Trace elements in human milk

by R.M. Parr*

Anyone who has had a baby recently, or who has followed newspaper articles about the feeding of babies, will be aware of the enormous importance now attached to breast-feeding. Medical authorities everywhere, supported by international organizations such as the World Health Organization (WHO), the United Nations Children's Fund (UNICEF), and the Food and Agriculture Organization (FAO), are making strenuous efforts to resist a growing tendency observed in many populations – young mothers abandoning breast-feeding in favour of other kinds of infant foodstuffs. These mothers are doing so not necessarily from choice but often because they are forced by the circumstances of their lives to leave their babies for long periods of time. Where they are rich enough – as in some industrialized countries – or when society is deliberately supportive – as in China – women are still able to breast-feed their babies successfully. In many developing countries however, poor women in mainly urban areas, in unsanitary conditions, with unclean water and insufficient money to buy enough breast-milk substitute, are failing to take full advantage of their potential to breast-feed their babies, or are weaning them too early, often with disastrous results.

A recent WHO/UNICEF publication expresses current thinking on this subject as follows: "Breast-feeding is an integral part of the reproductive process, the natural and ideal way of feeding the infant, and a unique biological and emotional basis for child development. This, together with its other important effects on the prevention of infections, on the health and well-being of the mother, on child spacing, on family health, on family and national economics, and on food production, makes it a key aspect of self-reliance, primary health care, and current development approaches. It is therefore a responsibility of society to promote breast-feeding and to protect pregnant and lactating mothers from any influence that could disrupt it."

That human milk is the *best* kind of food for a baby is, however, not quite as self-evident as it might seem. For example, it has been known for quite some time that milk is deficient in the minerals iron and copper, in the sense that it is not good to use it *indefinitely* as the sole source of nutrition of babies. Normal babies however, (but not premature ones) are born with adequate body-stores of these elements which can be drawn upon until dietary intakes increase after weaning. It is also not completely self-evident that *all* mothers should be capable of producing adequate milk. This may be true for a well-to-do mother who is healthy and consumes an adequate diet, but what about mothers from less privileged social groups?

It is a common observation that children in developing countries have a growth-rate similar to that of children in developed countries up to the age of about 3 to 4 months. Thereafter, their growth curve tends to flatten and diverges from that in developed countries. This observation has led many people to question the adequacy of breast-feeding in developing countries either because the volume of milk might be insufficient or because its nutrient composition might be inadequate. Indeed, it is an established fact that poor nutrition of the mother can in some circumstances lead to poor growth and development of the foetus, and to an insufficient quantity of milk.

In an attempt to provide some more definitive information on this question, the World Health Organization in 1976 decided to initiate a study dealing specifically with the volume and composition of breast-milk in a number of different countries. The constituents chosen for study included not only the major components such as protein, fat, and lactose, but also such minor and trace components as vitamins, pesticide residues and trace elements. It was in connection with the last of these, the trace elements, that the Agency became involved in the project. As will be explained later in this article, nuclear analytical techniques offer a very powerful means for determining the concentrations of minor and trace elements in biological materials such as human milk. Moreover, the Agency's laboratory has for many years been offering analytical quality-control services in exactly this field – the determination of trace elements – and was therefore in a very good position to arrange for analyses to be carried out by reliable and proven methodologies. However, before going into the details of this project and of the analytical techniques employed, it may be instructive first to make a digression to examine the role that trace elements appear to play in human nutrition.

Trace elements and human nutrition

The human body is composed of many elements; indeed as many as 81 of the 92 naturally occurring elements have been reported in some human tissues (Table 1). Most of the body, of course, is made up of the so-called major elements (e.g. oxygen, carbon, hydrogen) and minor elements (e.g. potassium, sodium, chlorine). But there are also many other elements that occur at only trace levels which, despite the low concentrations involved, are nevertheless of great importance

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Table 1. Total body content of some elements (ICRP reference man)

Element	Amount (g)	Per cent of total body weight
1. Oxygen	43 000	61
2. Carbon	16 000	23
3. Hydrogen	7 000	10
4. Nitrogen	1 800	2.6
5. Calcium	1 000	1.4
6. Phosphorus	780	1.1
7. Sulphur	140	0.20
8. Potassium	140	0.20
9. Sodium	100	0.14
10. Chlorine	95	0.12
11. Magnesium	19	0.027
12. Silicon	18	0.026
13. Iron	4.2	0.006
14. Fluorine	2.6	0.0037
15. Zinc	2.3	0.0033
16. Rubidium	0.32	0.00046
17. Strontium	0.32	0.00046
18. Bromine	0.20	0.00029
19. Lead	0.12	0.00017
20. Copper	0.072	0.00010
21. Aluminium	0.061	0.00009
22. Cadmium	0.050	0.00007
23. Boron	< 0.048	0.00007
24. Barium	0.022	0.00003
25. Tin	< 0.017	0.00002
26. Manganese	0.012	0.00002
27. Iodine	0.013	0.00002
28. Nickel	0.010	0.00001
29. Gold	< 0.010	0.00001
30. Molybdenum	< 0.0093	0.00001
31. Chromium	< 0.0018	0.000003
32. Caesium	0.0015	0.000002
33. Cobalt	0.0015	0.000002
34. Uranium	0.00009	0.0000001
35. Beryllium	0.000036	
36. Radium	3.1×10^{-11}	

for growth and development; indeed some of them are essential for life itself. The term *trace element* is usually applied to those elements which, at least in some tissues or body fluids, are found in concentrations below about 10 parts per million. In Table 1 all the elements below magnesium (i.e. beginning with silicon) are usually described as trace elements.

It has been known since the 17th Century that all human beings need iron if they are to survive, and iodine has also been recognized as an essential trace element since 1850. Most of our knowledge of trace elements, however, belongs to this century, particularly the last 20 years. Currently no fewer than 16 such elements are thought to be essential for humans (Table 2), though for some of them (e.g. arsenic, tin, and vanadium) the evidence is not direct but comes from animal experiments.

These elements play a variety of roles, serving in some cases as constituents of vital biological molecules (e.g. iron in haemoglobin, and iodine in thyroid hormones); alternatively they may form parts of enzymes or serve as cofactors for enzyme-mediated reactions. Despite the enormous advances in our knowledge of this subject in the past two decades, however, our understanding of many of them, particularly those whose essentiality has been established only rather recently, probably represents no more than the small tip of a very large iceberg.

As a practical matter iron deficiency anaemia, caused mainly by the consumption of food with a low availability of iron, is one of the world's most widespread nutritional deficiencies, affecting hundreds of millions of people. Iodine deficiency, leading to the development of goitre and cretinism, is also still a very widespread public health problem despite the fact that it can very easily and cheaply be prevented by iodine supplementation

Such deficiencies have been known for many years, but only recently has evidence started to emerge that other kinds of trace-element deficiency may also be widespread, in developed countries as well as in developing ones. Attention is being focused particularly at present on elements such as zinc, selenium, and chromium. Zinc deficiency is associated with growth depression, sexual immaturity, skin lesions, and depression of immunocompetence. Selenium plays an important role in animal nutrition; in humans it has been shown to be causally related to Keshan disease, an endemic heart disease affecting mainly young children in the Keshan province of China. Chromium appears to play a role in certain kinds of diabetes and may also be involved in the development of heart disease. Almost certainly, gross deficiencies of these trace elements hardly ever occur. However, the question remains as to whether marginal deficiencies might not be quite widespread, and whether these might not be having an important effect on various chronic diseases.

Not surprisingly, the health-food movement has enthusiastically embraced trace elements, and in several countries (particularly the USA) health-food shops are now promoting the sale of pills containing these elements. Accompanying literature lays claim to many beneficial effects, including improved resistance to infection, and protection from cancer and heart disease. Without doubt such claims are often exaggerated, though they are supported in part by persuasive, if not conclusive, scientific evidence.

Table 2. Classification of the essential trace elements

Element	Year of discovery	Function	Deficiency signs in humans	Occurrence of imbalances in humans
Iron	17th Century	Oxygen, electron transport	Anaemia	Deficiencies widespread; excesses dangerous in haemochromatosis; acute poisoning
Iodine	1850	Constituent of thyroid hormones	Goitre, depression of thyroid function, cretinism	Deficiencies widespread; excessive intakes may lead to thyrotoxicosis
Copper	1928	Oxidative enzymes; interaction with iron; cross-linking of elastin	Anaemia, changes of ossification; possibly elevated serum cholesterol	Deficiencies in the malnourished, and those receiving total intravenous feeding
Manganese	1931	Mucopolysaccharides metabolism, superoxide dismutase	Not known	Deficiency not known; toxicity by inhalation
Zinc	1934	Numerous enzymes involved in energy metabolism and in transcription and translation	Growth depression, sexual immaturity, skin lesions, depression of immunocompetence, change of taste acuity	Deficiencies in Iran, Egypt, in total intravenous feeding, genetic diseases, traumatic stress
Cobalt	1935	As part of vitamin B_{12}	Only as vitamin B_{12} deficiency	Inability to absorb vitamin B_{12}; low B_{12} intake from vegetarian diets
Molybdenum	1953	Xanthine, aldehyde, sulfide oxidases	Not known	Excessive exposure in parts of Soviet Union associated with goutlike syndrome
Selenium	1957	Glutathione peroxidase; interaction with heavy metals	Endemic cardiomyopathy (Keshan disease) conditioned by selenium deficiency	Deficiency and excess in areas of China; one case resulting from total intravenous feeding
Chromium	1959	Potentiation of insulin	Relative insulin resistance, impaired glucose tolerance, elevated serum lipids	Deficiency known in malnutrition, aging, total intravenous feeding
Tin	1970	Not known	Not known	Not known*
Vanadium	1971	Not known	Not known	Not known*
Fluorine	1971	Structure of teeth, possibly of bones; possibly growth effect	Increased incidence of caries; possibly risk factor for osteoporosis	Deficiency and excess known
Silicon	1972	Calcification; possibly function in connective tissue	Not known	Not known*
Nickel	1976	Interaction with iron absorption	Not known	Not known*
Arsenic	1977	Not known	Not known	Naturally occurring excesses known
Cadmium	1977	Not known	Not known	Not known*

* Naturally occurring imbalances are unknown, but excessive intake may arise in special circumstances, particularly through occupational exposure.

In many countries, supplementation of human diets with elements such as iron, iodine, and fluorine is already promoted by national health authorities and in at least one country (Sweden) indirect supplementation with selenium (via fertilizer applied to farmland) has recently been started. In some respects, farm animals are much better off than humans; their diets are much more frequently supplemented with trace elements! Before the reader rushes off to buy some trace-element pills, however, he or she should be forewarned that optimal health depends on the right balance of trace elements, and that too much of one can not only be toxic in itself but can also interfere with the metabolism of other elements. The best rule for avoiding trace-element deficiencies remains what has long been thought to be sound nutritional advice, namely that one should consume a well-varied diet, preferably with only a small proportion of highly refined food products such as sugar or white flour.

As far as infant foods are concerned, the main issue is whether formula products consumed by babies that are not breast-fed contain adequate levels of essential nutrients. As a World Health Organization expert committee put it in 1973: "Such formulations should contain all the essential trace elements ... at least in those levels that are present in human milk". But in order to implement this recommendation "it will be necessary to obtain additional information on the quantities of trace elements present in human milk." This, therefore, was the main aim of the research project described in this article. The project also had the supplementary aim of looking to see whether the concentrations of trace elements in human milk vary significantly with the socio-economic group or geographical origin of the mother.

Collecting the milk

Six collection centres were selected by the World Health Organization in countries representing several different degrees of industrial development, namely Guatemala, Hungary, Nigeria, Philippines, Sweden, and Zaire. The protocol for the study foresaw the desirability of collecting samples in each country from three different socio-economic groups: well-to-do (urban), poor (urban), and rural. (Some countries were not able fully to comply with this requirement.) Samples were collected from mothers in each study group by specially trained personnel who also kept records of relevant data on each mother and her child. For the study of some milk constituents (e.g. protein and fat), individual mothers were followed from the birth of their baby over the course of the lactation period so as to obtain information on the variations of nutrient intake of the baby as a function of its age. For the trace elements, however, this was not feasible for various reasons, and it was decided instead to study their concentrations in milk at just one particular time after the birth of the baby, namely at about three months. Information is available from other sources on the changes in composition of milk as a function of lactation time. At three months the milk is mature and its composition has reached fairly stable levels. Moreover, three months is a stage at which many mothers start to wean their babies. After this age, therefore, the baby's intakes of nutrients no longer depend exclusively on milk. For purposes of comparison, some commercially produced formula products for babies were also collected for analysis.

In parallel with this joint WHO/IAEA research project, analogous studies have also been supported by the Agency under its Research Contract programme. Comparable, and supportive, data have thereby been obtained from several other countries including particularly Chile, India, and Italy.

Analysis of trace elements

Responsibility for the analysis of trace elements was given to the Agency because of its long-term experience with and interest in this subject, particularly as regards the use of nuclear-based analytical techniques and analytical quality control. The elements selected for study included all the essential trace elements listed in Table 2, with the exception of silicon, together with some important toxic trace elements (antimony, lead, mercury and, if present at higher than natural levels, arsenic and cadmium). The minor elements calcium, chlorine, magnesium, phosphorus, potassium, and sodium were also included since they, like many of the trace elements, are also biologically essential and their analysis can be carried out by similar means; also there are important interactions between some of these elements that are potentially of interest. Altogether, therefore, 24 elements were included in this study.

Published values for most, if not all, of these elements can be found in the scientific literature and one might wonder, therefore, why it was necessary to carry out this study at all. The explanation is simply that some of the analyses are extremely difficult to perform properly, and it is only in recent years, and in very few laboratories, that reliable results have started to be obtainable. The scientific literature is therefore full of data that, even for just one element, seem to show differences covering several orders of magnitude. It is generally impossible to decide a priori whether these differences are real (i.e. representing biological or geographical variability) or whether they are simply the result of analytical error.

Abundant evidence that it is very often the latter, i.e. analytical error, is provided by the results of many intercomparisons conducted by the Agency's laboratory in recent years under its Analytical Quality Control Services programme. Figure 1, for example, shows results for the very important and widely studied trace element, copper, in a powdered cow's milk reference material prepared by the Agency. This is a homogeneous

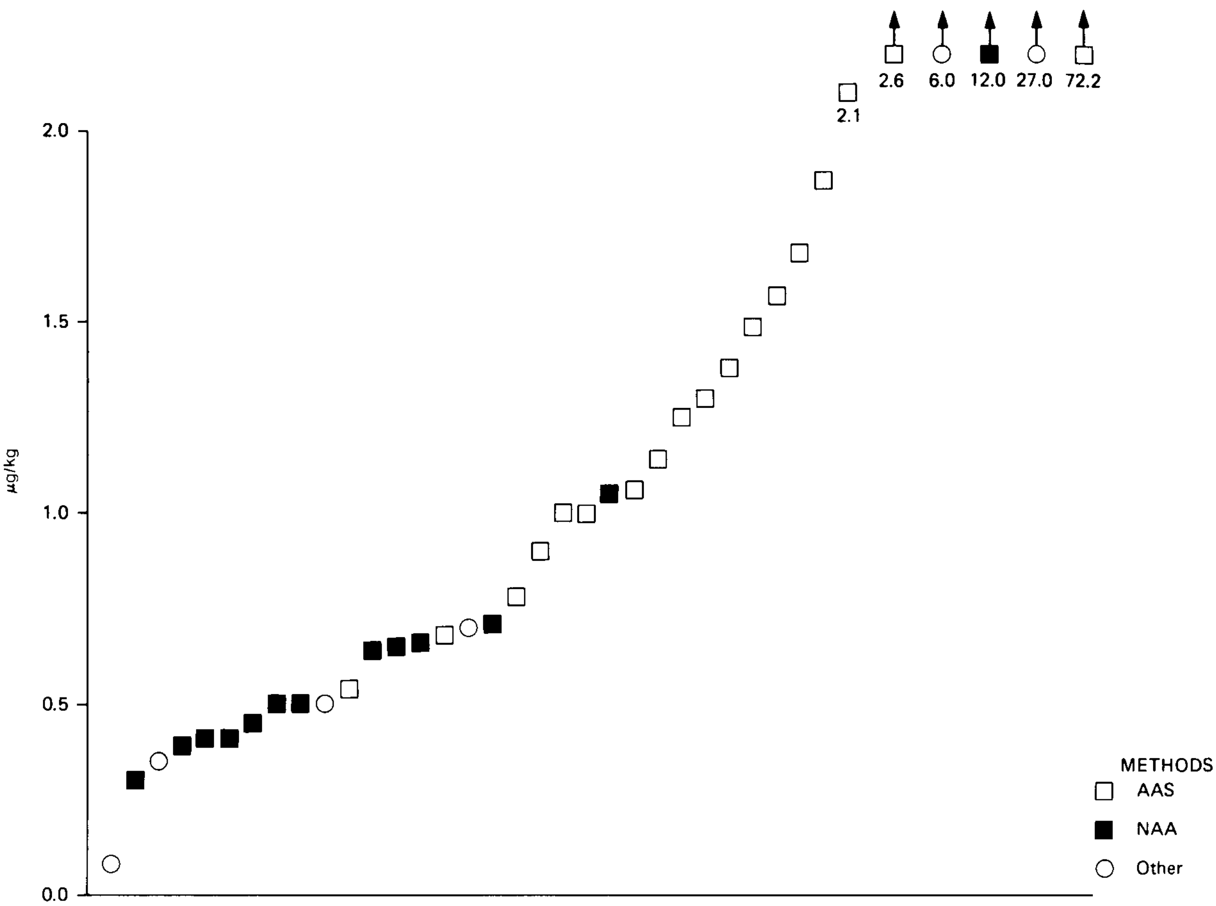


Figure 1. Results of an interlaboratory comparison for copper in a reference material made from cow's milk. Each point represents the mean value reported by a single laboratory. The results have been arranged from left to right in order of increasing value of the concentration. The main analytical methods used were atomic absorption spectrometry and neutron activation analysis.

powder, which obviously should have yielded the same result irrespective of who was analysing it and by what method. Nevertheless, the results reported cover a wide range (altogether a factor of 880 between the highest and the lowest) and on careful examination they reveal systematic differences depending on the analytical technique employed. The non-nuclear method of atomic absorption spectrometry yielded results that, on average, were approximately a factor of two higher than those obtained by the nuclear-based method, neutron activation analysis. Further work is still being done to demonstrate conclusively which of these values is correct, but it is already fairly clear that it is the lower one.

Scrupulous attention to analytical quality control is therefore a prerequisite for a study of this kind. To the knowledge of the author, this is the first time that such a study has been carried out for so many elements where quality control has been built into the programme from the very beginning. Only in this way is it possible to make meaningful comparisons between different countries and different socio-economic groups. Quality control has been achieved in this project firstly by making just one analytical laboratory responsible for each element (thus eliminating interlaboratory systematic errors), and secondly by the use of two specially prepared analytical quality control materials, one the cow's milk reference material referred to above, and the other a mixed pooled sample of human milk.

Nuclear analytical techniques, particularly neutron activation analysis (NAA), offer many advantages for a study of this kind. NAA is a method which depends on activating the sample (e.g. a specimen of human milk) by irradiating it with neutrons in a nuclear research reactor. Many of the atoms in the sample are thereby turned into radioactive isotopes which can either be measured directly using a suitable gamma-ray spectrometer (this version of the method is known as instrumental NAA), or they can be measured following a suitable radiochemical separation (radiochemical NAA). The amount of radioactivity thus recorded is a measure of the concentration of the original element in the sample. Particular advantages of the method include its high sensitivity and specificity (for many, though not all, of the elements of interest), and its relative freedom from contamination problems and matrix effects. Also it is a good multi-element method, which is an important

Table 3. Collaborating analytical laboratories, elements determined, and analytical methods

Analytical laboratory	Elements determined	Analytical method
IAEA, Vienna, Austria	Ca, Cr, K, Mg, Na	Atomic absorption spectrometry
	Cl	Instrumental NAA*
	Cd, Mo	Radiochemical NAA
Kernforschungsanlage (KFA), Jülich, FRG	Co, Fe, Hg, Sb, Se, Zn	Instrumental NAA
	Cu, Mn	Radiochemical NAA
Jožef Stefan Institute, Ljubljana, Yugoslavia	As, I, Sn, V	Radiochemical NAA
Institute of Science and Technology, Manchester, UK	Ni	Emission spectrometry (ICP-ES)
	Pb	Atomic absorption spectrometry
Helsinki University of Technology, Helsinki, Finland	F	Electrochemistry (ion-specific electrode)
Forschungsinstitut für Kinderernährung, Dortmund, FRG	P	Colorimetry

* NAA = neutron activation analysis.

consideration when one wishes to study as many as 24 elements in each sample.

NAA is not the optimal method for every element, however. For some elements (e.g. lead) it does not provide sufficient sensitivity, and for other elements (e.g. calcium) there are alternative methods available which are easier, quicker, and cheaper. For this reason the study as a whole involved the application of a variety of analytical methods, both nuclear and non-nuclear, only some of which are available within the Agency's own laboratory. The analyses have therefore been carried out by a collaborative effort involving several different laboratories (for details, see Table 3). In all this work, however, the Agency's laboratory has served as a co-ordinating centre by receiving the samples from the different study areas, homogenizing, drying, and aliquoting them, and sending the aliquots to the various analytical laboratories. The Agency's laboratory, in addition to doing many of the analyses itself, has also had overall responsibility for analytical quality control and for compiling and evaluating the results obtained.

Finally (or, more correctly, firstly), another important role of the laboratory has been to provide sample-collection kits for obtaining suitable human milk specimens for analysis. One of the important practical problems in this kind of research is that many of the elements of interest are present at such extremely low levels that the samples can easily be contaminated by the use of impure or inadequately-cleaned equipment. Specially prepared and cleaned collection vessels and specimen vials were therefore supplied to all the WHO collection centres (Fig.2). The sample-collection kits also included a special shampoo of low trace-element content for washing the breast prior to collection of the milk.

Are recommended dietary allowances correct?

Although most of the analyses required for this study have now been completed, the evaluation of the results, altogether about 8500 separate values, and preparation of the final report are still in progress. Enough evidence is already available, however, to show some very interesting differences between the different study areas and, in some cases, between different socio-economic groups within a single country. Results for manganese, for example, show very wide differences between the countries included in this study (Fig.3); the median concentrations for the Philippines and Sweden, respectively, differ by a factor of no less than 12. Many differences of this kind were observed (Table 4), with the Philippines rather consistently showing high levels of many of the elements of interest, probably associated with the relatively highly mineralized soils of this country. None of these high levels, however, appears to be of any particular concern because they do not exceed the concentrations presently considered to be consistent with good nutrition. Indeed it is somewhat more likely (but not yet proven) that some of the low levels observed in other countries may be of greater biological interest, as indicators of possible marginal trace-element deficiencies.

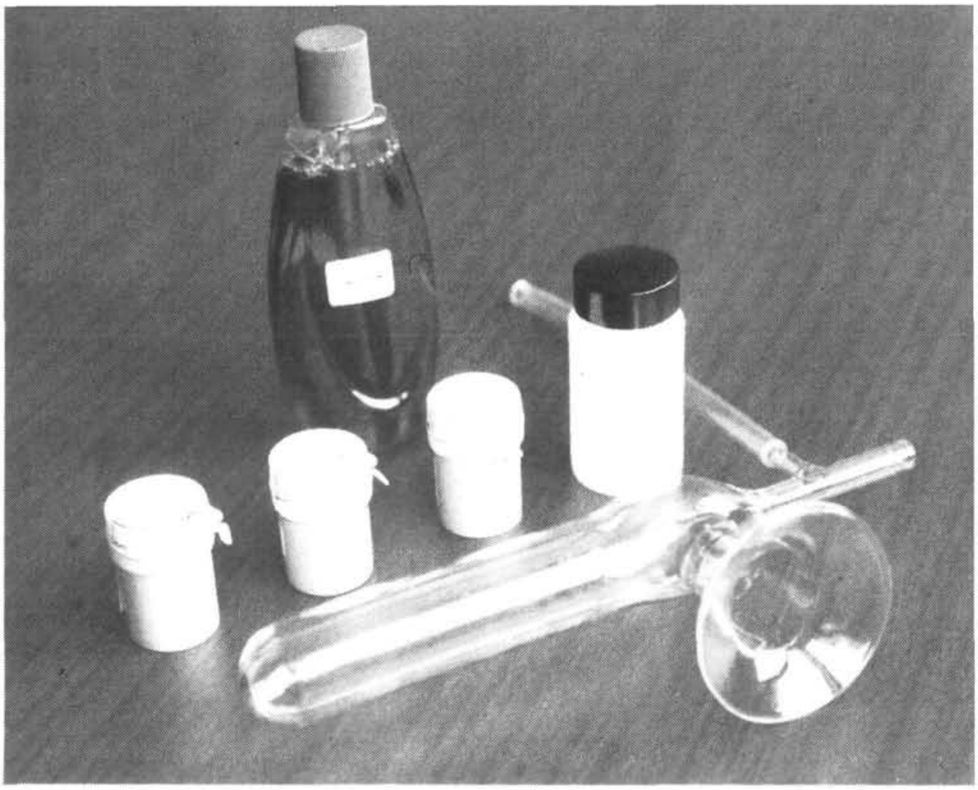


Figure 2. Collection kit for human milk samples comprising shampoo (of low trace-element content) for washing the breast, collection vessel (for connection to a suitable suction pump), and specimen vials. The collection vessel and specimen vials have to be precleaned to remove trace-element contamination.

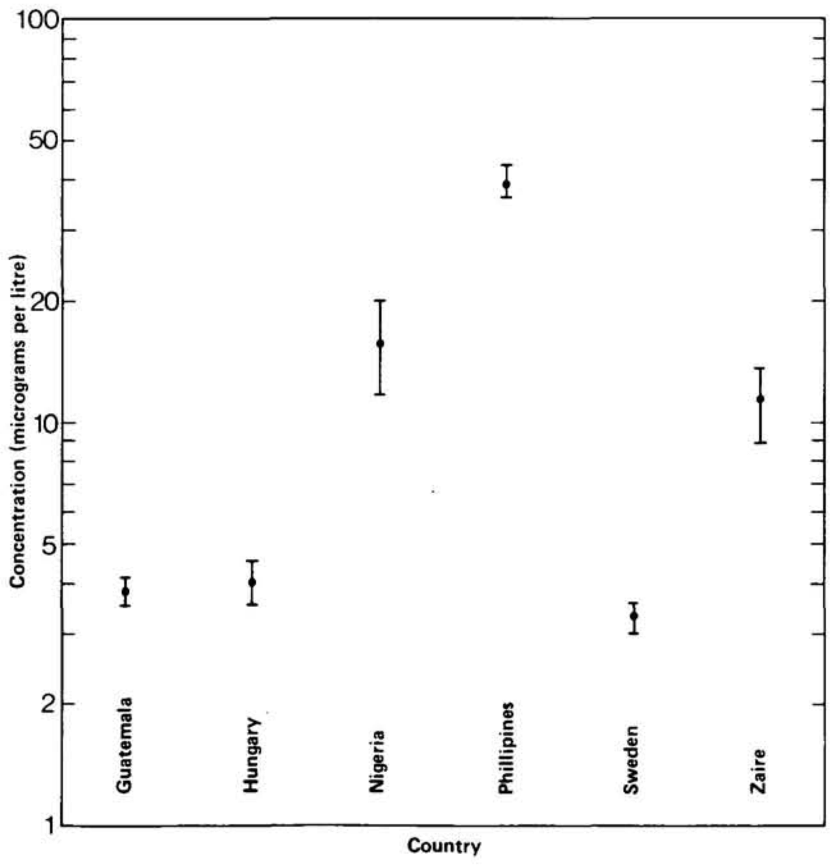


Figure 3. Manganese concentrations in human milk from different countries. The values plotted are the *median* concentrations (± 1 standard deviation of the median). Note that the concentration scale is logarithmic.

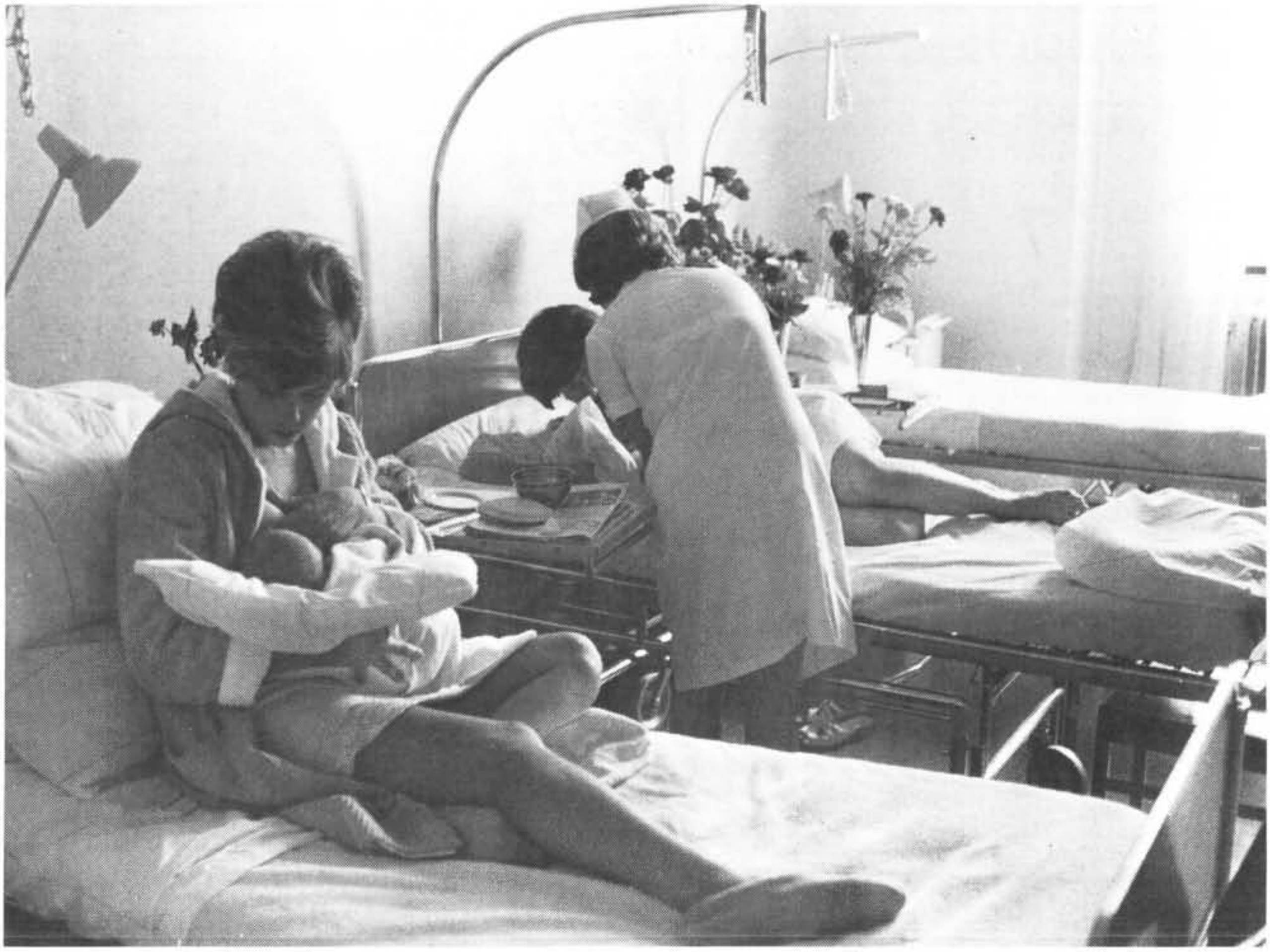
Table 4. Summary of geographical differences for the concentrations of selected elements in human milk.

Element	Guatemala	Hungary	Nigeria	Philippines	Sweden	Zaire
As				+		
Ca	+		−		−	
Cd			+	+		
Co		−	+	+		
Cr		−	+	+		
Cu	+	−	+	+	−	−
F	−			+		−
Fe	−	−		+		
Hg					+	
I						−
K		+			+	
Mg			−	−		+
Mn	−	−		+	−	
Mo				+		
Na				+		
Ni						−
Pb	−	+	−	+	+	−
Sb	−	−		+		
Se		−		+	−	
V				+		
Zn	+	−			−	
Net total	2−	6−	1+	13+	2−	4−

The values are compared to the means for all countries combined: + means relatively high; − means relatively low.

The main value of these data will probably be to throw new light on the nutritional requirements of young babies for trace elements. Recommended dietary allowances (RDAs) have been published recently by such bodies as the US National Academy of Sciences for 15 of the 24 elements included in this study, and are widely quoted and used in other countries. For some of the elements, however, there are astonishing differences between the actual intakes observed in this project and the presently used RDAs. For manganese, actual intakes (median values for the 6 different countries included in the project) varied between 2.5 and 25 μg/day whereas the RDA is 500 to 700 μg/day. Similarly, for iron, the actual intakes varied between 228 and 460 μg/day as compared with an RDA of 10 000 μg/day. Although the comparison of these figures is not altogether straightforward because of differences in bio-availability (e.g. an element such as iron is well absorbed from human milk but much less so from formula products) there nevertheless seem to be some significant differences between actual intakes and RDAs that need to be explained. This is a matter of no little importance since the commercial manufacturers of formula products for babies are starting to supplement their products with trace elements such as copper, iodine, iron, manganese, and zinc, at levels corresponding to the published RDAs. In this respect, therefore, formula products are beginning to have the appearance of being of better nutritional quality than mother's milk, which almost certainly cannot be true. It is expected, therefore, that this study will be useful in providing definitive new data for establishing the correct nutritional requirements of young babies for essential minor and trace elements.

Finally, we return to the question alluded to at the beginning of this article: why babies in some developing countries appear to thrive less well after the age of 3 to 4 month than babies in so-called developed countries. It now appears that the main reason for this is *not* a lack of milk or inadequate quality of milk, but rather inadequate supplementation and a heavy load of infectious diseases.



Mother's milk is generally accepted to be the best food for a baby. But do we know what the milk is made of? Nuclear techniques are helping supply at least part of the answer.

The arguments for encouraging breast-feeding are undoubtedly sound. Breast-milk is the most economic food for young babies and it, in addition, provides some immunological advantages. The advocates of prolonged breast-feeding seem, however, to underestimate the fact recognized by all mothers that, sooner rather than later, the maternal supply of breast-milk will cease to satisfy the growing demands of a healthy child and supplementation will be unavoidable. On average, such supplementation appears to be advisable from about 4 to 6 months of age onwards.

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