ESTIMATION OF THE GLOMERULAR FILTRATION RATE IN INFANTS AND CHILDREN USING IOHEXOL AND X-RAY FLUORESCENCE TECHNIQUE
Estimation of the glomerular filtration rate in infants and children using iohexol and X-ray fluorescence technique

by

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List of articles

This thesis is based on the following publications which will be referred to in the text by their Roman numerals:

**I** Stake G, Monn E, Rootwelt K, Grönberg T, Monclair T.
Glomerular filtration rate estimated by X-ray fluorescence technique in children: comparison between the plasma disappearance of [\(^{99}\text{Te}\)\(^{m}\)-DTPA and iohexol after urography.

**II** Stake G, Monn E, Rootwelt K, Golman K, Monclair T.
Influence of urography on renal function in children. A double blind study with metrizoate and iohexol.

**III** Stake G, Monclair T.

**IV** Stake G, Monn E, Rootwelt K, Monclair T.
A single plasma sample method for estimation of the glomerular filtration rate in infants and children using iohexol, II: establishment of the optimal plasma sampling time and a comparison with the [\(^{99}\text{Te}\)\(^{m}\)-DTPA method.

**V** Stake G, Monn E, Rootwelt K, Monclair T.
The clearance of iohexol as a measure of the glomerular filtration rate in children with chronic renal failure.
Introduction

Different methods may be used for determination of the glomerular filtration rate (GFR), which is considered to be the best single parameter for assessment of renal function [1].

In clinical practice the plasma concentration of creatinine \( (P_{cr}) \) is frequently used for estimation of the renal function in children. However, GFR may be reduced up to 50% before \( P_{cr} \)-values above normal are observed, as reported by Brøchner-Mortensen et al. [2] and illustrated in Figure 1 (own data).

Simple methods for estimation of GFR in children from a ratio between the body length and \( P_{cr} \) have been developed [3, 4], but the reliability of these methods has been questioned [5]. According to Schwartz et al. [6] more precise estimates may be obtained if the ratio also includes an age-dependent factor. Our results indicate, however, that the \( \text{age/body length}/P_{cr} \)-method is rather inaccurate (Figs. 2 a and b).

Mainly owing to the difficulties associated with quantitative, timed urine collection, estimation of the renal endogenous creatinine clearance is inaccurate [6, 7]. The method also overestimates GFR in patients with renal failure [8, 9]. Determination of the renal inulin clearance, "the golden standard" for GFR-measurements, is a laborious and time-consuming method necessitating continuous infusion of inulin over hours, timed urine collection, and multiple plasma samples, which is inconvenient for the child.

In later years GFR in children has therefore mostly been estimated following a single intravenous injection of a radioactive filtration marker such as contrast media (\( {\text{131I-diatrizoate, 131I-iothalamate}} \)) or metal chelates (\( {^{51}\text{Cr-EDTA or [99Tc]}^{m}}-\text{DTPA}} \)) [10-14]. The plasma elimination of the marker has been assessed either from plasma samples [15-18] or by external monitoring of the kidneys with a gamma camera [19].

![Graph with data points](image-url)

**FIG. 1.** The relation between plasma creatinine concentration and GFR in 205 infants and children. GFR may be reduced up to 50% before plasma creatinine concentration above normal is observed.
The original multiple plasma sample method, established by Sapirstein et al. [15], was later on simplified by Brochner-Mortensen [14], who also reduced the number of plasma samples necessary for the GFR-estimations. A further simplification is obtained when GFR is estimated from only one plasma sample, and several single sample methods were developed [17, 20 - 24]. In all of them, however, an empirical estimate for the distribution volume of the filtration marker must be used for the calculations, and the precision of the method depends on the size of the empirical volume used as well as the time interval between injection and plasma sampling [17].

\[
\frac{GFR_{\text{ref}} + GFR_{\text{Pcr formula}}}{2} \text{ (ml min}^{-1} \text{1.73 m}^{-2}\text{)}
\]

\[
\frac{GFR_{\text{ref}} + GFR_{\text{single sample}}}{2} \text{ (ml min}^{-1} \text{1.73 m}^{-2}\text{)}
\]

**FIG. 2.** The ratio between the reference GFR (GFR_{\text{ref}}) and GFR estimated by a) the formula of Schwartz et al. [6] using plasma creatinine concentration, age and body length of the patient (GFR_{\text{Pcr formula}}) and b) the single plasma sample iohexol method (GFR_{\text{single sample}}) against the average of the methods in 205 infants and children.

A rather inaccurate measure of GFR was obtained from the plasma creatinine method.
The development of methods for determination of iodine concentration in plasma samples, either with high-pressure liquid chromatography [25 - 27] or X-ray fluorescence technique [28, 29], has made it possible to use non-radioactive contrast media as filtration markers.

Although intravenous urography primarily is a method for morphologic study of the urinary tract, the X-ray films may also be used for evaluation of the functional state of the kidneys, especially if the relative kidney length [30] or parenchymal area [31] is determined. A requirement for performing such evaluations is high quality X-ray films, obtainable in quiet, well prepared children. Hydronephrotic, scarred, or contracted kidneys are often difficult to outline, and it is especially in these cases that more precise GFR estimates are needed. Some objections have also been raised against the accuracy of the renal length measurements [32].

Intravenous urography [33] as well as CT with organ enhancement will remain important diagnostic modalities for the years to come. By utilizing the contrast medium already injected for X-ray examination also for determination of GFR, a combined morphological and functional evaluation may be obtained, and an extra radionuclide study may be avoided.

Our interest in the X-ray fluorescence method was aroused because it might be combined with X-ray examinations and was technically simple. With the equipment at hand, GFR could be determined either from multiple plasma samples [29, 34 - 37] or from a single plasma sample [38, 39]. The latter method certainly seemed favourable for paediatric use. However, the single plasma sample method was primarily developed for adults, and a part of the present study was therefore carried out in order to adapt it for infants and children.
Aims of the present study

Before the contrast medium method could be introduced for measuring GFR in infants and children we wanted to:

1) decide if GFR was affected by the relatively high dose of the non-ionic iohexol routinely injected for X-ray diagnosis in children, and if valid GFR estimates could be calculated from the plasma disappearance of a contrast medium as previously shown in adults [29, 34 - 36] [Article I],

2) examine if sensitive renal function parameters: GFR as well as renal excretion of albumin, β₂-microglobulin, and alkaline phosphatase, were affected differently by iohexol and the ionic medium metrizoate [Article II],

3) establish a weight-related empirical formula for the distribution volume of iohexol in infants and children, adapt a single plasma sample method for determination of GFR [17] using iohexol, and evaluate the day-to-day variations of iohexol GFR in this age group [Article III],

4) test the precision of the GFR estimate - obtained by the 3-h single plasma sample iohexol method - in infants and children who were not included in the group of patients forming the basis for the method, and evaluate the optimal time interval between the injection of iohexol and the plasma sampling (= optimal sampling time) [Article IV], and

5) establish the optimal sampling time for estimation of the plasma elimination of iohexol in uraemic children and evaluate the usefulness of the single plasma sample method in these patients [Article V].
Summary of articles I - V

This study includes a total of 223 individuals, 108 girls and 115 boys, aged 2 days to 14 years. Their plasma creatinine concentration ranged from 15 μmol L⁻¹ to 615 μmol L⁻¹, and GFR from 4 ml min⁻¹ 1.73 m⁻² to 141 ml min⁻¹ 1.73 m⁻². The investigations are presented in 5 articles (I - V) and are summarized as follows:

I The total plasma disappearance of iohexol was determined by X-ray fluorescence technique following intravenous urography in 10 children aged between 2 and 13 years. For comparison the plasma disappearance of [⁹⁹Tc⁸⁶]-DTPA was estimated both 2 days before and simultaneously with the iohexol study. High correlations between the three sets of data were found, and no change in the GFR was detected following injection of the contrast medium. It was also found that reliable estimates of the GFR could be obtained from two plasma samples of 1 ml each, taken 3 and 4 h after the injection of the contrast medium.

II Thirty-two children aged between 1 and 14 years were given either metrizoate or iohexol for intravenous urography in a double blind study. Mild to moderate adverse events were observed in all patients receiving metrizoate (15/15) and in 4 receiving iohexol (4/17). Alkaline phosphatase concentration in urine was significantly increased 4 h after the injection of both media, but had returned to pre-injection levels 16 h later. The excretion of β₂-microglobulin and albumin was not altered. In 9 children of the metrizoate group and 11 of the iohexol group GFR was determined before urography by the single injection [⁹⁹Tc⁸⁶]-DTPA-technique and 3 to 4 h after urography by measuring the plasma disappearance of the contrast medium with X-ray fluorescence technique. No significant reduction of GFR was observed.

III This study was performed in order to develop a method for estimation of the GFR from a single plasma sample based upon the plasma disappearance rate of iohexol. The apparent distribution volume for iohexol was measured in 100 infants and children and used for establishment of a weight-related empirical formula for the distribution volume. Using the distribution volume obtained by this formula, a preliminary GFR was calculated from the iodine concentration measured in a plasma sample taken 3 h after injection of iohexol. When this estimate was corrected by another empirically established second degree correction factor, a high degree of agreement was found between a GFR reference method and the 3-h single plasma sample method. In another group of 13 children the 3-h single plasma sample GFR was estimated twice with a 2-days interval, and the day-to-day variations were found to be similar to those obtained with other standard methods.

IV In this study the validity of the empirical formulae established in Article III was confirmed in examinations in 143 patients. The results of the 3-h single plasma sample method were similar to those of a standard [⁹⁹Tc⁸⁶]-DTPA-method, and also to those of a two plasma sample iohexol method. Evaluation of the results obtained with plasma sampling 1, 2, 3 and 4 h after the injection of the contrast medium showed that the optimal sampling time for GFR-levels down to 20 ml min⁻¹ 1.73 m⁻² was about 3 h after the injection.

V The plasma clearances of [⁹⁹Tc⁸⁶]-DTPA and iohexol were estimated in 11 children with chronic renal failure for determination of the GFR. Equal values were obtained with the two substances provided plasma sampling was simultaneous, but when plasma was sampled within 3.5 h after injection of iohexol
and $^{99m}$Tc-DTPA the GFR was overestimated by more than 50%. For clearance values below $20 \text{ ml min}^{-1} \text{ m}^{-2}$, valid GFR estimates were obtained both from the two sample method with plasma samples taken 3 and 24 h after the injection of ioxixanol, and from a single plasma sample taken 24 h after the injection.
Clinical research in infants and children is associated with some practical problems, and attention is given to those related to the present study.

**Indwelling cannula**
Vein puncture in children is often difficult. We therefore decided to use one indwelling cannula for injection of \[^{99}\text{Tc}\text{m}]-\text{DTPA} and contrast medium, as well as for blood sampling (in the 14 youngest infants undergoing cardioangiography blood was sampled through a small endhole catheter in the inferior caval vein [III]). Special precautions were taken to minimize the risk of contamination with the marker substance by connecting the cannula with a three-way stopcock and using one opening for injection and the other for blood sampling, combined with repeated flushing of the device [40]. The risk of contamination is, however, completely eliminated when the contrast medium is injected in a peripheral vein through a small "butterfly" needle, and the plasma sample is taken by another, separate puncture. The latter method is now our routine procedure, and the sampling is preferably combined with blood testing for other purposes.

**Food and fluid intake before, during, and after the examination**
The children were encouraged to drink before the single dose injection of \[^{99}\text{Tc}\text{m}]-\text{DTPA}, but not during the sampling period, which in some of the patients took 4 - 5 h. Before intravenous urography, the children were fasted for 3 h, and they were allowed to eat and drink freely immediately after termination of the X-ray examination. Therefore, in those cases where GFR was determined by radionuclide and contrast medium simultaneously, somewhat different procedures were followed depending on what examination was the most important: the radionuclide study or the intravenous urography. We did not, however, observe any effect on the GFR estimates by the different hydration procedures used. No significant difference was found between

1) the GFRs determined by \[^{99}\text{Tc}\text{m}]-\text{DTPA} in 10 hydrated children on day 1 (radionuclide study) and those on day 3, when the same children had fasted for 3 h (intravenous urography combined with simultaneous injection of \[^{99}\text{Tc}\text{m}]-\text{DTPA} [I], and

2) the GFRs determined by iohexol in 13 hydrated children on day 1 (radionuclide study combined with simultaneous injection of a low dose of iohexol) and those on day 3 after 3-h fast (for intravenous urography) [III].

**Restriction of movements during the plasma sampling period**
The children were confined to bed during the radionuclide studies, as recommended by Brøchner-Mortensen [14] and Groth [18]. Initially we tried to keep them recumbent during the sampling period after the intravenous urography, but realized that after returning to the ward the children were either moving around in their beds or complaining of being kept away from the playrooms. Our planned restrictions were therefore soon abolished, an attitude which was supported by Tauxe who questioned the need for strict bed rest [21], and especially by Brauner and Westling who concluded that bed rest (in adults) was unnecessary [41].

**Contrast medium**
All contrast media are potentially nephrotoxic, but the renal affection is most frequent in adult patients, in some more or less well-defined high risk groups [42 - 45]. We have been unable to find any reports of GFR-depression caused by non-ionic contrast media in infants and children, and we did not detect any depressive effect on the GFR in the 30 children examined, neither with metrizoate nor iohexol [I and II].

The non-ionic contrast medium iohexol (Omnipaque®, Nycomed AS, Oslo, Norway) has been used for all routine intravascular con-
contrast injections in infants and children at our institution since 1983 [46]. At the start of our studies in 1985, the discussion about which contrast medium to prefer - ionic or non-ionic - was going on, and the ionic medium metrizoate (Isopaque® Nycomed AS, Oslo, Norway) was still extensively used. In spite of a large clinical experience surprisingly little information was available about the influence of contrast media on renal function in infants and children. The double blind study reported in Article II, comparing iohexol and metrizoate, was initially planned to be a more extensive one. It was, however, terminated after 32 examinations, mostly for ethical reasons, since the subjective adverse effects were rather pronounced in a high number of children. Not surprising [46], the adverse effects were almost entirely caused by metrizoate which was given to 15 children [46]. All other investigations in this study were performed with iohexol, and only GFRs determined with iohexol were used for establishment of the Formulae 3 and 5, as well as for testing of the validity of the single plasma sample method [IV and V].

The X-ray fluorescence analyser
The equipment used measured the iodine concentration in plasma by X-ray fluorescence technique [29]. It had a built-in computer which was programmed with Formulae 1 and 4, and the next model will also include Formulae 3 and 5 (see calculations). The data that have to be fed into the computer are age, body length and weight, amount of contrast medium, and time for injection and plasma sampling. The analyser can either operate on a single plasma sample basis, or with multiple plasma samples up to a total number of 8.

The accuracy of the measured iodine concentrations depends on several factors, among which the sample volume, counting time, and iodine concentration are important [35, 36]. Most of the patients in the present study were examined with the ELX 84 equipment (Elementanalys AB, Malmö, Sweden). It was adapted for samples of 1 ml, while the later model, Renalyzer PRX 90 (Provalid AB, Lund, Sweden), plasma samples of 2 ml had to be used. All plasma samples were counted for 5 min. The ELX 84 equipment had a detection limit of 0.033 mg I ml⁻¹, and only patients with an iodine concentration in the last plasma sample of more than 3 times the detection limit (i.e. 0.1 mg ml⁻¹) were included in the study. With the Renalyzer PRX 90, which has a detection limit of 0.014 mg I ml⁻¹, however, even plasma samples with an iodine concentration of 0.05 mg ml⁻¹ may be used for the calculations. In the total material the highest iodine concentration in the 3-h plasma sample was 4.051 mg ml⁻¹ and the lowest 4-h sample value was 0.077 mg ml⁻¹.

Noteworthy is also that the X-ray fluorescence method is inexpensive. With the equipment at hand and the extra cost is for the calibration fluid, which must be changed every second week, and the plastic tubes needed for the plasma.

Urine sampling
Timed, quantitative collection of urine without indwelling catheters is difficult. Even with adequate collection, interpretation of the clearance estimates may be complicated in patients with dilatation of their urinary tracts. Both the size of this urinary “dead space” and its influence on the GFR-determinations are difficult to assess [47]. This was the main reason why renal clearance estimations were not routinely included in the present study.
Calculations

Immediately after intravascular injection a marker substance leaves plasma both by glomerular filtration and by dispersion into the extravascular body fluid compartments. The amount of marker substance excreted during this early phase, the alpha-phase, is "lost" from the body fluids and consequently a somewhat lesser dose than injected is distributed in the body fluid compartments. Gradually the concentration in the plasma and the extravascular fluid equalizes, and the plasma elimination rate becomes constant (beta-phase). However, because of glomerular filtration the concentration in the extravascular fluid from then on remains somewhat higher than the plasma concentration [12]. The time needed for obtaining this "steady state" depends on GFR; the lower the GFR, the longer the time needed.

For determination of the plasma clearance two methods have been used; either a one- or a two-compartment model. For estimation of the plasma clearance of $^{99m}$Tc-DTPA the latter method, as described by Sapirstein et al. [15], was used. Two plasma samples were taken immediately after injection, during the alpha-phase (at 5 and 15 min), and 4 during the beta-phase (for the standard $^{99m}$Tc-DTPA-method at 120, 150, 180 and 210 min after the injection). For all patients a one-compartment model was used for estimation of the contrast medium GFR, with plasma samples taken during the beta-phase only [14].

In 18 children (10 included in Article I and 8 in Article V) a sufficient number of plasma samples were taken to permit the use of two-compartment model mathematics also for the iohexol data. The results were essentially the same, as illustrated in Figure 3.

![Figure 3](image-url)

**FIG. 3.** Relationship between iohexol GFR estimated by a two-compartment model (Sapirstein et al. [15]) and a one-compartment model (Brøchner-Mortensen [14]) against the average of the methods, in the 10 children described in Article I (one girl was examined twice), and 8 of the children described in Article V. Similar GFR-values were obtained with both methods, and for ethical and practical reasons the one-compartment model was used for estimation of the reference GFR.
When a one-compartment model is used, the multiple plasma sample clearance is estimated from the formula: 

\[ \text{GFR} = \frac{Q(0)}{C(0)} \times B \]  

(Fig. 4). However, since this clearance method does not include plasma samples taken in the alpha-phase, it will overestimate GFR. The multiple sample GFR of the contrast medium was therefore corrected by a reduction factor, as described by Brøchner-Mortensen [14]:

\[ \frac{Q_m}{C(0)} \times 0.99078 - \frac{Q_m}{C(0)} \times 0.001218 \]

Formula 1

The apparent distribution volume \( DV(a) \) (in ml) was estimated in 100 infants and children [III] according to the standard formula [48]:

\[ DV(a) = Q(0) : C(0) \]  

Formula 2

The linear regression calculated for the relationship between the body weight and the \( DV(a) \) [III] was used as the new empirical formula for calculation of the empirical distribution volume \( V \) (in ml):

\[ V = 231 \times \text{kg body weight} + 1215 \]  

Formula 3

A preliminary single plasma sample GFR \( (\text{GFR}_{ss}) \) was calculated according to Jacobsson's formula [17] and given in ml min\(^{-1}\) 1.73 m\(^2\):

\[ \text{GFR}_{ss} = \frac{1}{V + 0.0016} \times \ln \left( \frac{Q(0)}{V \times C(t)} \right) \]  

Formula 4

The relationship between the multiple plasma sample GFR \( (\text{GFR}_{ref}) \) and the preliminary 3-h single plasma sample GFR \( (\text{GFR}_{ss}) \) was curvilinear [III], and in order to obtain a final 3-h single sample GFR \( (\text{GFR}_{ss3h}) \) similar to the \( \text{GFR}_{ref} \), the preliminary 3-h single sample GFR \( (\text{GFR}_{ss}) \) had to be corrected by the factor:

\[ \text{GFR} = 180 - 14.1 \sqrt[3]{33 - \text{GFR}_{ss}^3} \]  

Formula 5

This factor was obtained by solving the equation for the relationship (second degree regression) between \( \text{GFR}_{ss} \) and \( \text{GFR}_{ref} \) [III].

The body surface area was estimated according to Haycock et al. [49].

\[ \text{FIG. 4. Schematic drawing showing the elimination curve for the one-compartment model used for estimation of both the plasma clearance of iohexol (GFR), and the apparent distribution volume for iohexol (DV(a)). The plasma elimination curve was determined by the iodine concentration measured in the plasma samples taken in the beta-phase and back-extrapolated to time zero (= time of iohexol injection).} \]

* \( Q(0) \) = injected amount of iodine in mg,  
\( C(0) \) = plasma iod\(^{+}-\) concentration in mg ml\(^{-1}\) at time zero (intercept of back-extrapolated monoexponential line with the ordinate on a semilogarithmic plot)  
\( B \) = slope of the monoexponential line described above (= plasma elimination curve)  
\( t \) = the time in minutes between injection of iohexol and blood sampling  
\( C(t) \) = plasma iodine concentration in mg ml\(^{-1}\) at time \( t \)  
\( DV(a) \) = apparent distribution volume (estimated from the plasma elimination curve) in ml  
\( V \) = empirical distribution volume (calculated from formula 3) in ml
Renal clearance studies

The initial version of Article V also contained renal clearance studies in 3 of the uraemic boys (patients 10 - 12). The clearances of inulin, endogenous creatinine, and iohexol were estimated with standard techniques. The plasma concentrations used were the arithmetic means for the sampling periods, and the renal iohexol clearance was corrected for urinary dead space according to Nosslin [47]. These investigations were mainly done in order to compare the results of the new method with the "golden standard" for GFR-determinations, the inulin clearance. The renal clearance of iohexol was similar to that of inulin, 10.1±0.9 and 10.5±1.0 ml min⁻¹ 1.73 m², respectively, with a difference of 0.4±1.4 ml min⁻¹ 1.73 m². The corresponding plasma clearance of iohexol was also similar, 10.1±1.2 ml min⁻¹ 1.73 m². The difference of 0±0.4 ml min⁻¹ 1.73 m² between the plasma - and renal clearances of iohexol indicates that there is no consistent extrarenal elimination of iohexol at low GFR in children, as has recently been suggested for adults [50].

In agreement with other reports, we also found that the renal creatinine clearance (16.2±2.1 ml min⁻¹ 1.73 m²) was about 50% higher than the inulin and iohexol clearances, a difference which is attributed to a relative increase in the tubular secretion of creatinine in patients with renal failure [8, 9]. Admittedly the number of children examined is small (3 patients), a fact which is mainly due to the difficulty of recruiting children for such investigations. Nevertheless, since each child served as its own control we considered that this part of the study still provided additional information.

General discussion

For ethical reasons the studies were to be conducted with the least possible discomfort to the patient. The X-ray and the radionuclide studies were the most important and had to be done according to our routine procedures. Therefore, a two-compartment model [51] had to be used for the [⁹⁹Tc⁶⁺]-DTPA-clearance, while the single plasma sample method implied the use of modified one-compartment model mathematics. Investigations necessitating extra injections of marker substances ([⁹⁹Tc⁶⁺]-DTPA or contrast medium) should be limited. As few plasma samples as possible should be taken, and preferably, used for determination of both radioactivity and iodine concentration. The GFR estimation with the X-ray fluorescence method should be supplementary.

Optimal time between injection of iohexol and plasma sampling

Both for the single and for the two sample methods the plasma must be sampled during the beta-phase, and the optimal time interval between injection and plasma sampling is influenced by GFR; the higher the GFR, the shorter the sampling time may be. In order to evaluate the optimal plasma sampling time for the single sample method [17], GFR was determined from samples taken at different times after the injection. When a 2-h single sample GFR was calculated from Formula 4 [subgroup 1 a, IV] and with the patient's apparent distribution volume (DVₐ), most of the points deviated from the average for all GFR-levels, while the 3-h and 4-h estimates were almost identical with the reference
FIG. 5. The ratio between the reference GFR (GFR$_{ref}$) and the uncorrected single plasma sample GFR calculated with the patient's apparent distribution volume (DV$_{(a)}$) against the average of the methods, a) from the 2-h and 3-h samples in 79 children (subgroup 1a, Article IV), and b) from the 3-h and the 5-h samples in 11 children presented in Article V (one boy was examined twice). The dashed line indicates equality. These figures emphasize the importance of the optimal sampling time. Even when the single sample GFR was calculated using the patient's DV$_{(a)}$, the time interval between the injection of iohexol and the plasma sampling should be longer than 2 h for all GFR-levels, and longest in patients with low GFR.

(Fig. 5a). In the 9 children with very low GFR [from Article V] the plasma elimination curves for iohexol indicated that steady state between plasma and extravascular fluid occurred after about 5 h. The points representing the GFR estimates calculated with the patient's actually measured apparent distribution volume (DV$_{(a)}$) and the single plasma sample taken after 3 h were scattered. Much better agreement with the reference GFR was obtained from the single samples taken about 5 h (Fig. 5b) and 8 h after the injection, respectively. Nearly identical estimates were obtained from the 24-h sample. These observations show that for estimation of the final single sample GFR, a 2-h interval between injection and plasma sampling is too short, even in patients with normal GFR. In uraemic children the sampling time should be longer than 5 h, while 3 h is considered the optimal time interval in all the other patients.
GFR-estimation with multiple plasma sample methods

The main purpose of the present study was to establish a single plasma sample method for estimation of GFR in infants and children using iohexol as marker substance. As pointed out by Jacobsson, a close relationship exists between the three factors: GFR, distribution volume, and time interval between injection of marker substance and plasma sampling [17].

However, the empirical formula for the distribution volume programmed in the early model of the X-ray fluorescence equipment was developed for adults [38], and it soon became apparent that it could not be used in infants and young children. A new formula for the empirical distribution volume therefore had to be established.

A prerequisite for development of the single plasma sample method was a valid reference GFR for each patient. Having established that the multiple sample iohexol clearance, estimated according to both one- and two-compartment models (Fig. 3), were similar to the \( ^{99}\text{Tc} \text{m}-\text{DTPA} \) clearance, the simpler, two plasma sample, one-compartment, iohexol method was chosen as the reference GFR both for practical and ethical reasons [1].

Apparent distribution volume

The apparent distribution volume \( (\text{DV}_{(a)}) \) estimated in 100 infants and children [III], was based on the iodine concentrations measured in the 2 plasma samples taken about 3 and 4 h after the injection of iohexol and calculated according to Formula 2. At least 3 factors may influence the size of the \( \text{DV}_{(a)} \): body weight, sex, and GFR.

Body weight: The average size of the \( \text{DV}_{(a)} \) for iohexol agreed well with the previously reported size of the extracellular fluid volume in children [48, 52]. It decreased from about 50% in neonates to about 25% in the older children.

Sex: While the extracellular body fluid compartments are different in adult men and women, it is generally accepted that before puberty no such difference exists [48, 52]. We could not find any sex difference in our material [III] and both girls \( (n=45) \) and boys \( (n=55) \) were therefore included when Formula 3 was established.

GFR-dependency: Mainly 2 factors influence the size of the \( \text{DV}_{(a)} \): 1) The "loss" of marker substance through the urine immediately after the injection (non-immediate mixing) results in a lesser dose than injected \( (\text{Q}_{(0)} \) being distributed in the body fluid compartments, and 2) the plasma, which constitutes only about 10 to 15% of the total extracellular body fluid volume, is continuously filtered by the kidneys, and a concentration gradient between the extra vascular fluid and plasma is maintained (non-uniform distribution [12, 17]). Accordingly, the higher the GFR, the larger is the loss of marker substance as well as the concentration gradient. The largest apparent distribution volume \( \text{DV}_{(a)} = \text{Q}_{(0)} \cdot \text{C}_{(0)} \) is therefore obtained in patients with normal renal function. This might also have been shown in Figure 2, Article III, if each patient's GFR had been included in the plot: the points in the lower part of the diagram represent children with reduced GFR, and the upper points those with normal renal function.

Empirical distribution volume

A basic difference exists between the multiple and the single plasma sample methods for GFR-measurement. Estimation from multiple samples includes the patient's apparent distribution volume, and the formula GFR = \( (\text{Q}_{(0)} \cdot \text{C}_{(0)}) \times \beta \) can be written GFR = \( \text{DV}_{(a)} \times \beta \) (Fig. 4). The single sample method is, on the other hand, based on an empirical estimate for the distribution volume (Formula 3). The relation between these 2 volumes is visualized in Figure 2, Article III, which also shows that \( \text{DV}_{(a)} \) estimated in patients with similar body weights varied up to about 50%.

The empirical formula (Formula 3) which was established from the relationship between \( \text{DV}_{(a)} \) and the body weight, thus compensates for the body weight, but not for different GFR levels. The fact that it was established from 100 patients with an average GFR of 78 ml min\(^{-1}\) 1.73 m\(^2\) is also indicated in Figure 4 a. Article III, where best agreement was obtained between the preliminary single sample GFR and the reference GFR for values about 50 to 100 ml min\(^{-1}\) 1.73 m\(^2\). For this GFR
range the single plasma sample method is less dependent on the size of the distribution volume than at low and high GFR values (Fig. 4 b, Article III).

*Single sample method, with GFR above 20 ml min\(^{-1}\) 1.73 m\(^2\)*

What is said above might give the impression that both the size of the empirical volume and the sampling time must be chosen according to the GFR-level. However, when the 3-h and 4-h preliminary single plasma sample GFRs were calculated with Jacobsson’s formula [17] and compared with the reference GFR, a curvilinear relationship was obtained [III]. The scatter of the points was more pronounced for the 4-h sample especially at GFR above 70 ml min\(^{-1}\) 1.73 m\(^2\), and hence 3 h was the optimal time for blood sampling in this group. When the correction factor is included (Formula 5) a final GFR value (GFR\(_{\text{ss}3\text{h}}\)) will be calculated directly by the analyser [III].

The results given in Article IV, revealed that the 2-h and 4-h final single plasma sample GFRs might be used. However, the single sample GFRs calculated with DV\(_{\text{a}}\) indicated that a sampling time of 2 h was too short, and since the correction factor was developed from the preliminary 3-h single sample GFR, we recommend that the sample is taken *about 3 h* after the injection.

*Single plasma sample in children with chronic renal failure*

As indicated above, and as shown in Article V, the optimal sampling time in uraemic children is considerably later than 3 h. For GFR below 20 ml min\(^{-1}\) 1.73 m\(^2\) a 24-h time interval between injection and plasma sampling sufficed for most of the patients, but for GFR below 5 ml min\(^{-1}\) 1.73 m\(^2\) even a later sampling time would be preferable [17, 24, 53]. It must be emphasized that the correction factor (Formula 5) established for the 3-h sample should not be used in these patients.
Conclusions

1) Urography with iohexol in children had no significant influence on the GFR. Valid GFR estimates were calculated from the plasma disappearance rate obtained from 2 plasma samples taken 3 and 4 h after the injection of iohexol [Article I].

2) Both iohexol and metrizoate caused a transitory, increased renal excretion of alkaline phosphatase. GFR as well as the excretion of albumin and β₂-microglobulin were unchanged [Article II].

3) Using the weight-related empirical distribution volume for determination of GFR from the plasma sample taken 3 h after the injection of iohexol, a high degree of agreement was found between the preliminary single sample GFR estimate and the reference, two plasma sample GFR. However, the relationship was curvilinear, and in order to obtain a value for the final 3-h single sample GFR equal to the reference GFR, the preliminary value had to be corrected by a second degree correction factor. The day-to-day variations of GFRs estimated with the iohexol methods were similar to those obtained with other standard methods [Article III].

4) In another group of infants and children, independent, but otherwise comparable to the patients who formed the basis for the single sample iohexol method, it was confirmed that valid GFR estimates were obtained from the 3-h single plasma sample. GFR determinations from 1-h, 2-h, and 4-h single samples further supported that the optimal sampling time in patients with GFR down to 20 ml min⁻¹ 1.73 m² was 3 h [Article IV].

5) In children with GFR below 20 ml min⁻¹ 1.73 m², valid GFR estimates were obtained by the two sample method with samples taken 3 h and 24 h after the injection of iohexol, as well as from the single plasma sample taken 24 h after the injection [Article V].
References


Article I
Glomerular filtration rate estimated by X-ray fluorescence technique in children: comparison between the plasma disappearance of $^{99}$Tc$^{m}$-DTPA and iohexol after urography

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The total plasma disappearance of the non-ionic contrast medium iohexol was determined by X-ray fluorescence technique following intravenous urography in 10 children aged between 2 and 13 years. For comparison the plasma disappearance of $^{99}$Tc$^{m}$-DTPA was estimated both 2 days before and simultaneously with the iohexol study. High correlations between the three sets of data were found and no change in the glomerular filtration rate was detected following injection of contrast medium. It was also found that reliable estimates of the glomerular filtration rate can be obtained from two plasma samples of 1 ml each, taken 3 h and 4 h after the injection of the contrast medium.

Key words: contrast media; iohexol; renal clearance; total plasma clearance; urography

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Urographic studies in children have mainly been focused on the diagnostic quality of the X-ray films and adverse reactions to the contrast media [1-6]. Knowledge about the effect of urography on renal function in children is, however, still limited. Estimation of the glomerular filtration rate (GFR), which is considered to be the best single parameter for assessing renal function [7], has been included in only a few studies [8, 9].

In adults, calculations based upon the plasma elimination curves of contrast media have been shown to provide an accurate and reliable estimate of the GFR [10-15]. At our centre children are often admitted both for urography and renography and we were therefore able to estimate the GFR by the plasma elimination of $^{99}$Tc$^{m}$-DTPA and contrast medium simultaneously.

The present study was performed to examine (1) whether the GFR in children is affected by the non-ionic contrast medium iohexol (Omnipaque®, Nycomed A/S, Oslo, Norway), and (2) if estimation of the GFR based upon the
Fig. 1. Plasma elimination curves (ln scale) in 10 children (one girl examined twice) after injection of $^{99m}Tc$-DTPA on day 1 (upper panel) and after simultaneous injections of $^{99m}Tc$-DTPA (middle panel) and tohexol (lower panel) on day 3.
Fig. 1. Plasma elimination curves (ln scale) in 10 children (one girl examined twice) after injection of $^{99m}$Tc-DTPA on day 1 (upper panel) and after simultaneous injections of $^{99m}$Tc-DTPA (middle) and iohexol (lower panel) on day 3.
and from five samples taken at about 150, 180, 210, 240 and 300 min.

All injections were given via an indwelling wrist or cubital vein cannula. The same cannula was used for the blood sampling taking special precautions to minimize the risk of contamination [19].

On day 1 the children were allowed to eat and drink before the examination but not during the sampling time. Before the urography on day 3 the bowel was emptied by a combination of a mild laxative and a rectal enema. The patients were fasting for 3 h before the injection of iohexol and isotope. After completing the urography, which usually took about 20 min and included abdominal compression, they were allowed to eat and drink freely.

Apart from a small additional dose of radioactive isotope and a few extra blood samples on day 3, all investigations were performed according to our routine procedures. The protocol was approved by the ethical committee of the hospital, and in each case informed consent was obtained from the parents. The GFR estimates are given in absolute values without correction for body surface.

All values are given as arithmetic means ±1 SEM. Linear regressions were determined according to the least squares method. The Wilcoxon's signed rank test for paired differences was applied for testing of significance. Using two-tailed test a probability of less than 0.05 was considered significant.

**RESULTS**

Following single injections the disappearance patterns with both dose levels of \[^{99}\text{Tc}\text{m}-\text{DTPA}\] were similar to that of iodine (Fig. 1). The disappearance curves from 150 min and onwards were monoexponential, as evidenced by a close to linear fall of the logarithms of the plasma iodine and \[^{99}\text{Tc}\text{m}-\text{DTPA}\] concentrations. The mean correlation coefficients for the linear regressions describing the final part of the disappearance curves at day 3 were \(-0.994±0.002\) for \[^{99}\text{Tc}\text{m}-\text{DTPA}\] and \(-0.993±0.002\) for iodine.

The average GFR estimated from the final slope of the iodine disappearance curves based on five observations (three observations in patient 10 and four observations in patient 11 (see Methods)) in each child was 50±9 ml/min. When GFR was calculated from only two iodine concentrations obtained at 180 and 240 min after the contrast injection, a 3±1% significantly
lower mean value was obtained, 49±8 ml/min (Fig. 2). The linear regression for this relationship was:

\[
\text{GFR}_{\text{iohexol (two plasma samples)}} = 0.98 \times \text{GFR}_{\text{iohexol (five plasma samples)}} - 0.5
\]

\(r=0.997\)

GFR calculated from the disappearance of \(^{99}\text{Tc}^{m}\)-DTPA did not change significantly from day 1 (47±7 ml/min) to day 3 (49±7 ml/min). The linear regression for this relationship was:

\[
\text{GFR}_{\text{(\(^{99}\text{Tc}^{m}\)-DTPA day 3)}} = 1.01 \times \text{GFR}_{\text{(\(^{99}\text{Tc}^{m}\)-DTPA day 1)}} + 1
\]

\(r=0.98\)

The linear regression for the relationship between GFR determined simultaneously with \(^{99}\text{Tc}^{m}\)-DTPA and iohexol based on two plasma samples was:

\[
\text{GFR}_{\text{iohexol}} = 1.11 \times \text{GFR}_{\text{(\(^{99}\text{Tc}^{m}\)-DTPA)}} - 6
\]

\(r=0.97\) (Fig. 3).

This regression line has a slope which does not deviate significantly from 1 and a y-intercept not significantly different from zero.

The mean GFR calculated from the plasma disappearance of iohexol (two plasma samples), 49±8 ml/min, was not significantly different from any of the \(^{99}\text{Tc}^{m}\)-DTPA estimates. The corresponding observations in each child are given in Table I.

**DISCUSSION**

The X-ray contrast media are excreted by glomerular filtration [20-23]. They satisfy the classical criteria for a glomerular filtration marker [24] with the possible reservation that they might affect the renal function. Both with cold [11, 15] and radioactive [25-32] iodine some of them have been used for determination of the GFR but in these studies only small amounts of contrast media were used. The potential nephrotoxicity is both dose- and medium-dependent [29-32]. Hence the possible toxic effects may not become apparent until X-ray examination with large doses is performed. In this respect children are especially interesting because they receive relatively high doses of contrast medium, both for urography [33] and cardioangiography [34].

In the present study, GFR was not significantly altered by administration of standard doses of iohexol. However, this finding must be interpreted with caution, as the number of patients in our study is small. Contrast-induced depression of the renal function is infrequent, but well known [31, 35]. Nevertheless, we believe that in the rare case with contrast-induced renal failure any currently employed method for estimation of the GFR, including the contrast medium method, would reveal a low value.

The correlation obtained between the GFR estimates calculated with the \(^{99}\text{Tc}^{m}\)-DTPA and the iohexol methods in this study also indicates that the latter can be taken as a valid measure of the GFR.

The present results are in accordance with studies in adults where high correlation between the total plasma clearances [12-14, 21] as well as the renal clearance [21] of \(^{51}\text{Cr}-\text{EDTA}\) and contrast media has been found. Estimates before and during the first 6 h after the injection of iohexol have also revealed that the renal clearance of the contrast medium, and hence the GFR, was not affected by doses up to 1500 mg l/kg body weight [21, 22, 36].

Since our \(^{99}\text{Tc}^{m}\)-DTPA method required multiple plasma samples, we took the opportunity to measure the iodine concentration in the same samples. Thereby the experimental data forming the basis for our iodine disappearance curves became more solid. The disappearance patterns indicated that the elimination rate was constant (monoeXponential) from 2.5 h after the injection and onwards. Samples taken at 3 h and 4 h therefore seem to be adequate for determination of the GFR. The fact that the GFR calculated from two plasma samples was 3% lower than from five samples is considered to be without biological significance because of the small difference and the very high correlation obtained between the two estimates (Fig. 2). Thus our findings confirm the results of others [14, 37] that with the one-compartment method two plasma samples are sufficient to obtain reliable estimates of the GFR. This is of practical importance, especially in children, in whom it is desirable that blood samples are as few and small as possible.
In this study different mathematics were applied for the two methods of estimating the GFR. For the iohexol method a one-compartment model was preferred mainly because of the advantage with fewer blood samples as outlined above. With $^{99m}$Tc-DTPA the two-compartment model of Sapirstein was used according to the routine procedure of our hospital [17].

Reproducibility testing for GFR estimations have mainly been done in adults, and rather large coefficients of variation have been found [11, 14, 36]. Since similar data in children are not available, comparison between methods have to be based upon information from adults. In our laboratory the total coefficient of variation is slightly above 10% for the $^{99m}$Tc-DTPA method, which is similar to the contrast media methods [11, 13]. Our data indicate that the precision of the iohexol method is probably equal to that of the $^{99m}$Tc-DTPA method also in children. This is not surprising when the similarity of the two techniques is taken into consideration.

REFERENCES

Glomerular filtration rate in children


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Article II
INFLUENCE OF UROGRAPHY ON RENAL FUNCTION IN CHILDREN

A double blind study with metrizoate and iohexol

G. Stake, E. Monn, K. Rootvelt, K. Golman and T. Monclair

Abstract

Thirty-two children were given either metrizoate or iohexol for urography in a double blind study. Mild to moderate adverse reactions were observed in all patients receiving metrizoate (15/15) and in 4 receiving iohexol (4/17). Alkaline phosphatase in urine was significantly increased 4 hours after the injection of both media, but had returned to pre-injection levels 16 hours later. The excretion of β2-microglobulin and albumin was not altered. In 9 children in the metrizoate group and 11 in the iohexol group the glomerular filtration rate (GFR) was determined before urography by the single injection 99mTc-DTPA-technique and 3 to 4 hours after urography by measuring the plasma disappearance of the contrast medium with the x-ray fluorescence technique. No reduction of GFR was observed.

Material and Methods

Thirty-two children admitted to the pediatric surgical department for treatment of urologic disorders were included in the study. Two boys were uremic with plasma creatinine concentrations of 264 and 363 μmol/l, respectively. The others were in good clinical condition. None of the patients had edema.

The bowel was emptied by a laxative (X-Prep, Nycomed, Norway) given on the day before and a rectal enema in the morning 3 to 6 hours before urography. About 3 hours prior to the urography, plasma creatinine concentration was measured, a urine sample was obtained, and in 20 children the GFR was determined with a single injection 99mTc-DTPA plasma disappearance method (18). The patients were encouraged to eat and drink before, but not during the radionuclide study.

All children underwent routine urography which was performed by the same radiologist (G. S.). Abdominal compression was used in 20 cases. No food or fluid was given during the last 3 hours before the contrast medium injection. After urography, which usually took about 20 minutes, the children ate and drank freely. According to a randomized, double blind protocol the patients received 2 ml/kg body weight of either Na/Ca/Mg-metzizoate (Isopaque 350, Nycomed) or iohexol (Omnipaque 350, Nycomed), both with iodine concentrations of 350 mg/ml.

The ages of the 15 children receiving metrizoate and the 17 children receiving iohexol were evenly distributed between 1 and 14 years (Fig. 1). There were 9 girls in the
metrizoate group, and 10 in the iohexol group. No information about the possible adverse reactions of the contrast medium was given to the children. The entire series of investigations was completed before the code was broken. All injections were given by an indwelling wrist or cubital cannula during one minute. The same cannula was used for the later blood sampling taking special precautions to minimize the risk of contamination (5).

Reactions to the injections were evaluated clinically by the same radiologist (G. S.), and arbitrarily classified as 'none', 'mild', or 'moderate'. There were no severe reactions. In the first reaction group the children did not experience any discomfort apart from the venipuncture and the abdominal compression. In the 'mild' group a mild heat sensation or a peculiar taste was experienced. One or several of the following reactions were recorded as 'moderate': pronounced heat sensation, headache, rash, nausea, sneezing or crying after the injection had started.

Blood samples were taken about 3 and 4 hours after the contrast medium injection, and the concentration of iodine in one ml of plasma was determined with the x-ray fluorescence technique (13). The plasma iodine disappearance curves obtained from these two samples were used to estimate the GFR according to a single compartment model as previously described (26).

Urine samples were obtained from all the patients about 3 to 4 hours after urography and on the following morning.

In 12 children who did not receive 99mTc-DTPA, determination of renal creatinine clearance had to be abandoned because of incomplete urine collections. However, in these patients the plasma creatinine concentration was measured in the same samples which were analysed for iodine and also in a plasma sample taken the next morning.

The creatinine concentrations in plasma and urine were determined with an AutoAnalyser. All urine samples were neutralized by adding NaOH or HCl, and immediately frozen at −70°C until the following analyses were performed: The concentration of albumin was measured by an immunologic method (7), β2-microglobulin by RIA (Pharmacia Diagnostica, Sweden) and alkaline phosphatase by an enzymatic colorimetric method (Boehringer Mannheim Diagnostica, West Germany). Since urine was not collected quantitatively, the amount of excreted substances was expressed per mmol creatinine.

The body surface area was determined according to Haycock et coll. (14), and the GFRs are given per 1.73 m² body surface. All values are given as arithmetic means and ranges. The Wilcoxon's signed rank tests were applied for testing of significance. Using the two-tailed test a probability of less than 0.05 was considered significant.

All investigations in this study were performed according to hospital routine procedures. The protocol was approved by the ethical committee of the hospital, and in each case informed consent was obtained from the parents.

**Fig. 1.** Age distribution in 19 girls and 13 boys receiving either iohexol (■) (n=17) or metrizoate (□) (n=15).

<table>
<thead>
<tr>
<th>Contrast medium</th>
<th>No. of patients</th>
<th>Reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>None</td>
</tr>
<tr>
<td>Metrizoate</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Iohexol</td>
<td>17</td>
<td>13</td>
</tr>
</tbody>
</table>

**Table 1**

Adverse reactions to metrizoate and iohexol

The pre-urography plasma creatinine concentration and the GFR were similar in the metrizoate and iohexol groups: in the metrizoate group 74 (range 42-264) μmol/l (n=15) and 94 (37-135) ml/min/1.73 m² (n=9), in the iohexol group 79 (48-363) μmol/l (n=17) and 88 (14-141) ml/min/1.73 m² (n=11).

Reactions to contrast medium. Injection of iohexol was significantly better tolerated than metrizoate. Adverse reactions were observed in all the children receiving metrizoate, but in only 4 in the iohexol group (Table 1).

**Effects of contrast media on GFR and the plasma concentration of creatinine.** Injection of either metrizoate or iohexol in a dose of 700 mg I/kg body weight had no significant influence on the GFR. When estimated from the iodine disappearance curve from 3 to 4 hours after the contrast medium injection, the average GFR in the metrizoate and iohexol groups was 97 (53-136) ml/min/1.73 m² and 85 (13-124) ml/min/1.73 m², respectively. The individual pre- and post-injection GFR in the two groups is shown in Fig. 2.

In the 12 children without GFR estimation before the urography, 6 receiving metrizoate and 6 iohexol, the plasma concentration of creatinine did not change throughout the investigation, neither within, nor between the two groups. The average creatinine concentrations (n=12) before the injection, at 3 and 4 hours (mean value) and at about 20 hours after the injection, were 79 (46-264) μmol/l, 80 (46-257) μmol/l and 80 (42-273) μmol/l, respectively.

**Effects of contrast media on the urinary excretion of markers for renal damage.** Neither metrizoate nor iohexol
**Table 2**

**Effect of metrizoate and iohexol, 0.7 g/kg body weight, on urinary excretion of albumin, β2-microglobulin and alkaline phosphatase in 32 children. Mean values and ranges**

<table>
<thead>
<tr>
<th></th>
<th>Albumin</th>
<th>β2-microglobulin</th>
<th>Alkaline phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>creatinine</td>
<td>creatinine</td>
<td>creatinine</td>
</tr>
<tr>
<td></td>
<td>(mg/mmol)</td>
<td>(μg/mmol)</td>
<td>(IU/mmol)</td>
</tr>
<tr>
<td>Metrizoate (n=15)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before injection</td>
<td>162 (1-626)</td>
<td>585 (5-8 258)</td>
<td>1.0 (0.3-2.3)</td>
</tr>
<tr>
<td>3 h after injection</td>
<td>199 (1-2 023)</td>
<td>386 (2-4 751)</td>
<td>1.6 (0.6-3.0)</td>
</tr>
<tr>
<td>Difference</td>
<td>NS</td>
<td>-199 (-3 507-81)</td>
<td>0.6 (-1.1-2.1)</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td></td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Iohexol (n=17)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before injection</td>
<td>67 (1-364)</td>
<td>411 (7-5 656)</td>
<td>0.8 (0.1-3.4)</td>
</tr>
<tr>
<td>3 h after injection</td>
<td>38 (1-152)</td>
<td>434 (0-6 222)</td>
<td>1.5 (3.3-9.8)</td>
</tr>
<tr>
<td>Difference</td>
<td>NS</td>
<td>-29 (-213-62)</td>
<td>0.7 (-2.9-9.2)</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td></td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

![Fig. 2. GFR determined in 20 children by the plasma disappearance of 99mTc-DTDP before urography, and by the plasma disappear­ance of contrast medium after urography. The dotted line denotes equality.](image)

The type of medium used (1). Injection of high osmolar, ionic media has caused severe (10, 29) and sometimes lethal complications in infants and children (19, 20). However, to our knowledge, no fatal reactions have so far been reported with the low osmolar, non-ionic media in the pediatric age group. We have used iohexol routinely for all intravascular applications in children since 1983 (24) and the present study confirms that iohexol causes fewer and milder adverse reactions than metrizoate.

In the present investigation we wanted to evaluate more specifically whether the two contrast media affect renal function differently. In order to minimize the risks of bias due to our documented preference for iohexol, we used a double blind study.

A rough estimate of the glomerular function can be obtained from the plasma creatinine concentrations (6). In our patients it was unchanged, as earlier reported, after injection of both metrizoate (25) and iohexol (27). Similar results were observed following cardioangiography in children, with the ionic medium diatrizoate (16).

A better estimate of the glomerular function can, however, be obtained from GFR measurements (5). GFR in children can be estimated with equal precision from the plasma disappearance curves of 99mTc-DTPA and iohexol (26). Others have shown that the plasma disappearance of metrizoate provides a valid estimate of GFR in adults (4, 13, 22).

In this study GFR was determined with the 99mTc-DTDP plasma disappearance method (18) before urography, and from the plasma disappearance curves of the contrast media after urography (26). No significant difference was observed between these two estimates irrespective of the contrast medium used.

In patients with impaired renal function high dose urography may aggravate the renal failure (23). However, no deterioration was observed in the two uremic boys. The
GFR was 14 ml/min/1.73 m² both before and after urography in one of them, and in the other the serum creatinine was 264 μmol/l before, and 273 μmol/l 20 hours after the urography.

Patients with renal tubular damage and individuals given nephrotoxic chemicals often experience increased amounts of low molecular weight proteins (2, 11, 15, 28). We found a transient increase in urinary alkaline phosphatase with both contrast media, but the magnitude of the increase was much smaller than reported in adults (15). This observation, together with the unaffected urinary excretion of albumin (11) and β₂-microglobulin (28), indicates that the contrast media had only a minor effect on the tubular cells in our patients.

In this material we were unable to find any discrepancies between the renal effects of metrizamide and iohexol. However, the latter was better tolerated and caused only negligible adverse reactions during the intravenous application. This is of practical importance, especially in children, to avoid apprehension in connection with renewed contrast studies. Contrary to others (17), therefore, we find there is good justification for using non-ionic contrast media in pediatric practice in spite of the extra cost.

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REFERENCES


Article III

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This study was performed in order to develop a method for estimation of the glomerular filtration rate (GFR) from a single plasma sample based upon the plasma disappearance rate of the non-ionic contrast medium iohexol. The apparent distribution volume for iohexol was measured in 117 infants and children and used for establishment of a weight-related empirical formula for the distribution volume. Using the distribution volume obtained by this formula, a preliminary GFR was calculated from the iodine concentration measured in a plasma sample taken 3 h after injection of iohexol. When this estimate was corrected by another empirically established correction factor, a high degree of agreement was found between a GFR reference method and the 3-h single plasma sample method. In another group of 13 children the 3-h single plasma sample GFR was estimated twice with a 2-day interval, and the day-to-day variations were found to be similar to those obtained with other standard methods.

Key words: apparent distribution volume; cardioangiography; urography

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Most methods for estimation of the glomerular filtration rate (GFR) are based on the disappearance rate of an injected filtration marker and determined by multiple plasma samples [1, 2]. However, GFR can also be calculated from a single plasma sample, provided that an estimate of the distribution volume for the filtration marker is available. By single plasma sample methods reliable estimates for GFR have been obtained with radionuclides both in adults [3-5] and in children [6].

The iodinated contrast media for intravascular injection are excreted by glomerular filtration. They have been used for determination of GFR.
by the X-ray fluorescence method [7] by multiple plasma samples [8–12], and in adults also from a single plasma sample [13].

For obvious reasons, few and small blood samples are especially desirable in the paediatric age group. Our intention was, therefore, to establish a reliable single plasma sample method for determination of GFR in infants and children based upon the plasma elimination of the much used non-ionic contrast medium iohexol (Omnipaque®, Nycomed A/S, Oslo, Norway).

In adult males the apparent distribution volume for iohexol is equal to the extracellular fluid volume, i.e. about one-quarter of the body weight [14]. However, in infants and children information about the distribution volume for iohexol was not readily available. Therefore, we had to establish a formula for a distribution volume which could be used for calculation of a single plasma sample GFR by a method originally developed for adults [5]. In addition we wanted to examine whether reliable estimates of the apparent distribution volume could be obtained with a small dose of iohexol [14] since routine examinations imply the use of contrast media over a wide dose range. Finally, the day-to-day variation of both the multiple and the single plasma sample methods was tested in a smaller group of patients by repeat examinations with 2 days interval.

MATERIALS AND METHODS

The material included 113 children, 82 of whom were admitted for urological disorders and 31 for congenital heart diseases. Some of the infants with heart disease were cyanotic. Otherwise all the patients were in stable clinical condition. All had normal height/weight proportions and none of them had oedema. Premature babies were not included.

Group 1 included 45 girls and 55 boys in whom 69 urographies and 31 cardioangiographies were performed. Their average age was 6 ± 1 years (range 2 days–14 years) (Fig. 1), body weight 23 ± 2 kg (range 2.6–63 kg) and plasma creatinine concentration 71 ± 4 μmol l⁻¹ (range 15–363 μmol l⁻¹).

The patients fasted for 3 h before the X-ray examinations. Those undergoing intravenous urography had their bowel emptied by a combination of a mild laxative and a rectal enema. Abdominal compression was routinely used. After the examinations they all were allowed to eat and drink freely.

Twenty-four of the patients undergoing cardioangiography received from two to four iohexol injections (mean 2.4 ± 0.1) at 8 ± 1 min intervals. All the others were given a single injection.

The amount of contrast medium injected was measured in the syringes used and varied between 210 and 2450 mg l⁻¹ kg⁻¹ body weight. With multiple injections the accumulated amount of iohexol was transformed to an equivalent single dose given at the time of the last injection according to Boijsen et al. [15].

Following the X-ray examinations two plasma samples were taken about 3 h and 4 h after the last injection of iohexol, and the iodine concentration in the plasma was measured by X-ray fluorescence technique [7] in plasma samples of 1 ml and with a counting time of 5 min [11].

Group 2 included nine boys and four girls with an average age of 5 ± 1 years (range 1–10 years), body weight 21 ± 2 kg (range 12–36 kg), and plasma creatinine concentration 88 ± 10 μmol l⁻¹ (range 54–169 μmol l⁻¹).

On day 1 they received an intravenous injection of iohexol, 175 mg l⁻¹ kg⁻¹ body weight (concomitant with a renal nuclear study). Two days later (day 3) an intravenous urography was performed with injection of iohexol 700 mg l⁻¹ kg⁻¹ body weight.

Before the injection of iohexol on day 1 the children drank freely, but they were fasting during the examination time of about 4 h. The intravenous urography was performed as described in group 1.

![Age and sex distribution in 55 boys (■) and 45 girls (□) in group 1.](image-url)
On day 1 four plasma samples were taken between 2 h and 4 h after the injection. On day 3, two plasma samples were taken about 3 h and 4 h after the injection. Their iodine concentration was measured by X-ray fluorescence technique, as described for group 1.

For all group 2 patients, and those in group 1 undergoing urography, a cubital indwelling cannula was used both for injection and blood sampling. Special precautions were taken to minimize the risk of contamination with the marker substance[16]. In the 14 youngest children undergoing cardioangiography blood was sampled through a 3F end-hole catheter with the tip located in the inferior vena cava. In the remaining children the blood samples were drawn from a cubital cannula.

Apart from the contrast medium injected simultaneously with the isotope (for the renal nuclear study) and the extra blood samples, all examinations were performed according to our routine procedures.

The body surface area was determined according to Haycock et al. [17], and all GFR estimates are given per 1.73 m² body surface. Values are given as arithmetic means ± 1 SEM and/or range. Linear regressions were determined according to the method of least squares. The Wilcoxon's signed rank test for paired differences was applied for testing of significance. Using two-tailed test a probability of less than 0.05 was considered significant. Agreement between two measurements was estimated by the method of Bland and Altman [18], and logarithmic transformed data were used for calculation of the 95% confidence interval [18].

The protocol was approved by the ethical committee of the hospital, and in each case informed consent was obtained from the parents.

Calculations

In group 1 both the apparent distribution volume for iohexol and the two plasma sample GFR (GFRsa) were determined from the iodine concentration measured in the two plasma samples taken about 3 h and 4 h after the injection.

The apparent distribution volume (V) was calculated according to the formula [19]:

\[ V = \frac{Q_{(0)}}{C_{(0)}} \]  

(1)*

and the reference GFR was determined according to an open one-compartment model as described by Brøchner-Mortensen [2]:

\[ \text{GFR}_{\text{ref}} = \left( \frac{Q_{(0)} \times \beta}{C_{(0)}} \right) \times 0.99078 - \left( \frac{Q_{(0)} \times \beta}{C_{(0)}} \right)^2 \times 0.001218 \]  

(2)*

The apparent distribution volumes were related to the body weight and subjected to linear regression analysis. The regression equation obtained was used as a formula for an empirical, weight-related estimate for the distribution volume.

A preliminary GFR (GFRsa) was calculated from the single plasma sample taken 3 h after injection of iohexol according to Jacobsson's formula [5]:

\[ \text{GFR}_{\text{sa}} = \frac{1}{V} \times \ln \left( \frac{Q_{(0)}}{V \times C_{(1)}} \right) + 0.0016 \]  

(3)*

Three values for GFRsa were obtained in each child by applying three different estimates for the distribution volume:

1. the empirical distribution volume estimated from the body weight,
2. the volume of estimate (1) increased by 50%, and
3. the volume of estimate (1) reduced by 50%.

The relationship between the GFRref and the preliminary GFRsa calculated by the empirical distribution volume could be expressed by a second degree polynomial. When this polynomial was solved for GFRref the equation

\[ Q_{(0)} = \text{injected amount of iodine in mg}; \]
\[ C_{(0)} = \text{plasma iodine concentration in mg ml}^{-1} \text{ at time zero (intercept of back-extrapolated mono exponential line with the ordinate on a semilogarithmic plot)}; \]
\[ \beta = \text{slope of the monoeponential line described above}; \]
\[ t = \text{the time in min between injection of iohexol and blood sampling}; \]
\[ C_{(1)} = \text{plasma iodine concentration in mg ml}^{-1} \text{ at time } 1; \]
\[ V = \text{distribution volume in ml.} \]
obtained was used to correct the GFR<sub>ref</sub>. By this empirical correction factor the final single plasma sample GFR (GFR<sub>s1h</sub>) is made equal to the GFR<sub>ref</sub>.

In group 2 two values for the apparent distribution volume as well as the reference GFR were obtained in each patient, on day 1 from the iodine concentration measured in the four plasma samples taken between 2 h and 4 h after injection of iohexol, and on day 3 from the iodine concentration measured in two samples taken about 3 h and 4 h after the injection. The 3-h single plasma sample GFR (GFR<sub>s3h</sub>) was estimated on both days.

RESULTS

In group 1 (n = 100) the reference GFR ranged from 14 to 126 ml min<sup>−1</sup> 1.73 m<sup>2</sup> (mean 78 ± 3 ml min<sup>−1</sup> 1.73 m<sup>2</sup>). The apparent distribution volume was about 50% of the body weight in the newborns, decreasing to about 25% in the older children. No difference between girls and boys was observed. The individual results are shown in Figure 2.

The linear regression for the relationship between the body weight and the apparent distribution volume (Fig. 3) was used for estimation of the empirical distribution volume, in ml (V):

\[ V = 231 \times \text{kg body weight} + 1215 \]  

(4)

The relationships between the reference GFR and the single plasma sample GFR calculated with the three different distribution volumes based on formula (4) are shown in Figure 4. A curvilinear regression for the relationship between the reference GFR and

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**Fig. 2.** Measured apparent distribution volume for iohexol in per cent of body weight in 55 boys (+) and 45 girls (○). (group 1). The dashed line indicates the values calculated by the empirical, weight-related, formula (formula 4).

**Fig. 3.** Relationship between the apparent distribution volume and the body weight in 100 infants and children (group 1).
Fig. 4. Relationships between the reference GFR and preliminary single plasma sample GFRs calculated with different estimates for the distribution volume. (a) The distribution volumes based upon body weight according to the empirical formula (formula 4). The dashed line denotes the regression line for the second-degree polynoma. (b) 150 (●) and 50 (+) Per cent of the volumes used in the panel a: ●=150%, + =50%. The dashed line denotes equality.

The preliminary GFR calculated with the distribution volume obtained from formula (4) was expressed by the correction factor (Fig. 4a):

\[ \text{GFR}_{\text{c}} = 1.8 \times \text{GFR}_{\text{ref}} - 0.005 \times \text{GFR}_{\text{ref}}^2 - 29 \]

Given that the final single sample GFR (GFR$_{\text{c,sh}}$) should be equal to reference GFR, the polynoma was solved for GFR$_{\text{ref}}$ = GFR$_{\text{c,sh}}$ and the equation obtained:

\[ \text{GFR}_{\text{c,sh}} = 180 - 14.1 \sqrt{133 - \text{GFR}_{\text{c}}} \]  

(5)

The difference between the mean values for GFR$_{\text{ref}}$ and GFR$_{\text{c,sh}}$ (calculated by the formulae 3, 4, and 5) was \(-1.4 \text{ ml min}^{-1} \text{ m}^{-2}\) with a 95% confidence limit from \(-2\) to \(1\%\) (Fig. 5).

In group 2 (\(n=13\)) the apparent distribution volume for iohexol found with a contrast medium dose of \(175 \text{ mg I} \text{ kg}^{-1} \text{ body weight}\) was not significantly different from that found with \(700 \text{ mg I} \text{ kg}^{-1} \text{ body weight}\) (6190±600 ml). The individual results are given in Table 1.

When measured by four plasma samples the day 1 GFR estimate (67±7 ml min$^{-1}$ 1.73 m$^{-2}$) was not significantly different from the two plasma samples day 3 estimate (66±7 ml min$^{-1}$ 1.73 m$^{-2}$).
G. Stake & T. Monclair

TABLE I. Age distribution and repeated estimates of the apparent distribution volume for iohexol and the glomerular filtration rate in 13 children (group 2)

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (years)</th>
<th>Apparent distribution volume for iohexol in ml</th>
<th>Glomerular filtration rate (ml min⁻¹ 1.73 m²⁻¹) determined by</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 1</td>
<td>Day 3</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>2609</td>
<td>3364</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>6971</td>
<td>5344</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>9967</td>
<td>9026</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>5556</td>
<td>7050</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>6847</td>
<td>8224</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>4249</td>
<td>3620</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>4395</td>
<td>5301</td>
</tr>
<tr>
<td>8</td>
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<td>8</td>
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<td>4396</td>
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<td>4689</td>
</tr>
<tr>
<td>13</td>
<td>4</td>
<td>8391</td>
<td>9756</td>
</tr>
</tbody>
</table>

The children received iohexol 175 mg l kg⁻¹ body weight on day 1 and 700 mg l kg⁻¹ on day 3. Both the apparent distribution volume and the multiple sample GFR were determined from four plasma samples on day 1 and from two plasma samples on day 3.

The day 1 3-h single plasma sample GFR (67±8 ml min⁻¹ 1.73 m²⁻¹) was not significantly different from the day 3 single sample estimate (66±6 ml min⁻¹ 1.73 m²⁻¹). The corresponding estimates in each child are given in Table 1.

DISCUSSION

For determination of a single plasma sample GFR an estimate of the apparent distribution volume for the GFR marker is needed [3–6]. In adults the apparent distribution volume for contrast media is equal to the extracellular body fluid volume; for iohexol it is on average 27% of body weight in adult males [14].

In children the extracellular fluid volume decreases from 40–50% of body weight in newborns, to 20–25% at puberty, without any sex difference [19, 20]. The measured apparent distribution volumes for iohexol in our patients agree fairly well with these figures, and we may assume that iohexol is also dispersed in a compartment about the size of the extracellular volume in infants and children.

The well-known inter-patient variation of the apparent distribution volumes does not appear to be associated with different dose levels of iohexol in adults [5, 14]. To evaluate this factor in children, two contrast medium doses—175 and 700 mg l kg⁻¹ body weight—were given to the same child (group 2), but similar apparent distribution volumes were obtained.

Although it is essential to know the distribution volume, at the same time calculation of GFR by a single plasma sample is rather insensitive to the precise size of this volume, as discussed by Jacobsson [5]. Hence, a rough estimate will suffice for the purpose, as illustrated in Figure 4 (b), which shows that acceptable values are obtained over a fairly large GFR range, even if the estimate of the distribution volume is increased or decreased by a factor of one-half. This may be partly explained by the fact that the distribution volume appears both in the numerator and the denominator in formula (3).

When the apparent distribution volume is determined from the plasma elimination curve, the volume is overestimated owing to the effect of non-uniform distribution; after equilibration the marker concentration is less in plasma than in the distribution volume as a whole [21]. This concentration gradient increases with increasing GFR, as does the overestimation of the distribution volume. The scatter of the points
shown in Figure 2, which reflects the inter-patient variation of the apparent distribution volume, is partly related to this phenomenon. However, since GFR is not known in advance, the influence of non-uniform distribution cannot be included in the empirical volume used in the single sample method. As pointed out by Chatterton [22], and also shown in Figure 4, the relationship between the reference GFR and the preliminary GFR will always be curvilinear. The deviation from the line of equality can be compensated for in several ways: Groth and Aasted [23] calculated a single plasma sample GFR by two different formulas, one for GFR above 40 ml min⁻¹ 1.73 m², and another if the first calculation indicated that GFR was between 15 ml min⁻¹ 1.73 m² and 40 ml min⁻¹ 1.73 m². However, we have chosen a formula (4) for estimation of the distribution volume, which describes the average apparent distribution volume for a given body weight. A preliminary calculated GFR has then been corrected according to its appropriate GFR level, by a correction formula (5), the main effect of which is to compensate for the influence of non-uniform distribution of the marker.

The precision of the single plasma sample method also depends on the timing of the blood sampling: the optimal sampling time is inversely related to GFR [5]. Accordingly, at low GFR Jacobsson recommended a sampling time of about 10 h [5]. Such a long interval is inconvenient in daily practice. However, if the single sample GFR is calculated by the plasma sample taken at about 3 h after injection and corrected according to formula (5), reliable estimates for the single sample GFR can be obtained for GFR values between 14 ml min⁻¹ 1.73 m² and 126 ml min⁻¹ 1.73 m², as shown in Fig. 5. The issue of the sampling time is further addressed in a separate article [24].

The concept of a single sample method for estimation of GFR is certainly open to discussion. The most obvious objection is the greater chance of error associated with the fact that one has to rely entirely upon a single plasma sample, as compared with the assumed safer multiple sample methods. However, in our daily work we have too often experienced that an attempted multiple sample test has left us with no result because of unsuccessful blood sampling. In the present study the repeatability of the multiple and single plasma sample methods was about equal, which indicates that the single plasma sample GFR is well-suited for routine use.

Oedematous patients and premature babies were not included in this study. For these children the formula for the distribution volume (4) will not be valid, and GFR should, therefore, be determined by other methods.

The agreement between the reference two plasma sample GFR, and the 3-h single plasma sample GFR in this study, was, however, obtained in the same group of patients that formed the basis for the formulae used in the calculation. In a further paper [24] we have applied these formulae to an entirely independent group of infants and children and a similar good agreement between the two GFR estimates was obtained.

REFERENCES


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Article IV
A single plasma sample method for estimation of the glomerular filtration rate in infants and children using iohexol, II: establishment of the optimal plasma sampling time and a comparison with the $^{99}\text{Tc}^m$-DTPA method

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The glomerular filtration rate (GFR) can be determined from the plasma disappearance rate of the non-ionic contrast medium iohexol. A preceding study established the empirical formulae enabling the development of a single plasma sample method for estimation of GFR in infants and children. In the present study the validity of these empirical formulae was confirmed in examinations in 143 patients. The results of the single plasma sample method were similar to those of a standard $^{99}\text{Tc}^m$-DTPA method, and also with those of a two plasma sample iohexol method. Evaluation of the results obtained with plasma sampling 1 h, 2 h, 3 h and 4 h after the injection of the contrast medium showed that the optimal sampling time was about 3 h after the injection.

Key words: iohexol; urography; renal function

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Utilizing the non-ionic contrast medium iohexol (Omnipaque®, Nycomed A/S, Oslo, Norway), we have adapted Jacobsson's single plasma sample method [1] for determination of the glomerular filtration rate (GFR) to infants and children [2]. In the present study the empirical formulae established in our preceding article [2] have been tested in an entirely independent group of patients.

With this method the single plasma sample is taken about 3 h after the injection of iohexol [2]. However, since single plasma sample methods depend on the sampling time [1, 3, 4] we have also compared the results obtained with plasma sampling after 1 h, 2 h, 3 h, and 4 h. In addition the iohexol single plasma sample method was compared with the well-established, multiple plasma sample $^{99}\text{Tc}^m$-DTPA method [5, 6].

MATERIALS AND METHODS

The material included 143 infants and children
admitted for urological disorders. They were in stable clinical condition with normal height/weight proportions and none of them had edema. Premature babies were not included.

Group 1 consisted of 46 girls and 50 boys undergoing urography with intravenous injection of iohexol. None of them were included in group 1 in the preceding article [2]. Their average age was 6±1 years (range 2-14 years) (Fig. 1), body weight 23±2 kg (range 4.1-68 kg) and plasma creatinine concentration 71±6 umol l⁻¹ (range 31-227 umol l⁻¹).

The patients had their bowel emptied by a combination of a mild laxative and a rectal enema. They were fasted for 3 h before the intravenous urography, but were allowed to eat and drink freely after the X-ray examination. Abdominal compression was used routinely.

The amount of iohexol injected was measured in the syringes used and varied between 170 mg I kg⁻¹ body weight and 1170 mg I kg⁻¹ body weight. Following intravenous urography the iodine concentration was measured by X-ray fluorescence technique [7]. The counting time was 5 min. In 79 of the children (subgroup la) a plasma sample was also taken about two hours after the injection; in addition 25 of the latter 79 children (subgroup lb) had another plasma sample taken already after about 1 h. These samples were used only for estimation of single sample GFR.

Group 2 included 18 girls and 29 boys with an average age of 5±1 years (range 1-13 years), body weight 22±2 kg (range 11-45 kg), and plasma creatinine concentration 85±9 μmol l⁻¹ (range 28-363 μmol l⁻¹).

Eleven children received a single intravenous injection of ⁹⁹mTc-DTPA (American Pentate II, Amersham International, Amersham, UK) 0.50 MBq kg⁻¹ body weight 4 h before a urography was performed with injection of iohexol 700 mg I kg⁻¹ body weight. Twenty-five children received simultaneous injections of ⁹⁹mTc-DTPA 0.50 MBq kg⁻¹ body weight and iohexol 175 mg I kg⁻¹ body weight, and 11 children received simultaneous injections of ⁹⁹mTc-DTPA 0.25 MBq kg⁻¹ body weight and iohexol 700 mg I kg⁻¹ body weight for intravenous urography. The injected amount of ⁹⁹mTc-DTPA was measured by counting and weighing. The radioactivity in six plasma samples of 2 ml taken about 5, 15, 120, 150, 180 and 210 min after the injection, was counted in a well scintillation counter.

The iodine concentration was measured by X-ray fluorescence technique [7] in a 1 ml plasma sample taken about 3 h after the injection of iohexol. Before the injection of the isotope the children drank freely, but they were fasted during the examination time. The intravenous urography was performed as described in group 1.

A cubital indwelling cannula was used both for injection and blood sampling. Special precautions were taken to minimize the risk of contamination with the marker substance [8]. Except for the contrast medium injected simultaneously with the isotope and the extra blood samples, all examinations were performed according to our routine procedures.

The body surface area was determined according to Haycock et al. [9], and all GFR estimates are given per 1.73 m² body surface. Values are given as arithmetic means ±1 SEM and/or range. The Wilcoxon's signed-rank test for paired differences was applied for testing of significance. Using two-tailed test a probability of less than 0.05 was considered significant. Agreement between two measurements was estimated by the method of Bland and Altman [10]. Logarithmic transformed data were used for calculation of the 95% confidence interval [10].

The protocol was approved by the ethical committee of the hospital, and in each case informed consent was obtained from the parents.

Calculations

The distribution volume, in ml (V), was calculated from the empirical formula [2]:

\[
V = \frac{1000 \times \text{GFR}}{\text{Clearance of iohexol}}
\]
In group I the iodine concentrations measured in two plasma samples taken about three and four hours after injection of iohexol were used for calculation of the reference GFR for iohexol (GFR$_{ref}$) according to an open one-compartment model as described by Brochner-Mortensen [11]:

$$GFR_{ref} = \left( \frac{Q_m \times \beta}{C_m} \right) \times (0.99078 - \left( \frac{Q_m \times \beta}{C_m} \right)^2) \times 0.001218$$  \hspace{1cm} (2)

The reference GFR in group 2 (GFR$_{w\text{Tc}\text{-DTPA}}$) was estimated from the plasma elimination curves of $^{99m}$Tc-DTPA by a two-compartment model as described by Sapirstein et al. [5, 6].

In all children a preliminary single plasma sample GFR (GFR$_{inj}$) was calculated according to Jacobsson’s formula [1]:

$$GFR_{inj} = \frac{1}{t} \times \ln \left( \frac{Q_m}{V \times C_{ti}} \right) + 0.016$$  \hspace{1cm} (3)

where:

- $Q_m$ - injected amount of iodine in mg,
- $C_{ti}$ - plasma iodine concentration in mg/ml at time zero (intercept of back-extrapolated monoeXponential line with the ordinate on a semi-logarithmic plot),
- $\beta$ - slope of the monoeXponential line described above,
- $t$ - the time in min between injection of iohexol and blood sampling,
- $V$ - distribution volume in ml.

and corrected, as previously described [2], by the formula:

$$GFR_{inj} = 180 - 14.1 \times \frac{133}{GFR_{inj}}$$  \hspace{1cm} (4)

RESULTS

In group 1 ($n=96$) the average reference, two plasma sample GFR (GFR$_{ref}$) was 77±3 ml min$^{-1}$ 1.73 m$^{-2}$ (range 13–125 ml min$^{-1}$ 1.73 m$^{-2}$).

The average 3 h single plasma sample GFR (GFR$_{3h}$) was 76±2 ml min$^{-1}$ 1.73 m$^{-2}$. The difference between the mean values for GFR$_{ref}$ and GFR$_{3h}$ was 0.3 ml min$^{-1}$ 1.73 m$^{-2}$ with a 95% confidence interval from -1 to 2$\%$ (Fig. 2).

The average 4 h single plasma sample GFR (GFR$_{4h}$) was 77±3 ml min$^{-1}$ 1.73 m$^{-2}$. The difference between the mean values for GFR$_{ref}$ and GFR$_{4h}$ was -0.4 ml min$^{-1}$ 1.73 m$^{-2}$ with a 95% confidence interval from -2 to 1.5$\%$ (Fig. 3).

In subgroup 1a ($n=79$) the average reference two plasma sample GFR was 79±3 ml min$^{-1}$ 1.73 m$^{-2}$ (range 16–125 ml min$^{-1}$ 1.73 m$^{-2}$). The corresponding 2 h single plasma sample GFR (GFR$_{2h}$) was 75±3 ml min$^{-1}$ 1.73 m$^{-2}$. The difference between the mean values for GFR$_{ref}$ and GFR$_{2h}$ was 4.4 ml min$^{-1}$ 1.73 m$^{-2}$ with a 95% confidence interval from -4 to 3$\%$, a significant difference with $p<0.05$ (Fig. 4).

In subgroup 1b ($n=25$) the average 1 h, 2 h, 3 h and 4 h single plasma sample GFRs expressed in per cent of the reference GFR were 67±5, 96±2, 101±1 and 103±2, respectively (Fig. 5). The 1 h single plasma sample GFR was significantly lower than all the other GFRs.

![Fig. 2](image-url)
Fig. 3 The ratio between the reference GFR (GFR<sub>ref</sub>) and the 4 h single plasma sample GFR (GFR<sub>pl</sub>) against the average of the methods in 96 infants and children (group 1).

Fig. 4 The ratio between the reference GFR (GFR<sub>ref</sub>) and the 2 h single plasma sample GFR (GFR<sub>pl</sub>) against the average of the methods in 79 infants and children (subgroup 1a).

Fig. 5 Corresponding single plasma sample GFRs related to the sampling time after injection of furosemide in 25 children (subgroup 1b).

estimates. The 2 h single plasma sample GFR was significantly lower than both the 3 h and 4 h single sample estimates, while it was no significant difference between the 3 h and 4 h single sample GFRs.

In group 2 (n=47) the average GFR determined with isotope technique (GFR<sup>151</sup>Cr-EDTA) was 86±4 ml min<sup>-1</sup> 1.73 m<sup>-2</sup> (range 14-141 ml min<sup>-1</sup> 1.73 m<sup>-2</sup>). The average 3 h single plasma sample GFR was 83±4 ml min<sup>-1</sup> 1.73 m<sup>-2</sup>. The difference between the mean values of the two GFR estimates was 3.2 ml min<sup>-1</sup> 1.73 m<sup>-2</sup> with
DISCUSSION

This study confirms that the GFR in infants and children can be determined from a single plasma sample taken about 3 h after an intravascular injection of the non-ionic contrast medium iohexol. The empirical formulae necessary for calculation of a single plasma sample GFR were established in a preceding study [2], and their validity is confirmed in the present study. In 96 new patients (group 1), none of whom were included in the material forming the basis for the formulae, a high degree of agreement was obtained with an established two plasma sample iohexol method [11]. In another group of 47 children (group 2) similar results were obtained when the 3 h single plasma sample GFR was compared with a standard $^{99m}$Tc-DTPA method [5, 6].

This study also shows that a sampling time at about 3 h can be used for GFR levels down to 25 ml mm$^{-1}$ 1.73 m$^{-2}$. When plasma was sampled 2 h after the injection of the contrast medium the single plasma sample GFR was significantly lower than the reference GFR; the underestimation of the GFR being especially pronounced at GFR levels below 50 ml mm$^{-1}$ 1.73 m$^{-2}$ (Fig. 4). The GFR estimate by the single 1 h plasma sample was clearly unusable.

The present method for GFR estimation was made possible by the development of an easily managed X-ray fluorescence analyser for determination of plasma iodine concentrations, the ELX 84, (Elementanalys AB, Malmo, Sweden) [7]. The later model, Renalyzer PRX 90, (Provalid AB, Lund, Sweden) has a built-in computer that holds all the necessary formulae, i.e. formulae (2) to (4) in the preceding article [2]. Hence, the only data that have to be fed into the computer are age, height and weight of the child, time for injection, amount of contrast medium given, and the time for plasma sampling. The analyser can either operate on a single plasma sample basis, or with multiple plasma samples up to a total number of eight.

The GFR is the best parameter for assessment of the renal function [12], and with the X-ray fluorescence analyser at hand it was easy to add an estimate of the GFR to the radiological examinations with intravascular injection of contrast media. Initially, a two plasma sample method for infants and children was established [13, 14], but the advantage of a single plasma sample method is evident. Vein punctures in children are annoying, and may be difficult. Multiple blood samplings often fail, even from indwelling cannulas. Although the same cannula can be used both for injection and blood sampling [8] the possibility of marker contamination of the plasma sample can never be excluded. It is, therefore, safer to inject and sample by independent punctures. Therefore, our present routine procedure is to inject the contrast medium in a peripheral vein through a small ‘butterfly’ needle, and to sample the plasma by another puncture, which is preferably combined with blood sampling for other purposes.

Our GFR determinations were initially confined to patients undergoing intravenous urography. Later we have included children receiving iohexol for organ enhancement.
during computed tomography. Most of these patients have malignant diseases that are treated with nephrotoxic drugs. A simple method for repeat estimation of the GFR is, therefore, of practical value. However, since adverse effects are rare with the non-ionic contrast media [14], we have now also started to perform renal function studies in patients in whom no X-ray examinations are done. These patients receive smaller amounts of the contrast medium. As shown in the present study (group 2), single sample GFRs with acceptable accuracy were obtained with an iodine dose of 175 mg kg\(^{-1}\) body weight. In our study the downwards limitation of the iohexol dose was caused by the detection limit of the ELX 84 equipment, 0.033 mg ml\(^{-1}\). It has, however, been shown that reliable GFR estimates can be obtained with much lower doses (about 20 mg I kg\(^{-1}\) body weight) when the iohexol concentration in plasma is determined with high-pressure liquid chromatography technique [15]. The newer PRX 90-analysers, which has a detection limit of 0.013 mg I ml\(^{-1}\) has been shown to provide reliable measurements down to plasma iodine concentrations of about 0.04 mg ml\(^{-1}\). From our data it can be calculated that patients with normal GFR receiving 50 mg I kg\(^{-1}\) body weight will have plasma iodine concentrations above this level 3 h after the injection of iohexol.

Both in the preceding article [2] and in this one the amount of data at very low GFR levels are sparse. There are few children with uraemia, and contrast medium examinations are rarely indicated in advanced renal failure. Very low GFR values should, therefore, for the time being, be interpreted with caution.

In this study we have included babies with body weights as low as 4 kg. Smaller babies, as well as oedematous patients have not been examined. Hence, we cannot appraise the accuracy of our method in such patients.

REFERENCES


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The clearance of iohexol as a measure of the glomerular filtration rate in children with chronic renal failure

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The plasma clearances of technetium-99m-labelled DTPA (99mTc-DTPA) and the non-ionic contrast medium iohexol were estimated in 11 children with chronic renal failure for determination of the glomerular filtration rate (GFR). Equal values were obtained with the two substances provided plasma sampling was simultaneous, but when plasma was sampled within 3.5 h after injection of iohexol and 99mTc-DTPA the GFR was overestimated by more than 50%. For clearance values below 20 ml min⁻¹ 1.73 m⁻², valid GFR estimates were obtained both from two plasma samples taken 3 h and 24 h after the injection of iohexol and from a single plasma sample taken 24 h after the injection.

Key words: clearance; glomerular filtration rate; iohexol; urography; X-ray fluorescence technique

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Jacobsson's method for estimation of the glomerular filtration rate (GFR) from a single plasma sample [1] has recently been modified for use in infants and children [2]. For GFR levels down to about 20 ml min⁻¹ 1.73 m⁻² it was shown that GFR could be determined from a plasma sample taken about 3 h after injection of the non-ionic contrast medium iohexol (Omnipaque, Nycomed, Oslo, Norway) [3]. In the present study we have evaluated the usefulness of the single plasma sample method in uraemic children.

Previous investigations [1, 4-6] have shown that during renal failure a later sampling time is necessary to obtain a correct GFR estimate. Therefore, one aim of this study was to establish the optimal time interval between iohexol injection and plasma sampling.

PATIENTS AND METHODS

Ten boys and one girl were included in the study. One boy (patient 7/10) was examined twice. None of the patients had oedema and all had normal body height/weight proportions. The average age was 9±1 years, body weight 27±3 kg and plasma creatinine concentration 399±48 µmol l⁻¹ (Table I).

The plasma clearance of 99mTc-DTPA (Amerscan Pentetate DII, Amersham International, Amersham, UK) was estimated in all the children (but only once in patient 7/10).
Table I. Age, body weight, plasma creatinine concentration and estimates of the plasma clearance of iohexol and \[^{99mTc}\]DTPA in 11 children

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (years)</th>
<th>Body weight (kg)</th>
<th>Creatinine concentration ((\mu mol l^{-1}))</th>
<th>Reference clearance (3 to 24 h)</th>
<th>Two samples (3 to 24 h)</th>
<th>Single sample 3 h corrected</th>
<th>24 h</th>
<th>Six samples (0.1) to (3.5) h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>47</td>
<td>554 (598)</td>
<td>4.1</td>
<td>4.1</td>
<td>8.2</td>
<td>2.2</td>
<td>10.4</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>21</td>
<td>615 (603)</td>
<td>5.7</td>
<td>5.6</td>
<td>10.8</td>
<td>4.6</td>
<td>9.3</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>19</td>
<td>523 (561)</td>
<td>6.7</td>
<td>6.4</td>
<td>15.3</td>
<td>6.1</td>
<td>10.1</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>27</td>
<td>665 (601)</td>
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<td>6.8</td>
<td>14.5</td>
<td>6.5</td>
<td>8.2</td>
</tr>
<tr>
<td>5</td>
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<td>312</td>
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<td>23.2</td>
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<td>363</td>
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<td>21.6</td>
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<td>31.1</td>
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<td>32</td>
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<td>29.5</td>
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<td>21</td>
<td>338 (317)</td>
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<td>23.3</td>
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<td>22.0</td>
</tr>
<tr>
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<td>11</td>
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<td>530 (539)</td>
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<td>7.7</td>
<td>16.1 (10.9)</td>
</tr>
<tr>
<td>12</td>
<td>14</td>
<td>32</td>
<td>518 (500)</td>
<td>10.5</td>
<td>10.1</td>
<td>13.5</td>
<td>10.8</td>
<td>18.0 (13.6)</td>
</tr>
</tbody>
</table>

*One patient was examined twice.
†Values in brackets are plasma creatinine concentrations 24 h after the injection of iohexol.
‡Values in brackets are calculated from six plasma samples taken from 0.1 to 20 h after the injection of \[^{99mTc}\]DTPA. The reference clearance was estimated from all plasma samples taken between 3 and 24 h after injection of iohexol. All clearances in ml min \(^{-1}\) 1.73 m\(^2\).
Altman [10], and logarithmic transformed data were used for calculation of the 95% confidence interval [10].

The protocol was approved by the ethical committee of the hospital, and informed consent was obtained from both the children and their parents.

Calculation:

The plasma clearance of $[^{99m}Tc]$DTPA was estimated from the plasma elimination curves by a two-compartment model as described by Sapirstein et al. [11] and Rootwelt et al. [12]. The multiple plasma sample clearance of iohexol was determined according to an open one-compartment model as described by Brøchner-Mortensen [13]:

$$ C_l = \left( \frac{Q_{10}}{C_{10}} \right) \times 0.99 \times 28 - \left( \frac{Q_{10}}{C_{10}} \right) \times 0.001218 $$

With this method six values were estimated in each child, depending on the sampling time and the number of plasma samples used for the calculations. The plasma clearance estimated from all samples taken between 3 and 24 h (6.3±0.3 samples, range 4-7) was used as the reference value ($C_{10}$). Clearances based on two plasma samples were calculated from the samples taken at 3 and 8 h, 3 and 24 h, 5 and 24 h and 8 and 24 h after the injection. The sixth value was estimated from the four samples taken at 120, 150, 180, and 240 min, which were also used for estimation of the standard $[^{99m}Tc]$DTPA clearance.

The single plasma sample clearance ($C_{1s}$) was calculated according to Jacobsson's formula [1]:

$$ C_{1s} = \frac{f}{t} \times \ln \left( \frac{Q_{10}}{V \times C_{1s}} \right) - \frac{0.0016}{V} $$

$Q_{10}$ - injected amount of iohexol in mg;
$C_{10}$ - plasma iohexol concentration in mg ml$^{-1}$ at time zero (intercept of back-extrapolated monoexponential line with the ordinate on a semilogarithmic plot);
$\beta$ - slope of the monoexponential line described above,
$t$ - the time in min between injection of iohexol and blood sampling,
$C_{1s}$ - plasma iohexol concentration in mg ml$^{-1}$ at time $t$;
$V$ - distribution volume in ml

An empirical estimate for the distribution volume ($V$) was calculated from the following equation [2, 3]:

$$ V = 231 \times \text{kg body weight} + 1215 $$

Single plasma sample clearances were calculated from the iodine concentrations measured in the plasma samples taken 3, 5, 8 and 24 h after the injection, without ($C_{1s}$) and with ($C_{1s}$) correction according to the equation [2, 3]:

$$ C_{1s} = 100 - 14.1 \sqrt{V(3-1)} - C_{1s} $$

RESULTS

The average reference iohexol plasma clearance ($C_{10}$) was 12.5±2.3 ml min$^{-1}$ 1.73 m$^{-2}$ (Table 1).

There was no significant difference between the simultaneously measured (within 3.5 h) clearances of $[^{99m}Tc]$DTPA (six plasma samples: 17.2±2.3 ml min$^{-1}$ 1.73 m$^{-2}$) and iohexol (four plasma samples: 15.9±2.3 ml min$^{-1}$ 1.73 m$^{-2}$). However, both these clearances were significantly higher than $C_{10}$ (Table 1). For $C_{10}$ levels below 20 ml min$^{-1}$ 1.73 m$^{-2}$ the clearance obtained with the standard $[^{99m}Tc]$DTPA method was 66±15% higher, and with the contemporaneous iohexol method 46±14% higher than $C_{10}$. With plasma sampling up to 20 h (patients 11 and 12) the $[^{99m}Tc]$DTPA results were about one-third higher than those obtained with the reference iohexol method (Table 1).

The clearance estimated from the two plasma samples taken 3 and 8 h after the injection (13.8±2.1 ml min$^{-1}$ 1.73 m$^{-2}$) was significantly higher than $C_{10}$. However, there were no differences between $C_{1s}$ and the estimates obtained from the two plasma samples taken 5 and 24 h (12.5±2.3 ml min$^{-1}$ 1.73 m$^{-2}$) and 8 and 24 h (12.6±2.4 ml min$^{-1}$ 1.73 m$^{-2}$) after the injection.

The two-sample clearance ($C_{1s,3-24h}$) estimated from the 3-h and 24-h plasma samples (11.9±2.1 ml min$^{-1}$ 1.73 m$^{-2}$) was significantly lower (5±1%) than $C_{10}$. The difference between the mean values of $C_{10}$ and $C_{1s,3-24h}$ was 0.5 ml min$^{-1}$ 1.73 m$^{-2}$ with a 95% confidence interval from -2 to 3%.

Both the 3-h single plasma sample clearance (0.1±3.2 ml min$^{-1}$ 1.73 m$^{-2}$) and the 3-h
corrected (by equation 3) single plasma sample clearance (17.6±2.0 ml min⁻¹ 1.73 m⁻²) were significantly different from $C_{\text{ref}}$. The 5-h single-sample value (7.6±3.4 ml min⁻¹ 1.73 m⁻²) was significantly lower than $C_{\text{ref}}$, while there was no significant difference between $C_{\text{ref}}$ and the 8-h single plasma sample clearance (11.3±3.1 ml min⁻¹ 1.73 m⁻²). When the 5-h and 8-h single plasma sample values were corrected according to equation (3), both corrected values were significantly higher than $C_{\text{ref}}$. 22.3±2.2 ml min⁻¹ 1.73 m⁻² and 24.6±2.0 ml min⁻¹ 1.73 m⁻² respectively.

There was no significant difference between the (uncorrected) 24-h single plasma sample clearance (12.5±2.5 ml min⁻¹ 1.73 m⁻²) and $C_{\text{ref}}$. The difference between the mean values of $C_{\text{ref}}$ and $C_{\text{ioh}}$ was 0.05±0.3 ml min⁻¹ 1.73 m⁻². The corrected (by equation 3) 24-h single plasma sample value (25.3±1.6 ml min⁻¹ 1.73 m⁻²) was significantly higher than $C_{\text{ref}}$.

In patients 1–4 and 10–12 (n=7) the plasma concentration of creatinine was similar before and 24 h after the injection of iohexol, 514±32 μmol 1⁻¹ and 522±37 μmol 1⁻¹ respectively (Table I).

**DISCUSSION**

It is well established that late plasma sampling is a necessity for correct estimation of the glomerular filtration rate when plasma disappearance methods are used in patients with renal failure [1, 4–6]. The present study confirms that this is true also in children.

At GFR levels below 30 ml min⁻¹ 1.73 m⁻² the standard [™Tc] DTPA method (with plasma samples taken within 3.5 h after the injection) overestimated the GFR by more than 50%. Thus the standard [™Tc] DTPA method should not be used for estimation of GFR in uraemic children. With later sampling times, however, the results in patients 11 and 12 indicate that the accuracy of the [™Tc] DTPA method increases. A similar conclusion can also be drawn from the results obtained with iohexol. When the four early plasma samples (2 to 3.5 h) were used the GFR was overestimated by nearly 50%. This indicates that the inaccuracy of the two sets of results are related to the early sampling time, and not to the marker substances themselves.

Our results confirm the importance of using an appropriate sampling time for the single plasma sample method: with decreasing GFR values a longer interval between the injection and the plasma sampling is needed. The results also show that correction equation 3, which was developed for a sampling time of about 3 h and for GFR levels above 20 ml min⁻¹ 1.73 m⁻² [2], cannot be used in uraemic children. It is further shown that when GFR is below 20 ml min⁻¹ 1.73 m⁻² the reference clearance time would be still later. This is illustrated in patient 1, in whom the considerably underestimated $C_{\text{ioh}}$ (Table I) probably indicates that the plasma had been sampled too early. According to Jacobsson [1] the optimal sampling time for the actual GFR level would have been 44 h.

The multiple plasma sample method has the advantage of being independent of the empirical formula (equation 2) for the distribution volume of iohexol [2, 3], and the present study confirms that two plasma samples suffice for the purpose [5, 8, 14], provided that the sampling time is appropriate for the actual GFR level. Thus, both the 5 h and 24 h, and the 8 h and 24 h clearances were almost identical to the reference iohexol clearance.

For practical reasons the 3-h and 24-h two plasma sample clearance ($C_{\text{ioh},24\,\text{h}}$) has been given special attention. The single plasma sample method for estimation of GFR levels above 20 ml min⁻¹ 1.73 m⁻² is based upon a sample taken about 3 h after the injection of iohexol [2, 3], and it is convenient to maintain 3 h as the time for the first plasma sampling in all patients. It is true that the 3-h (corrected) single plasma sample value should not be used at low GFR, however valid GFR estimates are obtained when the 3-h sample is combined with a later additional sample. Although the 3-h and 24-h clearance were significantly lower than the reference clearance the difference was only 5%, which is considered to be without clinical importance.

It is well established that contrast media may exert nephrotoxic effects [15]. But the frequency
with which they occur, the identification of the patients at risk and their predisposing conditions are matters of dispute [16–19]. However, children seem to be less prone to this complication, and we have not been able to find reports of severe nephrotoxic effects in this age group following injection of non-ionic contrast media. In our study the plasma concentration of creatinine was not significantly increased 24 h after the injection of iohexol in the seven patients examined. These children had an average GFR of 7.7 ml min⁻¹·1.73 m², and it can be calculated that a 50% GFR reduction of 24 h duration would have increased their plasma creatinine concentration by at least 250 μmol l⁻¹.

Children with chronic renal failure rarely undergo X-ray examinations with injection of contrast media. On the other hand, estimation of the plasma clearance of a contrast medium with X-ray fluorescence technique is easily performed and may be an alternative to the high-pressure liquid chromatography [20] or radionuclide methods [21]. However, because of possible nephrotoxic effects of iohexol [18], the dose should be kept as low as possible. A practical problem then arises as the dose necessary to ensure an adequate plasma iodine concentration 24 h after the injection depends on the GFR, which is to be measured. Calculated according to Jacobsson's formula [1] the iodine dose needed to obtain a concentration of 0.05 mg ml⁻¹, which is equivalent to three times the detection limit for the X-ray fluorescence equipment (Renalyzer PRX 90; Provalid AB, Lund, Sweden) [3], is shown in Figure 1. Although the plasma creatine concentration is generally a poor indicator of the GFR [22], it is more predictive in the low GFR range. Thus, in adults a concentration above 250 μmol l⁻¹ will indicate a GFR of less than 25 ml min⁻¹ [23] in our experience a plasma creatinine concentration of 200 μmol l⁻¹ in children will correspond to a GFR below 20 ml min⁻¹·1.73 m². Hence, for plasma creatinine concentrations above 250 μmol l⁻¹, and if the sampling time is planned to be 24 h, it is appropriate to use an iohexol dose corresponding to 175 mg l kg⁻¹ for a patient weighing 10 kg (corresponding to a 1 to 2-year-old child), linearly decreasing to about 100 mg l kg⁻¹ for a 50 kg patient (Fig. 1).

The results of the present study can be summarized in the following recommendations:

1. In children with a plasma creatinine concentration above 200 μmol l⁻¹ the plasma clearance of iohexol can be estimated from a single plasma sample taken 24 h after the injection.
2. If the plasma creatinine concentration is not

![Figure 1](image-url)
known in advance, and the corrected 3-h single plasma sample clearance (calculated from the equations 1–3) is below 25 ml min⁻¹ 1.73 m⁻², an additional plasma sample should be taken 24 h after the injection. Two valid estimates will then be obtained, the first from the 3-h and 24-h two plasma samples and the second from the 24-h single plasma sample.

REFERENCES


