MEASUREMENT OF THE GLOMERULAR FILTRATION RATE AND THE EFFECTIVE RENAL PLASMA FLOW USING SODIUM THIOSULPHATE AND *p*-AMINO-HIPPURIC ACID

BY

A. DICK AND C. E. DAVIES

From the Department of Medicine, University of Sheffield

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The clearance methods for the measurement of the glomerular filtration rate, the effective renal plasma flow, and the maximum tubular excretory capacity, which were originally developed by Professor Homer Smith and his associates, have found increased clinical application in America. These tests yield information of fundamental physiological significance, but they cannot be undertaken lightly because they are time-consuming both for patient and doctor. Many of the methods previously employed made use of a continuous drip infusion; either inulin or mannitol was employed to measure the glomerular filtration rate, and diodrast or *p*-amino-hippuric acid to measure the effective renal plasma flow and the maximum tubular excretory capacity. Recent modifications include the use of special infusion pumps which deliver at a constant rate so that equilibrium between injection and excretion can be attained (Earle and Berliner, 1946) and the adoption of a single-injection technique (Landowne and Alving, 1946; Josephson, 1947; and Newman and others, 1946).

Gilman and others (1946), while examining the mechanism of sodium thiosulphate excretion in the dog, found that the clearances of sodium thiosulphate and creatinine were identical. They assumed that sodium thiosulphate clearance was a measure of the glomerular filtration rate in the dog. Subsequently they measured simultaneously, in man, the clearances of sodium thiosulphate and inulin. Single large doses of the two substances were given intravenously (10 g. of inulin in 10 per

cent solution followed by 1 g. of sodium thiosulphate per 10 pounds of body weight). They found that the ratio of inulin to sodium thiosulphate excretion, as measured on declining blood concentrations, averaged 0.99, with a standard deviation of 0.08. These results were based on seventy-three observations; they assumed that sodium thiosulphate clearance was a measure of the glomerular filtration rate in man.

This single-injection method, while eliminating the technical disadvantages of a continuous infusion, does not permit observations to be made over a long period because the plasma concentration soon falls below the level at which it can be accurately estimated. It does not permit of simultaneous measurement of the effective renal plasma flow by *p*-amino-hippuric acid because the concentration of this substance in the plasma must be kept below 4 mg./100 ml. in order to ensure complete clearance in a single passage through the kidney (Goldring and Chasis, 1944). Lastly, the single injection of a large amount of sodium thiosulphate is liable to produce toxic effects; seven of the 26 patients studied by Gilman suffered nausea and vomiting.

The present work is an attempt to establish a reasonably simple method of measuring the glomerular filtration rate, using non-toxic doses of sodium thiosulphate, administered by continuous infusion. In ten normal male medical students the clearances of sodium thiosulphate and inulin have been measured consecutively; the drugs cannot be administered simultaneously in the same drip

because sodium thiosulphate precipitates inulin. In a further three students the glomerular filtration rate and the effective renal plasma flow have been measured simultaneously using sodium thiosulphate and sodium *p*-amino-hippurate. It is hoped that a description of the methods may be of some assistance to other workers.

Methods

The patient, who need not be fasting, is given adequate fluids (for example, 1 litre of water) in the hour preceding the test. A catheter of the Tieman's type, size No. 8, is passed and held in position by adhesive strapping; the bladder is allowed to drain continuously into a 500-ml. conical flask held at a suitable angle in a wooden stand placed between the legs. A special drip apparatus with small and large reservoirs for the priming and sustaining infusions is then set up and the needle introduced into an antecubital vein, the arm being held in position by a posterior splint. Before the infusion is begun, a sample of urine and 10 ml. of blood received into a heparinized screw-cap bottle are obtained for analysis as "blanks." The priming infusion is then commenced and administered in the course of a few minutes in order to obtain a suitable blood concentration, after which the sustaining infusion of about 500 ml. is continued at a rate of 4 ml. per minute. About half an hour is allowed for the diffusion of the drugs, after which three accurately timed collection periods of approximately 20 minutes each are used for the determination of the clearances. Continuous drainage of the bladder takes place during the collection period, at the end of which 20 ml. of saline and 20 ml. of air are injected to ensure that emptying of the bladder is as complete as possible, manual pressure being applied above the pubis. Three clearances of 20 minutes each are generally adequate, in which case the test can easily be completed within 2 hours, but the use of a sustaining infusion of 500 ml. allows two further clearances if required. Blood samples (of 10 ml. each) are withdrawn at the beginning and end of each collection period; the blood concentrations are plotted graphically against time and the concentration of each period is interpolated.

The Infusions

1. MEASUREMENT OF THE GLOMERULAR FILTRATION RATE

(a) *Inulin*.—A sterile 10 per cent solution as supplied by Messrs. Kerfoot was used, 25 ml. in the priming infusion and 75 ml. diluted to 500 ml. with normal saline in the sustaining infusion.

(b) *Sodium thiosulphate*.—A sterile 10 per cent solution (thiostab by Messrs. Boots) was employed, 18 ml. in the priming and 54 ml. diluted to 500 ml. in the sustaining infusion.

2. MEASUREMENT OF THE EFFECTIVE RENAL PLASMA FLOW

A 20 per cent solution of sodium *p*-amino-hippurate was prepared for intravenous use by dissolving 20 g. of the acid in normal sodium hydroxide and adjusting the volume to 100 ml. The pH was corrected to 7.2, the solution filtered through a Seitz filter, and 20 ml. (later 10 ml.) amounts placed in universal containers and sterilized by autoclaving.

In normal subjects at first we gave 4 ml. of the 20 per cent solution of sodium *p*-amino-hippurate for the priming infusion and 16 ml. diluted to 500 ml. with normal saline for the sustaining infusion. Latterly we used half this dosage because, even with concentrations of 1 mg. per 100 ml. or less, accurate determinations can be accomplished. In patients with obvious renal impairment the dosage must be reduced; the urea clearance test might be of help in determining a suitable dose.

3. SIMULTANEOUS MEASUREMENT OF THE GLOMERULAR FILTRATION RATE AND THE EFFECTIVE RENAL PLASMA FLOW

In the combined test the priming infusion contains 18 ml. of a 10 per cent sodium thiosulphate solution and 2 ml. of a 20 per cent sodium *p*-amino-hippurate solution, and the continuous infusion 54 ml. of thiosulphate and 8 ml. of hippurate diluted to 500 ml. with normal saline.

Analytical Methods

1. INULIN

No method for the determination of inulin has proved entirely satisfactory. Alving and others (1939) described a method for determination of inulin at relatively low concentrations (for example, 4 μ g. or more per ml.). This method requires the use of special stoppered tubes to prevent evaporation and to withstand changes of temperature. In the presence of inulin a blue colour develops with alcoholic diphenylamine in hot acid solution. A further improvement was devised by Harrison (1942) in which the diphenylamine reagent was dissolved in a mixture of glacial acetic and concentrated hydrochloric acids and it was not necessary to heat in closed tubes. With both these methods fermentation with yeast is not essential, but both plasma and urine "blanks" should be determined. These methods were not entirely satisfactory; the reagent blank using B.D.H. "analar" reagents was appreciable, and Prof. Homer Smith (personal communication) suggested purification of diphenylamine by recrystallization from 70 per cent alcohol and treatment with norit, which leaves the crystals pure white.

It was decided to use an alternative method giving the Seliwanoff reaction; the following method, devised by Mr. S. W. Cole and used by Bacon and Bell (1948), was employed and found to be entirely satisfactory. We preferred to heat the tubes at 80° C. for 40 minutes in order to ensure maximum development of colour.

Reagents.—The reagents were:

Acid cadmium sulphate: $\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ 17.34 g.; $\text{N-H}_2\text{SO}_4$, 84.55 ml.; water to 500 ml.

1.1 *N*-sodium hydroxide.

0.15 per cent resorcinol (A.R.) in absolute ethanol.

Concentrated hydrochloric acid (A.R.) containing 7.5 mg. ferric chloride per litre.

Inulin—standard solutions containing 0.5, 1.0, 1.5, 2.0, 2.5 mg./100 ml.

The plasma proteins were precipitated by taking 5 vol. of distilled water, 3 vol. of acid cadmium sulphate, 1 vol. of plasma, and 1 vol. of 1.1 *N*-sodium hydroxide (dilution 1:10). After they have stood for a few minutes the specimens are centrifuged, and if necessary the filtrate can be further diluted.

To 4 ml. of plasma filtrate or urine suitably diluted or standard solutions of inulin was added 6 ml. of resorcinol reagent, and the solution was mixed. 6 ml. of concentrated hydrochloric acid reagent was added and mixed, and the tubes were heated at 80° C. for 40 minutes. After being cooled, the solutions were compared in a photo-electric colorimeter using an Ilford spectrum blue filter No. 602. Either glass-stoppered pyrex tubes or universal screw-cap containers could be used; both withstood changes of temperature without breaking. If an Evans type EEL colorimeter is used the tubes should be stoppered, otherwise the fumes of hydrochloric acid affect the selenium cell; with this colorimeter an Ilford minus red filter was used.

2. SODIUM THIOSULPHATE

The method employed was that of Newman and others (1946). Using thiosulphate the plasma proteins cannot be precipitated by cadmium hydroxide, so it is necessary to use a different method from that for either inulin or *p*-amino-hippurate.

Reagents.—The reagents were:

0.01 *N*-potassium iodate (0.3567 g. per litre).

10 per cent potassium iodide (freshly prepared).

0.01 *N*-sodium thiosulphate (2.5 g. per litre).

1 per cent starch solution.

2 *N*-hydrochloric acid.

‡ *N*-sodium tungstate.

‡ *N*-sulphuric acid (not ‡ *N* as stated in original paper).

(a) *Plasma.*—Proteins were precipitated by adding 2 ml. of plasma to 14 ml. of distilled water and adding 2 ml. each of ‡ *N*-sodium tungstate and ‡ *N*-sulphuric acid. After the mixture had stood for a few minutes a clear supernatant fluid was obtained on centrifugation. To 10 ml. of filtrate were added 10 ml. of *N*/100-potassium iodate and 2 ml. of 2*N*-hydrochloric acid, and the mixture was allowed to stand for five minutes. Then 2 ml. of 10 per cent potassium iodide were added and the liberated iodine was titrated immediately with standardized *N*/100 thiosulphate.

The calculation of plasma concentration in mg. per cent was:

$$(\text{standard titration—unknown titration}) \times \frac{1.58}{8} \times \frac{10}{\text{St'd tit.}} \times \frac{100 \times 10}{\text{ml. of filtrate aliquot}}$$

(b) As large an aliquot of urine as possible (5 to 10 ml.) was used and made alkaline with a few drops of *N*-sodium hydroxide using phenolphthalein as indicator. 25 ml. of *N*/100 potassium iodate were added, followed by 2 ml. of potassium iodide and 2 ml. of hydrochloric acid. The liberated iodine was titrated immediately with *N*/100 thiosulphate using starch as indicator.

Calculation of urine concentration in mg. per cent was:

$$(\text{standard titration—unknown titration}) \times 1.58 \times \frac{25}{\text{St'd tit.}} \times \frac{100}{\text{ml. of urine aliquot}}$$

3. PARA-AMINO-HIPPURIC ACID

The method used was a modification of the Bratton and Marshall (1939) method as is fully described by Goldring and Chasis (1944).

Reagents.—The reagents were:

Acid cadmium sulphate and 1.1 *N*-sodium hydroxide as for estimation of inulin.

1.2 *N*-hydrochloric acid.

Sodium nitrite, 100 mg./100 ml. (prepared freshly every few days).

Ammonium sulphamate 500 mg./100 ml. (prepared every two weeks).

N-(1-naphthyl)-ethylene-diamine-dihydrochloride 100 mg./100 ml. (kept in dark bottle).

A stock solution of *p*-amino-hippuric acid containing 2 mg./100 ml., from which a series of standard solutions containing 0.01 to 0.25 mg./100 ml. are prepared.

The plasma proteins were precipitated by taking 10 vols. of distilled water, 3 vols. of acid cadmium sulphate, 1 vol. of plasma, and 1 vol. of 1.1 *N*-sodium hydroxide (dilution 1:15). After being allowed to stand for a few minutes the specimens were centrifuged, and if necessary the filtrate was diluted. To 10 ml. of plasma filtrate, diluted urine, or standard solutions were added 2 ml. of 1.2 *N*-hydrochloric acid and 1 ml. of sodium nitrite solution. The tubes were well shaken and allowed to stand for five minutes, after which 1 ml. of ammonium sulphamate and after three minutes 1 ml. of *N*-(naphthyl)-ethylene-diamine-dihydrochloride were added. The contents were well mixed, and after they had stood for at least 10 minutes comparison was made in a photo-electric colorimeter using a green filter (such as Ilford tricolor green). The mg. per cent of *p*-amino-hippuric acid were read from a curve plotted from known concentrations.

Results

1. A COMPARISON OF INULIN AND THIOSULPHATE CLEARANCES IN 10 NORMAL MALES

In Cases 1 to 8 the thiosulphate was given first, and in Cases 9 and 10 inulin was followed by thiosulphate. Two clearance periods of 20 minutes were used for each substance; the results obtained

TABLE I
COMPARISON OF INULIN AND SODIUM THIOSULPHATE CLEARANCES IN TEN NORMAL MALES

Case no.	Age	Height and weight	Inulin clearance ml. per min.	Thiosulphate clearance ml. per min.	Mean inulin clearance corrected to 1.73 sq. m.	Mean thiosulphate clearance corrected to 1.73 sq. m.
1	20	5 ft. 6 in. 10 st. 1 lb.	a. 168 b. 165	164 165	166.5	164.5
2	32	6 ft. 2 in. 11 st. 8 lb.	a. 132 b. 138	121 113	119	103
3	19	5 ft. 10 in. 11 st.	a. 204 b. 206	208 170	190	176
4	19	6 ft. 1 in. 11 st. 12 lb.	a. 154 b. 154	160 143	134	131
5	26	5 ft. 8 in. 12 st.	a. 148 b. 148	145 156	135	137
6	18	5 ft. 9½ in. 11 st. 1 lb.	a. 169 b. 191	177 178	168	165
7	20	5 ft. 9 in. 10 st. 6 lb.	a. 113 b. 104	113 117	104	111
8	25	5 ft. 10½ in. 10 st. 8 lb.	a. 144 b. 140	142 136	133	131
9	31	5 ft. 10 in. 9 st. 13 lb.	a. 158 b. 164	165 154	158	157
10	34	5 ft. 7 in. 10 st. 3 lb.	a. 166 b. 166	176 180	175	164
Mean ..			—	—	144	146

are shown in Table I. The clearances were corrected to a surface area of 1.73 sq. m. using Du Bois tables. There is a close relationship between inulin and thiosulphate clearances in normal individuals; the mean clearance for inulin was 144 ml. per minute and for thiosulphate 146 ml. per minute.

The total plasma concentration of thiosulphate was 12 to 15 mg./100 ml. and of inulin 20 to 25 mg./100 ml. With these amounts accurate determinations are possible and the end point of the thiosulphate estimation is sharply definable. Both plasma and urine "blank" determinations were made; the mean plasma level of "thiosulphate" was about 4 mg./100 ml. and of "inulin" 2 mg./100 ml. The concentration of the urine will obviously affect such "blanks"; the specimen should be obtained under similar circumstances to those during the clearances. The concentrations of both thiosulphate and inulin in the urine were very low and may therefore be neglected.

2. THE COMBINED TEST TO MEASURE SIMULTANEOUSLY THE GLOMERULAR FILTRATION RATE AND THE EFFECTIVE RENAL PLASMA FLOW

This was carried out in three healthy male medical students; the results are shown in Table II.

The mean clearances for normal males given by Goldring and Chasis (1944) are 697 ml. per minute for the effective renal plasma flow and 131 ml. per minute for the glomerular filtration rate, giving a filtration fraction of 0.19. In normal individuals the range is wide but the results obtained agree closely with the American workers' larger series. The effective renal plasma flow of Case 1 was low; this particular student was very apprehensive and we are inclined to believe his effective renal plasma flow was diminished on this account while his filtration rate remained normal.

Discussion

It was our intention to employ a simple test for the study of separate aspects of renal function, and

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**TABLE II
THE RESULTS OF THE COMBINED TEST TO MEASURE SIMULTANEOUSLY THE GLOMERULAR FILTRATION RATE AND THE EFFECTIVE RENAL PLASMA FLOW
IN THREE NORMAL MALES**

Case No.	Age	Height and weight	Collection period (min.)	Urine vol. (ml.)	Sodium p-amino-hippurate					Sodium thiosulphate					Filtration fraction
					Urine conc. (mg./100 ml.)	Mg./min. excreted	Plasma conc. (mg./100 ml.)	Clearance (ml./min.)	Clearance ml./min. corrected to 1.73 sq. m.	Urine conc. (mg./100 ml.)	Mg./min. excreted	Plasma conc. (mg./100 ml.)	Clearance (ml./min.)	Clearance ml./min. corrected to 1.73 sq. m.	
1	19	5 ft. 8 in. 9 st. 10 lb.	20	88	406	17.8	3.17	560		325	14.25	10.4	137		0.27
					530	14.9	2.97	500		495	13.75	9.5	145		
					520	14.4	2.88	500		465	12.80	9.1	141		
								520	525				141	142	
2	22	6 ft. 2 in. 11 st. 6 lb.	17	66	446	17.4	2.15	817		358	13.9	8.0	174		0.21
					364	19.4	2.03	962		274	14.4	7.6	189		
					240	24.0	2.47	970		157	15.7	8.1	194		
								916	795				186	165	
3	26	5 ft. 10½ in. 11 st. 8 lb.	20	100	298	14.9	1.98	756		329	16.45	10.4	158		0.21
					300	15.0	1.98	756		327	16.35	10.2	160		
					304	14.5	2.06	710		294	14.70	9.6	154		
								740	675				157	143	

we hope this test may be extended for routine clinical use.

The methods already described for the accurate measurement of the glomerular filtration rate and the effective renal plasma flow have several disadvantages, especially when applied to man. Inaccurate collection of urine constitutes one of the main sources of error. In order to obtain reasonable accuracy it is necessary to perform two or three serial collections of urine, employing continuous drainage through an indwelling catheter and washing out the bladder at the end of each collection period with saline and air. The time of the collection period can, of course, be accurately measured by a stopwatch. The time devoted to the collection of blood and urine samples is considerable, but three clearances of about 20 minutes each can be completed within two hours. Apart from the necessity of catheterization, the discomfort to the patient, involving one venepuncture in one arm for the infusion, and four venepunctures in the other arm, is not excessive. None of our patients complained of undue discomfort, and none suffered toxic symptoms from sodium thiosulphate.

We conclude that the method we have outlined employing sodium thiosulphate and *p*-amino-hippuric acid for the measurement of the glomerular filtration rate and the effective renal plasma flow gives accurate results by means of chemical analyses which are within the scope of most laboratories.

Summary

1. A comparison of inulin and sodium thiosulphate clearances in 10 normal males, and the results of the combined test to measure simultaneously the glomerular filtration rate, and the effective renal plasma flow in three normal males are presented.

2. Using a continuous drip infusion sodium thiosulphate appears to offer a reliable means for the determination of the glomerular filtration rate; it is cheap and non-toxic, and the estimation is simple and accurate.

3. Sodium thiosulphate does not affect the excretion of sodium *p*-amino-hippurate.

We wish to thank those students who volunteered as subjects for the tests. We are indebted to Dr. J. S. D. Bacon for advice on the estimation of inulin, and to Dr. A. Jordan for helpful criticism. Thanks are due to Mr. A. F. Nicholls for technical assistance.

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