A Computerized Cumulative Integral Method for the Precise Measurement of the Glomerular Filtration Rate[1] (36630)

JOHN D. HARRIES,[2] RICHARD R. MILDENBERGER, ALFRED S. MALOWANY, AND KEITH N. DRUMMOND

Department of Nephrology, McGill University-Montreal Children's Hospital Research Institute, and the Department of Electrical Engineering, McGill University, Montreal, Canada

The classical method for measuring the glomerular filtration rate (GFR) is the constant infusion technique utilizing inulin as proposed by Shannon and Smith (1) in 1935. This method requires prolonged iv infusion and bladder catheterization. Because it is a difficult procedure to carry out, especially in the pediatric age group, less accurate tests such as the endogenous creatinine clearance are often substituted. With the advent of immunosuppressive drug therapy for renal disease, kidney transplantation and other new forms of treatment, an accurate and reproducible method for determining GFR has become a matter of fundamental importance for the assessment of the course of the patient's illness.

In this paper a new method for the accurate determination of GFR is presented. The theoretical basis begins with the original formulation of Austin, Stillman and Van Slyke (2) as applied to a freely filtered, nonreabsorbable molecule such as inulin (3) or iothalamate (4, 5). The mathematical relationship used to calculate the GFR by the constant infusion technique is:

$$\text{GFR} = \frac{\text{urine concn of inulin} \times \text{urine vol/min}}{\text{plasma concn of inulin}} = \frac{\text{amount of inulin in urine}}{\text{plasma concn of inulin} \times \text{time}} = \frac{A_u}{C_p \cdot t}$$

There are several errors inherent in this technique especially if the plasma concentration of inulin is not constant. It is difficult to predict a rate of infusion which will provide a constant plasma level, especially in patients with impaired renal function, and thus changing plasma concentrations may occur. In order to minimize the effect of changing plasma concentrations short urine collection periods of 20–30 min are commonly used. This, however, enhances the errors introduced by residual bladder inulin storage (6) and by collection delay due to transit time of the filtrate from glomerulus to bladder (7).

In the above formula the denominator $C_p \cdot t$ may be considered as the area under the plasma concentration (C_p) curve from time t_1 to t_2, the period of the urine collection, as is shown in Fig. 1, and we may rewrite the formula to give

$$\text{GFR} = \frac{\text{amount of inulin in the urine from } t_1 \text{ to } t_2}{\text{area under the } C_p \text{ curve from } t_1 \text{ to } t_2}.$$

Since the area under any curve is the integral of the function it is no longer necessary to consider this formula over short intervals of time where C_p remains relatively constant; this relationship is true whether the function C_p is constant or not. If one measures the quantity of inulin in the urine over a certain time interval and can, for this period, describe the area under the plasma concentration curve regardless of its shape, as shown in Fig. 2, then the GFR may be calculated from the relationship:

[1] Aided by Term Grant MT-1579 from The Medical Research Council of Canada and a grant from The National Research Council of Canada. We acknowledge the assistance and encouragement of Dr. Peter Nash, Director of the Scientific Division, Abbott Laboratories Limited, Montreal, Quebec, Canada.

[2] Present address: The Department of Anesthesia, Winnipeg General Hospital, Winnipeg, Manitoba, Canada. Dr. Harries was a Medical Research Council of Canada Fellow during the period of this study.

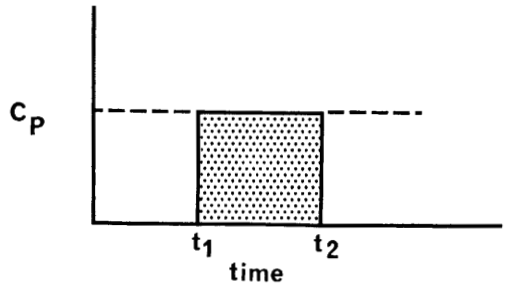

FIG. 1. In the constant infusion method $C_p \cdot t$ represents the shaded area under the C_p curve between t_1 and t_2.

$$\text{GFR} = \frac{\text{quantity of inulin in the urine } (t_1 \rightarrow t_2)}{\text{area under the } C_p \text{ curve } (t_1 \rightarrow t_2)},$$

$$i.e., \quad \text{GFR} = \frac{[A_u]_{t_1}^{t_2}}{\int_{t_1}^{t_2} C_p dt}.$$

Since inulin and iothalamate are not metabolized and are excreted only by the kidney, at infinity the amount in the urine (A_u) must be equal to the amount injected (A_0):

$$i.e., \quad \text{GFR} = \frac{A_0}{\int_0^{\infty} C_p dt}.$$

This is shown in Fig. 3. Previous authors have attempted to calculate GFR from blood sampling alone after a single bolus injection of inulin (8) or iothalamate (9). Errors are introduced, however, as attempts are made to predict the integral of C_p to infinity on the basis of limited plasma sampling.

Aware of the disadvantages of the techniques employing constant infusion or plasma sampling only, we developed a new method based on the integral formulation just presented. One or two urine samples are collected over a sufficiently long interval to minimize errors introduced by bladder storage and transit time. A single injection of inulin or iothalamate is given at the start of the test and several blood samples are obtained to give plasma concentration points. A computer programmed to fit a curve to these raw data points using the method of least squares calculates the area under that curve.[3] The GFR is computed using the integral formulation:

$$\text{GFR} = \frac{\text{amount of tracer in urine to time } t}{\text{integral of the curve describing the plasma fall-off of tracer to time } t}$$

$$= \frac{[A_u]_0^t}{\int_0^t C_p dt}.$$

We refer to this as the cumulative integral method (CIM). It should be emphasized that the C_p curve is being measured by proper sampling and that no predictions of the shape of the C_p curve are required in this CIM calculation. We have also used this same plasma fall-off curve extrapolated to infinity to estimate the GFR without urine collection—this method we call the plasma predictive method (PPM).

Materials and Methods. Eleven patients ranging in age from 8–30 years with normal or impaired renal function were studied using inulin[4] and/or sodium iothalamate-^{125}I.[5] In 4 patients classical constant infusion tests were done on the same or subsequent days using 30 min collection periods.

Inulin was administered as a single dose of 75 mg/kg over 60 sec; time zero was taken as 15 sec after the start of the injection. After premedication with Lugol's solution iothalamate-^{125}I was administered in the same manner in a dose of 5 μCi in patients 10 years and under, and 10 μCi for those over 10 years. In the later studies the iothalamate was given over 15–20 sec and time zero was taken when half of the dose was given. The same solutions given to the patient were used to prepare the standards. The amount given to the patient and for the standard was determined by weighing the syringe prior to and following delivery. Blood samples were obtained at about 2, 6, 20, 60, 120, 180 and 240 min after the start of the test. Prolonged sampling was carried out on 2 patients.

The early rapid fall-off component must be included to evaluate properly the integral of C_p. Samples were taken from the arm oppo-

[3] Copies of this program may be obtained from: The Computer Department for Health Sciences, Faculty of Medicine, University of Manitoba, 753 McDermot Avenue, Winnipeg 3, Manitoba, Canada.

[4] Inulin 10%, Arnar Stone Laboratories, 601 E. Kensington Rd., Mt. Prospect, IL.

[5] Iothalamate-^{125}I Glofil, Abbott Laboratories Limited, P.O. Box 6150, Montreal, Quebec, Canada.

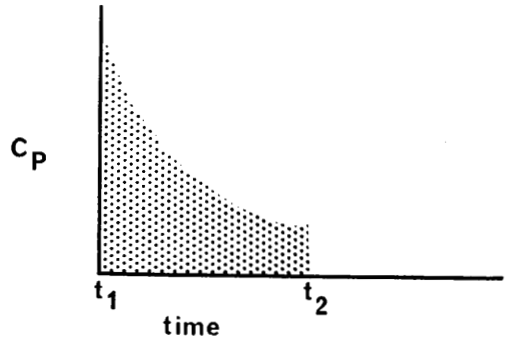

FIG. 2. During the period of urine collection t_1 to t_2 the shaded area under the C_p curve represents the integral of the function $C_p dt$; i.e., $\int_{t_1}^{t_2} C_p dt$.

site the one in which inulin or iothalamate had been injected using an indwelling No. 21 scalp vein needle.

Inulin was determined in blood and urine using an autoanalyzer designed by the authors which makes use of a modification of the hydrochloric acid thiobarbituric acid method of Galli (10). Over the range of plasma and urine values obtained our method gave reproducible values within 3% limits.

One milliliter samples of serum, urine and a standard weighed amount of the diluted injected solution containing iothalamate-^{125}I were counted in a well-type counter for 10–20 min. The cpm per ml of plasma, the cpm excreted in the urine and the total cpm injected were then calculated. In our laboratory the counted values are reproducible within 2% limits.

In order to increase the volume of urine and hence minimize bladder storage error patients were asked not to void for about 2 hr before the test and were encouraged to drink water. For any voiding the larger the urine volume the less significant becomes any possible residual. Furthermore, the cumulative aspect of urine tracer measurement in the CIM ultimately reduces to an insignificant level error introduced by bladder residual. Urine specimens were obtained by voiding around 20 min after the start of the test and then hourly. It should be emphasized that neither blood nor urine collections need be taken exactly at the specified times since use of the integral formulation permits flexibility in the time of sampling, especially as regards the urine.

Results. The results of the studies in the 11 patients are given in Table I.

As shown, the CIM gives a low early estimate of GFR using either inulin or iothalamate and the values rise to a stable level by 120 min in patients with normal filtration and by 180 min when the GFR is markedly reduced. We consider the GFR estimates to be correct when 2 consecutive estimates are within the 5% limits of precision accounted for by variation due to analysis and computation.

The PPM estimates are initially very high but approach the CIM values within 2 hr

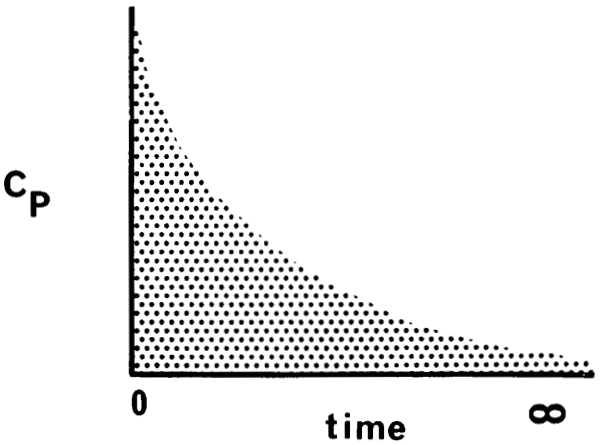

FIG. 3. Following a single injection of a nonmetabolized tracer, as infinity is approached A_u approximates A_0. The shaded area represents the predicted integral of C_p to infinity, $\int_0^{\infty} C_p dt$.

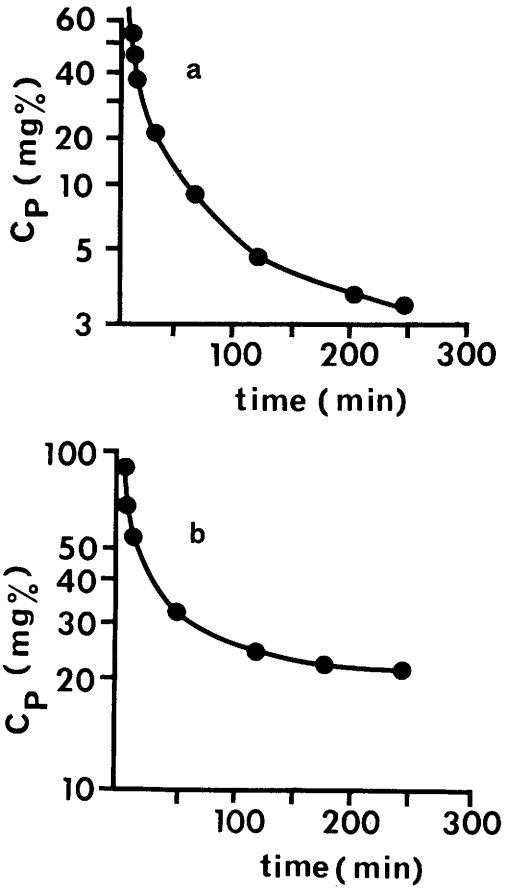

FIG. 4. Comparison of plasma fall-off curve of inulin following a single injection in patient 10, whose GFR is normal (a) with that in patient 1, whose GFR is markedly reduced (b).

when the GFR is normal, but the period is much prolonged if the GFR is reduced. When the GFR is normal and prolonged sampling using inulin is done, the accuracy of the chemical analysis is unacceptable because of the low plasma concentration.

Figures 4a and 5a present the data from a patient with a normal GFR. In this case the loading dose of 10 μCi iothalamate-^{125}I equaled a measured quantity of 3.975×10^6 cpm. One half of these counts were recovered in the urine in 60 min and 78% in 4 hr. Considering the GFR to be 111 ml/min (av of 3 constant infusion values) the initial CIM value using 3 blood samples (3, 5, 20 min) and a single urine sample (24 min) resulted in a computed GFR of 104 ml/min. However, if these data are cumulated using another blood and urine sample at 120 min, a value within 5% of the constant infusion result is obtained. This additional collection time allows errors attributable to bladder storage, transit time and arteriovenous (A-V) concentration differences to become insignificant and demonstrates the value of the integral approach, rather than artificially separating the data into individual collection periods.

As discussed before, the initial PPM estimate is high because the prediction of the area under the curve to infinity during the rapid C_p fall-off phase (due to equilibration and excretion) is much less than the true area. Thus substituting the predicted area from PPM values in the equation:

$$\text{GFR} = \frac{\text{amount of tracer injected } (A_0)}{\text{predicted area under the } C_p \text{ curve at infinity}},$$

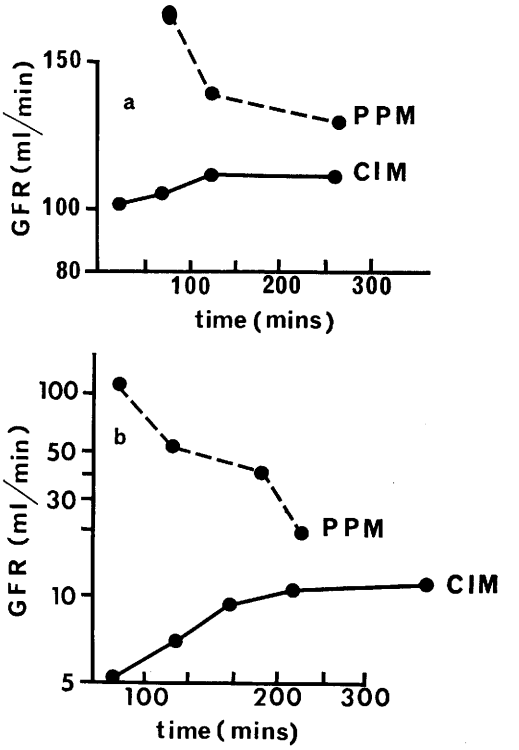

FIG. 5. Comparison of the computed GFR in Cases 10 and 1 (a) and (b). Note that in Case 10, (a), whose GFR is normal, the values both by the PPM and CIM stabilize much earlier than is the case when the GFR is low.

E I. Comparison of GFR Values Obtained Using Constant Infusion Technique, Cumulative Integral Method (CIM), and Plasma Prec
od (PPM).

ase	Tracer amount[a]	Constant infusion[b]		CIM		PPM		% of recov
		Period	GFR[c]	Time[d]	GFR[c]	Time[d]	GFR[c]	%
1	Ioth 4.9418	I	9.8	23	5.0	40	100	50
		II	10.3	153	8.4	100	50	
		III	10.8	210	10.5	220	20	
				361	10.5			
2	Ioth 3.735			30	3.3	160	16	20
				126	6.5	250	14	
				255	6.8			
				383	6.8			
3	Ioth 1.969	I	6.0	31	3.7	30	62.8	19
		II	7.5	103	3.8	181	9.3	
		III	5.0	181	4.2			
				249	4.3			
4	Ioth 3.918			17.4	100	125	170	83
				125	120	277	127	
				218	123			
5[e]	a. Ioth 3.678	I	134	75	126	131	146	74
		II	137	108	133	258	144	
				168	136			
	b. Inulin 3522	I	134	32	135	93	179	80
		II	137	87	136	120	175	
				123	138			
6	Inulin 4680			66	120	25	230	86
				117	124	100	140	
				246	118	160	122	
				422	123			
7	Ioth 3.225			43.2	21.5	61	27.2	34
				129.7	27	120	24.6	
				233.7	22.6	240	22.2	

TABLE I. (Continued).

ase	Tracer amount[a]	Constant infusion[b] Period	GFR[c]	CIM Time[d]	GFR[c]	PPM Time[d]	GFR[c]	% of recove %
8	Ioth 4.999			44	14.3	60	29.8	26
				114	15	117	28.8	
				226	14.3	210	21.2	
				272	14.2	262	19.8	
9	Ioth 4.918			30.49	16.6			29
				110.19	16.1	112	31.9	
				212.03	15.8	207	21.2	
				259.56	15.8	252	24.7	
0[e]	a. Ioth 3.975	I	107	24.7	104	60	170	78
		II	114	64.6	107	122	140	
		III	112	122	114	254	132	
				254	112			
	b. Inulin 2645	I	107	24.7	105	122	169	88
		II	114	64.6	109	357	120	
		III	112	122	108			
				254	111			
1	Ioth 3.833			73	10	73	32	47
				120	18.6	173	28	
				173	19.0	384	25	
				296	19.3			

= iothalamate-^{125}I. The number is the number of cpm injected (A_0) (millions). Inulin dose (mg).
collection periods in which the constant infusion method was used were of 30 min duration.
R (ml/min) uncorrected for surface area.
e (min).
ases 5 and 10 simultaneous iothalamate and inulin studies were done.

gives an overestimation of the GFR. Later this prediction becomes more accurate as slower modes in the plasma fall-off curve appear. Indeed we detected at 240 min the emergence of yet a slower mode of fall-off which if included in the PPM would lead to a further increase in the predicted area under the plasma fall-off curve.

In Figures 4b and 5b an example of data from a patient with reduced GFR is given to demonstrate the difficulty of the PPM technique. Twenty hours were required to attain 50% recovery of the injected dose of inulin in this case. The PPM estimate was found to be extremely sensitive to small errors in the C_p data. Obviously it would be difficult to detect small changes in C_p due to the slow rate of excretion if samples were taken for just 4 hr. For example it was computed in this patient that changing the last C_p data point only 0.4% (from 25.7 to 25.8) changed the PPM estimate from 20 to 10 ml/min. This extreme sensitivity to data errors demonstrates that any estimate based on plasma sampling alone is unreliable in cases where the GFR is markedly reduced. As expected, the CIM estimate required a somewhat longer sampling time than in patients with a normal GFR to attain a constant value, since the urine excretion rate of inulin was slow; by 3 hr however, a consistent and accurate estimate of GFR was obtained.

Discussion. This is the first description of a method for the determination of GFR using a computer calculated integral of the area under the plasma fall-off curve and the cumulated urine collection following a single injection of a suitable tracer. This method overcomes errors introduced by transit time, bladder storage and A-V concentration differences into other techniques for GFR determination. The significance of these errors is now discussed.

Transit time. If a constant plasma concentration of inulin or iothalamate were suddenly established the tracer first appears in the urine in 2–3 min but a constant rate of excretion does not occur before 20 min as shown by Bradley, Leifer and Nickel (7). These authors consider this effect to result from a distribution of transit times in nephrons of various lengths. However, if the cumulative period of urine collection is long enough the error introduced by this factor can be shown mathematically to become insignificant.

Bladder storage. The repeated short urine collection periods used in the constant infusion and other techniques make complete emptying of the bladder difficult. With cumulative urine collection and the possibility of starting the test with residual urine in the bladder this error becomes insignificant.

A-V concentration differences. Truninger, Donath and Kappeler (11) noted errors in excess of 10% in GFR estimation due to A-V differences when sampling for short periods after a single iv injection. With a single injection the arterial concentration is initially higher but soon the tissue spaces are equilibrated and the venous concentration becomes higher because of augmentation of capillary blood by tracer returning from the extravascular space. We have estimated that sampling for 1 hr reduces this error to 5% and for 2 hr to 2–3%.

Techniques using plasma sampling alone such as the 2 compartment model of Sapirstein *et al.* (12) may be extended to give the general equation for the plasma concentration fall-off surve $[C_p(t)]$ as follows: $C_p(t) = Ae^{-at} + Be^{-\beta t}$. . . . Integrating this formula to obtain the area under the curve to infinity gives the integral at infinity $[I(\infty)]$ as follows: $I = A/a + B/\beta$. . . . It can be seen that each mode of $C_p(t)$ contributes a term to the integral. If a slow mode escapes detection, either because C_p is too low to be determined accurately or because plasma sampling is done for too short a period, a significant underestimation of the integral $C_p(t)$ is made. This can consistently result in overestimation of the GFR (13).

Using the method described in this paper we have shown that collecting urine for 2 hr and dividing its content of tracer by the computed area under the curve described by the plasma concentration fall-off for the same period results in an accurate measurement of GFR. There are no predictions since the C_p curve is properly sampled by measured data points. A detailed discussion of the mathematical derivations is presented in a

subsequent paper (14).

Conclusions. A computerized cumulative integral method was developed for the precise determination of the glomerular filtration rate following a single injection of tracer using the integral formulation:

$$GFR = \frac{\text{cumulative amount of tracer in urine to time } t}{\text{integral of the curve describing the plasma fall-off of tracer to time } t} = \frac{[A_u]_0^t}{\int_0^t C_p dt}.$$

This method eliminates errors due to bladder storage, transit time and A-V concentration differences. The accuracy of this method results from several factors including a sound theoretical basis and the use of the computer to curve fit and calculate the cumulative area under the curve.

With blood sampling at 2, 6, 20, 60, and 120 min and urine specimens at 15–30 min and 100–130 min an accuracy within 5% can be expected in individuals whose GFR is 50% of normal or more. An additional blood and urine sample at 180 min is needed when the GFR is below this level.

Summary. A new computerized method for the accurate determination of the glomerular filtration rate (GFR) is presented. It is based on an integral formulation which expands the collection period and minimizes the errors of transit time, bladder storage and A-V concentration differences. A single injection of tracer, followed by serial sampling of blood and a single urine sample taken over 120 min will give an accuracy of within 5% in individuals whose GFR is 50% of normal or more. An additional blood and urine sample at 180 min is occasionally required when the GFR is below this level. The use of a labeled tracer makes this test a practical simple method for evaluation of the GFR in clinical practice.

1. Shannon, J. A., and Smith, H. W., J. Clin. Invest. **14**, 393 (1935).

2. Austin, J., Stillman, H. E., and Van Slyke, D. D., J. Biol. Chem. **46**, 91 (1921).

3. Hendrix, J. P., Westfall, B. B., and Richards, A. N., J. Biol. Chem. **116**, 735 (1936).

4. Sigman, E. M., Elwood, C. M., and Know, F., J. Nucl. Med. **1**, 60 (1965).

5. Griep, R. J., and Nelp, W. B., Radiology **93**, 807 (1969).

6. Mills, J. N., Thomas, S., and Yates, P. A., J. Physiol. (London) **129**, 408 (1955).

7. Bradley, S. E., Leifer, E., and Nickel, J. F., *in* "Ciba Foundation Symposium on the Kidney" (A. Lewis, ed.). Churchill, London (1954).

8. Alving, A. S., and Miller, B. F., Arch. Intern. Med. **66**, 306 (1940).

9. Sakai, T., Leumann, E. P., and Holliday, M. A., Pediatrics **44**, 905 (1969).

10. Galli, M., "A Colorimetric Determination of Inulin in Blood and Urine." Interim reprint of text as presented: 1964 Technicon Int. Symp., New York 1964.

11. Truninger, B., Donath, A., and Kappeler, M., Helv. Med. Acta **34**, 116 (1967).

12. Sapirstein, L., Vidt, D., Mandel, M., *et al.* Amer. J. Physiol. **181**, 330 (1955).

13. Blaufox, M., and Merrill, J., Nephron **3**, 274 (1966).

14. Mildenberger, R. R., Harries, J. D., Drummond, K. N., and Malowany, A. S., Med. Biol. Engin. (in press).

Received Mar. 3, 1972. P.S.E.B.M., 1972, Vol. 140.