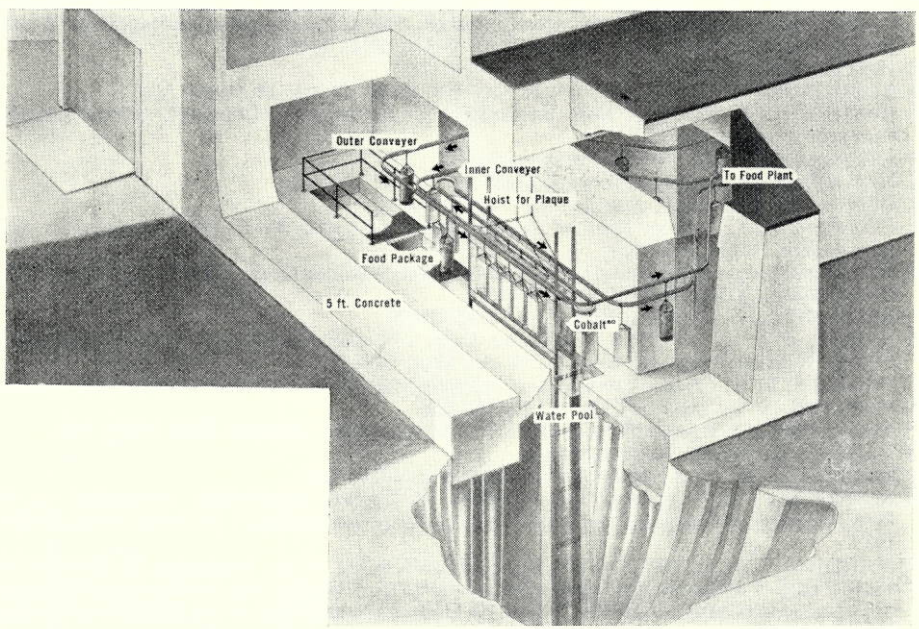


Fig. 6
Artist's conception of Food Process Development Irradiator

The largest gamma-radiation source now contemplated, is the Food Process Development Irradiator (*cf.* Fig. 6), now being designed by Curtiss-Wright Corporation for the Quartermaster Corps of the United States Army. This unit, which is modified from the High Intensity Food Irradiator to come in line with the changing needs of the Quartermaster, will use approx. 1 Mc of cobalt-60 and is scheduled for operation in the summer of 1962. It is primarily intended as a research facility and will have a flexible rod-source arrangement to allow study of different source and conveyor geometries. However, it will be used to irradiate production quantities of food at all doses from disinfestation levels up to 5 Mrad.

From the research studies already made, it is clear that irradiation may provide an answer to some of the problems of food losses from insect infestation. Radiation sources are readily available for further studies of the important species at a research or pilot-plant level. The recent fall in the price of cobalt-60 favours the economics of radiation disinfestation and should encourage the responsible authorities to give full support to this technique.

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DISCUSSION

P. B. CORNWELL (United Kingdom): Proof of the pudding being in the eating, I think the value of the interesting designs to which Dr. Brownell has referred will emerge at a point in the future when we have experimented with them and convinced commercial interests that they are suitable for the job.

Now there are one or two points I should like to take up. The first is the proposed use of radioactive gases. Let us face it: if we are going to pump a gas at all, why not pump a fumigant, which has no hazard of radioactivity and which is yet reasonably efficient? My second point has a bearing on our Chairman's opening remarks, when he referred to the enormous pest problems facing the under-developed countries. Why are these problems so immense? Is this not partly the result of inadequate education in techniques of insect control and of lack of adequate storage facilities, plus of course, in many cases, temperatures which lead to rapid reinfestation? I therefore dare to prophesy that when the under-developed countries undertake programmes for building *adequate* storage facilities, losses of stored food products will fall considerably. Hence this leads me to suggest that we should be encouraging the irradiation process in those under-developed countries which have obtained or can obtain adequate storage facilities.

They must also be in a position to afford the irradiation process. We have been listening for many years to confident forecasts that the price of this or that radiation-emitting isotope will drop to so many cents per curie, so that perhaps disinfestation by irradiation may be competitive economically. But, in fact, there has been no real change over the last five years in the selling price of cobalt or estimated cost of caesium production. In the United Kingdom we still consider it uneconomical to attempt to extract caesium. Using the by-products of nuclear reactors also requires, of course, a close degree of proximity between the reactors and grain storage installations, and this is obviously not the case at the moment.

Finally, I should like to stress a point I made earlier on, namely that we should make detailed studies of present methods of handling infestable commodities and attempt to integrate irradiation processes with least disturbance to existing facilities. I am convinced that this is the most rewarding approach.

L. E. BROWNELL: I mentioned fission gases only because very large quantities of activity are necessary for food sterilization, and it has been thought that food processing plants using irradiation may be able to utilize these gases at some future time when homogeneous reactors have been perfected. At present we are pumping fumigants through our grain-storage facilities in the United States; we may spend $50000 at one grain terminal on the operation, and yet still we lose grain. So even in an advanced country, with fully adequate storage facilities, losses occur. Present methods are unsatisfactory for other reasons also. For example, there are residual toxins left in the grain after fumigation. Sometimes, when shipment is necessary before the expected date, consignors must either face an expensive airing process or ship the grain with a level of toxic materials above that permitted by the Food and Drug Administration, in the hope that it will be sufficiently ventilated by the time of arrival. If not, it is rejected for human consumption and must be sold as animal feed, at a loss of half the price. A number of grain-handling companies are extremely concerned about this situation and are therefore interested in trying irradiation, particularly in warm areas where they now fumigate two or three times a year—as opposed to once a year or once every 18 months in the United Kingdom or the northern United States, for example.

Now, as far as the correct design goes, I simply tried to show some of the effects of geometry and why this flexible unit, which offers many alternative geometries, is an interesting research tool.

P. B. CORNWELL: May I briefly outline how we at Wantage think our own thoughts on sources for the irradiation of grain. I have emphasized previously our feeling that cobalt-60—in fact, isotope sources in general—are not practicable for disinfesting grain, the reasons being both the high capital cost, enough to deter the stoutest-hearted business man, and operating costs which are well above those for fumigation. Although this may be contrary to Dr. Brownell's views, I do believe that we can be successful in the disinfestation of grain by using the new high-voltage electron machines at present being developed. In fact High Voltage Engineering Corporation have developed, and have in the testing stage at the moment, machines which can treat grain at 200 t/h with 16500 rep at a cost comparable to or undercutting insecticidal treatment. In the near future we hope to be engaged at Wantage in pilot-plant studies with a machine of this type, principally to investigate conveyor systems to afford the required penetration for this kind of treatment.

L. E. BROWNELL: Although cobalt-60 is high-priced at present, we believe we could carry out irradiation with it at a price of two cents per bushel, on a 24 h/d, 300 d/yr operating basis. We estimated, with reference to an actual plant that handles, if I remember correctly, 10000 bushels an hour at full load, that we could perform disinfestation at a cost of one cent per bushel, using zirconium-niobium loaded three times annually. The company was actually interested in doing this as soon as approval from the Food and Drug Administration to irradiate food could be obtained.

Now as far as electron-beam accelerators are concerned, I made a study of the situation for the Hanford people in connection with a review of the future of the caesium market, and one of the problems is the purely mechanical one of the limited speed of the conveyor system. Since we run into induced radioactivity above 8 MeV, we are limited to, say, 5-MeV machines, which means a very narrow band of grain. This in turn calls for very high conveyor-speed in order to get adequate capacity. As a report published by Atomics International in June 1958 confirms, this precisely is the limiting factor: one cannot get a high enough conveyor-speed consistent with keeping the grain from flying off. With the gamma-irradiator, on the other hand, one can irradiate in great volume and so get the large capacity.

P. B. CORNWELL: We intend to convey grain for electron treatment, pneumatically or by a system of free-fall. I would agree with Dr. Brownell that it is out of the question to use a belt conveyor for treatment with electrons. Using pneumatic handling, the density of the grain is reduced to about a sixtieth of the static bulk density, permitting the penetration of electrons; with the currently available pneumatic conveyors, using air speeds of 100 mile/h, it is possible to convey grains at extremely high speeds. Furthermore the high-voltage machines we have in mind provide dose rates in the range of 6 to 7 million rep/s. Thus, with a combination of pneumatic conveyors or free-fall systems with such very high dose-rates, it is possible to convey the grain and achieve the required dose.

L. E. BROWNELL: There is no doubt that the issue between accelerators and isotopes is still a live one, and the food irradiation facilities for the Quartermaster Corps will have one of each type.

THE CHAIRMAN (D. W. Jenkins, United States of America): Much good work is going on in several countries on the effects of radiation on insect pests. I have asked Dr. Andreev to be kind enough to review what is happening in this field in the USSR, but he tells me that he has not got adequate notes with him at the moment on the work in progress. I will therefore request Dr. Cornwell to give us a brief résumé of the work Drs. Peredelskii, Rumiantsev and others are doing in the Soviet Union.

P. B. CORNWELL: I have before me abstracts of three Soviet papers on the biological effects of radiation on insects infesting cereal commodities. From the first, by Peredelskii, I quote the conclusion that "5000 r used against the rice weevil is very close to the sterilizing dose, and this value could be used in technological calculations on irradiators designed to disinfest grain on State granary conveyors". I am not sure that I agree with the application of this low dose of 5000 r since it will certainly allow some progeny to be produced. It is also stated that: "Preliminary calculations show that it is theoretically and technically possible to design gamma-ray units for large-scale sterilization of moving grain."

The second paper is by Topchiev *et al.* and was presented at the second United Nations International Conference on the Peaceful Uses of Atomic Energy in 1958. 10000 r is considered quite sufficient to kill and completely sterilize weevils, and there is also a statement in the paper that an installation has been designed and is to be put into operation in the USSR in that year, having a capacity of 500 kg of grain (i.e. 0.5 t) per hour.

The third paper, by Sumarokov, is on radiobiology and deals principally with "delayed death" of weevils subjected to irradiation. It gives a very interesting account of radiation effects at different levels of oxygen tension, pointing out the greater resistance of weevils under conditions of oxygen depletion.

S. V. ANDREEV (Union of Soviet Socialist Republics): I should like to offer a few comments in connection with Dr. Brownell's second paper.

In the USSR we have paid great attention to pest control, particularly in recent years when over 30 million hectares of virgin lands have been brought under cultivation. According to the most recent information at my disposal, a large-scale elevator plant is being planned where grain will be treated by the continuous process.

With regard to the economic aspects of the matter, there is much discussion in our own as in other countries, but it would appear that one of the economically most advantageous methods is the use of high-frequency waves which cause thermal selective processes in living organisms. Insects have, of course, an almost 100% moisture content, and these thermal processes occur much more rapidly in insects than in grain, which has a moisture content of 12 to 15%. This principle is yielding very effective results.

The work going on in numerous countries in this field does indicate that atomic energy is at last really being used for rational purposes, in this case the preservation of the fruits of human toil.

M. S. QURAISHI (Pakistan): I was greatly interested by Dr. Andreev's references to energy radiations other than nuclear ones. We did some work on the effects of ultrasonics on *Aëdes aegypti* eggs and housefly pupae. We found that after exposure to a frequency of 0.5 MHz, the percentage of eggs hatching out increased in comparison with controls and that the time taken for hatching was also reduced.

The effects on housefly pupae were somewhat similar to those induced by irradiation as reported by Dr. Nair, *these proceedings*, p. 207. I wonder whether anyone would like to comment on the effects of ultrasonics on insects and insect eggs, and on how these effects compare with those of radiation.

THE CHAIRMAN: If there are no further remarks which participants wish to make in this connection, we will now proceed to consider another aspect of insect control, namely, the actual elimination of insect species. This, I think, is a particularly exciting aspect, and I believe we are on the threshold of really spectacular results.

Until recently we have had the idea that the only good insect pest was a dead one or at all events one that was not present to disturb us. This being so, classical methods of

control consist firstly of quarantine, by which the attempt is made to keep insects out of certain areas of particular importance, and secondly, of using insecticides to kill insects. Much has been achieved by this latter method, in particular. However, mass use of insecticides, in addition to leading us to neglect appropriate measures in other directions—draining swamps, and so on—and to bringing about the elimination of numerous parasites and predators which formerly helped us to maintain control, has also conjured up the enormous problem of resistance. We thus find ourselves on what one might term an insecticide treadmill, using ever more toxic insecticides, ever more varied types of insecticide, ever greater quantities of insecticides—a treadmill off which we fain would step, yet dare not.

Consideration of the small size and enormous reproductive capacity of insects suggests the idea that if only we could find a way of applying these outstanding characteristics of insects against themselves, in judo fashion, great results might be achieved. And this has actually happened, as we are going to hear shortly. There are, in fact, about five different methods by which insects can be used against themselves. The first consists of the release of males rendered sterile by irradiation. The second is to employ genetic characteristics such as sterile hybrids, using male factors such as those that have been found by Craig in *Aëdes aegypti*, and dominant lethals or a series of recessive lethals. Very interesting potentialities exist here for changing even the behaviour pattern of insects by genetic means. The third method calls for the use of chemicals to bring about sterilization, including various radiomimetic and other substances which have been reported on recently. Under this head also comes the use of hormones, to which Drs. Williams and Wigglesworth have referred, as a means of upsetting the growth cycle, preventing diapause, and so on. The fourth method consists of releasing parasites and pathogens along with sterile insects themselves, which helps in the work of elimination. The fifth and last method consists of population substitution or replacement. Here genetics and selection are called into play to breed species less liable to attack man or his livestock or crops. This method is particularly suitable for use in cases—which have arisen—where we find we are unable to eliminate insects completely and have to find a way of living with them.

Returning to the first method, I think everybody has heard about the screwworm project, which is undoubtedly one of the outstanding entomological accomplishments of the century. We shall now hear something more of this in the course of the next paper.

SECTION 2

USING INSECTS AGAINST THEMSELVES

RESEARCH ON RADIATION IN INSECT CONTROL

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Abstract — Résumé — Аннотация — Resumen

Research on radiation in insect control. A summary of the development and application of the sterile-male release technique, in which gamma-radiation was used as the sterilant, in the eradication of the screwworm, *Callitroga hominivorax* (Cqrl.), is presented. Preliminary laboratory and field results on the application of this same technique to the control or eradication of mosquitoes, *Anopheles quadrimaculatus* (Say), and fruit-flies,—the Mediterranean fruit-fly, *Ceratitis capitata* (Weid.), the oriental fruit-fly, *Dacus dorsalis*, (Hendel) and the melon fly, *Dacus curcurbitae* (Coq.)—are presented. Results are also given showing the lethal effects of radiation on some insects of medical importance, including the body louse (*Pediculus humanus humanus* L.), the housefly (*Musca domestica* L.), the American cockroach (*Periplaneta americana* (L.)), the German cockroach (*Blattella germanica* (L.)), the firebrat (*Thermobia domestica* (Pack.)), the bed bug (*Cimex lectularius* L.) and the Pharaoh ant (*Monomorium pharaonis* (L.)).

Recherche sur l'emploi des rayonnements dans la lutte contre les insectes. Le mémoire donne un aperçu de la mise au point et de l'application de la méthode du lâcher de mâles stérilisés par les rayons-gamma dans la destruction de la lucilie bouchère (*Callitroga hominivorax* [Cqrl.]). Il indique également les résultats préliminaires de recherches faites au laboratoire et dans des champs d'essai sur l'application de la méthode pour combattre et exterminer certains moustiques (*Anopheles quadrimaculatus* [Say]), les mouches à fruit telles que la mouche méditerranéenne (*Ceratitis capitata* [Weid.]), la mouche orientale (*Dacus dorsalis* [Hendel]) et la mouche du melon (*Dacus cucurbitae* [Coq.]). Enfin, le mémoire contient des données qui attestent les effets létaux de l'irradiation sur plusieurs insectes présentant un intérêt médical, y compris le pou (*Pediculus humanus humanus* [L.]), la mouche commune (*Musca domestica* [L.]), la blatte américaine (*Periplaneta americana* [L.]), la blatte germanique (*Blattella germanica* [L.]), la thermobia commune (*Thermobia domestica* [Pack.]), la punaise (*Cimex lectularius* [L.]) et la fourmi de Pharaon (*Monomorium pharaonis* [L.]).

Исследования в области применения радиоизотопов и радиации для борьбы с насекомыми. В докладе дается обзор развития и применения метода выпуска стерильных мужских особей, при котором гамма-радиация использовалась в качестве стерилизатора для уничтожения кольцевого червя *Callitroga hominivorax* (Cqrl.). В докладе приводятся также предварительные результаты лабораторных и полевых исследований по применению этого метода для контроля или уничтожения москитов *Anopheles quadrimaculatus* (Say) и фруктовых мух — средиземноморской фруктовой мухи *Ceratitis capitata* (Weid.), восточной фруктовой мухи *Dacus dorsalis* (Hendel) и дынной мухи *Dacus curcurbitae* (Coq.). В докладе приводятся также результаты, свидетельствующие о смертельном действии радиации на некоторых насекомых, имеющих медицинское значение, включая нательную вошь (*Pediculus humanus humanus* L.), домашнюю муху (*Musca domestica* L.), американского таракана (*Periplaneta americana* (L.)), немецкого таракана (*Blatella germanica* (L)), (*Thermobia domestica* (Pack)), постельного клопа (*Cimex lectularius* L.) и муравья-фараона (*Monomorium pharaonis* (L)).

Investigaciones sobre el empleo de las radiaciones en la lucha contra los insectos. En la memoria se reseña la preparación y aplicación de la técnica de suelta de machos esterilizados con radiaciones gamma para erradicar la *Callitroga hominivorax* (Cqrl.). Se indican igualmente los resultados preliminares obtenidos en el laboratorio y sobre el terreno acerca de la aplicación de esta técnica para combatir y erradicar ciertos mosquitos [*Anopheles quadrimaculatus* (Say)], y algunas moscas de la fruta como la mosca mediterránea [*Ceratitis capitata* (Weid.)], la mosca oriental [*Dacus dorsalis* (Hendel)], y la mosca del melón [*Dacus curcurbitae* (Coq.)]. Se exponen los efectos letales de las radiaciones sobre algunos insectos de importancia médica, en especial el piojo vulgar [*Pediculus*

humanus humanus (L.)], la mosca común [*Musca domestica* (L.)], la cucaracha americana [*Periplaneta americana* (L.)], la cucaracha alemana [*Blattella germanica* (L.)], la [*Thermobia domestica* (Pack.)], la chinche [*Cimex lectularius* (L.)] y la hormiga faraónica [*Monomorium pharaonis* (L.)].

I. Introduction

The impact of radiation and radiosiotopes on studies related to the control of insects has been very great over the past decade. Radioactive tracers have been employed to study the complexities of insect toxicology, physiology, behaviour, and genetics. Radioisotopes have allowed us to advance our knowledge in these fields and have contributed greatly to studies on the mode of action of insecticides, mechanism of resistance, development of systemic insecticides, ecology of insects, and insecticide residues. The eradication of the screwworm (*Callitroga hominivorax* (Cqrl.)) from the southeastern United States of America by rearing and releasing flies sterilized by gamma-radiation has successfully demonstrated a new concept of insect control in which radiation is employed. Studies on the lethal effects of radiation on insects suggest another approach in which radiation is used directly to kill insects such as those found in stored products. The Entomology Research Division of the United States Department of Agriculture has worked with several applications. It is the purpose of this paper and the companion article on p. 93 to summarize the research and development of the sterile-male release technique and the lethal effects of radiation on some insects affecting man.

II. Eradication or control by release of sterile males

As early as 1937 KNIPLING [9] suggested the possibility of controlling insects by inducing sterility in the male insect. He elucidated [8] the theories and applications of this concept and emphasized the need for a thorough knowledge of the biology and behaviour of insects considered to be controlled by this method. The development of this technique has been made possible by the use of gamma-radiation produced by cobalt-60. For the method to be successful, large numbers of insects must be reared, rendered sterile, and released into a wild population so that the ratio of released sterile males to wild fertile males is sufficient to overpower the wild population. Both the density of the wild population and its biotic potential become extremely important factors. For example, if sufficient sterile males could be released to reduce the overall viability of eggs from wild females to one-fifth (theoretically a ratio of 5 sterile males to every wild male) and the biotic potential of the species were 5, then no reduction would occur since the effect of induced male-sterility would be just balanced by the biotic potential.

With any insect one naturally appraises the situation in terms of requirements that must be met if the technique is to be at all practical in application. The requirements to consider seem to be rather severe and are listed as follows:

(1) An economical method of rearing large numbers of insects must be available, or at least the problem must be reasonably amenable to development.

(2) The insect must be of a type that can be readily dispersed by aircraft and other means, and the males must have the ability to search effectively for the opposite sex and mate in competition with native males.

(3) The sterilizing procedure must not adversely affect mating behaviour or injure the males appreciably.

(4) The species to be controlled must have a comparatively low population or be subject to reduction by insecticide or other means. Advantage may be taken of seasonal fluctuations, since most insects are less numerous at some seasons of the year than others.

(5) The area to be treated must be reasonably well protected against reinfestation, preferably isolated by water, mountains, or other barriers.

(6) The males to be released must not be harmful to man, animals or plants.

(7) Mechanical and low-cost means of separating the sexes before release must be available in cases where the female is harmful. For example, to release female mosquitoes in large numbers would create an intolerable condition for man and animals within the area. This problem could be exceedingly difficult, although progress has been made in mechanically separating mosquito pupae.

(8) A thorough knowledge of the habits and ecology of the insect is essential.

Research and development on the technique of releasing sterile males, with radiation as the means of sterilization, has followed a general pattern. In cage tests with a laboratory strain of insects, a species is exposed to different dosages of X- or gamma-radiation to determine the minimum amount of radiation necessary to sterilize the insect without impairing its vigour and longevity. Success in the preliminary phase of observation leads to a determination of the competitive ability of sterilized and normal males. Different ratios of sterile males, normal males, and normal females (for example, 1:1:1, 3:1:1, 5:1:1, and 10:1:1) are chosen and the insects allowed to cohabit cages; thus the reduction in egg-viability is determined. Experiments to determine how many times males and females mate are also conducted. Finally, with the successful conclusion of this type of experimentation, field trials are initiated.

III. Eradication of the screwworm from the southeastern United States of America

The screwworm proved to be ideally suited to the application of the sterile-male release technique. A considerable amount of basic information on the biology and behaviour of this pest was available. BUSHLAND and HOPKINS [3] [4] showed that males could be sterilized with 5000 r, but adult females exposed at that dosage were still fertile. Exposure of 5-d-old pupae proved much more satisfactory since males could be sterilized with 2500 r and females with 5000 r. Up to the lethal dose of 20000 r there was little difference in longevity between irradiated and normal flies.

Observations and experiments showed that the female mated only once, but that males mated repeatedly. Only infertile eggs resulted from a mating of a fertile female and a sterile male and the sterile male was shown to be competitive with the normal male. Subsequently field tests were initiated.

The first of the field tests was conducted on the small island of Sanibal, 2 miles off the west coast of Florida. Here sterilized males were released at the rate of 100/mile^2 per week. Within 8 weeks the percentage of viable egg-masses collected fell from 100 to 25% and subsequently the original population seemed to be near extinction. However, one single fertile egg-mass was found during the 12th week after releases had been started. Because of the possibility of reinfestation from the mainland or nearby islands, the experiment was moved to the completely isolated island of Curaçao, with an area of 170 mile^2 and lying 40 miles off the coast of Venezuela. The Netherlands Antilles Government agreed to join in the experiment. Rearing and sterilizing of the flies were done at Orlando, Florida, and pupae were shipped by air to Curaçao. Within 24 h of emergence, flies were released by air. Those released at the rate of 100/mile^2 per week during April and May of 1954 caused

15% of the native flies to lay infertile eggs. The release rate was increased to 400/mile² per week in August 1954. The results obtained are shown in Table I.

TABLE I
RESULTS OF RELEASING 400 STERILE MALES PER MILE²/WEEK

Date		Number of fertile egg masses found	Number of sterile egg masses found	Per cent sterile
August	9—15	15	34	69
	23—29	17	38	69
September	6—12	7	42	86
	13—19	3	23	88
	20—26	0	10	100
October	4—10	0	0	—

The last fertile egg mass was collected during the week of September 13. Two sterile egg masses were collected on November 4 and 11. By releasing and maintaining a dominant population of sterile males on the island, eradication was brought about in 5 months or in 4 generations of the insect. This experiment was summarized by BAUMHOVER *et al.* [1].

In the United States of America the screwworm was present in the southeastern and southwestern parts of the country. The population in the southeast was separate and distinct from that in the southwest. Since that in the southeast overwintered only in Florida, there was a good opportunity for eradication by the sterile-male release technique. The screwworm caused losses to livestock estimated at $10 million per year in Florida and $20 million per year in southeastern United States. Since the attempt at eradication would involve an area over 300 times as large as the island of Curaçao, large-scale techniques had to be developed. A 2000-mile² test-area was marked off on the east central coast of Florida (BAUMHOVER *et al.*, [2]). Approximately 500 sterile males were released per square mile per week which required the rearing of 2 million flies per week. Egg-mass collections in the treated area declined from a weekly average of 41 per station during the first 2 months to 11 in the 12th week. At the end of 3 months, 70% of the egg masses were sterile. There was a continued high population in the check station north of the test area, and a decline in those stations to the south and west.

Convinced of the applicability of the technique, the legislatures of the Federal Government and the State of Florida appropriated the money for conducting the eradication campaign in the southeast. For this purpose a fly-rearing factory was built at Sebring, Florida, in a surplus aeroplane-hangar. A maximum production of 70 million flies per week was obtained with an average of 50 million sustained during the campaign. A floor plan of the assembly-line techniques used is given by JEFFERSON [7]. The magnitude of the operation is reflected in the weekly food-requirements for rearing 50 million flies, as shown below:

Ground meat	40 t
Water	9600 gal
Beef blood	4500 gal
Plasma	65 gal
Honey	35 gal

This campaign was so successful that the screwworm was eradicated completely and the fly factory closed down less than 2 years after the start of the programme.

IV. Studies of the sterile-male technique with *Anopheles quadrimaculatus* (Say)

The successful development of the sterile-male technique with the screwworm fly suggested its promise for other species. Among these would be certain anopheline mosquitoes. These mosquitoes do not pass the winter in the egg stage, and consequently spring populations are usually low. Even if not suitable by itself, the method might be useful in combination with others or at a time of unfavourable biotic balance to the species. Much effort is devoted to anti-malarial campaigns. Sterilized insects might be released to eradicate a population already depleted by other control measures. The survivors in the native population could be reached more certainly by the released insects than by any other means. Laboratory-rearing procedures are available and it seems probable that mass-rearing and irradiation techniques could be developed.

DAVIS *et al.* [6] in preliminary studies on the development of the sterile-male technique with *quadrimaculatus* showed that a laboratory strain of this insect could be sterilized. Dosages of 8865 to 12900 r applied in the pupal or adult stage produced sterility in both sexes. Sterilized females mated to normal males produced no eggs, whereas normal females mated with sterile males produced a normal number of eggs but none hatched. When sterile males were introduced into caged populations of normal males and normal females at ratios of 4:1:1 or less, usually no reduction in the total number of viable eggs was produced, but at ratios of 6:1:1 and 10:1:1 there was a reduction of about 80%. With these promising results the research programme on the sterile-male technique with *quadrimaculatus* has been expanded to include further laboratory studies and field tests. It has been shown that motile sperm are produced and transmitted to females by sterile males. No gross differences between normal and sterile males have been found with regard to sperm production, sperm motility, or mating behaviour. The production of standardized, sexually vigorous males on a large scale is indicated to be a problem, since sexual vigour and longevity have shown considerable variations in control studies. Studies of this species, particularly with regard to mating behaviour, male behaviour and flight range, biotic potential, and population levels and fluctuations are being conducted along with a field experiment in which sterile males are being released into a wild population.

V. Studies on the sterile-male technique with fruit-flies

In 1956 STEINER and CHRISTENSON [11] reported studies on the lethal and sterilizing effects of radiation on three species of fruit-flies; the oriental (*Dacus dorsalis* Hendel), the Mediterranean (*Ceratitis capitata* Wied.), and the melon (*Dacus cucurbitae* Coq.). They showed that sterility could be introduced into males when pupae were irradiated with dosages of 2500 to 10000 r. The induced sterility was proportional to the dosage; however, increased sterility with higher dosage levels was accompanied with increased mortality and abnormalities. The most practical dosage was from 6700 to 8400 r. However, at this dosage some fertile eggs were obtained from females mated to irradiated males 24 to 44 d old. The females of the three species named are known to mate several times; however, in small-cage tests it was found that egg fertility was inversely proportional to the ratio of sterile to normal males. Consequently, one can counteract multiple mating by releasing very large numbers of sterile males. Members of the Hawaii Fruit Fly Investigations Laboratory of the US Department of Agriculture have carried out many tests with regard

to the development of the sterile-male technique. These include the gathering of data on the biology of the insect, mass-rearing, sterilizing, and release methods. The entomologists at this laboratory are now carrying out eradiction tests on islands in the southwestern Pacific. The Mexico Fruit Fly Investigations Laboratory, USDA, in co-operation with the Mexican government, has conducted similar developmental research on the sterilization of the Mexican fruit-fly (*Anastrepha ludens* Loew). Studies have shown results similar to those found with the other fruit-flies and this work has progressed to a field-development stage.

VI. Lethal effects of radiation on insects of medical importance

The availability of a cobalt-60 source and interest in the effect of radiation on insects led COLE *et al.* [5] to study the lethal effects of gamma-radiation on the body louse (*Pediculus humanus humanus* L.), American cockroach (*Periplaneta americana* L.), German cockroach (*Blattella germanica* L.), the firebrat (*Thermobia domestica* Pack.) bed bug (*Cimex lectularius* L.), Pharaoh ant (*Monomorium pharaonis* L.), and housefly (*Musca domestica* L.). These research-workers exposed all the insects at different stages to gamma-radiation and observed mortalities after 24 and 48 h. The 24-h LD_{50} values for adults are given in Table II.

TABLE II

LD_{50} VALUES FOR INSECTS EXPOSED TO γ-RADIATION FOR 24 h

Species	LD_{50} (r)	
	Females	Males
American cockroach	48000	50000
German cockroach	72000	76000
Firebrat	98000	98000
Housefly	110000	72000
Bed bug	150000	160000
Body louse	180000	170000
Pharaoh ant	190000	140000

A comparison of the LD_{50}'s obtained with the above insects with those presented by PLOUGH [10] for the hamster, 1000 r; the rabbit, 750 r; the mouse, 650 r; the dog, 550 r; and man, 400 r demonstrates that the above-named insects are much more resistant to radiation than the mammals. SULLIVAN and GROSCH [12] studied the effects of radiation on adult wasps and noticed this difference. They pointed out that insects are less susceptible since they have no replaceable epithelium, mucous membranes, or no hematopoietic or lymphatic systems. Furthermore the adult-insect body is principally composed of fat, muscle, and nerve tissues that, in mammals, are fairly radiation-resistant. In addition, the insect is covered by a cuticle. Only tissues undergoing rapid division in insects (the gonads) are highly susceptible to radiation. There appears to be a correlation between the size of the insect and the amount of radiation required to kill it—the larger the insect the lower the LD_{50}. This correlation seems to be an anomaly in that smaller insects may absorb less of the administered dosage than large ones. However, differences of species may be the cause.

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DISCUSSION

THE CHAIRMAN (D. W. Jenkins, United States of America): There are three questions I should like to put to Dr. Weidhaas: first, what was the total number of screwworm flies released in Florida? Secondly, what were the livestock losses due to screwworm fly in the whole southeastern area? Thirdly, what was the total cost of the entire elimination project?

D. E. WEIDHAAS: If my memory serves me correctly, the total number of insects released was of the order of 6000 million. In Florida the depredations are estimated to have caused 10 million dollars' worth of damage per year; if one includes the southeastern States, the figure rises to $25 million/yr. The cost of the programme was of the order of $8 million. I think all will agree that this represents quite a good return on the investment.

P. J. DEORAS (India): In the spectacular nature of the results it has achieved, the technique which we have heard described is reminiscent of the advent of DDT in 1945. There is, however, an important difference: we have learned from the unexpected phenomenon of resistance-development that control is a highly complex operation and that a thorough study of the behaviour, biology and ecology of the pest is an essential factor in achieving good results. I feel that the success of this new technique is due in large measure to the importance which has now been accorded to these aspects of the problem.

D. E. WEIDHAAS: Indeed, for the application of this technique very many factors are important, apart from those of total population and biotic potential. In fact, many of these factors might also be termed limitations of the technique itself, and indicate already that it will not be a universal panacea. We shall need to take our studies even further.

J. A. QAYYUM (Pakistan): What precautions are you taking to prevent reinfestation by screwworm fly once it has disappeared?

D. E. WEIDHAAS: A quarantine station has been set up and consignments of animals have to go through it. A case was recently reported of screwworm detected in a greyhound dog arriving in Florida. This case in itself did not lead to any danger of reinfestation, but indicates that we have nonetheless a serious problem here.

R. M. PATEL (India): Do you think that the disappearance of screwworm fly could be attributed to some factor other than the release of sterile males, i.e. to some natural factor? There are cases of pests disappearing for several seasons and subsequently re-appearing.

D. E. WEIDHAAS: No, I do not think there is any possibility of this having occurred. During both the campaign itself and all the field trials, traps were operated throughout the entire area to collect the fly, and measurements were made of the sterility induced in the population. We could clearly see the sterility pattern in the wild fly, which is pretty conclusive, I think.

THE CHAIRMAN: There is also the fact that we had an excellent natural control in the form of the high level of fly population which persisted in Texas, while the fly was eliminated in Florida.

P. B. CORNWELL (United Kingdom): I was interested in your attempts to correlate radiation susceptibility with the size of the different species of insect. In your experiments I agree that differences in susceptibility of species rather than of size confuse the issue. It may be of interest to mention that we have imported about 40 wild strains of the grain weevil from different parts of the world, tested their susceptibility against average adult weight, and found no significant correlation between susceptibility and size.

Turning now to another point, you indicate in your slide that the species can acquire no immunity. Is anyone aware of research being carried out on the possible development of resistance in insects subjected to substerilizing doses?

D. E. WEIDHAAS: I know of none myself. The statement about non-immunity was, of course, made in connection with the release of completely sterile males.

P. PÉLEGRIN (France): To be effective, need the sterile-male release method be confined to insects which mate only once? One rather gets this impression from the literature.

D. E. WEIDHAAS: No, I think on the contrary that the laboratory work with the fruit-fly is a definite indication that the method will also work with multiple-mating insects. Fruit-flies are, of course, known to mate many times, and yet, in the laboratory, if sterile males are made to cohabit with normal males and normal females a reduction in egg viability can be shown. It seems to me—and I suppose this will have to be proved for any particular species—that if the sperm of irradiated insects is equally competitive with that of normal ones, then multiple-mating should really have no influence.

THE CHAIRMAN: I might add a comment on some work that has recently been done with the tsetse fly. It has been found that, although the tsetse fly mates more than once, the sperm that is put in the receptacle during the first mating is that which determines fertility. Thus a normal female inseminated by a sterile male will produce only sterile offspring, even though she has mated with other, normal males.

P. B. CORNWELL: Donnelly has presented some interesting observations (unpublished) pertinent to this question of multiple mating. In the blow fly, *Lucilia sericata* he found that insemination of sperm is apparently followed by a secretion of the male accessory glands which coagulates to form a plug. It would certainly be of interest to see to what extent this feature is characteristic of other species, since such plugs may well prevent the introduction of further sperm.

D. E. WEIDHAAS: We are beginning to undertake studies on the reproductive systems of our mosquitoes. Among the interesting discoveries made is the fact that our *Anopheles* mosquitoes have one spermotheca which is, of course, full when the insect is mated, while our *Aëdes* species mostly have three spermotheca, two of which are full and one empty. We have run some sterilization tests with *Aëdes aegypti* and found that we could obtain partial sterilization. We have still a tremendous amount to learn about insect reproduction.

P. PÉLEGRIN: If I understood correctly, your insect-breeding plant has been closed down. What would happen if the operation has to be repeated two or three years hence?

D. E. WEIDHAAS: Perhaps I did not make myself quite clear. The plant was "put in moth balls", meaning that it would be available for further use if required—either in Florida again or elsewhere in the South-East.

I should like to make just one comment in connection with chemical sterilants, to which Dr. Jenkins referred a while back. Assume for example that an insecticide and a sterilant can each account for 90% of a population, the former of course by killing, the latter by inducing sterilization in 90% of individuals. Where insecticide has been used the 10% survivors still represent a viable population capable of reproduction, and through the development of resistance will have become harder to eliminate. The 10% unaffected by the sterilant are, however, in quite a different situation; their numbers will be still further reduced by mating with individuals of the sterile sector, and, on the basis of the 90% sterilization potential which we have taken as an example, the odds are 9 to 1 that mating will render more individuals sterile. Instead of a 90% reduction of the population, obtained with insecticides, a 99% reduction will, in fact, have been achieved with sterilants. This again illustrates the deadly effectiveness of the self-eradication principle.

THE CHAIRMAN: I might add that at the conference on entomology held at Atlantic City last week one participant reported some excellent results with the use of chemical sterilants against houseflies.

NOTE SUR LES POSSIBILITÉS DE LUTTE AUTRES QUE LES INSECTICIDES

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Abstract — Résumé — Аннотация — Resumen

Control other than by insecticides. A new method of control by irradiation of the males has recently been successfully tried in the United States. It would be very interesting—and seems feasible—to apply this method to the *Dysdercus* problem in tropical countries. The breeding and basic experimental work could be done in Metropolitan France, the effects of the irradiation could be propagated by suitably attracting the populations and as a result of their own movements. The results, if successful, would be of considerable economic value to the Overseas States.

Note sur les possibilités de lutte autres que les insecticides. Une nouvelle méthode de lutte par irradiation des mâles a récemment fait ses preuves aux Etats-Unis; il serait très intéressant et il paraît possible de partir de cette donnée pour l'appliquer au problème des *Dysdercus* en pays tropicaux. Elevage et expérimentation de base sont possibles en métropole, attractifs et mouvements des populations simplifieraient la mise en pratique de l'irradiation. L'intérêt économique final serait considérable pour les Etats d'Outre-Mer, en cas de réussite d'un tel projet.

Замечания о возможностях борьбы с насекомыми помимо инсектицидов. В последнее время в США испытан новый метод борьбы с насекомыми при помощи облучения самцов; было бы весьма интересно и возможно исходить из этих данных и применить этот метод к *Dysdercus* в тропических странах. Разведение и эксперименты возможны во Франции; привлекающие средства и движение популяций упростили бы применение облучения. Конечные экономические выгоды были бы значительны для других стран, если бы подобный проект закончился успешно.

Nota sobre la posibilidad de luchar contra los insectos con medios distintos de los insecticidas. La eficacia de un nuevo método de lucha contra los insectos basado en la irradiación de los machos ha quedado demostrada recientemente en los Estados Unidos; sería muy interesante poder aplicarlo al problema de los *Dysdercus* en los países tropicales, donde es posible que dé buenos resultados. Las primeras operaciones de cría y experimentación podrían llevarse a cabo en Europa; los movimientos de las poblaciones de insectos, atraídas mediante cebos adecuados, simplificarían la irradiación. En caso de que el proyecto tenga éxito, el método presentaría en definitiva considerables ventajas económicas para los países de ultramar.

Généralités

C'est un souci tenace chez certains entomologistes, et pas seulement ceux de la «vieille école», d'échapper à l'extension tyrannique des traitements chimiques qui s'est réalisée depuis une quinzaine d'années grâce à la découverte des insecticides synthétiques. Sans reprendre tous leurs arguments, citons simplement, en défaveur des traitements chimiques: leur fréquent danger pour l'homme et les vertébrés, la destruction aveugle des utiles aussi bien que des nuisibles, et l'accoutumance plus ou moins rapide de certaines espèces à un produit ou même à une classe entière de produits.

Dans les contrées tropicales «sous-développées», le prix de revient des traitements est un autre obstacle majeur, pour des cultures de faible rendement ou de productivité économique

marginale. Cependant, les traitements chimiques sont en voie d'extension pour quelques cultures industrielles de bonnes conditions techniques. Même ici, les aléas demeurent grands: pannes d'appareils, d'avion, météorologie défavorable au moment de l'application, manque de personnel spécialisé, etc.

Les méthodes évoquées sous le terme général de «lutte biologique» connaissent un renouveau d'intérêt, et les recherches ont été étendues à de nouveaux chapitres: ainsi, l'étude des maladies bactériennes et à virus chez les insectes est en passe de recevoir des applications pratiques étendues et fort prometteuses. Jusqu'alors, on recherchait des méthodes où l'homme, après avoir introduit un facteur nouveau (entomophage, maladie) dans une région naturelle, laissait ensuite évoluer l'équilibre, sans intervenir continuellement.

I. Lutte par stérilisation des mâles

Depuis peu sont apparues des techniques qui, s'appuyant essentiellement sur la connaissance des facteurs biologiques, laissent cependant opérer un facteur physique ou chimique que l'homme maintient en permanence ou réintroduit fréquemment. Parmi quelques exemples, le plus démonstratif est celui de la lutte contre les mouches, *Callitroga hominivorax*, vivant dans les plaies des mammifères en Amérique centrale, et causant aussi de graves pertes à l'élevage dans le sud des Etats-Unis, au Mexique etc. Des études biologiques minutieuses ont d'abord montré que les femelles de ces diptères ne s'accouplaient qu'une fois. On a ensuite envisagé de lâcher des mâles stériles au moment de l'accouplement. Enfin, on a mis au point l'irradiation par une bombe au cobalt, des pupes de ces mouches, élevées en grand nombre au laboratoire, afin de lâcher des adultes stériles (mais pouvant s'accoupler) ou porteurs de «mutations chromosomiques» conduisant à l'avortement chez les générations suivantes. Après une réussite éclatante dans l'île de Curaçao, on étudie l'extension de cette lutte aux régions continentales.

Ce succès est certainement attribuable tout d'abord à l'effort scientifique et financier que les laboratoires des Etats-Unis ont pu fournir, ensuite à certaines particularités biologiques de l'espèce en cause, notamment le fait qu'elle parasite des animaux vivant pour la plupart en domestication et près de l'homme. En conséquence, la transposition directe de la méthode à d'autres problèmes parasitaires paraît être exceptionnelle.

Reprenons les points délicats

1. L'accouplement unique de la femelle, qui fut le point de départ des recherches, n'est pas, comme on pourrait le croire, une donnée nécessaire. En effet, pourvu que les spermatozoïdes «irradiés» aient gardé une mobilité normale (ce qui peut être ajusté assez facilement, semble-t-il), peu importe, pour le but final, que la réduction soit due à une proportion X de femelles sans descendance dans la population des mouches, ou à la même proportion X d'ovules voués à l'avortement dans l'ensemble des mouches. On peut donc espérer arriver à des résultats pratiques dans la généralité des cas.
2. La libération d'une quantité considérable de mâles traités (50 millions par semaine) impose des installations énormes pour l'élevage artificiel des mouches, et un personnel extrêmement nombreux. Les mêmes contingences, transposées à des parasites de végétaux, sembleraient impossibles à réaliser.
3. Les résultats ont été très démonstratifs en raison de leur rapidité: réduction des populations de parasites en peu de semaines, éradication probablement totale en quelques mois. Il n'est certes pas indispensable que les résultats soient aussi complets et aussi rapides, pour être extrêmement intéressants.

II. Projet d'étude sur *Dysdercus*

Dysdercus est un hétéroptère; les nombreuses espèces du genre sont répandues dans les zones intertropicales ou subtropicales du monde entier. Le plupart d'entre elles sont plus ou moins inféodées aux malvales, et certaines sont extrêmement nuisibles aux cultures cotonnières, surtout en Afrique noire et en Asie tropicale. La pullulation de ces hémiptères s'établit en hors-saison sur des plantes herbacées ou arborescentes de la brousse ou de la forêt, et, au moment de la formation des capsules, on assiste à une migration parfois formidable des adultes vers les champs de cotonniers. Ces insectes sont nuisibles directement par leurs piqûres nutritielles, mais surtout indirectement par l'inoculation de maladies cryptogamiques (champignons du genre *Nematospora* et voisins, bactéries, etc.) amenant une pourriture totale du contenu des capsules. Les dégâts peuvent être considérables même avec des populations assez faibles, et ils sont d'autant plus graves qu'ils se produisent sur la récolte à un stade avancé, à une époque où une nouvelle fructification ne peut plus s'établir. La lutte chimique se révèle parfois décevante, non pas parce que l'insecte est spécialement résistant aux insecticides (l'action directe de l'H.C.H., de la dieldrine, du parathion, etc. est excellente) mais parce que le renouvellement continu des flots d'invasion maintient un niveau assez élevé pour faire des dégâts indirects considérables, bien que les migrants meurent quelques heures après leur arrivée dans un champ traité de façon convenable.

Les entomophages s'attaquant à *Dysdercus* sont assez rares, limités aux zones humides, et en général peu efficaces; ils sont au moins aussi sensibles aux insecticides que *Dysdercus* lui-même. Les maladies de ces insectes ne sont pas connues, mais il n'y a pas eu jusqu'à présent d'épidémies observées, ni sur des plantes hôtes sauvages, ni dans les populations établies dans les cotonniers.

Ainsi, ni la lutte biologique classique ni le renforcement des traitements chimiques ne sont assurés d'apporter une solution toujours pleinement satisfaisante. N'y a-t-il pas une indication intéressante pour chercher une éventuelle application de la lutte par irradiation à ces insectes qui sont d'une importance économique très grande, à la fois pour des vastes régions où la culture cotonnière ne peut supporter les frais des traitements insecticides et pour d'autres où ces derniers n'arrivent pas à résoudre totalement ce problème?

Un facteur favorable complémentaire réside dans l'attraction que certains extraits de fruits de végétaux peuvent exercer sur des adultes ailés. Des essais préliminaires effectués à Madagascar ont montré que les tourteaux industriels provenant de l'huilerie locale traitant les graines de baobab attiraient les insectes sexuellement mûrs à des distances de plusieurs centaines de mètres. Au lieu de créer des élevages artificiels très onéreux, on pourrait donc utiliser cette attraction pour obtenir de grandes quantités d'insectes venant de la nature, qui pourraient être traités pour ainsi dire automatiquement dans une installation comportant attractif et générateur de radiation; ils seraient ensuite relâchés en temps utile, c'est-à-dire avant la génération qui fournira les migrants vers les cotonniers.

Enfin, signalons que les élevages sont relativement faciles à réaliser en climat tempéré, et que le laboratoire d'entomologie appliquée du Muséum maintient des souches de *D. fasciatus* depuis plusieurs années.

Il semblerait donc possible d'entreprendre un programme préliminaire portant sur les points suivants:

1. Amélioration des techniques d'élevage en métropole, constitution d'élevage des diverses espèces nuisibles au laboratoire.

2. Etude préliminaire des doses et modalités d'irradiation nécessaire et des effets sur les générations présentes ou ultérieures, sur le plan génétique des populations—sur ces élevages de laboratoire.
3. Action éventuelle de l'irradiation sur les champignons et bactéries véhiculés par les insectes (action directe et mutagène).
4. Etude plus approfondie des attractifs, extraction et isolement des substances chimiques responsables, etc.
5. Etude préliminaire de la dynamique des populations dans la nature, sur les plantes de brousse et sur les champs, étude poussée des facteurs de migration.
6. Esquisse préliminaire de l'installation de traitement automatique dans la nature, à installer tout d'abord près d'une station de recherches cotonnières.

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DISCUSSION

J. E. CASIDA (United States of America): Is the chemical nature of the attractant known?

P. PÉLEGRIN (France), who presented the paper: I think I am right in saying that the chemical nature of the attractant is not known at present.

P. J. DEORAS (India): Some work has been done in India which indicates that chemical attractants can be used to attract female insects for oviposition or males for mating. For example, it was found that the chemical Indol attracted the females of *Dacus cucurbitae*, and that fermenting oil-cake was an attractant for oviposition by females of *Musca nebulo*. The whole question of chemical attractants, particularly for the control of the housefly, is extremely interesting, and I feel sure that our Chairman can throw much light on it.

THE CHAIRMAN (D. W. Jenkins, United States of America): The subject of attractants is an enormous one, and so it is scarcely possible to go into it in detail here. Certain attractants have been developed for houseflies and mosquitoes; in the case of the former, for example, a certain form of peptone and similar compounds have been found to be highly effective. In the case of mosquitoes, much work has been done on attraction; some successful methods are, in fact, not chemical, such as the use of tuning forks and of animals put out as attractants. Among chemical, methods the release of carbon dioxide from dry ice has been found to be highly attractive. Other chemicals are being studied but none has yet been fully proven as far as I know, although there have been some promising reports recently.

I would conclude that, in general, attractants have a great value in conjunction with the use of radioisotopes and radiation. I think it would be possible to use mobile radiation units in the field to sterilize various types of insect which have been artifically attracted into the vicinity. It may even be possible to use such mobile radiation units where mosquitoes or houseflies are breeding in numbers, and irradiate them before using them in the sterile-male release technique.

P. B. CORNWELL (United Kingdom): During the course of studies at Wantage we have been interested in the possible use of sterile-male release for control of insect pests in cotton. Suggestions have been raised with the Empire Cotton-Growing Corporation in the United Kingdom but I think it is felt that the biology of the species, particularly *Dysdercus fasciatus*, is not really suited to this technique.

In the paper just given, I understand that the author is suggesting that irradiation might be carried out in the field at points where the seeds attract the insects. If this is so, I rather doubt the feasibility of this approach; success with sterile-male release, surely, depends on the irradiation of material at a critical stage of development and with a critical dose. Similar suggestions have been raised with other species for the use of irradiation in the field at points where the insects accumulate; for example, rhinoceros beetle (*Oryctes rhinoceros*), a pest of coconut palms, is attracted to and breeds in trash in the soil, and it has been suggested that radioisotopes might be poured into these trash tips and irradiation thus carried out under field conditions. But I think this would prove an unprofitable aid to the sterile-male technique.

E. J. VEVAI (India): *Dysdercus cingulatus*, known as red cotton bug, is very common in India, and I have observed that it is strongly attracted to *Hibiscus esculentus* and other *hibiscus* species. I wonder whether this could be used as a trap crop? It certainly has one drawback: it is a preferential host for other cotton pests.

P. PÉLEGRIN: Mr. Delattre's studies concerned *Dysdercus fasciatus*, and so I am unfortunately unable to comment on this.

ON THE ROLE OF LETHAL MUTANTS IN THE CONTROL OF POPULATIONS

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Abstract — Résumé — Аннотация — Resumen

On the role of lethal mutants in the control of populations. Population control by release of irradiated males requires that the sperm must be damaged by radiation. The type of damage induced by radiation imposes a restriction on which species may be controlled because if the sperm are functionally damaged by radiation, then for effective control, the females must be monogamous. If dominant lethality is induced in sperm then either polygamy or monogamy may prevail.

It is generally accepted that dominant lethal events are induced in sperm at doses much lower than those required to hamper sperm function or cause sperm inactivation. With *Drosophila* it is possible to test directly the effect of releasing irradiated males into an artificial population where polygamy is the rule.

Preliminary experiments have been performed under conditions of unlimited production of offspring.

It appears that radiation induces dominant lethality in sperm, and the sperm that bear dominant lethals are able to compete successfully with normal sperm. A series of tests are currently under way to ascertain the degree of induced dominant lethality and sperm inactivation at different X-ray dosages.

A series of experiments are outlined in a general discussion of the possible use of dominant and recessive lethals for bringing about collapse of artificial and natural populations.

Le rôle des mutants létaux dans la lutte contre les insectes. Pour que le lâcher de mâles irradiés puisse contribuer à la lutte contre la prolifération des insectes, il faut que le sperme ait été endommagé par les rayonnements. Les espèces contre lesquelles on peut lutter par ce moyen dépendent du genre de dommages radioinduits. En effet, si seule la fonction spermatique est atteinte, l'irradiation ne sera un moyen de lutte efficace que si les femelles sont monogames; si au contraire on provoque une létalité dominante dans le sperme, les femelles peuvent être polygames ou monogames.

On admet généralement que les phénomènes de létalité dominante interviennent dans le sperme à des doses sensiblement inférieures aux doses nécessaires pour modifier la fonction spermatique ou rendre le sperme inactif. La drosophile permet de constater directement les effets du lâcher de mâles irradiés dans une population artificielle dans laquelle la polygamie est la règle.

Les auteurs ont fait des essais préliminaires dans des conditions de reproduction illimitée.

Il est apparu que les rayonnements provoquent une létalité dominante dans le sperme, et que les spermes à mutants létaux dominants ne le cèdent en rien aux spermes normaux. Les auteurs font actuellement des essais pour déterminer le degré de létalité dominante radioinduite et le degré d'inactivation du sperme, selon la dose de rayons X.

Le mémoire présente les résultats des expériences faites et donne un exposé général des diverses façons dont on peut tirer parti des mutants létaux dominants ou récessifs pour décimer des populations artificielles ou naturelles.

О роли летальных мутантов в контроле над видами. При осуществлении контроля над видами при помощи выпуска облученных мужских особей необходимо, чтобы сперма была повреждена радиацией. Тип повреждения, вызванного радиацией, налагает ограничение в отношении возможности контроля над видами, поскольку, если радиация вызвала нару-

шение функций спермы, для обеспечения эффективности контроля необходимо, чтобы женские особи были моногамны. Если в сперме образована доминирующая летальность, то возможно преобладание либо полигамии, либо моногамии.

Общепризнано, что случаи доминирующей летальности вызываются в сперме дозами значительно меньшими, чем дозы, требуемые для нарушения функций спермы или для прекращения ее жизнедеятельности. При использовании дрозофилы возможно непосредственно исследовать влияние выпуска облученных мужских особей в искусственный рой, где полигамия является правилом.

Предварительные опыты были проведены в условиях неограниченного воспроизводства потомства.

Оказалось, что радиация вызывает в спермах доминирующую летальность, и спермы, несущие дозы доминирующей летальности, способны успешно конкурировать с нормальными спермами. В настоящее время проводится серия опытов с целью установления степени обрузуемой доминирующей летальности и прекращения жизнедеятельности спермы при различных дозах облучения рентгеновскими лучами.

Результаты наших опытов будут представлены вместе с общим обзором возможного использования доз доминирующей и уменьшающейся летальности для уничтожения искусственных и естественных роев.

Función de las mutaciones letales en la lucha contra los insectos. Para que la suelta de machos irradiados pueda contribuir a la lucha contra la proliferación de los insectos, es preciso que el esperma sufra daños como consecuencia de la irradiación. El tipo de daño radioinducido limita el número de las especies que pueden combatirse, porque en los casos en que la irradiación causa daños funcionales en el esperma, las hembras han de ser monógamas para que la lucha tenga eficacia. Si se inducen en el esperma mutaciones letales dominantes, puede predominar la poligamia o la monogamia.

Se admite generalmente que las mutaciones letales dominantes son inducidas en el esperma mediante dosis muy inferiores a las necesarias para entorpecer la formación del esperma o producir su inactivación. Empleando *Drosophila*, es posible comprobar directamente la influencia de la suelta de machos irradiados sobre una población artificial en la que predomina la poligamia.

Se han efectuado experimentos preliminares en condiciones que favorecían la producción ilimitada de progenie.

Se ha comprobado que la irradiación induce mutaciones letales dominantes en el esperma, y que los espermas que han sufrido esas mutaciones son capaces de competir con éxito con los espermas normales. Se está llevando a cabo una serie de experimentos para averiguar el grado de letalidad dominante inducida y de inactivación del esperma con diferentes dosis de rayos X.

Se presentan los resultados de estos experimentos, junto con un estudio general de la posible utilización de las mutaciones letales dominantes y recesivas destinadas a provocar el exterminio de las poblaciones naturales y artificiales.

I. Introduction

KNIPLING [1] has discussed the possibility of controlling insect and other animal populations through application of the following principle: introduction of a number of sexually vigorous, sterile males into the natural population will have a greater influence in reducing its biotic potential than if the same number were eliminated by destruction or removal. He has also discussed the need for studying physical and chemical means which may produce sterility in various types of animals. The eradication of the screwworm from Curaçao and Florida serves to underscore the essential correctness of his principle.

One of the present authors [2] has pointed out that where males are irradiated and released in the field, the restriction of monogamy in females of a species is not a requirement for controlling population-size, since sterility of the males (*sensu stricto*) is not necessarily the radiation effect which causes the population decline. KAUFMAN and WASSERMAN [3] have indeed shown that dominant lethals are induced by X-rays in the screwworm. Even

with multiple matings by every female, the population collapse would be as inevitable and rapid as when the females are monogamous.

II. Dominant lethals and sterility

Probably the most important effect of radiation is the induction of dominant lethal mutations in the sperm, not male sterility. For illustration, let us consider an insect population made up of ten males and ten virgin females. Ninety irradiated males are introduced into this population. The females mate only once. For this simple example, let us assume that nine of the females will mate with irradiated males and produce no viable offspring, and that one will mate with a normal male and produce normal offspring. 100% of the eggs from one female and 10% of the total batch of eggs will survive. In this case, it will not matter whether the sperm contain dominant lethals or the males are made sterile.

Now consider the same conditions, but let every female mate ten times. Of her ten mates, assume nine have been irradiated and have sperm containing dominant lethals and one has normal sperm. 10% of the eggs from each female and therefore 10% of the batch will survive; this result is identical to the outcome predicted from strict monogamy, even though polygamy is the case here. On the other hand, in the polygamous case, if the irradiated males are sterile, 100% of the total batch of eggs survive.

It is obvious that, if radiation primarily induces dominant lethal mutations in the sperm, the results are identical whether female monogamy or promiscuity obtains. In practice, one can, of course, imagine circumstances whereby monogamy or polygamy could influence the rate of decline, and according to the circumstances, polygamy actually could be a necessary requirement for population collapse. For instance, if selective mating of brothers and sisters occurs when adults from one clutch emerge simultaneously (as with certain wasp species), then further matings to irradiated non-brothers would be required for successful population-control by the irradiation-of-male method.

It is generally known that at levels of radiation of about 10 kr, dominant lethal events are induced in over 99% of the sperm, both in the fly *Drosophila* and the wasp *Habrobracon*. However, to obtain complete killing of the sperm, radiation levels of about 200 kr are required [4] [5]. It has been observed in *Drosophila* that dominant lethals are induced in mature sperm and spermatocytes in later stages of spermatogenesis, and that after these cells are exhausted a period of sterility sets in, from which, at doses of about 10 kr, the flies never recover [6]. The process of sperm exhaustion following irradiation requires about a week of continuous multiple matings, but *Drosophila* males that have not been mated for 19 d after irradiation still have sperm reserves containing dominant lethals [7]. With the simple cytological procedures now available for determining, at different doses of radiation the components of dominant lethality [8] and sterility [6], there should be little difficulty in determining dose-effect relations for any insect.

Furthermore, it seems likely that collapse of populations from chronic or acute irradiation is principally a reflection of the sensitivity of the dominant lethality component of the mutation spectrum. The general problem of eradication of populations by introduction of irradiated males is, therefore, closely related to the problem of population collapse from induction (by radiation) of dominant lethality *within* a population.

III. Experimental plan

Since the analytical and experimental procedures we are using with *Drosophila* populations are applicable to almost every other insect that can be reared in the laboratory, these methods are briefly described even though they have not yet been implemented in detail.

It is expected that a number of parameters affecting population size could be determined and that on this basis equations could be derived that show the most efficient radiation dose for males, the most effective time interval for the introduction of new males, and the optimal number of males introduced. Accordingly, experiments are under way to assess the optimal ratio of normal males to males which carry different amounts of dominant lethals or are sterile. Other parameters, such as the sensitivity of the gametes to radiations (dominant and recessive lethal mutability), the effective lifetime of females, and the lifetime of unirradiated and irradiated males, are already known or can be easily determined.

The second phase of the programme will be instituted on completion of data-processing to find how closely the observed results fit the expected results. If unforeseen parameters are uncovered (if population decline is slower or more rapid than expected), these will be studied with the second series of experiments.

The second series will consist in adjusting the parameters (radiation dose, number of males released, and intervals of release) so that the number of fertile flies will become constant and remain stabilized.

From this study it is expected that formal equations can be established from which one could learn many basic genetic features of natural populations on the verge of extinction; these equations also could serve as a basis for estimating parameters necessary for the eradication of any insect pest.

The third phase of the programme will be to use the data revealed by eradication from irradiation and release of males to estimate the effects of acute and chronic radiation directly upon populations of *D. melanogaster* without the introduction of irradiated males.

This programme could then fit in neatly with a separate programme being initiated to determine the effects of the high doses of radiation necessary for exterminating populations without the addition of individuals from outside. The components of the mutation spectrum that are effective in such an extermination (radiation-induced dominant lethality, recessive lethality, and subvital mutations) will be computed. These data will be used to aid in estimating radiation hazards to human populations.

IV. Recessive lethality and female sterility

It is of interest to consider briefly the possible consequences of introducing recessive lethal genes or female sterility genes into a natural population. With the successive introduction of large numbers of males, heterozygous at several loci for these genes or types of similar genetic character, it is inevitable that the reproducing population size would be depressed. Elimination of the population by this type of control-measure would proceed more slowly than after the introduction of males containing dominant lethals, and would reach equilibrium after each group of genetic defectives had been introduced. Final collapse of the population could not result directly from interference with its genetic structure but would depend upon extraneous factors such as the inability of mating pairs to find one another after the population declines to a certain critical size.

V. Distortion of segregation ratios

Several genetic and physiological situations are known which have one characteristic in common, namely that of distorting the normal, mendelizing, 1:1 ratio of gametes or of altering zygotic ratios. Two examples will be discussed which might possibly be used for the control and eradication of natural populations, once their characteristics become fully understood.

The first example concerns the existence of aberrant sex ratios in the genus *Drosophila*, caused by factors inherited in the cytoplasm [9] [10] or nucleus [11] [12]. Disproportionate segregation should eventually result in the population becoming either all male or all female; extermination would follow. Of such cases discovered in nature, small micro-environmental differences [13] or genetic factors working in the opposite direction [12] [14] tend to keep the sex-ratio factors from obliterating a population. For a population to survive, and thus for the condition to have been discovered in the first place, the sex-ratio factors must, of course, be mild and subject to selection pressures.

It is possible that sex-ratio distorters coming by chance into a genotype have been powerful enough to cause the extinction of a species. When better understood, sex-ratio distorters could become a useful weapon for the control of economically undesirable species that could not otherwise be touched.

The second example is that of distortion of segregation, which does not necessarily involve the production of unequal numbers of gametes deriving from nuclei at the opposite ends of the spindle (meiotic-drive chromosomes [15]). This provides a mechanism whereby a few individuals introduced into a population will have their chromosomes pass into the genetic make-up of the entire population without necessarily harming it. If, on the other hand, recessive genes for female sterility were on the chromosome exhibiting meiotic drive, then, as this chromosome sweeps through the population, the homozygous females would be useless for further propagation, but the males would still be produced in disproportionate numbers and eventually every female would become sterile. At present not enough is known about the characteristics of the meiotic-drive chromosomes to construct them at will, but meiotic-drive phenomena are being investigated vigorously [16]. It is possible that meiotic drive has been a potent evolutionary force [15] which could be controlled in order to exterminate economically undesirable populations.

VI. Summary

Radiation induces dominant lethal mutations in sperm. It therefore can be shown that monogamy is not requisite for eradicating a population through the introduction of irradiated males.

An outline is presented for experimental analysis of population collapse by the irradiation-of-male method where females mate more than once.

Possible effects on populations of release of males containing recessive lethal mutations or mutations for female sterility are briefly discussed. The possibility of genetic induction of population extinction is explored.

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PART III

SOME INSECT PROBLEMS IN TROPICAL COUNTRIES

THE SCOPE OF THE USE OF RADIOISOTOPES AND RADIATION SOURCES IN ENTOMOLOGY IN PAKISTAN

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Abstract — Résumé — Аннотация — Resumen

The scope of the use of radioisotopes and radiation sources in entomology in Pakistan. In Pakistan, as in many other countries, there is a growing realization of the benefits which may be derived from the application of radioisotopes and radiation sources in different branches of agriculture. The complete eradication of the screwworm fly which was estimated to cost the cattle breeders in the United States of America 20 million dollars each year has definitely given stimulus to the application of nuclear energy in the field of entomology. In Pakistan work has not, so far, been taken up in this direction. But it is hoped that with the establishment of two Agricultural Research Centres at Lahore and Dacca by the middle of next year, the use of radioisotopes in entomological research and the eradication of insects to protect crops and farm animals will be started. Some of the problems which deserve the special attention of entomologists in Pakistan are the conditions required for the effective use of insecticides, and the possibility of using radioactive methods in toxicological studies on insects and rodents. At present there is a trend in Pakistan as elsewhere to concentrate on the chemical control of different insects. For this purpose all the newly manufactured non-systemic and systemic insecticides are being brought into use. The use of radioisotopes can greatly assist in studies on the mode of action of all such insecticides, as, for instance, their penetration and decomposition in different organs and tissues of insects. In addition, similar radioactive techniques may be used to measure the time lag between the application of a lethal dose of an insecticide and its effects on insects and rodents.

The study of migratory habits of insects like *Sylepta derogata* F., *Apis dorsata* F. and *Apis indica* F. can also be made effectively and efficiently with the help of radioisotopes.

It would be of particular interest if radiation sources could be used to control crop borers, fruit-flies and stored-grain pests.

Perspectives d'emploi des radioisotopes et des sources de rayonnements en entomologie au Pakistan. Au Pakistan, comme dans beaucoup d'autres pays, on se rend de mieux en mieux compte des avantages que l'on peut tirer de l'emploi des radioisotopes et des sources de rayonnements dans divers secteurs de l'agriculture. L'extermination de la lucilie bouchère, dont les ravages entraînaient pour les éleveurs américains des pertes annuelles évaluées à 20 millions de dollars, a donné une nette impulsion à l'emploi de l'énergie atomique en entomologie. Le Pakistan n'a pas encore entrepris de travaux dans ce sens. L'auteur espère cependant qu'après la création de deux centres de recherches agricoles à Lahore et à Dacca, dont l'achèvement est prévu pour le milieu de l'année prochaine, il sera possible d'employer les radioisotopes dans les recherches entomologiques et dans la lutte contre les insectes qui nuisent aux récoltes et au bétail. Parmi les questions qui doivent retenir tout spécialement l'attention des entomologistes pakistanais, l'auteur signale, d'une part, les conditions nécessaires à l'emploi efficace des insecticides et, d'autre part, la possibilité d'appliquer la radioactivité dans les études toxicologiques sur les insectes et les rongeurs. A l'heure actuelle, on observe au Pakistan, comme dans de nombreux autres pays, une tendence à s'attacher surtout à la lutte contre divers insectes par des moyens chimiques. A cet effet, on a mis en œuvre tous les nouveaux insecticides, systémiques ou autres. L'emploi des radioisotopes peut grandement faciliter l'étude du mode d'action des divers insecticides, notamment de leur pénétration et de leur décomposition dans certains organes et tissus des insectes. En outre, on peut utiliser des méthodes analogues pour mesurer le décalage dans le temps entre l'application d'une dose létale d'un insecticide et ses effets sur les insectes et les rongeurs.

On peut également étudier efficacement, à l'aide des radioisotopes, les habitudes migratoires

d'insectes tels que *Sylepta derogata* F., *Apis dorsata* F. et *Apis indica* F.

Il serait particulièrement intéressant de pouvoir utiliser les sources de rayonnements dans la lutte contre les artisons des cultures, les mouches à fruits et les calandres du blé en silo.

Масштабы использования радиоизотопов и радиационных источников в энтомологии в Пакистане. В Пакистане, как и во многих других странах, растет понимание того, какие блага могут быть получены в результате применения радиоизотопов и радиационных источников в различных отраслях сельского хозяйства. Полное уничтожение мухи кольцевого червя, которая, как подсчитано, ежегодно обходилась животноводам США в 20 млн. долл., определенно стимулировало использование ядерной энергии в области энтомологии. До настоящего времени в Пакистане работа в этом направлении еще не начиналась. Однако предполагается, что после создания двух исследовательских сельскохозяйственных центров в Лахоре и Дакке в середине следующего года будет положено начало использованию радиоизотопов для энтомологических исследований и уничтожения насекомых с целью защиты посевов и домашних животных. Условия, необходимые для эффективного использования средств против насекомых, и возможность использования радиоактивных методов в токсикологических исследованиях насекомых и грызунов являются частью проблем, которые заслуживают специального внимания энтомологов в Пакистане. В настоящее время в Пакистане, как и во многих других странах, существует тенденция сосредоточить усилия на химическом контроле различных насекомых. С этой целью применяются все вновь произведенные несистематические и систематические средства против насекомых. Использование радиоизотопов может оказать большую помощь в исследованиях характера воздействия всех этих средств против насекомых, например, в том, что касается их проникновения и разложения в различных органах и тканях насекомых. Более того, аналогичные радиоактивные методы могут быть использованы для измерения промежутка времени между введением летальной дозы средства против насекомого и его воздействием на насекомых и грызунов.

С помощью радиоизотопов может быть проведено эффективное и действенное исследование миграционных инстинктов таких насекомых, как *Sylepta derogata* F., *Apis dorsata* F. и *Apis indica* F.

Особый интерес представляла бы возможность использования радиационных источников для контроля точильщиков посевов, мух-вредителей фруктовых деревьев и вредителей, водящихся в зернохранилищах.

Alcance de las aplicaciones entomológicas de los radioisótopos y de las fuentes de radiación en el Pakistán. En el Pakistán, como en otros muchos países, se perciben mejor cada día los beneficios que puede reportar la aplicación de los radioisótopos y de fuentes de radiación en distintas ramas de la agricultura. La erradicación completa de la mosca *Callitroga hominivorax*, que, según algunos cálculos, causaba anualmente a los ganaderos de los Estados Unidos daños por valor de 20 millones de dólares, ha dado un firme impulso a la utilización de la energía nuclear en la esfera de la entomología. En el Pakistán no se ha iniciado hasta ahora trabajo alguno en ese sentido. No obstante, se espera que, con la creación de dos centros de investigaciones agronómicas en Lahore y Dacca, prevista para mediados del año próximo, comenzará el empleo de los radioisótopos en las investigaciones entomológicas y la erradicación de insectos con objeto de proteger las cosechas y los animales de granja. Entre las cuestiones que merecen especial atención por parte de los entomólogos del Pakistán, figuran las condiciones necesarias para utilizar con eficacia los insecticidas y la posibilidad de emplear indicadores radiactivos en los estudios toxicológicos sobre insectos y roedores. En la actualidad, tanto en el Pakistán como en otros muchos países, se tiende a dar preponderancia a la lucha contra distintos insectos mediante productos químicos. Con este objeto se están aplicando todos los insecticidas de fabricación reciente, tanto sistémicos como no sistémicos. El empleo de los radioisótopos puede facilitar considerablemente los estudios sobre el modo de acción de todos estos insecticidas, por ejemplo, su penetración y descomposición en distintos órganos y tejidos de insectos. Además, pueden utilizarse técnicas similares para medir el intervalo que transcurre entre la aplicación de una dosis letal de un insecticida y el momento en que empieza a ejercer sus efectos sobre los insectos y roedores.

También es posible estudiar eficazmente, con ayuda de los radioisótopos, los hábitos migratorios de insectos tales como la *Sylepta derogata* F., la *Apis dorsata* F. y la *Apis indica* F.

Sería muy interesante poder utilizar fuentes de radiación para luchar contra el barrenillo de las cosechas, la mosca de los frutales y el gorgojo de los cereales almacenados.

Pakistan is predominantly an agricultural country, and more than 80% of the population derives its livelihood either directly or indirectly from agriculture. With the rapid increase in population there is a great need to increase the yield of food crops, and all-round efforts are being made to increase the food production by adopting modern scientific methods in agriculture. The most immediate need is to take care of what is already being produced in the country. According to conservative estimates 10 to 15% of the damage to the agricultural produce in Pakistan is being caused by insect pests. This loss sometimes goes up to 80% or even more in areas where the pests appear in epidemic form. Every possible effort is being made to keep these pests under control by keeping abreast of the latest control measures but intensive and continuous research is essential to meet the growing needs of an expanding agricultural economy. As in many other countries there is a growing realization in Pakistan of the benefits which may be derived from the application of radioisotopes and radiation sources in different branches of agriculture. The tangible results achieved in the United States of America in completely eradicating pests like the screwworm fly has given a definite stimulus to the application of nuclear energy in the field of entomology.

In Pakistan no work has, so far, been taken up in this direction but the Pakistan Atomic Energy Commission is making every effort to encourage the application of atomic science to agricultural research. The training of a sufficient number of agriculturists in basic isotope techniques and agricultural isotope experimentation is being arranged in the United States of America, the United Kingdom and at the CENTO Institute of Nuclear Science, Teheran, (Iran), with a view to enabling them to undertake serious research on problems dealing with different aspects of agriculture including entomology. In addition, a reactor is being built near Rawalpindi along with supporting laboratories, and its completion is expected by the end of next year. It is also hoped that two research centres at Lahore and Dacca will be established by the middle of next year to facilitate further research work with radioisotopes. The Pakistan Atomic Energy Commission has further decided to establish a well equipped laboratory and a gamma-garden at the Punjab Agricultural College, Lyallpur, to initiate the use of radioisotopes in agricultural research.

As far as the subject of entomology is concerned, the application of atomic science is likely to take two main forms: one using radioisotopes and the radiological technique as a research tool, the other using radiation techniques for the control of insect pests. At this stage it is rather difficult to evaluate the economic potentialities for Pakistan of the use of radioisotopes as a research tool in entomology; judging by the progress achieved in some countries such research would by very worthwhile.

The problems which deserve to be investigated by entomologists in Pakistan with the help of radioisotopes include a study of the migratory habits of insects like *Sylepta derogata* F., *Apis dorsata* F. and *Apis indica* F.

Sylepta derogata F. is encountered abundantly in the Daphar forest plantation (Gujrat district), and is a constant threat to the adjoining cotton crop to which it migrates from the forest during the rainy season. The population fluctuations and the migration of the pest from the forest area to the cotton fields and back can be followed more effectively by labelling the insects with a suitable radioisotope. Migration of *Apis dorsata* F. from the sub-mountainous tracts of West Pakistan to the plains during spring and their return in the

summer, and that of *Apis indica* F. from high altitudes to lower altitudes during the winter still remain to be investigated.

At present, there is a trend in Pakistan as in many other countries to concentrate upon the chemical control of various insects, and all newly manufactured synthetic and systemic insecticides are therefore being brought into use. The use of radioisotopes can go a long way in studying the mode of action of all such insecticides, and their penetration and decomposition in different insect organs and tissues, etc. Furthermore, the time taken by the various insecticides after administration of their lethal doses to produce certain effects on the different systems of insects and rodents can easily be studied with radioisotopes.

On the control side there is great scope for the use of radioisotopes in the sterilization of stored grain pests. The total annual production of cereals in Pakistan was estimated at about 13.4 million tons in 1958—59. If, during storage, 5% is consumed by insects it would entail a wastage of 670000 t, amounting to one crore rupees. As such, the problem is of great economic importance, and detailed studies are necessary to determine the extent to which radiation sources might be employed for its solution. It would also be worthwhile trying to control crop borers and fruit-flies by means of radioisotopes and radiation sources. For instance, the rice-stem borer (*Schoenobius incertellus* Wlk.) alone is responsible for destroying an average of about 0.8 million tons of rice per year in Pakistan and if only this pest could be eradicated by means of the sterile-male technique it would indeed represent a great achievement.

Summary and conclusions

There is considerable scope for the application of radioisotopes and radiation sources in entomological research in Pakistan. Such research should be considered as practical, and of potential productive interest since it may ultimately contribute towards a saving of millions of rupees by eradicating certain crop pests from Pakistan. The Pakistan Atomic Energy Commission is fully alive to the situation and is making every effort to ensure the success of this programme.

DISCUSSION

P. J. DEORAS (India): I should like to hear participants' views on the doses necessary for disinfesting stored products under Indian conditions.

P. B. CORNWELL (United Kingdom): Yesterday I mentioned a dose of 16500 rep (i.e. 16000 rad) as being adequate for sterilization of large populations of grain weevils. We have every reason to believe that this dose will be equally efficient against most other pests infesting grains, rice and perhaps pulses. We have done a considerable amount of work in the United Kingdom on the effects of doses up to one million rad on wheat, and there is every indication that we have a very large safety margin before the onset of adverse effects on baking and manufacturing properties. I must emphasize, however, that one cannot generalize about the effects of radiation on food products. Each case must be individually tested.

P. J. DEORAS: Thank you very much for this valuable information. The only thing that still worries me a little is the adequacy of these doses under our local conditions, where we normally have no silos and must store grain either in bulk in ordinary godowns or in bags.

P. B. CORNWELL: In determining the general applicability of doses we are primarily concerned with the susceptibility of the insect. It is obviously important to test not only

laboratory in-bred strains, ideal for research purposes, but also wild strains from storage in various parts of the world. As I said during previous discussions, we have in fact experimented with about 40 strains of grain weevil. While there are slight differences in their susceptibility to the lethal effect of gamma-radiation, we have no indication at the moment that they differ in their response to sterilization.

R. M. PATEL (India): In India much damage and loss are caused by stem borers, for which no effective method of control has yet been found. I should therefore like to appeal to radiation and radioisotope research-workers to give some attention to this problem.

THE FUTURE OF RADIOISOTOPES IN INSECT-CONTROL INVESTIGATIONS IN THE PHILIPPINES

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Abstract — Résumé — Аннотация — Resumen

The future of radioisotopes in insect-control investigations in the Philippines. Agriculture is the major industry in the Philippines, yet the production of the staple food and other basic necessities is very low, and insufficient to meet the local demand. As a result, the country imports annually millions of pesos' worth of rice, corn and other agricultural products.

One of the major causes of the low production of agricultural crops in the Philippines is the enormous damage caused by insect pests on both the standing crop and the stored product. It is estimated that about 15% of the product is lost to insects, which amounted to more than 269372000 pesos for the six major crops of the country in the 1958—1959 season.

Not a single insect pest affecting Philippine agriculture has been studied thoroughly. The bulk of investigations on insect pests in this country bears on life-history, morphology, host survey, and control. Control measures employed at present, particularly the use of chemicals, are based mainly on results of investigations in foreign countries, which may account for protection being ineffective, so far. Much essential information regarding pests, particularly their ecology, is needed. The difficulties of undertaking investigations on ecology of insects in this country, prior to the advent of radioisotopes, are mainly responsible for the almost complete lack of knowledge in this field.

The use of insecticides in insect control in the Philippines is limited not only by inadequate information on the bionomics of the pests but also by the toxicity of these chemicals to humans. Detection and assay of insecticide residues on treated foodstuffs with the use of tagged insecticides will enable the formulation of effective control measures in order to protect the consumer.

In the light of present knowledge, the use of radiation for the control of insects affecting Philippine agriculture can be investigated. In the Philippines, shelled corn, clean rice, mango, and other foodstuffs cannot be stored longer than three months without extensive damage by stored-product insects. The use of radiation-sterilized males as a possible means of control in this country can also be investigated.

Perspectives d'emploi des radioisotopes dans les études sur la lutte contre les insectes aux Philippines. L'agriculture est la principale activité des Philippines; cependant, la production des aliments de première nécessité et autres produits de base est très faible et insuffisante pour satisfaire les besoins du pays. De ce fait, le pays importe chaque année du riz, du maïs et d'autres produits agricoles pour une valeur de plusieurs millions de pesos.

L'insuffisance de la production aux Philippines est due surtout aux énormes dégâts causés aux récoltes sur pied ou en silo par les insectes nuisibles. Les pertes correspondantes pour la saison de 1958/59 sont estimées à environ 15% de la production, soit plus de 269372000 pesos pour les six cultures principales du pays.

Aucun des insectes qui nuisent à l'agriculture philippine n'a encore été étudié à fond. La majeure partie des études que les Philippines ont faites sur ces insectes portent sur leur développement, leur morphologie, leurs hôtes et les mesures de lutte. La campagne menée actuellement, notamment l'emploi de produits chimiques, s'inspire avant tout des résultats d'enquêtes faites à l'étranger, ce qui explique peut-être pourquoi cette protection est inefficace. On a encore besoin de beaucoup de renseignements essentiels sur les insectes nuisibles, particulièrement sur leur écologie. Ce sont principalement les difficultés auxquelles se heurtaient les études sur l'écologie des insectes aux Philippines, avant l'invention des radioisotopes, qui expliquent l'absence presque complète de données dans ce domaine.

Si l'on n'emploie les insecticides que dans une mesure limitée aux Philippines, c'est non seulement en raison de l'insuffisance de renseignements sur la bionomie des insectes, mais aussi parce que ces

produits sont toxiques pour les êtres humains. Grâce à l'emploi d'insecticides marqués, la détection et le dosage des résidus d'insecticides sur les aliments traités permettront d'exercer un contrôle efficace en vue de protéger le consommateur.

A la lumière des connaissances actuelles, il est possible d'envisager l'emploi des rayonnements dans la lutte contre les insectes qui nuisent à l'agriculture aux Philippines. Dans ce pays, on ne peut emmagasiner du maïs égrené, du riz décortiqué, des mangues ou d'autres produits alimentaires pendant plus de 3 mois sans risquer que ces produits ne soient très endommagés par les parasites. Dans cet ordre d'idées, on peut également envisager d'utiliser des mâles stérilisés par irradiation pour combattre la prolifération des insectes aux Philippines.

Будущее радиоизотопов в исследованиях по борьбе с сельскохозяйственными вредителями на Филиппинах. Сельское хозяйство на Филиппинах является ведущей отраслью, но производство главных продуктов питания и основных предметов первой необходимости находится на очень низком уровне и не удовлетворяет в достаточном количестве местных нужд. В результате этого страна ежегодно расходует миллионы песо на ввоз риса, зерна и других сельскохозяйственных продуктов.

Одной из основных причин получения низкого урожая сельскохозяйственных продуктов на Филиппинах является нанесение огромного вреда сельскохозяйственными вредителями как растениям на корню, так и собранному урожаю. Подсчитано, что за сельскохозяйственный сезон 1958—59 года потеряно урожая около 15% из-за вредителей, наличие которых превысило P 269, 372, 000 по шести основным сельскохозяйственным культурам.

Ни один из сельскохозяйственных вредителей на Филиппинах не был достаточно изучен. Наибольшая часть исследований в области сельскохозяйственных вредителей в этой стране проводится по биоэкологии, морфологии, исследованию растений, на которых живут паразитирующие организмы, а также по мерам борьбы. Используемые меры борьбы, в особенности с применением химикатов, основаны главным образом на результатах исследований в других странах; эти химикаты, возможно, не представляют собой эффективной защиты. Требуются более существенные данные относительно вредителей, в особенности по экологии. Трудности проведения исследований по экологии вредителей на Филиппинах до введения радиоизотопов объясняются в основном почти полным отсутствием знаний в этой области.

Использование инсектицидов для борьбы с вредителями на Филиппинах ограничено не только из-за недостаточных сведений относительно экологии вредителей, но также из-за токсического воздействия этих химикатов на человека. Определение и опробование остатков инсектицидов на обработанных пищевых продуктах при помощи инсектицидов даст возможность сформулировать эффективные меры по защите потребителя этих продуктов.

Использование радиации по борьбе с вредителями, поражающими урожай на Филиппинах, можно исследовать и при существующих знаниях. На Филиппинах нельзя хранить на складах неочищенное зерно, очищенный рис, манго и другие продукты более трех месяцев без того, чтобы эти продукты в значительной степени не подверглись уничтожению амбарными вредителями. На Филиппинах в качестве возможных средств борьбы можно исследовать облучение мужских особей вредителей на предмет их стерилизации.

Porvenir de los radioisótopos en las investigaciones sobre la lucha contra los insectos realizadas en las Filipinas. Si bien la agricultura constituye la base de la economía filipina, la producción de alimentos y otros artículos de primera necesidad es muy escasa y no alcanza a satisfacer la demanda nacional. Como consecuencia de este hecho, el país debe importar anualmente arroz, trigo y otros productos agrícolas por valor de varios millones de pesos filipinos.

El bajo rendimiento de los cultivos en las Filipinas se debe ante todo a los enormes daños causados por las plagas de insectos en las cosechas, tanto en el campo como almacenadas. Se estima que las pérdidas ocasionadas por los insectos en el curso de la temporada 1958—1959 se elevaron a cerca del 15%, o sea, a más de 269 372 000 pesos para los seis cultivos principales del país.

Aún no se ha estudiado a fondo ninguno de los insectos que afectan a la agricultura de las Filipinas. La mayor parte de las investigaciones realizadas en este país sobre los insectos dañinos han versado sobre su evolución y morfología, así como sobre las plantas huéspedes y los métodos de lucha más

adecuados. Los procedimientos empleados en la actualidad para combatir los insectos, especialmente los que utilizan productos químicos, están basados ante todo en los resultados de las investigaciones efectuadas en otros países, lo cual puede ser la causa de que las medidas de protección hayan resultado tan ineficaces hasta ahora. Es preciso obtener muchos datos esenciales sobre los parásitos y especialmente sobre su ecología. Las dificultades con que tropezaban las investigaciones sobre la ecología de los insectos en este país, antes de descubrirse los radioisótopos, explican la falta casi absoluta de conocimientos en esta esfera.

El empleo de insecticidas en las campañas de lucha contra los insectos realizadas en las Filipinas se ve restringido no sólo por la insuficiencia de informaciones sobre la bionomía de los insectos, sino también por la toxicidad que esos productos químicos presentan para los seres humanos. La detección y análisis, con ayuda de insecticidas marcados, de los residuos de insecticidas en los productos alimenticios tratados permitirá elaborar procedimientos eficaces de lucha contra los insectos, con objeto de proteger al consumidor.

Partiendo de los conocimientos que se poseen en la actualidad, es posible estudiar el empleo de las radiaciones en la lucha contra los insectos y sus consecuencias en la agricultura de las Filipinas. En este país ya no es posible almacenar durante más de tres meses maíz desgranado, arroz pulido, garbanzos y otros artículos alimenticios sin correr el riesgo de que las plagas agrícolas dañen seriamente los productos almacenados. También se puede investigar la posibilidad de utilizar machos esterilizados por irradiación como posible medio de lucha contra la proliferación de los insectos.

Introduction

The economy of the Philippines is mainly geared to the agriculture of the country. The Government as well as the people are largely dependent upon agricultural pursuits for their support. Of the total area of the Philippines under cultivation (7003600 hectares), about 80% is devoted to the production of the staple crops and the major export products, namely, rice, corn, coco-nut, sugar cane, abaca, and tobacco, [1] [2], (Table I). The remaining 20% is planted to fruit trees, root crops, vegetables, ramie and other fibre plants, and other minor crops.

The production of the basic agricultural necessities of the people is very low and insufficient to meet the local demand. As a result, the country imports annually millions of pesos' worth of rice and corn, the staple food of the people, to ward off starvation. Other agricultural products, such as onion, potato, garlic, tobacco, fruits, etc., which could also be produced commercially in this country are also imported in large quantities.

One of the important causes of the low production of crops in the Philippines is the damage inflicted on these crops by insect pests. A conservative estimate of the annual damage to crops by insect pests is about 15%. Many of these insects are so destructive that they constitute a constant threat to production. The more important insect pests of the major and some of the minor crops and the extent of the damage they cause will be reviewed below to allow a better appreciation of the need for effective control measures so as to prevent or at least reduce to the minimum their ravages, and thereby increase production.

Rice

Rice is the most important crop of the country. It is the staple food of over three-quarters of the population. The Philippines, however, is among the countries with the lowest yield, producing an average of only about 27.5 cavans of palay per hectare, compared to 32 for Thailand, 61 for Taiwan, 36 for Java, and 120 for Italy [1]. Because of this low production, the country imports millions of pesos' worth of rice annually. In 1957, the total rice-import amounted to 120834 t (metric) or 2 157 750 cavans of clean rice [2].

The low production of rice is attributed partly to ravages caused by insect pests, the most destructive being the stem borers of which there are four species; the oriental migratory locust, *Locusta migratoria manilensis* (Meyen); the rice armyworm, *Spodoptera mauritia* (Bois); and the rice bug, *Leptocorisa acuta* (Thunb.). Two species of leafhoppers, *Nephotettix apicalis* (Motsch.) and *N. bipunctatus* (Fabr.), and about half a dozen other species at times cause serious injury to the rice plant but are ordinarily minor pests. The migratory locust is only a threat to crops in areas near its permanent breeding ground and within range of the migrating adults. On the basis of the latest available statistics (Table I), a conservative estimate of the damage by insects to the 1958—1959 rice crop amounted to about 650205 t (metric) of palay, valued at 124479741 pesos. Various estimates, some as high as 30% or more, have been reported particularly during serious outbreaks of these insects. For the last three years the situation has become aggravated by outbreaks of rats in some sections of the country, particularly in Mindanao, which consume whatever is left by the insects.

TABLE I

AREA PLANTED, PRODUCTION, VALUE OF PRODUCE, AND ESTIMATED LOSS DUE TO INSECTS, CORRESPONDING TO THE 1958—1959 CROP SEASON OF THE MAJOR AND FOOD CROPS

Crop	Area (hectares)	Production (metric tons)	Value (pesos)	Loss due to insects (metric tons)	Loss due to insects (pesos)
Rice	2970770	3684503[1]	705385200	650205	124479741
Corn	1335860	1015911[2]	132516300	179278	23385229
Coco-nut	1006100	1121993[3]	255382500	197998	45066852
Sugar Cane	252160	1806777[4]	344851100	318843	60856076
Abaca	192540	111453	39435600	12383	4381766
Tobacco	90990	51719[5]	63482200	9127	11202741
Fruits[6]	366330	672575	96160600	118690	16969518
Root Crops[6]	291700	1292000	80384600	228000	14185518
Vegetables[6]	175660	231300	63016200	40818	11120506

[1] Palay
[2] Shelled corn
[3] Copra and desiccated coco-nut
[4] Centrifugal and raw sugar
[5] Virginia and native tobacco
[6] Based on the 1957 figures

Stem borers are perennial pests of rice in the Philippines. Four species are known to infest the crop in this country, namely, *Schoenobius incertulas* (Walker), *Chilo suppressalis* (Walker), *Sesamia inferens* (Walker), and *Scirpophaga innotata* (Walker). The first two species are those most commonly encountered in the field, and the most destructive. It is difficult to give an accurate estimate of the damage caused by these insects because of the growth characteristics of the rice plant and the nature of the injury caused by the insects. When rice plants are infested, the affected tillers are killed and replaced by new ones. This sets back and impairs the development of the plant considerably. Repeated attacks by various generations of insects, therefore, will have a telling effect on the yield. Late infestations, particularly those occuring immediately before the bolting stage are rendered visible by the appearance of "white heads" or empty panicles. This damage represents only part of the total injury to the plants. Very often the "white head" is used as the sole basis for estimating damage caused by rice stem-borers.

Present knowledge about the rice stem-borers in the Philippines is very limited. With the exception of a few published studies [4] [7] [18] [19] [23], and the incidental inclusion in reports of fragmentary data or references to these insects, no thorough study has been published. Basic knowledge of the ecology of these pests is badly needed for a better understanding of the seasonal abundance, population trends, dispersion, migration, feeding, mating and oviposition habits, and the number of broods and generations per year. A thorough understanding of the nutritional requirements of the insects may shed light on the cause of varietal susceptibility of rice to the different species of stem borers. It is hoped that radioisotopes will contribute a great deal in the solution of these problems.

Milled rice is susceptible to stored-grain insects, particularly to the rice weevil, *Sitophilus oryza* (L.) and the rice moth, *Corcyra cephalonica* (Staint.). However, the damage caused by these insects is greatly minimized by storing uncleaned rice or palay, and milling it only as the demand warrants.

Corn

Corn is the staple food of about 21% of the population. It grows in all regions of the archipelago. The most destructive pests of this crop are the corn borer, *Pyrausta salentialis* (Sn.) and the corn earworm, *Heliothis armigera* (Hubner) [25] [26]. The corn borer is the more destructive of the two species. During heavy infestations, as much as 50% of the crop is destroyed. In some localities complete destruction of the crop has been reported during seasons of extremely severe infestation. At other times, almost complete absence of the insect has been noted. The biology of the corn borer has been studied by BULIGAN [3]. The corn earworm has been reported more numerous on the dry-season crop than on the wet-season in some sections of the country. This could be due, at least in part, to the crop coinciding with the growing season of the other major hosts of this pest, such as tomato, cabbage, eggplant, cotton, and other crops.

The enormous damage caused by these insects on corn every year deserves careful consideration. No extensive ecological investigation has been conducted in this country on these insects. Investigations to determine the causes of population fluctuations, extent of dispersion and migration, and other factors affecting the insects will greatly contribute to the formulation of effective control measures against these insects. Radiotracers will be invaluable in such investigations. Studies on the effects of irradiation on the reproduction of the species may throw light on the possible use of radioisotopes in the biological control of these and other species of insect pests.

One of the major factors responsible for the wide fluctuation in the price of shelled corn in the Philippines is the susceptibility of the grain to the ravages of insects, so that the produce could not be stored for any length of time without extensive damage to the grain. According to LABADAN and VIADO [6], a conservative estimate of at least 5% of the grain is lost to insects in three months of storage. After six months, the shelled corn lost about 17 wt.% as a result of insect damage, and 75% of the remaining grains were already damaged. The most destructive of these species are the rice weevil, *Sitophilus oryza* (L.), the lesser grain borer, *Rhizopertha dominica* (F.), and the rice moth, *Corcyra cephalonica* (Staint.). VIADO and LABADAN [24] investigated the use of DDT-treated containers for the control of storage insects of corn. The use of irradiation for the control of these pests and the application of radiotracers in studies of their bionomics will open a new field of research on the control of these destructive pests.

Coco-nut

For many years the coco-nut has been the principal dollar-earning crop of the country. In recent years, this crop has been threatened by the coco-nut cadang-cadang, a yellowing disease which has been killing many thousands of trees in the Bicol area during the last few years. The disease appears to be spreading to other localities. Its cause is still unknown but a possibly viral origin or malnutrition have been suggested. There appears to be more support for the viral nature of the disease. Studies on the physiology and nutrition of the coco-nut with the use of tracer atoms may eventually lead to correct diagnosis and control of the disease. If it is caused by a virus, it may be transmitted to healthy trees by vectors which are, most likely, insects or other arthropods. Transmission studies with suspected vectors will be facilitated with the use of radiotracers.

The black or rhinoceros beetle, *Oryctes rhinoceros* (L.), and the Asiatic palm weevil, *Rhynchophorus ferrugineus* (Olivier) are very destructive to coco-nut in some sections of the country. Trees attacked by the Asiatic palm weevil seldom recover. The coco-nut leaf miner, *Promecotheca cumingii* (Baly) and the Florida red scale, *Chrysomphalus aonidum* (Linn.), which are ordinarily minor pests of the coco-nut, at times become major pests. UICHANCO [10] [13] [14], and VIADO and BIGORNIA [21] conducted studies on coco-nut insects. Investigations on the ecology of these insects may contribute immensely to the formulation of measures for their control.

Copra constitutes the bulk of the coco-nut export of the Philippines. The market for this product abroad has been threatened by the copra beetle, *Necrobia rufipes* (De G.). The declining reputation of Philippine copra on the world market as reflected in the low grade of copra exported during the past few years has been blamed on this destructive pest. This insect seems to prefer copra with a high moisture content, characteristic of copra obtained from insufficiently mature nuts and/or copra which has not been thoroughly dried. Very little is known about the bionomics of this insect particularly with regard to its flight range, dispersion, migration, seasonal abundance, etc. Other copra pests which at times become numerous and destructive, and deserve study are *Dermestes sp.*, *Ephestia cautella* (Wlk.), *Oryzaephilus surinamensis* (Linn.), and *Tribolium castaneum* (Herbst.).

Sugar cane

Sugar is one of the principal exports, and a major dollar-earner for the country. The sugar industry is the most highly developed of the agricultural industries of the Philippines. It is, however, faced with perennial insect problems which hold no promise of an early solution. The more important of these insect problems are the gray borer, *Argyroploce schistaceana* (Snellen), which bores into and kills the young shoots of the plant; the leafhopper, *Perkinsiella vastatrix* (Breddin), which is a vector of the sugar-cane mosaic; the white grubs, which feed on the underground portions of the plant, of which *Leucopholis irrorata* (Chevr.), and *Lepidiota blanchardi* (Dalla Torre) are the most important; and a score of other insects. URBINO [15], UICHANCO [11] [12], and VIADO [16] conducted studies on major sugar-cane insects. It is estimated that these insects cause the industry an annual loss of millions of pesos. Radioisotopes will play a major role in the ecological studies of these insects.

Abaca

The abaca industry is one of the four major dollar-earning industries of the country. It is, however, declining rapidly. The most destructive disease of the abaca plant and the

factor responsible for the rapid decline of the industry after the last war, is the abaca mosaic. It is the consensus of opinion among abaca planters that, unless something is done, the disease will ruin the industry completely. This disease is transmitted from diseased to healthy abaca plants by such insect vectors as *Aphis gossypii* (Glover), *Rhopalosiphum nymphaeae* (Linn.), *R. prunifoliae* (Fitch), and *Proutista moesta* (Westwood). Another species of aphid, *Aphis maidis* (Fitch) transmits corn mosaic to abaca. KENT [5] reviewed the investigations on this disease in the Philippines. There are other insects which affect the abaca but their injury to the plant is relatively small. Studies on the feeding and other habits of these vectors will be facilitated with the use of radiotracers, thereby making possible the formulation of more effective control measures.

Tobacco

Tobacco is one of the major dollar-earning crops in the Philippines and a major source of revenue for the government. In 1956 alone the government collected 105 151 513.68 pesos in the form of specific taxes from locally produced tobacco and its products. The production of native varieties for cigar and Virginia varieties for cigarette manufacture is low, considering the large amount of cigarette and leaf tobacco imports into the country.

The low production of this crop and the poor quality of the product is attributed in part to the damage caused by several species of insects, the tobacco budworm, *Heliothis armigera* (Hubner), the semi-looper, *Plusia chalcytes* (Esper), and the cutworm *Prodenia litura* (Fabr.), all of which are leaf feeders, being the most destructive. VIADO *et al.* [29] investigated the control of these insects with organic insecticides. These insects constitute a limiting factor in the culture of wrapper tobacco. At times, the stem borer *Gnorimoschema heliopa* (Low) and *Phaneroptera furcifera* (Stål) cause damage of serious proportions. Stored tobacco leaves and manufactured products are damaged and rendered unmarketable by the cigarette beetle, *Lasioderma serricorne* (Fabr.).

With the use of radioisotopes, present knowledge of the bionomics of these insects, particularly those of ecology, will be considerably enriched, thereby contributing to the speedy solution of the problem of controlling these pests. Radioisotopes will also allow investigations to be made on the use of irradiation for the control of the cigarette beetle and other tobacco insects.

Other crops

Banana, mango, pineapple, jackfruit, citrus, lanzon, and papaya are the commercially grown fruits of the country. The most widely grown fruit which is still without any insect pest of economic importance, is the banana. The twig borers, *Niphonoclea albata* (Newm.) and *N. capito* (Pascoe) and the mango hopper, *Idiocerus clypealis* (Lethiery) are the mango insect-pests of economic importance. White grubs, particularly those of *Leucopholis irrorata* (Chev.), *Lepidiota blanchardi* (Burm.), and *Holotrichia spp.* are the most destructive insect pests of pineapple in the Philippines. The damage caused by these soil-inhabiting insects is detected late, at a stage when the affected plants can usually no longer be saved. The worst insect enemy of the jackfruit is the fruit fly, *Dacus umbrosus* (Fabr.). Citrus has two common destructive insects, the rind borer, *Prays sp.* and the green bug, *Rhynchocoris longirostris* (Stal.). SAN JUAN [8] and VIADO *et al.* [28] conducted studies on the rind borer. Two species of bark borers, *Prasinoxena sp.* and *Cossus sp.* are very detructive to lanzon [20]. The former is the more common, hence the more destructive of the two species. Many trees are killed during heavy infestation. Papaya is grown both for vegetable and for table fruit. It has no insect pest of economic importance.

Sweet potato, cassava, and gabi are the most important root crops in the Philippines. They are used to supplement rice and corn in places where production of the staple crops is low or limited by environmental factors.

The most destructive insect of sweet potato in this country is the sweet-potato weevil, *Cylas formicarius* (Fabr.). During heavy infestation, as much as 75 to 100% of the harvest is infested. Infested roots are unsuitable for human consumption or animal feed. Not much is known about this most destructive pest of sweet potato or its control. The cassava has no known pest of economic importance in this country.

Tomato, mango, eggplant, beans, cabbage, Irish potato, onions, pechay and gourds (squash, upo, ampalaya) are the common vegetables grown in the Philippines. Each of these crops has its insect pests. Of the polyphagous species infesting most of these crops, the cutworm, *Prodenia litura* (Fabr.), and the tomato worm, *Heliothis armigera* (Hbn.) are the most destructive and most common. VIADO [17] and VIADO and ESTIOKO [22] conducted investigations on insects infesting tomato and eggplants. The most destructive pest of cabbage and pechay at high elevations is the imported cabbage worm, *Pieris canidia* (Sparrman) [25]. This species is confined only to the higher altitudes (Mountain Province). On the lowland the most destructive pests of these two crops is the cabbage moth, *Crocidolomia binotalis* (Zeller). *Prodenia litura* (Fabr.) and *Heliothis armigera* (Hbn.) are also important pests of these crops in the lowlands.

The most destructive insects of beans, peas, and mango in addition to *P. litura* and *H. armigera*, are various species of bugs, which feed on the young pods and shoots. The dried seeds in storage are very susceptible to the ravages of the bean weevil, *Bruchus chinensis* (Linn.). The onion thrips, *Thrips tabaci* (Lind.) is the most destructive insect of onion in the Philippines. Young plants when severely affected fail to reach maturity. Late crops are most badly affected by this insect.

Gourds (squash, upo and ampalaya) are subject to the depredations of the fruit-fly *Dacus cucurbitae* (Coq.), the most economically important insect pest of these crops. During severe infestations, an entire crop may be wiped out.

Insect pests have been causing tremendous losses to crops since the advent of Philippine agriculture. The progress of our knowledge on the effective control of these pests is limited by inadequate information on their bionomics. Conventional methods used in the study of the bionomics of insect pests are not only tedious but also give unreliable results, so that other methods must be sought. With the advent of radioisotopes, many investigations which might only be undertaken with extreme difficulty or not at all by conventional methods, could be pursued with relative ease by means of these new research-tools.

One of the principal drawbacks in the use of insecticides for the control of insect pests on food crops is the fact that insecticides are toxic not only to insects but also to humans. The amount of toxic residues present on the edible portions of those food crops treated with insecticides to control insects is a hazard to health. Investigations using insecticides tagged or labelled with radioisotopes will make possible the formulation of schedules of insecticide application which will avoid the accumulation of toxic amounts of the poison on the crop at harvest, whilst at the same time ensuring effective control of the pests.

The discovery of the use of irradiation for the control of insect pests opens a new field of investigation. Sublethal doses of irradiation which render insects sterile have found successful application in the control of the screwworm in the United States.

It is hoped that with the use of radioisotopes a better understanding of these pests in the Philippines will be reached so that more effective control-measures may be formulated. Radioisotopes will open a new horizon for entomological research in the Philippines.

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DISCUSSION

P. J. DEORAS (India): This interesting enumeration of the various types of damage done to different crops in the Philippines suggests the question whether any work is being done anywhere on the use of isotopes for estimating and analysing damage caused by insects and rodents to growing crops or to stored products.

J. DE WILDE (Netherlands): At the Plant Breeding Institute of the German Democratic Republic's Academy of Agricultural Sciences, I have seen a very interesting arrangement using a Co^{60} source to measure crop density, crop growth and loss by defoliators. The source was placed in the centre of a circular experimental field, and counter tubes were arranged so that they could be moved along the periphery. For cases where one wants to keep the living plant *in situ* and still make measurements, this seems a very interesting idea.

A. R. GOPAL-AYENGAR (India): I think it is well to bear in mind that radioisotopes are not a universal panacea. Where a better method of doing a job exists, that method should be used, and the temptation to bring in isotopes at all costs resisted.

THE CHAIRMAN (K. K. NAIR, India): I think Dr. Gopal-Ayengar's remark is most apposite.

TRAVAUX DE RECHERCHES UTILISANT LES ISOTOPES ET LES RAYONNEMENTS NUCLÉAIRES EN ENTOMOLOGIE APPLIQUÉE EN FRANCE ET DANS LES PAYS ASSOCIÉS

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Abstract — Résumé — Аннотация — Resumen

Applied entomology research using isotopes and nuclear radiations in France and the associated countries. The paper is in two parts. The first part deals with work done or being done in Upper Volta, on the Ivory Coast, at Bures-sur-Yvette (S. et O.) and at Bondy, using radioisotopes for labelling sand-flies, ants *Apis mellifica* and *Aphis leguminosae* and secondly, with research projects in tropical regions, in tropical Africa and in France in connection with the labelling of *Perkinsiella*, Trichogramma, *Locusta migratoria* and certain mosquitoes or their predators.

The second part is concerned with research on the sterilization of insects (*Apis mellifica, Ephestia kühniella, Calandra granaria, C. oryzae, Trogoderma, Acanthoscelides obtectus, Rhizophertha, Gnathocerus, Tenebrio* and *Sitotroga cerealella*) and the cytological effects of ionizing radiations on the gonads of *Calandra granaria.*

Travaux de recherches utilisant les isotopes et les rayonnements nucléaires en entomologie appliquée en France et dans les pays associés. Dans une première partie, l'auteur passe en revue les travaux, effectués ou en cours en Haute-Volta, en Côte-d'Ivoire, à Bures-sur-Yvette (S. & O.) et à Bondy, qui utilisent des radioisotopes pour le marquage de simulies, de fourmis, d'*Apis mellifica* et d'*Aphis leguminosae*, ainsi que les projets de recherches en régions tropicales, en Afrique tropicale et en France pour le marquage de *Perkinsiella*, de trichogrammes, de *Locusta migratoria* et de certains moustiques ou de leurs prédateurs.

Dans la seconde partie, il mentionne les études poursuivies sur la stérilisation des insectes (*Apis mellifica, Ephestia kühniella, Calandra granaria, C. oryzae, Trogoderma, Acanthoscelides obtectus, Rhizopertha, Gnathocerus, Tenebrio* et *Sitotroga cerealella*) et l'action cytologique des radiations ionisantes sur les gonades de *Calandra granaria.*

План исследовательских работ с помощью изотопов и ядерного излучения, которые могут быть проведены в области прикладной энтомологии во Франции и в странах французского сообщества. Доклад состоит из двух разделов. В разделе А описываются работы, проведенные или проводящиеся в Верхней Вольте, Береге Слоновой кости, в Бюр-сюр-Иветт (департамент Сена и Уаза) и в Бонди с помощью радиоизотопов для мечения соответственно мошек, муравьев, обыкновенных медоносных пчел (*Apis mellifica*) и тлей (*Aphis Leguminosae*), а также исследовательские проекты в тропических районах, тропической Африке и во Франции для мечения *Perkinsiella*, трихограмм, азиатской саранчи (*Locusta migratoria*) и некоторых комаров или их истребителей.

В разделе В описываются исследовательские работы по стерилизации насекомых (обыкновенные медоносные пчелы, *Ephestia kühniella*, амбарные долгоносики, ризовые долгоносики, *Trogoderma, Acanthoscelides obtectus, Rhizopertha, Gnathocerus*, хрущаки и зерновая моль), а также исследовательские работы по цитологическому действию ионизирующей радиации на гонады амбарного долгоносика.

Plan de las investigaciones de entomología aplicada, basadas en el empleo de los isótopos y las radiaciones nucleares, susceptibles de emprenderse en Francia y en los países asociados. El presente trabajo se divide en dos partes. La sección A se refiere en primer término a los trabajos terminados o en curso de ejecución en el Alto Volta, sobre la Costa del Marfil, en Bures sobre el Yvette (Departa-

mento de Seine et Oise) y en Bondy, en los que se utilizan radioisótopos para marcar insectos tales como la mosca negra (Simulies), hormigas, *Apis mellifica* y *Aphis leguminosae*, respectivamente, y, en segundo término, a proyectos de investigación en regiones tropicales, en el Africa Tropical y en Francia, basados en la marcación de los insectos *Perkinsiella, Trichogramma, Locusta migratoria* y de ciertos mosquitos o de sus entomófagos.

La sección B trata de estudios relacionados con la esterilización de insectos (*Apis mellifica, Ephestia kühniella, Calandra granaria, C. oryzae, Trogoderma, Acanthoscelides obtectus, Rhizopertha, Gnathocerus, Tenebrio* y *Sitotroga cerealella*) y de estudios sobre los efectos citológicos de las radiaciones ionizantes sobre las gónadas de la *Calandra granaria*.

El autor cita los nombres de los investigadores y las publicaciones correspondientes a cada grupo.

Utilisation des isotopes

TRAVAUX EFFECTUÉS OU EN COURS

Haute-Volta

Utilisation du phosphore-32 pour le marquage de simulies (diptères nématocères), vecteur en Afrique tropicale de filarioses (onchocercose). Lâchers expérimentaux d'insectes, en vue de contrôler leur zone de dispersion.

Dans le cadre de l'Office de la recherche scientifique et technique d'outre-mer (ORSTOM), Section d'entomologie médicale: Centre Muraz (Bobodioulasso). Chercheur: M. Mortreuil.

Côte-d'Ivoire

Utilisation du phosphore-32 pour le marquage de fourmis qui, en Afrique tropicale, interviennent pour la protection et la diffusion de cochenilles (*Pseudococcus brevipes*), vecteur d'une maladie à virus de l'ananas (Wilt de l'ananas). Recherches entreprises en Côte-d'Ivoire en vue de préciser la zone d'activité des fourmis de diverses espèces, et leur zone de dispersion à partir de la fourmilière.

Dans le cadre de l'ORSTOM. Centres de l'Institut d'études et de recherches tropicales (IDERT) (Adiopodoumé) et de l'IFAC (station de l'Anguedou). Chercheurs: M. Mortreuil, Mlle Brader.

Bures-sur-Yvette (*Seine-et-Oise*)

Utilisation de l'or-198 pour le marquage d'abeilles domestiques (*Apis mellifica*) en vue de préciser les conditions de pollinisation et de la rumination sociale.

Dans le cadre de l'Institut national de la recherche agronomique (INRA) et du Commissariat à l'énergie atomique (CEA): Centre de recherches apicoles de Bures-sur-Yvette. Chercheurs: MM. Lecomte et Courtois. Publication: *C. R. Acad. Sci.* (*Paris*) **247** (1958) 147—9.

Bondy (*Seine*)

Utilisation du phosphore-32 pour le marquage de pucerons (*Aphis leguminosae*), vecteurs en Afrique tropicale d'une maladie à virus de l'arachide (rosette de l'arachide). Essais préliminaires dans un laboratoire métropolitain (afin de préciser les doses utilisables, durées, rémanence). Dans le cadre de l'ORSTOM: Institut d'études et de recherches tropicales (IDERT). Chercheurs: MM. Mortreuil et Réal (entomologiste).

PROJETS DE RECHERCHES

Madagascar

Dans le cadre de l'ORSTOM et de l'IRAM (Institut de recherches agronomiques tropicales de Madagascar).

a) Utilisation d'isotopes pour le marquage de *Perkinsiella* (homoptères), insecte vecteur d'une maladie à virus de la canne à sucre (maladie de Fidji). Recherches en vue de préciser les conditions de dispersion du vecteur, et les modalités de l'infection. Recherches biologiques préalables en cours. Ce problème peut intéresser l'Ile Maurice, la Réunion. Chercheur: M. Sigwalt.

b) Utilisation d'isotopes pour le marquage de trichogrammes (hyménoptères), parasite des œufs d'un papillon dont la chenille est mineuse de la canne à sucre (Borer de la canne à sucre). Recherches en vue de préciser les conditions de dispersion et de survie des hyménoptères auxiliaires utilisés industriellement dans la lutte biologique contre le borer. Recherches biologiques en cours et très avancées. Chercheurs entomologistes: MM. Caresche, Brenière, Ravelojoana.

c) Utilisation d'isotopes pour le marquage d'acridiens migrateurs (*Locusta migratoria*) dans la zone des aires grégarigènes du sud de Madagascar. Recherches en vue de préciser, en dehors des périodes de pullulation, le comportement et la diffusion des larves et adultes de la forme solitaire. Marquage des adultes avant la ponte en vue d'observer la diffusion des larves et des adultes de la génération qui en découle. Centre de recherches anti-acridien de Betioky-Sud. Chercheur entomologiste: M. Têtefort.

Afrique tropicale

Problèmes non suffisamment précisés, mais pouvant être envisagés.

Etudes concernant la biologie de certains moustiques ou de leurs prédateurs.

Etudes concernant la biologie des insectes supposés vecteurs d'une maladie à virus du cocotier au Togo (maladie à Kaincopé).

Etudes concernant la biologie de fourmis Magnans.

Etudes concernant la biologie de certains insectes dans le cadre de recherche écologique de la voûte forestière.

France

Etudes concernant le marquage du puceron (spécialement *Myzodes persicae*), vecteurs de viroses de la pomme de terre. Recherches biologiques récentes sur ce problème. Dans le cadre de l'INRA. Entomologiste: M. Cairaschi.

Stérilisation des insectes

a) Etudes de doses stérilisantes sur *Apis mellifica* (Abeille domestique). Dans le cadre de l'INRA (laboratoire de recherches agricoles de Bures-sur-Yvette). Chercheurs: MM. Courtois et Lecomte. Publications: Rapport CEA n° 1377, (1959) 285—290.

b) Etudes sur les doses stérilisantes à l'égard d' insectes des denrées (Teigne de la farine (*Ephestia kühniella*)), *Calandra granaria* et *C. oryzae*, *Trogoderma*, Bruche du haricot (*Acanthoscelides obtectus*), *Rhyzopertha*, *Gnathocerus*, *Tenebrio*, *Sitotroga cerealella*). Recherches en vue de préciser les doses minima utiles pour assurer la protection des denrées stockées. Dans le cadre INA (Paris) et Conservatome (Lyon). Chercheurs: MM. Pesson, Vidal et Brunelet.

c) Etudes sur l'action cytologique des radiations ionisantes sur les gonades de *Calandra granaria*. Dans le cadre INA (laboratoire de zoologie, Paris). Chercheurs: MM. Pesson et Vernier.

DISCUSSION

W. KLOFT (Federal Republic of Germany): I should like to add that Mme Alibert-Berthot is carrying out radioisotope studies on the biology of various termites in the laboratory of Professor Grassé in Paris. Mme Alibert-Berthot began her studies in our laboratory at Würzburg and is now continuing them in Paris.

P. PÉLEGRIN (France), who presented the paper: Thank you for this addition to our list.

CONCLUDING DISCUSSION

CHAIRMAN: J. HALBERSTADT (IAEA, Scientific Secretary)

The CHAIRMAN: Ladies and gentlemen, we have come to the end of our work here. But before we close the proceedings I should like to ask some of the participants to summarize the salient points of the week's activities. I will first call on Dr. Winteringham to give a brief résumé of what we have been discussing in the fields of insect physiology and biochemistry and resistance problems.

F. P. W. WINTERINGHAM (United Kingdom): We have heard about two distinct types of application of radioactive isotopes at this most interesting Symposium. One type comprises the more direct application to problems of control, as in ecological studies, and the other the more fundamental applications at laboratory level to problems of research, and it is not possible in a few words to do justice to the great effort which has obviously been made by several of the individual speakers. I will, therefore, just attempt to catalogue some of the salient points.

We heard from J. E. Casida how OP-compounds labelled with P^{32} and other isotopes were being used very effectively for studying metabolism in plants. B. W. Arthur similarly discussed and reviewed the use of labelled OP-compounds, particularly Dipterex, for studying their metabolism in insects, and in both cases this work has led to some important conclusions about the persistence and effective toxicity of these substances when used in the field. D. F. Heath described some preliminary work with Cl^{36}-labelled insecticides of the Dieldrin type, and showed how this technique was being used effectively for studying the all-important problems of mammalian toxicology. T. L. Hopkins discussed briefly the significance of the metabolism of the insecticide Dipterex in resistant and susceptible strains, and how such studies are giving us important information on the mechanisms of resistance—in many cases an essential step in the development of effective counter-measures. I explained the technique developed by my colleagues, particularly in England, known as the labelled pool technique, by which effects of poisons, and possibly ecological factors, can be studied on the insect by labelling the insect and its various metabolic pools. J. E. Treherne explained how radioactive isotopes are being used for studying the central nervous system in the insect, which we know to be so important in the action of many insecticides. R. G. Bridges explained how P^{32} was used in studying lipid metabolism, a comparatively new field in insect biochemistry, and mentioned briefly some results which may prove to be highly relevant to the problems of resistance. Next we had, also under the same heading, a paper by H. Huque on the persistence of P^{32} in cockroaches representing a very important series of measurements, for if we are going to use P^{32} for studying the behaviour of these insects we must know how persistent the label itself is going to be. Then W. Kloft described how important it is to consider the various geometrical and physical factors in interpreting the relation between the amount of radioactive isotope present in an insect and the actual response one gets on the detector. I think many of us were particularly pleased to hear this, because too often it is assumed that a particular radioactive isotope administered to an insect will give the same sort of response irrespective of the various physical conditions involved — and nothing could be further from the truth. C. J. Banks gave us an extremely interesting account of his field-work in studying the behaviour of aphids, their food intake, excretion and so on, and lastly, a similar paper was given by W. Kloft on the uptake of phloem sap by aphids in the field and in the laboratory.

I think we were all very sorry that our friends from Japan, T. Fukuda and C. Tomizawa, were absent and, also, that the paper by Karlson and his colleagues could not be presented at the Symposium. Nevertheless, I think that as far as biochemistry and physiology are concerned,

we have had convincing evidence of how effectively radioisotopes can be used, especially at the research level. However, I would be the last to say — and I think we have heard several speakers express the same sentiment — that they are a universal panacea in the research laboratory. They are not. These tools are most effectively used in conjunction with other methods. Even since radioisotopes have been introduced — and they have sometimes been used, say, for solving particular analytical problems — other methods have been evolved which do not call for the use of radioisotopes, but which are even more effective. I can think of one illustration. For some time we in England used the method of labelled pools for determining relative concentrations of ATP in insects. Two of my younger colleagues are now using the firefly enzyme lysepherine-lysepherase complex for determining ATP with, I should say, many times the sensitivity of the radioactive-tracer technique. We must recognize this sort of development.

Well, Mr. Chairman and ladies and gentlemen, at all events I feel that this has been a very instructive Symposium, and as far as insect biochemistry is concerned, I consider it has been a very representative one.

The CHAIRMAN: Thank you very much, Dr. Winteringham. I am particularly happy that you mentioned the potentialities of other techniques as I consider it to be one of the purposes of this type of meeting to compare the merits of the various techniques available.

May I now ask Dr. Cornwell to summarize the situation as far as disinfestation of stored products is concerned.

P. B. CORNWELL (United Kingdom): The actual number of papers on radiation disinfestation has been comparatively small, but indirectly, through the discussions, we have received ample evidence of the need for improving our present methods of controlling insects in stored commodities. We have heard from J. A. Qayyum, G. B. Viado and P. J. Deoras of losses in various parts of the world. We have been given an insight into the nature and operation of radiation disinfestation plants that may soon be available, L. E. Brownell, in particular, giving us a detailed account of ideas on this subject in the United States, and we have had contributions on radiation susceptibility studies from K. K. Nair and from myself. The Symposium has done much to indicate what can and what cannot be done in the field of radiation disinfestation; we have come to recognize that radiation disinfestation is an elaborately conceived technique which requires the concurrence of various favourable factors for it to be applied in practice. Instead of becoming infected with excessive enthusiasm and optimism regarding the future of radiation disinfestation, I believe that we should take the view that, for the moment at least, we are likely to achieve better control measures by concentrating on more efficient application of conventional insecticides and on improving storage facilities for our commodities in various parts of the world. Where large losses to commodities occur, it is often the problem of reinfestation which is of vital importance and here, as we well know, radiation offers no solution, whereas insecticides giving residual protection are available. This, of course, is not to say that we should run to the opposite extreme and allow our research to become infused with a spirit of pessimism. The study of radiation disinfestation is a comparatively new subject. At this stage our attitude should be one of sound realism, bearing in mind that irradiation may have its place as an adjunct to existing control measurements but not as a replacement for them. We are all aware of the need for improved methods, and one of the things the Symposium has indicated is that there are in different parts of the world a number of people very interested in the subject who will actively pursue their investigations on it. The fact that we have now met and know one another will be a powerful stimulus to such work. I would perhaps point out here that we may have the possibility of combining a number of the

techniques which we have been discussing: disinfestation with the principle of sterile-male release with a view to preventing reinfestation, and the possibility of tracer techniques to determine the distribution of sterile insects in relation to the probability of insects with residual fertility mating and thus bringing about reinfestation.

We realise also that, when radiation disinfestation is applied commercially we shall be treating commodities which will pass from one part of the world to another in international trade. The problem will therefore arise of international clearance of irradiated foods for human consumption, and it is, I think, the duty of international organizations such as OEEC, FAO and the IAEA to lend their aid in this respect.

In conclusion, then, I feel that this has been a very successful Symposium and one from which participants have derived the maximum benefit. This happy result is due in large measure to the excellence of the organization for the meeting.

The CHAIRMAN: Thank you, Dr. Cornwell. May I also ask you, Dr. Jenkins, to summarize for us your impressions of the meeting, especially in the fields of particular interest to yourself.

D. W. JENKINS (United States of America): A few years ago, when residual insecticides were introduced we thought that we had gained the upper hand in the struggle against insects; however, species at first susceptible to given insecticides developed resistance, new species replaced others that had been effectively controlled, and the present situation seems to be that all our moves are met by countermoves on the part of the insects.

The field of ecology is an enormously wide one, and an enormous number of studies are required. I do not think that there is, as yet, sufficient general recognition of the fact that, in these ecological studies, extremely thorough basic research is required before it is possible to carry out field-work effectively and accurately. F. P.W. Winteringham has stressed the need for basic research in his own field, and I would emphasize the importance of this in ecology also.

We have heard, during the Symposium, of the value of radioisotopes in connection with insecticides; of particular further interest were the contributions dealing with the new "self-eradication" approach, making insects act against themselves. The paper introduced by D. E. Weidhaas on the use of the sterile-male technique in the United States concerned a successfully completed project, thus positively proving the efficacy of this method. I believe that in the future we shall hear a great deal about the other four "self-eradication" methods, genetics and so on, to which reference has been made. I would enter a very strong plea that before these are actually applied on a large scale, ecological studies be carried out in some detail; otherwise we shall have a repetition of what has occurred in the case of parasites and predators, when release of the latter has been followed by nil results, because we do not know enough about either the parasites and predators or the host insect to be controlled.

We heard very briefly about the subject of genetics. This is one which is going to be extremely important, and radiation will be of great value here. I am indeed very sorry that A. A. Buzzati-Traverso and R. C. von Borstel were not able to attend because the subject of dominant lethals and related topics is one which I feel has a great potential, and I know that very stimulating discussions would have taken place had they been here.

We did not take up certain very interesting items such as the possibility of increasing the resistance to cold of insects for release, thus rendering them better capable of competing with the insects to be controlled. Another aspect which deserves more attention, as P. B. Cornwell has just suggested, is the possibility of combining a number of techniques such as insecticide control to reduce the population to a low level and the sterile-male technique or genetic factors to eliminate the residue.

Finally, we have heard some extremely interesting reports by S. V. Andreev of work being done in the USSR.

I feel that one of the most successful features of the meeting has been the opportunity it has afforded specialists from all over the world to meet one another personally, and to assist one another's work by the direct exchange of ideas. I am convinced that this contact, now established, will bear even richer fruit in the future, and the International Atomic Energy Agency has rendered us all a great service in sponsoring the meeting.

In conclusion, as the last among the visitors to speak, I should like to express my own appreciation, and that of the other participants, of the excellent facilities, the hospitality and the co-operation which have been offered to us by everyone here in India.

A. R. GOPAL-AYENGAR (India): Mr. Chairman, ladies and gentlemen: I should just like to say how much I, as representing the host Government, have enjoyed participating in this Symposium and how effectively, I feel, the diverse aspects of radiation and radioisotopes in relation to entomology have been brought into relief by all the participants. From among those manifold aspects I will single out only one: I was very pleased to hear P. B. Cornwell stressing the need to take into account a wide variety of factors before we can make any attempt to solve the disinfestation problem or indeed many of the other entomological problems that are confronting us. I am reminded in this connection of the solution facetiously proposed by Sir William Bragg, many years ago now, for the problem of whether the nature of light should be considered in terms of waves or in terms of corpuscles; if I remember aright, he suggested that we should follow the wave theory on Mondays, Wednesdays and Fridays and the corpuscular theory on Tuesdays, Thursdays and Saturdays. Although meant as a joke, this was rather wise counsel I think, and one which we may follow to some extent by synthesizing and integrating various approaches to the problems of entomology.

On behalf of the Government of India and the Indian Atomic Energy Commission I should like to express our deep gratitude and thanks to the International Atomic Energy Agency and to the participants who came from far and near to make this Symposium such an outstanding success.

K. K. NAIR (India): On behalf of the Atomic Energy Establishment, Trombay, I should like to thank the International Atomic Energy Agency for having held the Symposium in Bombay, and, in particular, to express our gratitude to J. Halberstadt, our Scientific Secretary, for his untiring labours before and during the proceedings. To us in this country, the Symposium has given an excellent opportunity of coming into closer contact with eminent scientists from various parts of the world, and of discussing our problems with them. We are all agreed, I am sure, that the Symposium has done much to define and clarify the potentialities of radioisotopes and radiation in entomology.

The CHAIRMAN: Before closing the meeting I should like to thank the Government of India and the Indian Atomic Energy Commission for their kind invitation to hold the Symposium in India and for everything they have done for us. Our thanks are due in the first place to Dr. Bhabha as Chairman of the Commission; to A. R. Gopal-Ayengar, for the great interest he has shown in the meeting, and to Mr. Gulrajani to whom we owe the smooth organization which has been such a help to us. In this I include, of course, Mr. Gulrajani's entire staff, and those who volunteered their assistance in some capacity. We are grateful to the participants for the papers they have contributed and for their active participation in the discussions, and to all Chairmen of sessions for their work as discussion leaders. The discussions were, I think, extremely stimulating. I hope that in perhaps three or four years' time we may be able to meet again to discuss what has been achieved in the meantime, achieved perhaps through studies prompted by our exchanges here this week, and to talk over the fresh problems that will have arisen in the interval.

I thank you one and all, and I now declare the meeting closed.

SYMPOSIUM
ON RADIOISOTOPES AND RADIATION IN ENTOMOLOGY
HELD IN BOMBAY, 5—9 DECEMBER 1960

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Session 4	S. V. Andreev	All Union Research Institute for Plant Protection, Leningrad
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