Upsala J Med Sci 83: 53–63, 1978

The Usefulness of ^{125}I-sodium Iothalamate as a GFR-indicator in Single Intravenous Injection Tests

TORGNY GROTH and BERTIL TENGSTRÖM

From the Group for Biomedical Informatics, Uppsala University Data Center, Uppsala, and District General Hospital, Skövde, Sweden

ABSTRACT

Clearance of sodium iothalamate was estimated from plasma elimination curves obtained from 90 patients having varying renal function, after a single intravenous injection of ^{125}I-sodium iothalamate. The usefulness of sodium iothalamate as a GFR-indicator with this technique was tested by a strict statistical comparison with conventional inulin clearance as a reference. The regression line, covering the clearance range 2–163 ml min^{-1}, $Cl_{inulin} = 1.08 \cdot Cl_{sodiumiothalamate} - 3.7$, ($n = 84$, $r = 0.85$, SEE = 2.3 ml · min^{-1}) was not significantly different from the identity line, which means (i) that extrarenal elimination could not be detected, and (ii) that I^{125}-sodium iothalamate should be regarded as a useful glomerular marker for single intravenous injection studies.

An open two-compartment model of mamillary type was found to give an adequate representation of the plasma disappearance curve. The results are not critically dependent on the choice of approximating function. The method by Sapirstein et al., the method by Nosslin and a power-law method were used for comparison and were found to give the same results. However, the present type of curve fitting analyses requires frequent blood sampling or external counting over a long period in order to give reliable estimates of GFR. This circumstance makes these methods less attractive for clinical use.

INTRODUCTION

The glomerular filtration rate (GFR), which is one important measure of the renal function, is commonly considered to be determined accurately by inulin clearance. An attractive alternative to the classical method (35, 36) is to estimate GFR from the plasma disappearance curve after a single intravenous injection of a suitable substance (for a survey, see e.g. (8, 12)).

Evidence is available that sodium iothalamate, a substance originally introduced as an X-ray contrast medium, fulfils the requirements which can be demanded of a good GFR-indicator in the classical sense (cf. (37)). Clearance studies with sodium iothalamate in man using (i) the classical method with constant infusion and urine collection (9, 11, 22, 23, 25, 33, 34, 2, 15, 22), and using (ii) a single *subcutaneous* injection technique with urine collection (1, 18) have shown good agreement between the clearance of sodium iothalamate and inulin.

No direct evidence is available that sodium iothalamate is a good GFR-indicator in tests based on a single *intravenous* injection, although good correlations to endogenous creatinine clearance (9, 31) and to continuous infusion clearance of iothalamate (29) have been reported. Accurate estimation of GFR from the disappearance curve after a single intravenous injection requires that the substance used is not eliminated extrarenally. This has been questioned for sodium iothalamate (cf. (18)), but, to the authors' knowledge, it has not been adequately investigated so far. In passing it may be mentioned that in experiments with aglomerular fish and in stop-flow studies in dogs, no extrarenal elimination was found, nor was any significant renal tubular secretion or reabsorption demonstrated (16).

The primary aim of the present investigation was to perform a test of the usefulness of sodium iothalamate as a GFR-indicator in single intravenous injection tests, by comparing GFR as estimated from the plasma disappearance curve with conventional inulin clearance. Within this scope a comparison is made, from a theoretical and numerical point of view, between various methods of analysing plasma disappearance data in terms of renal clearance.

EXPERIMENTAL METHODS

Ninety adult patients with varying renal function were studied. Inulin clearance, Cl_{in}, was determined with constant infusion technique. The collection of urine was carried out via an indwelling catheter during three 20-minute periods. The clearance values ranged between 2 and 163 ml/min (cf. Fig. 3).

Inulin in plasma and urine was determined with a diphenylamine method, essentially according to Corcoran & Page (13). The standard error of the triplicate determinations of inulin clearance in the same subject was 6.4 ml/min, corresponding to an average coefficient of variation of 0.094.

Simultaneous with the start of the inulin infusion, 10 μCi of sodium iothalamate-^{125}I (The Radiochemical Centre, Amersham, England) diluted with 2–4 ml of physiological saline was given as a rapid intravenous injection in one arm. Plasma samples were collected from the opposite arm 5 minutes after the injection and at 30 minute intervals during 4 hours. In 3 cases the initial phase, 0–10 minutes, was investigated in greater detail. The radioactivity of 2 ml plasma samples was determined in a gamma radiation spectrometer.

According to the manufacturer the labelled sodium iothalamate contains less than 1% of free iodine and is stable for at least 3 months. In order to block possible thyroidal uptake of radioactive iodine the patients were given capsules containing 80 mg of potassium iodide the night before the investigation.

THEORY

Methods for calculation of clearance

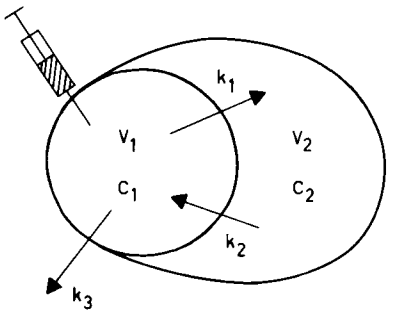
According to the Stewart-Hamilton dye dilution formula (cf. (41, 42)) total plasma clearance is theoretically calculated from the ratio between the dose given, Q, and the area under the plasma concentration curve

$$\text{clearance}=\frac{Q}{\int_0^\infty C(t)\,dt} \tag{1}$$

For practical application of this formula a reliable estimation of the integral $\int_0^\infty C(t)dt$ requires consideration of (i) the spacing in time of the experimental points on the plasma curve, and (ii) the choice of approximating function.

Simple numerical integration routines, such as the trapezoidal method and Simpson's method, require that the plasma curve is followed for "infinite time" (or until the plasma concentration is less than a small preset value). This is not an attractive alternative. Useful integration procedures must be based on mathematical functions, which approximate the measured plasma concentration data satisfactorily and which can be used to extrapolate the same data reliably outside the experimental time domain. For this purpose it is necessary to make assumptions about the processes underlying the distribution and elimination of the test substance.

The Integral Method of Nosslin (26, 27) calculates total plasma clearance from the area under the normalized plasma curve and the "plasma volume":



Volumes of distribution: V_1 , V_2
Concentration of tracer: C_1, C_2
Rate constants: k_1, k_2, k_3

Fig. 1. The open two-compartment model and symbols used in eqs. (8–11).

$$\text{clearance}=\frac{V}{\int_0^\infty C'(t)\,dt} \tag{2}$$

V=plasma volume

$$C'(t)=C(t)/C_0 \tag{3}$$

$$C_0=Q/V \tag{4}$$

i.e. the substance is postulated to distribute primarily in the plasma volume. The area can be estimated in various ways, e.g. from the exponential components of the curve as analysed by peeling-off methods:

$$C'(t)=\sum_i A_i' e^{-\gamma_i t};\ (\sum_i A_i'=1) \tag{5}$$

$$\int_0^\infty C'(t)=\sum\frac{A_i}{\gamma_i} \tag{6}$$

The total distribution volume:

$$D=V\frac{\Sigma(A_i'/\gamma_i)^2}{(\Sigma A_i'/\gamma_i)^2} \tag{7}$$

So far, sums of negative exponentials have mostly been used in the analysis of single-injection curves, a choice which has been guided by the well-known circumstance that these functions build up the analytical solutions of the kinetic equations of compartmental models.

The Present Compartmental Analysis of the plasma elimination curve of iothalamate is based on an open two-compartment model of the mamillary type (Fig. 1). Models of catenary type, i.e., with compartments connected in series, seem less plausible from physiological considerations, although there is a lack of more quantitative data for a direct test of various possible compartmental models (cf. (14)).

The following conventional (but still questionable) assumptions are made: (i) the mixing of tracer within each compartment is very rapid compared with the elimination from and exchange between the two compartments, resulting in a homogeneous distribution of tracer for both

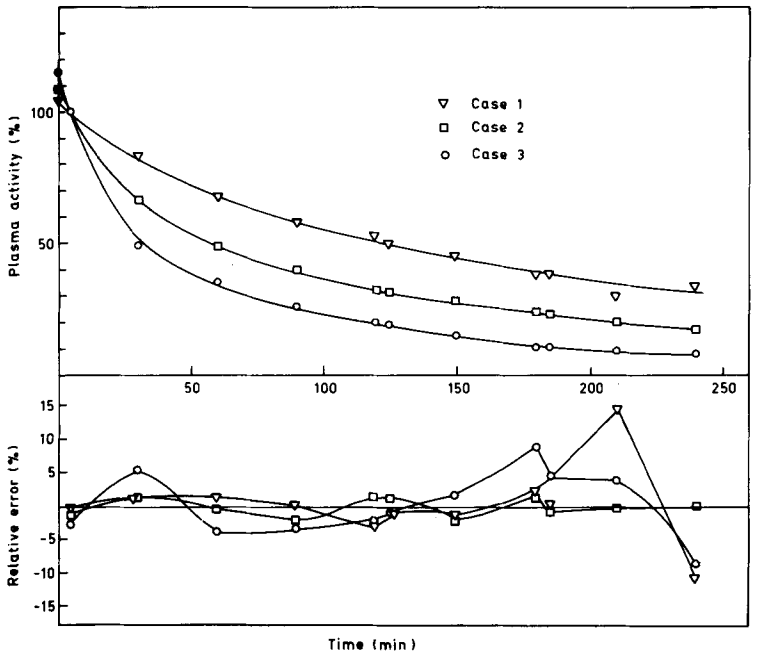


Fig. 2. Plasma disappearance data from three typical single injection experiments with ^{125}I-sodium iothalamate. The symbols denote the experimental points in percentage of the concentration of the first sample (t=5 min). The virtual initial concentrations (filled symbols) were calculated by exponential extrapolation. The drawn lines illustrate the theoretical curves as calculated from the two-compartment model and fitted to the experimental data in a least-square sense.

compartments; (ii) the flux of tracer leaving a compartment is proportional to the concentration of tracer in that compartment, i.e. the rate constants k_i, (min^{-1}) i=1–3 are lumped parameters including transport by bulk flow, diffusion and other possible transport processes which can be described in this way; (iii) the rate constants and the volumes of distribution are constant in time.

The kinetic equations for the model are

$$\frac{dC_1}{dt}=k_1(C_2-C_1)-k_3C_1 \quad (8)$$

$$\frac{dC_2}{dt}=k_2(C_1-C_2) \quad (9)$$

with the starting conditions

$$C_1(0)=C_0 \quad (10)$$

$$C_2(0)=0 \quad (11)$$

(for symbols see Fig. 1)

The virtual initial concentration C_0 was estimated from the first two points (t=5 min and 30 min) by exponential extrapolation to zero time. The eqs. (8, 9) can be solved easily by both analytical and numerical integration (cf. (7)), giving the time course of the concentration of tracer in the two compartments. A fourth order Runge-Kutta method was used here with a step length Δt=1 min. The rate constants, k_1, k_2 and k_3, were estimated by fitting the integrated function C_1 (eq. 8) to the plasma tracer data (normalized to 100 at t=5 min) in a least-square sense.

The primary volume of distribution, V_1, was estimated as the ratio between the given dose (10 μci (7.68±0.41)×10^6 cpm) and the virtual initial concentration of tracer, as calculated by extrapolation, to zero time (see Table I and discussion below). Clearance is then given by the product k_3V_1 (ml/min). The secondary volume of distribution, V_2, was estimated from the steady-state condition $k_1V_1=k_2V_2$.

The Method of Sapirstein et al. (32) is based on a two-compartment model of the same kind as described above. However, the plasma elimination curve is separated in two exponential terms by peeling-off analysis anf distribution volumes and GFR are then calculated from explicit expressions, viz.

the plasma elimination curve: $C(t)=A_1e^{-\gamma_1 t}+A_2e^{-\gamma_2 t}$ (12)

the glomerular filtration rate : $G=\frac{I\gamma_1\gamma_2}{A_1\gamma_2+A_2\gamma_1}$ (13)

where I=initial dose of tracer.

The primary volume of distribution: $V_1=\frac{I}{A_1+A_2}$ (14)

The intercompartmental clearance: $\alpha=\frac{V_1(A_1\gamma_1+A_2\gamma_2)}{A_1+A_2}-G$ (15)

The second volume of distribution: $V_2=\frac{\alpha G}{V_1\gamma_1\gamma_2}$ (16)

"Random-walk" models have also been proposed for studying the removal of tracers from the blood stream (see, e.g., (3, 4, 5, 39, 40)). The simple interpretation in terms of physiological compartments is replaced by the concept that individual test molecules move through the physiological system in a kind of a "random-walk" manner. The passage times of the molecules should then follow a gamma distribution;

$$C(t)=At^{-\alpha}e^{-\beta t} \quad (17)$$

Table I. *"Best" estimates of the parameters in the two-compartment model for three typical cases*

	Case 1	Case 2	Case 3
$k_1(min^{-1})$	0.004±0.004	0.011±0.001	0.020±0.004
$k_2(min^{-1})$	0.02±0.02	0.024±0.004	0.031±0.007
$k_3(min^{-1})$	0.0050±0.0004	0.0086±0.0004	0.0155±0.0009
V_1 (ml)	8 100±400	7 400±400	7 100±400
V_2 (ml)	$0.20 \cdot V_1$	$0.46 \cdot V_1$	$0.65 \cdot V_1$
Clearance			
$k_3 \cdot V_1$ (ml/min)	40±4	63±5	110±9

The analysis was performed by fitting this function to the plasma data in the interval t=5–240 min. The area was calculated by numerical integration of the fitting function over the interval t=5–2 000 min and by adding an estimate of the initial part (a triangle of base 5 min and height C^*).

In the present study preference was given in the first place to the compartmental analysis approach and the other alternatives were used for comparative purposes.

RESULTS

Adequacy of the two-compartment model

The multiexponential appearance of the plasma elimination curve suggests models with at least two mixing compartments in order to reach compatibility with the data. The compatibility of the two-compartment model with the tracer data of three typical cases is illustrated in Fig. 2. The corresponding "best" estimates with standard errors are given in Table I. As can be seen from the standard errors, the parameters are well determined by the data, i.e. the proposed two-compartment model is an *adequate* model for the three cases studied. The elimination rate constant, k_3, is the parameter, which is best determined by the data. A manual peeling-off analysis of three cases (4, 5 and 6), which were studied in greater detail during the first 10 min after tracer injection, revealed three exponential terms (see Fig. 5). However, a three-compartment model is not an adequate description, since one single plasma disappearance curve does not permit a confident estimation of all the parameters of such a complex model (cf. also (21, 24)). A two-compartment model is, on the other hand, simple enough to be well determined by the data and complex enough to give compatibility to the data (cf. Fig. 4).

Table II. *Estimates of the glomerular filtration rate according to different methods for the analysis of plasma elimination curves*

Methods	Estimates of GFR (ml/min)		
	Case 1	Case 2	Case 3
Two-compartment model; direct fit	40±4	63±5	110±9
Method of Sapirstein et al.	42	63	114
Nosslin's method	42	61	113
"Random-walk" model	42	63	107
Conventional inulin clearance	46	69	163

Comparison of various methods

In Table II, estimates of GFR are compared as calculated from the two-compartment analysis, the methods by Sapirstein et al. (32) and Nosslin (26), and by using a gamma distribution as approximating function ("random-walk" model).

The differences between the estimates of GFR are not significant and the choice of method does not seem critical for the analysis of data of the type represented by the three cases chosen (cf. page 59). By expressing the elimination rate constant of the present two-compartment model in terms of the intercepts and slopes from a peeling-off analysis

$$k_3=\frac{\gamma_1\gamma_2(A_2+A_1)}{A_1\gamma_2+A_2\gamma_1} \qquad (18)$$

$\text{GFR}=k_3 \cdot V_1=\frac{I\gamma_1\gamma_2}{A_1\gamma_2+A_2\gamma_1}=G$ according to Sapirstein et al. (32), it is obvious that this method and Sapirstein's method are equivalent from the theoretical point of view. The difference between the two methods is only due to the uncertainty introduced by the numerical methods. Furthermore, it can be noted that for a two-exponential peeling-

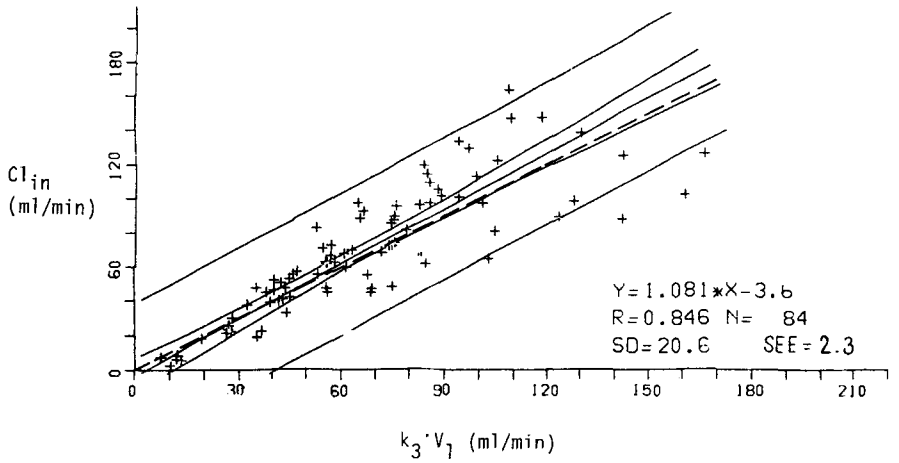


Fig. 3. Graphical illustration of the relation between inulin clearance (normalized to 1.73 m²) and the theoretical measure k_3V_1 as estimated for the patients examined. The crosses (+) indicate patients included in the study (estimated V_1+V_2 <34% body weight), and the lines indicate the regression line with its 95% confidence region, and the 95% confidence region of the points. The identity line is dashed.

off analysis and using V_1 as an estimate of the "plasma volume", Nosslin's (27) method is equivalent to the method by Sapirstein et al. (32) concerning total clearance and total volume of distribution.

Comparison between theoretical measures of GFR and inulin clearance

The validity of the theoretical measure of glomerular filtration rate, as calculated from the two-compartment model (k_3V_1), was tested by comparison with standard inulin clearance. The statistical analysis was made on data from 90 patients with certain constraints. Thus patients with a calculated GFR greater than 180 ml/min were not included. These cases are beset with the greatest uncertainty in the calculations of k_3V_1, which is reflected in extreme estimated values of the total distribution volume (greater than 34% of body weight, compared with the average of about 18–20%, Fig. 6). The regression analysis was performed with due consideration of the errors in both the ordinata and the abscissa; in this case $\sigma_x \simeq \sigma_y$.

The correlation between standard inulin clearance and the estimated GFR was rather good, with a coefficient of correlation $r=0.85$, a standard deviation of an observed inulin clearance (S.D.) equal to 20.6 ml/min, and a standard error of the estimated GFR (SEE) equal to 2.3 ml/min, $n=84$. The regression line, $Cl_{in}=1.081\ (k_3V_1)-3.7$, is not signif-

Table III. *"Best" estimates of the parameters in the two-compartment model for three typical cases where data from the initial part, $t \leq 5$ min, are available*

	Data	k_1 (min^{-1})	k_2 (min^{-1})	k_3 (min^{-1})	V_1 (ml)	$k_3 \cdot V_1$ (ml/min)	Cl_{in} (ml/min)	Total volume of distr. $V_1(1+\frac{k_1}{k_2})$ (ml)
Case 4	$t \geq 5$ min	0.021	0.028	0.008	9 746	82	69	$1.77 \cdot V_1 = 17\ 250$
	$t \geq 1$ min	0.238	0.097	0.023	4 009	91		$3.44 \cdot V_1 = 13\ 800$
Case 5	$t \geq 5$ min	0.024	0.021	0.016	6 222	99	116±19[a]	$2.15 \cdot V_1 = 13\ 400$
	$t \geq 1$ min	0.101	0.052	0.026	4 029	105		$2.94 \cdot V_1 = 11\ 800$
Case 6	$t \geq 5$ min	0.012	0.027	0.011	9 452	103	91	$1.45 \cdot V_1 = 13\ 700$
	$t \geq 1$ min	0.139	0.107	0.020	5 258	106		$2.30 \cdot V_1 = 12\ 100$

[a] Not measured because of experimental difficulties, estimated to 116±19 ml/min.

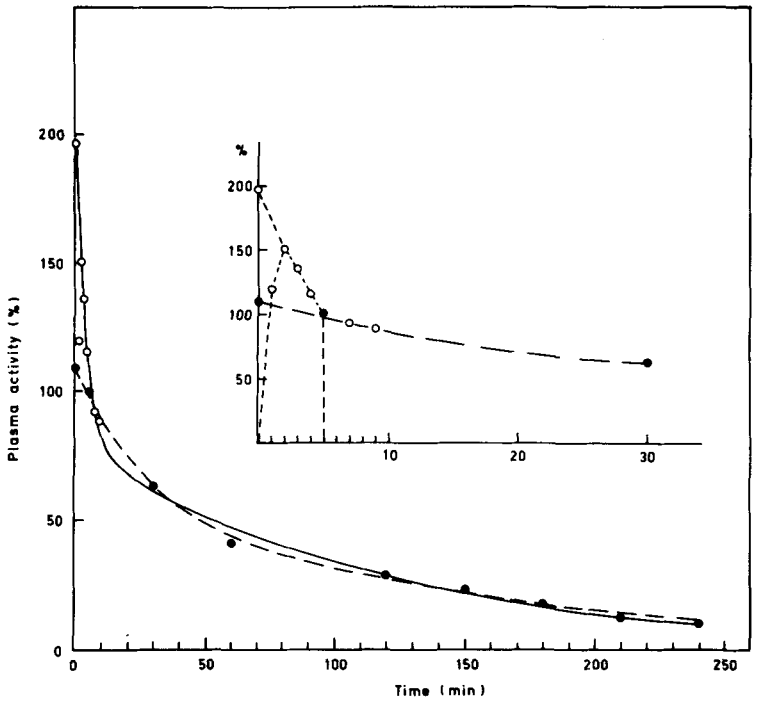


Fig. 4. A comparison between theoretical curves from the two-compartment model as fitted to data $t>5$ min (●) and to additional data from the initial phase of mixing (○). The virtual initial concentration is indicated on the ordinata, as estimated by backward exponential extrapolation. The experimental data (case 6) are expressed in percent of the concentration at $t=5$ min. The inserted figure illustrates three ways of estimating the area under the initial part of the curve (i) by the trapezoidal rule; (ii) by using data for $t \geq 5$ min and exponential extrapolation backwards and (iii) by extrapolation of data for $t=$ 0–5 min.

icantly different from the regression line, $Cl_{in}=0.904\ (k_3V_1)+8.3$, $r=0.85$, S.D.=19.7 ml/min, $n=84$, SEE=2.2 ml/min, that is obtained when the error of the calculated GFR is neglected. This is commonly done for reasons of simplicity in the calculations, but is not correct from a statistical point of view. The regression line is not significantly different from the identity line in either of these two versions of analysis as judged from the 95% confidence limits of the regression lines (see Fig. 3).

As a further illustration of the influence of the choice of statistics, it may be mentioned that a simple regression analysis ($\sigma_x=0$) on 82 cases with a calculated GFR less than 150 ml/min gave the result $Cl_{in}=1.008\ (k_3V_1)+2.5$, $r=0.87$, S.D.=18.4 ml/min, SEE=2 ml/min.

By normalization of inulin clearance to 1.73 m^2 body surface area, the correlation to the correspondingly normalized measure of GFR ($=k_3$, i.e. with the primary distribution volume normalized to unity) was improved; $r=0.90$, S.D.=11 ml/min/1.73 m^2, SEE=1.2 ml/min/1.73 m^2, $n=82$. This improvement may indicate that the calculated V_1-values are poor estimates of the primary distribution volume, and that the true value of this volume is better correlated to body surface area.

Sources of error and uncertainty in the estimation of GFR and distribution volumes from a single injection curve

The variance of GFR as estimated from eq. (1) is equal to the sum of the variances of the injected dose and the estimated area. The former should be kept within reasonable limits by a careful injection

Table IV. *"Best" estimates of the parameters in the "random-walk" model for three typical cases*

$C(t)=At^{-\alpha}e^{-\beta t}$ $t>5$ min	Case 4	Case 5	Case 6
A	1.53±0.05	2.01±0.13	1.40±0.06
α	(2.64±0.17)10^{-1}	(4.37±0.35)10^{-1}	(1.86±0.24)10^{-1}
β	(2.66±0.38)10^{-3}	(2.92±0.91)10^{-3}	(6.12±0.61)10^{-3}
GFR (ml/min)	73	90	102

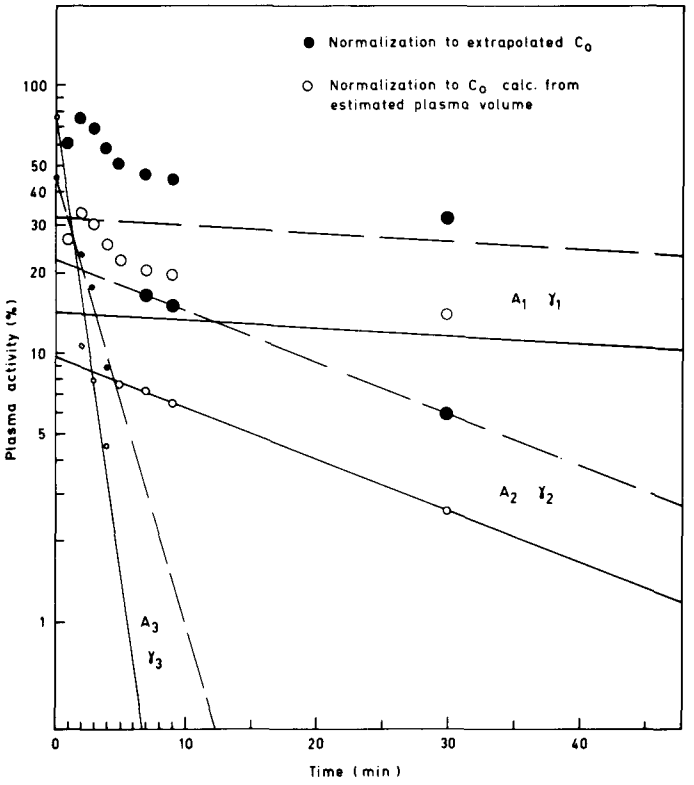


Fig. 5. Plasma disappearance data from one (no. 6) of the three cases (4, 5 and 6), which were studied in more detail during the first ten minutes after the injection. The data have been normalized 1) to the virtual initial concentration C_0 as estimated by extrapolation (●) and 2) to C_0 calculated from the estimated plasma volume (○) according to Nosslin et al. (26, 28). The lines indicate the three exponential terms, which can be resolved in a peeling-off analysis by eye. As can be seen, the latter procedure of normalization intrudes on the nature of the primary data and influence heavily the estimated slope (λ_3) and intercept (A_3) of the third exponential component unless a fourth exponential is introduced (cf. 28).

of the tracer; the latter, which will be investigated in greater detail in the following, is dependent on the choice of the approximation-extrapolation function and the spacing in time of the experimental points. The 'initial' concentration, C_0, (as calculated by exponential extrapolation of the plasma curve to zero time) and V_1 (as calculated from the 'initial' concentration of tracer in plasma and the injected dose) are critically dependent on the availability of data from the first few minutes. Systematic changes are also introduced in the estimates of rate constants, clearance, and the total volume of distribution, if data from the initial phase ($t<5$ min) are included in the analysis. This can be seen in Table III which summarizes the results from an analysis of cases 4, 5 and 6 mentioned above (see Fig. 4). The changes in V_1 and k_3 counteract (cf. (9), p. 176) and therefore the change in the product k_3V_1, is comparatively small, though still large enough (3–10%) to be considered as a significant systematic error (cf. the standard deviations of the estimated GFR in Table II).

In order to estimate the possible error caused by the extrapolation of the plasma curve backwards to zero time, the area under the initial part ($t=0$–5 min) of the curve (for the three cases 4, 5 and 6) was calculated by using (i) the trapezoidal rule, (ii) extrapolation using data $t\geqslant 5$ min, and (iii) extrapolation using data $t\leqslant 5$ min (see Fig. 4). When compared with the reference value from (i), the procedure (ii) resulted in an underestimation of the total area by 0.5–1%, while procedure (iii) gave an overestimate of the same area by 1–1.5%, i.e. negligible errors.

It may be noted in passing, that the procedure to calculate the "initial concentration" C_0 from the injected dose and the plasma volume (and to express the concentrations in fractions of C_0, as suggested by Nosslin (27, 28)) cannot be regarded as a proper transformation of the experimental data. This procedure mostly intrudes into the inherent nature of the primary data (see Fig. 5), since the initial distribution volume of the substance V_1 (as reflected in the initial maximum concentration) is usually considerably larger than the plausible plasma volume (e.g., predicted from the body weight; cf. Table V and Fig. 6).

The choice of approximating function may play a decisive role in the estimation of GFR. This is especially true when the plasma curve has not been

Table V. *Estimates of distribution volumes for the cases 4, 5 and 6*
Plasma and extracellular volumes, as estimated from body weight, are given for comparison

	Body weight (kg)	V_1 (l)	Total volume of distr. (l)	Plasma vol. 4% of b.w. (l)	Extracell. vol. 10% of b.w. (l)
Case 4	75.0	4.0	13.8	3.0	14.3
Case 5	60.6	4.0	11.8	2.4	11.5
Case 6	57.4	5.3	12.1	2.3	10.9

followed for a sufficiently long time, but should be considered also in cases when the sampling period is nearly complete. This can be seen in Table IV summarizing the result of using a gamma distribution to fit the data of cases 4, 5 and 6 over the time interval 3–240 min.

The gamma distribution gives an adequate description of the plasma data for $t \geq 5$ min, but is inadequate when data from the initial part are included in the analysis. The GFR values in Table IV were calculated by adding the integral of the gamma distribution from $t=5$ to 'infinity' ($t=2\,000$ min) and the area under the initial part $t=0$–5 min as estimated by the trapezoidal rule.

It can be noted that the differences between these values and the corresponding estimates of GFR by the two-compartment model (see Table III), are of a systematic nature and may be as high as 15–20%. The influence of the arterio-venous concentration difference has not been investigated in the present paper (cf. Discussion).

DISCUSSION

The usefulness of ^{125}I-sodium iothalamate as a GFR-indicator with single intravenous injection technique has been evaluated by statistical comparison of a direct estimate of GFR from the plasma elimination curve with conventional inulin clearance.

The result of such a regression analysis depends on

(i) the validity of the model concepts applied in the analysis of the plasma elimination curve

(ii) the adequacy of the mathematical model and the experimental data; besides

(iii) the (validity and) reliability of the reference value, and

(iv) the design of the regression analysis itself with regard to the number of cases, and the range and errors of the correlated variables.

A compartmental model with two mixing compartments in parallel was found to describe the plasma data adequately, and the validity of the concepts behind this approach is corroborated, to some extent, by plausible estimates of the distribution volumes of the tracer. As can be seen in Table V the primary volume of distribution is, for the cases 4, 5 and 6, significantly larger than the plasma volume as predicted from the body weight (cf. Fig. 6), but seems reasonable in comparison with the extracellular volume estimated in the same way (plasma volume *c.* 4% and extracellular volume *c.* 19% of body weight). This is further illustrated in Fig. 6, which gives the frequency distribution of the estimated volumes. As pointed out by many authors (e.g. 14, 20, 32, 38) it may well be that the calculated distribution volumes have no true anatomical counterparts. However, the figures in Table V and Fig. 6 suggest the following identification of the compartmental model in physiological terms: The GFR indicator is initially distributed in plasma and parts of the interstitial fluids. The third exponential component observed in the peeling-off analysis, with a slope indicating a rapid 'phase' of mixing, probably reflects the mixing within this pool. The second distribution volume, V_2, mainly represents the rest of the interstitial fluids.

As can be seen from Table V and as confirmed by regression analysis on the total patient material, V_1 as estimated from the virtual initial concentration and injected dose, is not related to body weight ($V_1=0.107$ bw$+1.20$, $r=38$, S.D.$=3.25$, $n=90$). The same is true of the total distribution volume ($V_1+V_2=0.201$ bw-0.20, $r=42$, S.D.$=5.5$, $n=90$). This may be due to inter- and intra-individual variations in glomerular filtration rate, capillary permeability and possibly extrarenal clearance.

A so-called 'random walk' model was also found to be adequate for most of the cases in the present investigation. However, for three cases (nos. 4, 5

and 6), in which data from the initial phase were included, this model was incompatible. The validity of the 'random-walk' model is difficult to evaluate as it does not provide parameters which can be interpreted in simple physiological concepts and measured by independent methods. This model is also less attractive from the computational point of wiew, both with regard to parameter estimation and integration, and preference is therefore given to the compartmental model, which may even be evaluated by manual methods (32). It may be of interest to note that the method by Nosslin (27), often referred to as model-independent, is equivalent to the two methods using compartmental analysis in the case of the plasma curve being separated in two exponentials and the "plasma volume" being calculated from the virtual initial concentration (p. 2).

The arterio-venous concentration difference has been measured in dog and in man after a single injection of iothalamate (34); the V/A ratio started below 1.0 in the initial phase, 0–10 min, and then increased to a rather constant value of about 1.08 in man. No doubt, the difference is a major source of error in the calculation of clearance when this is based on venous blood samples taken during a short time interval, but the error may be neglected when sampling is extended over a sufficiently long time (6, 19, 27). Truniger et al. (38) and Chantler et al. (10) reported an underestimate of ^{151}Cr-EDTA clearance of 11%, when venous samples were used. In the first case this is most likely due to the short sampling time, 8–70 min, and in the latter case to the use of the approximate slope-intercept method based on data between 120–140 min.

The somewhat low correlation (r=0.85–0.87) between the estimate of GFR from the compartment model, k_3V_1, and inulin clearance is probably mainly due to the error in the inulin clearance determinations (9.4% coefficient of variation), but may partly be due to the errors introduced in the estimate of V_1 by incomplete plasma data from the initial phase of mixing.

It should also be noted that the design of the regression analysis is important for the outcome. Errors in each one of the correlated variables should be duly considered and the number of cases must be sufficiently large to reduce the influence of chance. Other investigations based on regression analysis should be judged with this in mind. In our case the choice of statistics is not critical. Different designs all ended up with 95% confidence limits of the regression line covering the identify line, which implies that there is no significant difference between standard inulin clearance and iothalamate single-injection clearance in the present study.

To summarize, it may be concluded that

(i) ^{125}I-sodium iothalamate seems to be a useful glomerular marker for single intravenous injection studies, as judged by the present analysis of plasma elimination curves from 90 patients with varying renal function and using conventional inulin clearance as an absolute reference for a statistical comparison;

(ii) A single plasma elimination curve does not allow a strict choice between different hypothetical models. From the computational point of view preference is given to a simple two-compartment model, even though 'random-walk' models may be more realistic with regard to the basic assumptions

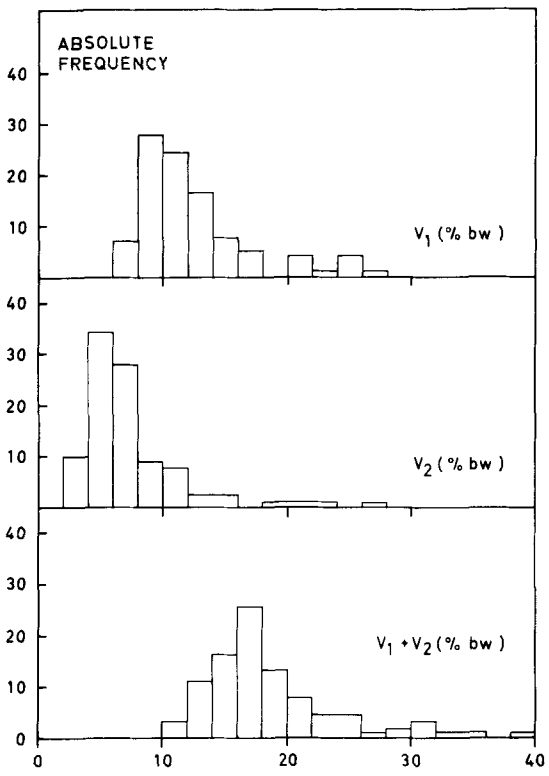


Fig. 6. Frequency distributions of the estimated primary (V_1), secondary (V_2) and total (V_1+V_2) distribution volumes (in percent of body weight) as defined by the two-compartment model. In the lower histogram three extreme cases are missing with a total distribution volume of 44, 46 and 51% of body weight, respectively.

concerning the processes of distribution of the tracer;

(iii) The present type of curve fitting analysis requires frequent blood sampling or external counting over a wide time interval in order to give reliable estimates of the glomerular filtrationrate. This circumstance makes these methods less attractive for clinical use. Simplified procedures have been discussed in a separate paper (17).

ACKNOWLEDGEMENT

The assistance of Mr Hans Falk in the statistical computations is gratefully acknowledged.

REFERENCES

1. Adefuin, P. Y., Gur, A., Siegel, N. J., Spencer, R. P. & Hayslett, J. P.: Single subcutaneous injection of iothalamate sodium I^{125} to measure glomerular filtration Rate. JAMA *235:* 1467, 1976.
2. Anderson, C. F., Sawyer, T. K. & Cutler, R. E.: Iothalamate sodium I-125 vs. cyanocobalomin CO-57 as a measure of glomerular filtration rate in man. JAMA *204:* 105, 1968.
3. Anderson, J., Osborn, S. B., Tomlinson, R. W. S. & Weinbreu, I.: Some applications of power law analysis to radioisotope studies in man. Phys Med Biol *8:* 287, 1963.
4. Anderson, J., Osborn, S. B., Tomlinson, R. W. S. & Wise, M. E.: clearance curves for radioactive tracers —sums of exponentials or powers of time? Phys. Med. Biol. *14:* 498, 1969.
5. Beck, J. S. & Rescigno, A.: Calcium kinetics: The philosophy and practice of science. Phys Med Biol *15:* 566, 1970.
6. Bergner, P.-E.: Tracer dynamics and the pool-sizes and turnover factors in metabolic systems. J. Theoret Biol *6:* 137, 1964.
7. Benyon, P. R.: A review of numerical methods for digital simulation. SIMULATION *11:* 219, 1968.
8. Blaufox, M. D.: Measurement of renal function with radioactive materials. *In* Progress in Nucl Med *2:* 9, 1972.
9. Cangiano, J., Genuth, S., Renerts, L. & Berman, L.: Simplified measurement of glomerular filtration rate. Invest Urol *9:* 34, 1971.
10. Chantler, C., Garnett, E. S., Parsons, V. & Veall, N.: Glomerular filtration rate measurement in man by the single injection methods using ^{51}Cr-EDTA. Clin Sci *37:* 169, 1969.
11. Cohen, M., Smith, F., Mindell, R. & Vernier, R.: A simple, reliable method of measuring glomerular filtration rate using single, low dose sodium iothalamate I^{131}. Pediatrics *43:* 407, 1969.
12. Cohen, M. L.: Radionuclide clearance techniques. Seminars in Nucl Med *4:* 23, 1974.
13. Corcoran, A. C. & Page, I. H.: Applications of diphenylamine in the determination of levulose in biological media. J Biol Chem *127:* 601, 1939.
14. Donath, A.: The simultaneous determination in children of glomerular filtration rate and effective renal plasma flow by the single injection clearance technique. Acta Paediat Scand *60:* 512, 1971.
15. Elwood, C. M. & Sigman, E. M.: The measurement of glomerular filtration rate and effective renal plasma flow in man by iothalamate ^{125}I and iodopyra ^{131}I. Circulation *36:* 441, 1967.
16. Griep, R. & Nelp, W.: Mechanism of excretion of radioiodinated sodium iothalamate. Radiology *93:* 807, 1969.
17. Groth, T. & Tengström, B.: A simple method for the estimation of glomerular filtration rate. Scand J Clin. Lab Invest *37:* 25, 1977.
18. Israelit, A. H., Long, D. L., White, M. G. & Hull, A. R.: Measurement of glomerular filtration rate utilizing a single subcutaneous injection of ^{125}I-iothalamate. Kidney Int *4:* 346, 1973.
19. Ladegaard-Pedersen, H. J.: Measurement of extracellular volume and renal clearance by a single injection of inulin. Scand J Clin Lab Invest *29:* 145, 1972.
20. Ladegaard-Pedersen, H. J. & Engnell, H. C.: A comparison between the changes in the distribution volumes of inulin and ^{51}Cr-EDTA after major surgery. Scand J Clin Lab Invest *35:* 109, 1975.
21. Llaurado, J. G. & Smith, G. A.: Some observations on the effects of time curtailing on the parameters of decaying exponentials fitted to biomedical data. Biomed Comput *2:* 265, 1971.
22. Maher, F. T., Nolan, N .G. & Elveback, L. R.: Comparison of simultaneous clearances of ^{125}I-labelled sodium iothalamate (Glofil) and of inulin. Mayo Clin Proc *46:* 690, 1971.
23. Malamos, B., Dontas, A. S. Koutras, D. A., Marketos, S., Sfontouris, J. & Papanicolaou, N.: ^{125}I-sodium iothalamate in the determination of the glomerular filtration rate. Nucl Med *6:* 304, 1967.
24. Mancini, P. & Pilo, A.: A computer program for multiexponential fitting by the peeling method. Comp Biom Res *3:* 1, 1970.
25. Mogensen, C. E.: Glomerular filtration rate and renal plasma flow in short-term and long-term juvenile diabetes mellitus. Scand J Clin Lab Invest *28:* 91, 1971.
26. Nosslin, B.: Mathematical model of plasma turnover determined with ^{131}I-labelled protein. *In* Metabolism of Human Gamma-globulin (γ_{35}-globulin) (ed. S. B. Andersen, p. 115. Blackwell, Oxford, 1964.
27. Nosslin, B.: Determination of clearance and distribution volume with the single injection technique. Acta Med Scand *179:* Suppl. *442,* 97, 1965.
28. Nosslin, B. & Pihl, B.: The single injection technique for determination of renal clearance. IV. Clearance calculations and kinetics of the test substances. *In* Studies on the Single Injection Technique for Determination of Renal Clearance (thesis). Studentlitteratur, Lund, Malmö, 1973.
29. Pihl, P.: The single injection technique for determination of renal clearance. V. A comparison with the continuous infusion technique in the dog and in man.

In Studies on the Single Injection Technique for Determination of Renal Clearance (thesis). Studentlitteratur, Lund, Malmö, 1973.
30. Pihl, B.: The single injection technique for determination of renal clearance. II. Experimental studies on methodological problems. *In* Studies on the Single Injection Technique for Determination of Renal Clearance (thesis). Studentlitteratur, Lund, Malmö, 1973.
31. Sakai, T., Leumann, E. & Holliday, M.: Single injection clearance in children. Pediatrics *44:* 905, 1969.
32. Sapirstein, L., Vidt, D., Mandel, M. & Hanusek, G.: Volumes of distribution and clearance of intravenously injected creatinine in the dog. Amer J Physiol *181:* 330, 1955.
33. Sigman, E., Elwood, C., Reagan, M., Morris, A. & Catanzaro, A.: The renal clearance of I^{131} labelled sodium iothalamate in man. Invest Urol *2:* 432, 1965.
34. Sigman, E., Elwood, C. & Knox, F.: The measurement of glomerular filtration rate in man with sodium iothalamate ^{131}I (Conray). J Nucl Med *7:* 60, 1966.
35. Smith, H. W.: The Kidney. Oxford University Press, New York, 1951.
36. Smith, H. W. Principles of Renal Physiology. Oxford University Press, New York, 1956.
37. The Lancet, Leading article. Vol. ii, p. 276, 1965.
38. Truniger, B., Donath, A. & Kappeler, M.: Simplified clearance techniques. The single injection method and its modifications. Helv Med Acta *34:* 116, 1968.
39. Wise, M. E., Osborn, S. B., Anderson, J. & Tomlinson, R. W. S.: A stochastic model for turnover of radio calcium based on the observed power laws. Math Biosci *2:* 97, 1971.
40. Wise, M. E.: The evidence against compartments. Biometrics *27:* 262, 1971. See also discussion, Biometrics *27:* 97, 1971.
41. Zierler, K. L.: Equations for measuring blood flow by external monitoring of radioisotopes. Circ Res *16:* 309, 1963.
42. Zierler, K. L.: Theory of use of indicators to measure blood flow and extracellular volume and calculation of transcapillary movement of tracers. Circ Res *12:* 464, 1963.

Received May 20, 1977

Address for reprints:

Docent Torgny Groth
Gruppen för biomedicinsk systemanalys
Uppsala Datacentral
Box 2103
S-750 02 Uppsala
Sweden