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TECHNICAL NOTE

An accurate practical method for estimating GFR in clinical studies using a constant subcutaneous infusion

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Glomerular filtration rate (GFR) is the standard measure of renal function and serial measurements often are used to describe the course of renal disease in clinical studies including controlled clinical trials. Serum creatinine (S_{Cr}), commonly used to estimate renal function, is now recognized as insufficiently accurate for this purpose [1, 2]. The reference standard for estimating GFR is the renal clearance of inulin (C_{In}). The plasma C_{In}, derived from steady state level and input rate, is equally suitable [3]. Although inulin is the ideal marker, isotopic and unlabeled iothalamate are both used to estimate GFR from the renal [4–6] or plasma [5, 7, 8] clearance of iothalamate (C_{Io}). Even these clearance methods, however, are sufficiently time consuming and expensive to constitute an impediment to accurately estimating GFR in clinical studies, particularly in children.

Plasma clearance is measured either by giving a single bolus injection of a marker and measuring its fractional disappearance rate from plasma [9] (single-injection plasma clearance) or by infusing a marker to a steady state and measuring its input rate divided by its plasma concentration [10] (steady-state plasma clearance). Either of the two plasma clearance methods is preferred in children or patients with voiding dysfunction because collecting timed urine samples as required for renal clearances often is impractical.

We describe a method for measuring the steady-state plasma C_{Io}. Iothalamate is delivered subcutaneously from a very accurate portable pump at a constant rate for a calculated minimum infusion time. Clearance is calculated from input rate and the steady state plasma level.

Methods

Principle

The method uses a constant infusion of iothalamate to achieve a steady-state plasma concentration (S_{Io}). Unlabeled undiluted iothalamate is delivered subcutaneously, rather than intravenously, at a constant rate from the small MiniMed portable pump (MiniMed Technologies, Sylmar, California, USA) which commonly is used to deliver insulin to patients with diabetes. The delivery rate is accurate (±2%), the infusion comfortable, and the uptake steady. A programmable pocket calculator or personal computer calculates the loading dose, infusion rate and minimum infusion time. After the infusion is started, patients go about their usual activities until a blood sample is drawn once the minimum infusion time has elapsed (usually 6 to 24 hours); the infusion is then discontinued and the pump recovered. Plasma C_{Io} is calculated from the rate of iothalamate input divided by S_{Io} when S_{Io} is at a steady state. Criteria for establishing steady state conditions are defined in the **Appendix.**

Infusion

Iothalamate meglumine (Malinkrodt Medical Inc., St. Louis, Missouri, USA; brand name, Conray 60) (~450 mg/ml of iothalamic acid) is drawn into a 3 ml syringe that accompanies the pump. The pump is programmed to deliver iothalamate at a constant rate (between 0.002 and 0.7 ml per hour with an accuracy of ±2%) through tubing and a 26 gauge silastic catheter provided with the pump (Fig. 1). The catheter usually is placed into abdominal subcutaneous tissue over a guide and affixed to the skin with a butterfly. The infusion runs for the calculated minimum infusion time or longer.

Procedure

All protocols were approved by the Committee on Human Research, UCSF. Each subject, patient or guardian read and signed the appropriate consent form.

The patient's age, weight, height, and S_{Cr} were used to calculate the loading dose, the patient's estimated GFR from a modification of the Ht/S_{Cr} equation [11] (which we term the Ht/S_{Cr}-GFR), the infusion rate and the minimum infusion time. For adults an alternate method was used to estimate GFR (**Appendix**). For children up to twelve years of age we also estimated normal GFR for age and weight [12].

A suitable dilution is made for the loading dose. The pump is programmed to set the infusion rate. The syringe and tubing are loaded with undiluted iothalamate, care being taken to be sure that connections are tight and the line is cleared of air bubbles. A sample of the undiluted infusate and a serum blank (S_0) are obtained. The loading dose is given, the pump is activated at a relatively high infusion rate to maintain the needle and catheter

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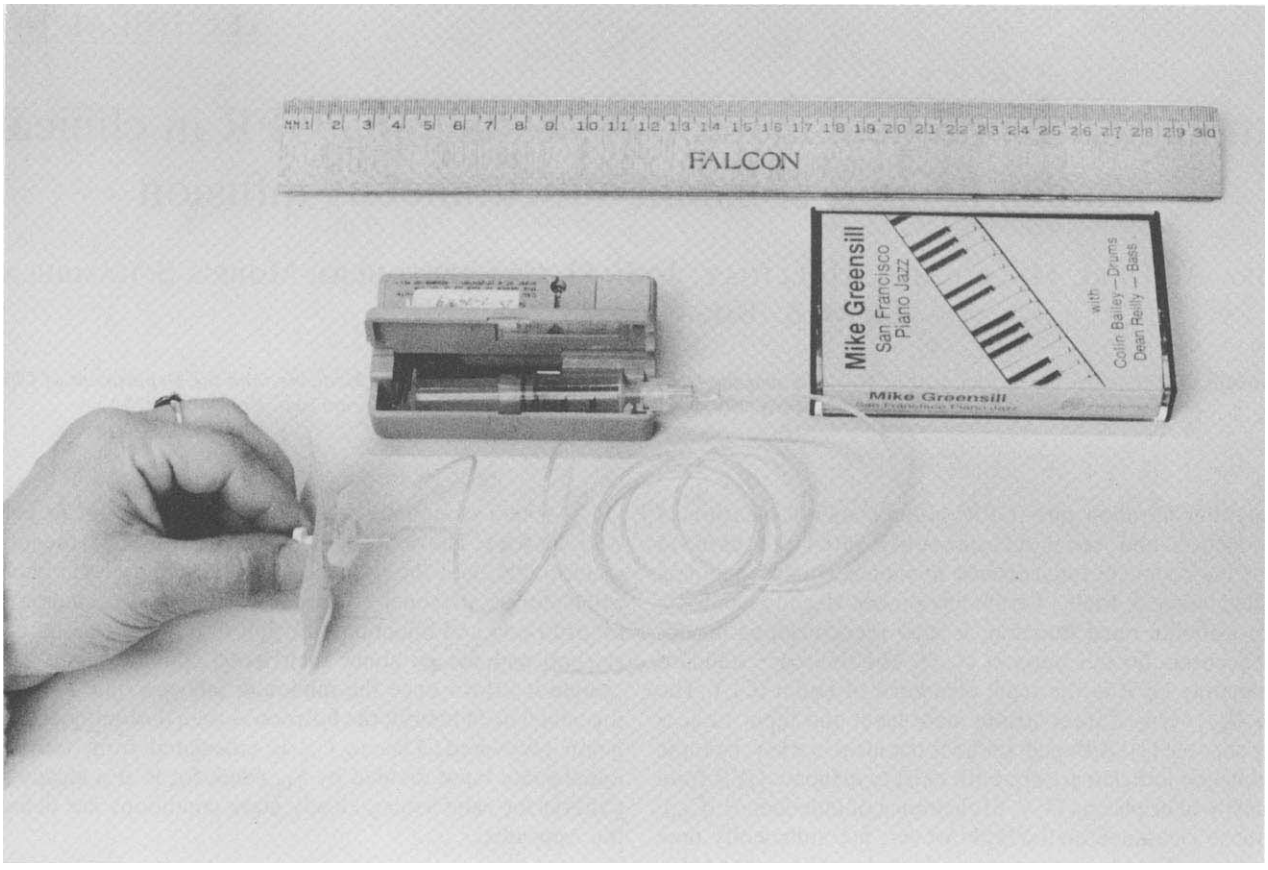

Fig. 1. *The MiniMed pump, the line, and the 26-gauge silastic catheter with stylet used to insert catheter.* The stylet is removed and the butterfly fixes the hub of the catheter to the skin. The audiocassette is displayed to illustrate the size of the pump.

clear of any air bubbles, the catheter is inserted into the abdominal subcutaneous tissue and fixed with a butterfly, and the pump is reset to the calculated infusion rate. A second serum sample (S_1) is taken one hour post-loading dose to compare with the steady-state serum sample (S_{Io}); S_{Io} is obtained at a convenient time after the minimum infusion time has passed. The serum samples and a dilution of the infusate sample are analyzed for iothalamate.

Analysis

Iothalamate is determined by high pressure liquid chromatography using a reverse phase column (C_{18} 5 μ Ultrasphere) with a mobile phase of water:acetonitrile:phosphoric acid (990:10:0.5) at pH 2.65 and a flow rate of 1.5 ml/min. UV absorbance is monitored at 236 nm; the retention time is 13 minutes [13].

Calculations

The absolute C_{Io} (ml/min) is calculated from the following equation:

$$C_{Io}\ (\text{ml/min}) = \frac{\text{Infusion}_{Io}\ (\text{mg/ml}) \cdot \text{Infusion rate (ml/hr)}}{S_{Io}\ (\text{mg/ml}) \cdot 60\ \text{min/hr}} \quad (1)$$

The result may be adjusted to standard surface area (ml/min/1.73 m^2) and, for children, to percent normal (% nl) for age and weight as we have described previously [11]. Detailed calculations for these purposes are included in the **Appendix.**

Results

We did three studies to validate this method:

1. Purpose and protocol

The first study had four objectives: to determine whether uptake was steady; to compare the time required to achieve steady state without giving a loading dose with that when a loading dose was given; to evaluate reproducibility of the method repeated in the same subject; and to determine whether the subcutaneous infusion of undiluted iothalamate was well tolerated.

Each of four normal male volunteers (aged 21 to 32) underwent a subcutaneous infusion begun without any loading dose; blood samples were obtained periodically for up to 18 hours after starting the infusion. Each volunteer was restudied within two weeks, at which time the protocol was the same except that a loading dose (2 mg/kg) was given.

Results. The uptake curve of iothalamate in the four instances where no loading dose was given was similar to the curve that would be obtained from a constant intravenous infusion begun at zero concentration (Fig. 2). The time required to achieve a steady state was shortened when a loading dose

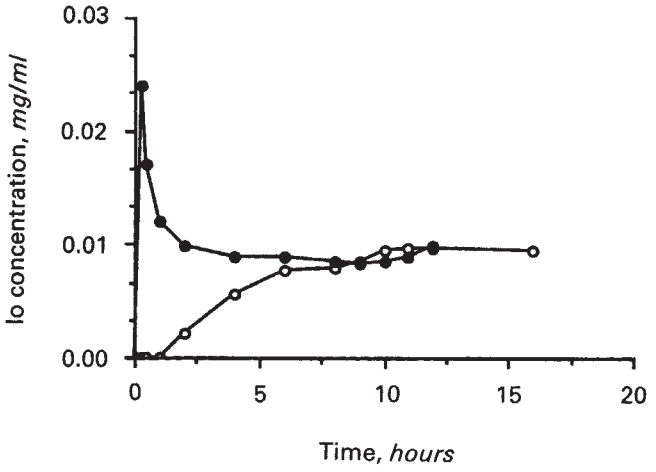

Fig. 2. *The S_{Io} curves of one of the subjects in the first study showing the plasma uptake curve when no loading dose was given (○) and when a loading dose was given (●).* The uptake to steady state when no loading dose was given was the same as one would see if iothalamate was given intravenously except for a small initial delay. The loading dose shortened the time to near steady state.

Table 1. GFR measurement with and without an i.v. priming dose of Iothalamate in four healthy men

Subject no.	C_{Io} without i.v. priming dose *ml/min*	Time to S_{Io} (*N*) *hr*	C_{Io} with i.v. priming dose *ml/min*	Time to S_{Io} (*N*) *hr*
1	122	8 (2)	120	6 (4)
2	117	8 (3)	98	1 (10)
3	122	8 (6)	123	2 (8)
4	138	6 (7)	140	1 (9)
Mean ± SD	124.8 ± 9.1	7.5 ± 1	120.3 ± 17.3	2.5 ± 2.3
Mean difference ± SD		4.5 ± 4.9		

Plasma clearance of Io was measured using an extended subcutaneous infusion (s.c.) infusion of Io, first without; and then one week later, with an i.v. priming dose of Io.

Time to S_{Io}: Hours needed to reach steady state plasma Io levels, with a coefficient of variation of < 10% between blood samples. (*N*) = Number of blood samples at S_{Io}.

was given. There was good agreement when a study was repeated in the same subject (Fig. 2, Table 1). The steady state was maintained when the infusion was extended (Fig. 3). None of the subjects experienced any pain, discomfort or skin reaction at the site of the infusion or any evidence of an allergic reaction; this also has been the case for all other subjects and all patients we have studied.

2. Purpose and protocol

The second study compared the plasma C_{Io} with the renal C_{Io} and the renal C_{In} measured simultaneously. Five normal male subjects aged 21 to 34 were admitted to the Clinical Research Center, SFGH and a subcutaneous infusion of iothalamate was begun in the evening; they were fasted overnight. At 8 a.m. each subject was given a loading dose of inulin (50 mg/kg) and 10 ml/kg water to drink. In each a constant infusion of inulin was started to sustain a serum level of 0.15 mg to 0.20 mg/ml. Throughout the study each subject drank a volume of water equal to the volume of urine passed plus insensible water loss. Following a 90-minute equilibration period four to six spontaneously voided timed urine samples were collected; these were bracketed by blood samples. The renal clearances of iothalamate and inulin were calculated for each period and the average was used for that clearance. Plasma C_{Io} was calculated as described.

Results. The ratios of the plasma C_{Io} to that of its renal clearance and to the renal C_{In} are illustrated in Table 2. Neither differed significantly from 1.0.

3. Purpose and protocol

The third study evaluated the practicality of this method for measuring the steady-state plasma C_{Io} in children with varying degrees of renal impairment. A C_{Io} was measured in association with other clinical studies in twenty-five patients who ranged in age from six months to nineteen years of age, had evidence of renal disease, and were being followed by the pediatric nephrology or urology service. Twenty-three were admitted to the Pediatric Clinical Research Center, UCSF and the infusion was started; the pump was secured in a cloth pouch. Two had the infusion started as outpatients; the patients then were sent home with instructions to maintain usual activities and return the next day when the final blood sample was obtained and the pump recovered. We also did the following: (a) calculated the ratio of the C_{Io} to Ht/S_{Cr}-GFR in all twenty-five patients to determine whether that ratio was greater than 0.6 in accordance with the assumptions underlying the calculation of the minimum infusion time as described in the **Appendix.** (b) We measured the ratio of S_I to S_{Io} in fourteen patients; (c) measured the plasma C_{In} simultaneously in five patients; and (d) obtained in eight patients, in whom an extended study was practical, five or more blood samples that were drawn over a period of 12 to 50 hours following the bolus dose; all these samples were obtained after the minimum infusion time had passed. The coefficient of variation (c.v.) of these multiple samples for each patient was considered an index of variability during the steady state.

Results. The studies were done without complication. In one case a connection was not tight and the study had to be repeated. The C_{Io} is expressed as the absolute GFR, GFR adjusted to standard body surface area, and as % nl GFR for age and weight [11] (Table 3). We also found that: (a) the $C_{Io}/(Ht/S_{Cr}$-GFR) was >0.6 in every case (Table 3); (b) the ratio of S_I to S_{Io} was between 0.6 and 2.4 in all but one of 14 instances where it was determined; (c) the ratio of the C_{Io}/C_{In} for the five patients who had both done was 1.01 ± 0.14 (mean ± SD); and (d) the c.v. for the serial serum S_{Io} values of eight children who had five or more samples obtained at steady state averaged 6.2% (Table 4).

Discussion

Previous work and our findings support the use of the steady-state plasma C_{Io} as an accurate estimate of GFR as long as steady state conditions can be verified. Cole and her colleagues [10] have shown that the steady-state plasma C_{In} in children is equal to its renal clearance, and Mak et al [5] have shown the steady-state plasma C_{Io} in children is the same as the renal C_{Io} and C_{In}. One of us (MAH) and others [14] have

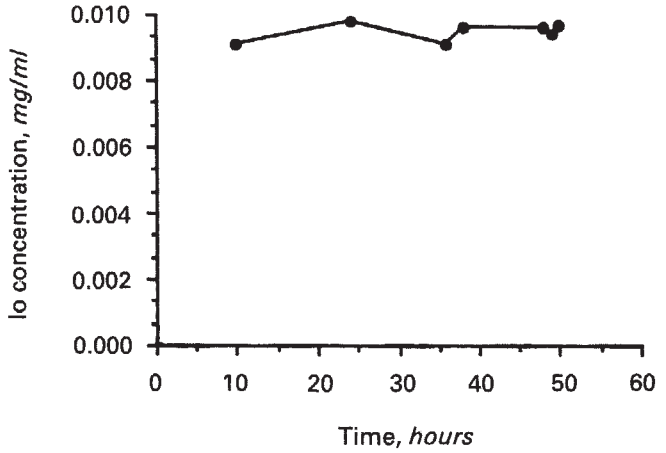

Fig. 3. *The stability of S_{Io} is illustrated in a patient infused for 52 hours (study 3).* The t½ calculated to be 7 hours. The Approx GFR (Ht/S_{Cr}) predicted the C_{Io} closely following a bolus dose, steady state reached promptly.

reported steady-state plasma C_{Io} was the same as the steady-state plasma C_{In} when both were infused intravenously. In this study we confirm that the steady-state plasma C_{Io} and the renal C_{Io} and C_{In} were the same when the iothalamate was given subcutaneously as described, and the inulin was given intravenously to five volunteers with normal renal function and five patients with reduced renal function.

This method has several features that make it suitable for use in children in whom the serial estimate of GFR is indicated. It is practical: undiluted iothalamate is delivered subcutaneously at a slow constant rate by a portable, accurate infusion pump; the patient is free to follow usual activities after the hour required to initiate the study and returns only to have the steady state sample (S_{Io}) drawn and the pump recovered. It is safe: the iothalamate is not radioactive; the risk of an allergic reaction is very low and the patients are under observation for one hour after being given the loading dose to check for this possibility.

The method accurately measures the plasma C_{Io}. We used two strategies to affirm that we had reached a steady state which we defined as a value that was within 100 ± 10% of true steady state and did so within the calculated minimum infusion time (usually between 6 to 24 hours following the loading dose). First, we gave a loading dose (initially 2 mg/kg) calculated so that the priming level, S_1, would likely be between 0.6 and 2.4 of the steady state level (S_{Io}), and verified that it was in thirteen of fourteen cases. In the one exception serial S_{Io} values obtained after the calculated minimum infusion time had elapsed established that a steady state had been reached. Using a loading dose that meets the criteria shortens the minimum infusion time by approximately a third. [We now double the loading dose (4 mg/kg; **Appendix**) to be more confident that S_1 will be between 0.6 and 2.4 of S_{Io} and routinely check to see that it is]. Second, we estimated a t½ for a clearance that would be 60% of Ht/S_{Cr}-GFR and used this to derive a minimum infusion time (2.5 · est t½). The t½ we estimated would exceed the true t½ so long as C_{Io} (Ht/S_{Cr}-GFR) exceeded 0.6; it did in all cases (Table 4). When these conditions are met the true minimum infusion time to steady state is less than the calculated one.

Table 2. Comparison of the plasma clearance of Iothalamate with the renal clearances of Iothalamate and inulin in five healthy men

Subject no.	PC_{Io}	RC_{Io}	RC_{In}	PC_{Io}/RC_{Io}	PC_{Io}/RC_{In}
1	180	165	173	1.09	1.04
2	125	135	123	0.93	1.02
3	153	148	153	1.03	1.00
4	143	153	169	0.93	0.90
5	132	120	126	1.10	1.05
Mean ± SD				1.02 ± 0.08	1.00 ± 0.06

Abbreviations are: PC_{Io}, plasma clearance of Iothalamate; RC_{Io}, renal clearance of Iothalamate; RC_{In}, renal clearance of inulin.

To further demonstrate whether a steady state has been reached an additional sample may be drawn; it is well to wait at least another one fourth of the estimated t½ [15]. This often is impractical when studies are done in children or in an outpatient setting. In the eight children in whom we obtained additional samples for that purpose, we verified that we achieved and sustained a steady state. The other checks we describe make the test redundant. When results are not within the limits we have set, the study should be repeated.

Several clinics, recognizing that the estimation of GFR from S_{Cr} is inadequate for clinical follow up, have adopted clearance methods that use an isotope as a marker and either measure renal clearance or single-injection plasma clearance. A few pediatric clinics use the steady-state plasma C_{In} for this purpose. Each of these methods requires the patient to be in clinic for from 5 to 8 hours and requires a comparable amount of staff time. For patients or parents of children this often means a missed day of work. Single-injection plasma clearances are less accurate [16]. The direct cost for each clearance that was reported to us in an informal survey varied between $150 to $400. We calculated costs for the method we describe to be less than $100 under comparable conditions (>30 tests per year for 5 years); this estimate includes amortizing the HPLC equipment and the pumps. In cases where it is inconvenient for a patient to return to the central clinic for the S_{Io} sample, it would be practical to have the sample drawn in a laboratory near to the patient and have the sample and pump returned by a commercial delivery service.

This method makes it practical and economical to make accurate estimates of GFR serially in children either in a single center doing >30 tests per year or multiple centers participating in a controlled clinical trial in which the total averages >50 per year over a five year period. The procedure can be done on an outpatient or inpatient basis without requiring that staff be specially trained and dedicated to that method. The cost per test projects to be less than the reported costs of present methods. The accuracy in our hands is equal to that reported for the other methods being used. These considerations lead us to conclude that this method makes accurate serial estimates of GFR practical in patients, particularly in children and others in whom the collection of timed urine samples is problematic.

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Table 3. Clearance data on twenty-five patients

Pt. no.	Age *years*	Ht *cm*	Wt *kg*	SA m^2	nl GFR *ml/min*	S_{Cr} *mg/dl*	Ht/S_{Cr}-GFR *ml/min*	C_{Io} *ml/min*	C_{Io}/SA *ml/min/1.73 m^2*	%nl GFR	$C_{Io}/(Ht/S_{Cr}\text{-GFR})$
1	0.67	66.0	5.9	0.33	19.0	0.5	11.4	8.7	45.4	45.8	0.76
2	0.92	70.5	9.1	0.43	27.1	0.4	19.7	16.0	64.4	59.0	0.81
3	1.08	73.6	11.4	0.49	32.3	0.6	15.8	22.3	78.2	69.1	1.42
4	1.58	78.6	10.5	0.48	33.3	0.3	33.0	25.0	89.2	75.0	0.76
5	2.58	88.9	14.7	0.61	44.9	0.8	17.6	40.2	114.0	89.5	2.28
6	3.67	81.5	12.3	0.54	44.1	1.1	10.3	9.4	30.4	21.3	0.91
7	5.17	109.2	18.0	0.74	58.0	0.7	29.9	40.0	93.8	69.0	1.34
8	7.08	113.0	19.1	0.77	63.8	0.7	32.4	41.3	92.5	64.7	1.27
9	9.42	132.1	26.7	0.98	79.0	0.9	37.6	44.0	77.4	55.7	1.17
10	10.25	123.8	24.3	0.91	77.1	3.3	8.9	7.8	14.8	10.1	0.88
11	0.58	68.0	8.9	0.42	24.5	0.6	12.3	11.0	45.4	44.9	0.89
12	12.00	155.6	49.5	1.46	107.5	0.9	65.8	89.0	105.2	82.8	1.35
13	19.00	130.0	30.0	1.04	95.5	1.2	29.3	26.3	43.7	27.5	0.90
14	4.17	88.9	12.0	0.55	44.8	3.2	4.0	2.9	9.2	6.5	0.73
15	6.08	115.0	20.7	0.81	64.2	0.5	48.6	41.3	88.0	64.3	0.85
16	2.00	78.5	10.0	0.47	34.1	1.0	9.6	8.5	31.2	24.9	0.88
17	0.42	50.0	3.4	0.22	11.2	0.2	14.4	10.6	83.0	94.4	0.74
18	6.08	111.7	22.2	0.83	66.4	0.8	30.3	33.7	69.9	50.8	1.11
19	0.92	73.0	10.1	0.46	29.0	0.8	10.9	8.8	33.0	30.4	0.80
20	2.42	92.0	17.0	0.67	47.8	0.5	32.0	33.3	86.2	69.7	1.04
21	14.00	100.0	68.0	1.46	125.2	0.8	47.4	87.0	103.3	69.5	1.84
22	9.92	131.0	47.0	1.33	101.6	1.0	45.3	53.8	70.0	52.9	1.19
23	12.00	152.0	38.4	1.26	96.9	0.8	62.5	88.4	120.9	91.2	1.41
24	14.58	102.5	70.5	1.50	127.8	0.6	66.6	76.0	87.7	59.5	1.14
25	4.83	84.0	10.7	0.50	43.6	0.6	18.3	17.9	61.6	41.0	0.98
Avg	6.06	98.78	22.82	0.77	60.0	0.91	28.6	33.7	69.5	54.8	1.10
SD	5.14	27.31	17.99	0.37	32.8	0.73	18.3	26.3	30.1	24.0	0.36

Table 4. Summary statistics of S_{Io} on 8 patients who had more than five values over 12 to 50 hours

N	Avg of S_{Io} *mg/ml*	SD of Avg	CV *%*
5	0.55	0.03	4.6
5	1.41	0.07	5.0
7	1.03	0.14	13.3
5	1.12	0.05	4.5
7	0.95	0.03	2.7
5	0.96	0.06	6.3
5	1.40	0.05	3.3
5	1.44	0.13	9.3

N, number of blood samples at S_{Io}; data are mean ± SD 6.13 ± 3.54.

Appendix

The loading dose (4 mg/kg when ECF volume is estimated to be 200 ml/kg) is calculated to achieve a concentration of iothalamate at one hour that exceeds the anticipated steady state S_{Io} if C_{Io} equals Ht/S_{Cr}-GFR.

$$\text{Loading dose (mg)} = 0.02\ \text{(mg/ml)} \cdot \text{ECF vol (ml/kg)} \cdot \text{body wt (kg)} \quad (2)$$

The infusion rate is calculated to sustain a steady state S_{Io} of 0.02 mg/ml if the measured GFR (C_{Io}) equals the Ht/S_{Cr}-GFR. So long as C_{Io} varies between 0.6 and 2.4 of the Ht/S_{Cr}-GFR the infusion time as calculated is sufficient for serum iothalamate to achieve a steady state concentration as we define it.

$$\text{Est GFR (ml/min)} = 0.45 \cdot [\text{Ht (cm)}/S_{Cr}\ \text{(mg/dl)}] \cdot (\text{SA}/1.73\ m^2) \quad (3)$$

We use the constant 0.45 for all ages rather than to introduce age-dependent higher values as recommended by the original authors because this increases the likelihood that the C_{Io} will be >60% of the Ht/S_{Cr}-GFR. Taking 60% of the Ht/S_{Cr}-GFR to calculate the estimated t½ assures that the true t½ will be less so long as the C_{Io} is >60% of the Ht/S_{Cr}-GFR; the infusion time under these conditions will exceed 2.5 true t½s (see equation 4). Surface area (SA) used in the equation may be derived from a nomogram or from the height/weight equation described by Haycock and his associates [17]:

$$\text{SA} = 0.024265 \cdot \text{weight}^{0.5378} \cdot \text{height}^{0.396}$$

It is convenient to include the latter as part of the computer program. Other formulas are used for estimating GFR in adults [18].

The infusion rate (ml/hr) is calculated from the term:

$$Ht/S_{Cr}\text{-GFR (ml/min)} \cdot 0.02\ \text{mg/ml} \cdot 60\ \text{min/hr} \cdot (1/450\ \text{mg/ml})$$

Using a priming dose so that the ratio of S_1 to S_{Io} will be between 0.6 and 2.4 reduces the minimum infusion time by shortening the number of true t½s required to reach the steady state.

The minimum infusion time is based on an assumption that C_{Io} is at least 60% of the est GFR and that the ratio of S_1 to S_{Io} is between 0.6 and 2.4.

$$\text{Minimum infusion time} = 2.5 \cdot \ln 2/[0.6 \cdot (Ht/S_{Cr}\text{-GFR})/\text{ECF}] \quad (4)$$

If the patient is edematous a larger ECF volume must be used in the equations.

If total infusion volume (infusion rate · infusion time) exceeds 3 ml arrangements must be made to refill the syringe. This is unlikely to be required except in patients who have very low clearances and are in the adult weight range mandating a long infusion time or where, for convenience, an extended infusion time is planned. Patients can be trained to refill the syringe.

Normal GFR for age and weight for children up to twelve years of age is calculated from the child's age and weight according to the following equations:

$$\text{nl GFR (age \& wt)} = z^3 + 0.1293 \cdot z,$$

where

$$z = 0.7434 + [0.6956 \cdot \log (5 + \text{age})] + (1.47 \cdot \log \text{wt}).$$

Age is in months and weight is in kg.

Percent normal for age and weight (% nl) is computed from the equation:

$$\%\text{nl GFR} = (\text{C.Io/nl GFR}) \cdot 100\%$$

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