A Dye Dilution Method of Measuring Renal Blood Flow in Man, with Special Reference to the Anuric Subject.* (27914)

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Current methods of measuring renal blood flow in man are based on the Fick principle. These procedures include the gas diffusion technics, using nitrous oxide or radioactive krypton, and the excretion methods, using paraaminohippurate (PAH) or radioactive Diodrast. The excretion methods cannot be used in anuric subjects, and, even in the presence of urine flow, they yield inaccurate values when the extraction of the test substance is very low. In acute renal failure, only the gas diffusion technics have proved to be reliable. They have, however, some disadvantages.

Recently we have used a dye dilution method, based on the Stewart-Hamilton principle (3,10), to study renal circulation in man. Our first results are presented here.

Material and methods. Eleven subjects, either with normal kidneys or suffering from renal disease, were examined by both the dye dilution technic and the conventional PAH-method(8) on the same occasion and results compared. As the dye method measured the flow in only *one* kidney, the following equation was used for calculation of PAH-values:

$$\text{Renal blood flow (one kidney)} = \frac{C_{PAH}}{E_{PAH}\,(1 - Hc) \cdot 2}$$

where C_{PAH} = PAH-clearance
E_{PAH} = PAH-extraction ratio
Hc = Hematocrit

In addition, the dye dilution technic alone was applied to 4 anuric patients with acute renal failure. Renal oxygen consumption was also determined in these patients.

Technic of the dye dilution method. This method requires catheterization of the renal artery and of the renal vein. The renal artery was catheterized with a Seldinger catheter inserted into the femoral artery according to the percutaneous technic devised by Oedman(7), the renal vein, with a No. 9 Cournand catheter from the antecubital vein(11). Good visualization was ensured by use of a roentgen image amplifier. The position of the catheters was considered to be satisfactory when their tips appeared to lie in the main stem of each vessel. Two different technics were tried. a) *Single injection technic.* According to the Stewart principle, the amount of dye injected into the renal artery is equal to the amount which leaves the kidney by the renal vein, provided no recirculation occurs during the time of sampling (10).

Let I = quantity of dye injected (mg)
Q_R = quantity of dye leaving kidney (mg)
F = renal blood flow (ml/min)
R = concentration of dye in renal vein (mg/ml)

If $I = Q_R$ and $Q_R = F \int_0^t R\,dt$,

$$F = \frac{I}{\int_0^t R\,dt}$$

or, using Hamilton's equation(3):

$$F = \frac{60 \cdot I}{S}$$

in which S = area under the recorded dye concentration curve in the renal vein (sq cm)

Procedure. Renal venous blood was withdrawn at a constant rate (14-29 ml/min) with a pump and passed through the cuvette of a modified oximeter ("Norman dye assembly," manufactured by New Electronic Products Ltd., London). As soon as stabilization of the base line was obtained, an accurately measured quantity of dye (1-2 ml of a 0.1 to 0.2% solution of Evans blue) was injected rapidly into the renal artery. The dye concentration curve in the renal vein was recorded by the dye assembly (Fig. 1). Calibration was performed according to the procedure devised by Sparling(9). While

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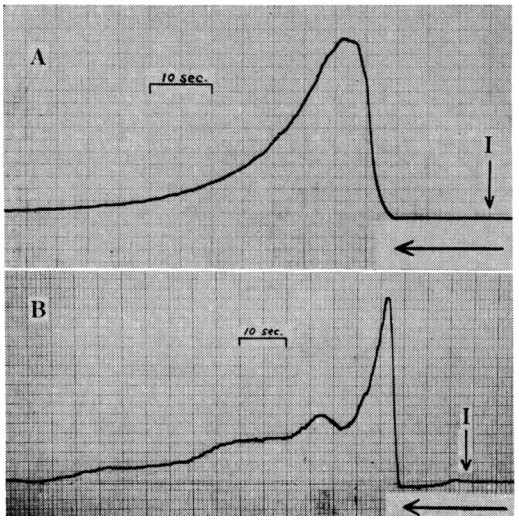

FIG. 1. Two examples of dye dilution curves recorded in the renal vein. I = Dye injection into renal artery. Catheter passage time was 9 sec in case A, 10 sec in case B. Curve A was obtained in an anuric patient (Table I, No. 12), curve B in a normal subject (Table I, No. 3).

blood was drawn at the same rate as for determining the renal blood flow, a known amount of dye was injected into the recording system and a calibration curve was recorded. The whole surface under the flow and calibration curves was measured, and renal blood flow was calculated from the equation

$$F = \frac{I \cdot s \cdot f}{S \cdot i}$$

where f = rate of flow through the recording system (ml/min)
i = quantity of dye used for calibration (mg)
s = area under the calibration curve (sq cm)

The "appearance time" of the dye was calculated by subtracting the catheter passage time from the measured value. An attempt was made to estimate the "mean transit time" and the "effective renal blood volume." As catheter sampling, however, resulted in a marked distortion of the curves, the data were not considered to be reliable. b) *Technic with constant rate of dye injection.* As recirculation of the injected dye occurs, the amount of dye entering the kidney is equal to the sum of injected plus recirculated quantities(10).

Procedure. A solution of dye was injected into the renal artery by a constant speed injector. After 5-10 minutes, simultaneous blood samples of 10 ml were taken from the renal venous catheter and from the femoral artery. Several pairs of samples were withdrawn at different blood levels of dye. After centrifugation the dye concentrations in plasma were determined by colorimetry. Renal blood flow was calculated from an equation similar to that used by Gibbs *et al.* (2) for measuring cerebral circulation:

$$F_{Plasma} = \frac{D}{R_P - A_P} \text{ or}$$

$$F_{Blood} = \frac{D}{(R_P - A_P)(1 - Hc)}$$

where D = constant rate of dye injection (mg/min)
A_P = concentration of dye in arterial plasma (mg/ml)
R_P = concentration of dye in renal venous plasma (mg/ml)
Hc = hematocrit

Results are summarized in Table I.

Discussion. Most experiments performed with the *continuous injection technic* yielded erratic results. Only 3 values have been recorded in Table I. In the remaining instances, there was no measurable arteriovenous difference, or, the arteriovenous difference varied with increasing blood dye concentrations. The reasons for this are not clearly understood. Variable absorption of the dye by the renal parenchyma might represent one factor. With the *single injection technic* the results were more encouraging. The flow values presented in Table I are averages of successive determinations (usually 3 to 5), whose reproducibility was satisfactory. Comparison of the dye and PAH methods shows acceptable agreement for the whole series (average dye/PAH ratio 0.95). In individual cases, however, marked discrepancies were observed (range of the dye/PAH ratios 0.48-1.56). It is hoped that further improvement of the dye method will result in a better correlation. We should like

TABLE I. Comparison of the Dye and PAH Methods of Measuring Renal Blood Flow in 11 Patients. Blood flow and oxygen consumption in 4 anuric subjects.

		Renal blood flow (one kidney)					Oxygen	
		Dye dilution method						
Patient	Diagnosis	Single injection, ml/min	Continuous infusion, ml/min	PAH method, ml/min	Dye/PAH ratio, —	Dye appearance time, sec	a.-v. difference, vol %	Consumption (one kidney), meq/min
1	Normal	1090		698	1.56	1.5		
2	"	563		868	.65	4.5		
3	"	668		1100	.61	4.5		
4	"	400		552	.73	4.5		
5	" *	1305		1305	1.00	<1.0		
6	"	374		780	.48	1.5		
7	"	590		706	.84	1.5		
8	Nephrosclerosis		280	269	1.05			
9	"	432	290	383	1.13 (.75)	4.5		
10	Pyelonephritis	173		208	.83	1.0		
11	"	267		176	1.52	3.0		
Avg cases 1-11					.95			
12	Shock kidney (anuria)	316	184			6.5	1.45	.82
13	*Idem.*	215				4.0	1.51	.58
14	Hg-poisoning (anuria) & pyelonephritis	106				1.0	.30	.06
15	Hg-poisoning (anuria)	538				<1.0	0	0

* Pyrogenic reaction.

briefly to review the causes of error possibly involved in this series.

Influence of the procedure itself on renal circulation. The presence of a catheter within the renal artery may reduce the blood flow. If the tip lies in a small branch, vascular spasms may be elicited. If it lies only a few centimeters away from the aorta, however, interference with the renal circulation is presumably not too important. The procedure requires withdrawal of relatively large amounts of blood, and this may cause renal vasoconstriction. To compensate for the blood loss, a blood transfusion was given during the procedure to most patients. Occasionally, the amount transfused exceeded the quantity lost, resulting in transient hypervolemia and possibly renal hyperemia. Hyperemia attributable to pyrogenic reaction (8) was seen in one instance. Thus, the patients were not all studied under basal conditions.

Errors involved in determining PAH-blood flow. A source of error lies in the fact that the PAH-blood flow for the catheterized kidney has been considered to be half of the total blood flow. This is probably not entirely true, especially in renal disease. In a few subjects, the urine flow was very low at the time the PAH-clearance was measured (less than 0.5 ml/min). In those patients some PAH extracted from the blood was possibly stored within the urinary tract, resulting in unduly low PAH-flow values.

Errors involved in the dye method. Reliable flow values can be obtained only if complete mixing of the dye with the blood is achieved within the renal vessels. If the tips of the catheters are located correctly, this assumption probably holds. If the dye is injected into the main stem of the renal artery, and the tip of the venous catheter lies in a branch of the renal vein, the values are still reliable. If the dye is injected into a branch of the renal artery, or into an accessory artery, there are 3 possibilities: (a)

The venous catheter lies in the main stem of the vein: the blood passing through it comes from the whole kidney, but mixing is likely to be incomplete and the flow values are not reliable. (b) The venous catheter lies in a branch draining the injected territory: only a sectorial flow is measured. (c) The venous catheter lies in a branch of the vein not draining the injected territory: no dye concentration curve can be recorded. We believe that in some of our experiments, an incorrect position of the catheter accounted for the observed discrepancies between PAH and dye methods. A certain amount of the injected dye is taken up by the renal tissue, as can be shown in animal experiments. Under normal conditions, this quantity is probably small. It may possibly increase in cases with tubular necrosis and/or interstitial infiltration, thus resulting in unduly high flow values.

Animal experiments have disclosed marked differences in flow rates through the various parts of the kidney(4). The shape of our dilution curves suggests that the largest fraction of the injected dye passes very rapidly through the renal vessels, presumably through the cortex. In most curves the peak concentration of the dye was recorded 4-8 seconds after its appearance, while the slope of the descending limb was more variable. In some cases it fell rather rapidly (within 10 seconds) in a roughly exponential manner. In other instances the fall was slower and irregular. Occasionally a second and even a third wave was seen, suggesting either recirculation of the dye or, more likely, different rates of flow through the kidney. We believe that recirculation does not contribute significantly in producing high secondary waves for the following reason: the amount of injected dye is small (1-3 mg) and after its first passage through the kidney it is diluted at least 10 times within the body circulation before it reaches the renal vein for the second time. As the length of the venous catheter results in a marked distortion of the curves, a more detailed analysis of their shape is difficult. It is still uncertain whether it is better to measure the whole area under the curve, thus including recirculated dye, or to measure only the area under a "primary" curve constructed according to Hamilton's method(3), thus leaving out the fraction of dye circulating more slowly. In this series, the former procedure was used. Only if recirculation were really an important factor would this method yield unduly low values for the renal blood flow. On the contrary, measuring only a part of the surface under the curve would almost certainly result in unduly high flow values. It is also very important to obtain good calibration curves. In the present series they were not always satisfactory. This fact may partly explain the scatter of the data.

Results in anuric subjects. If the dye method can be considered reliable, the flow values measured in 4 anuric subjects are of special interest. They compare favorably with data obtained by other investigators using gas diffusion methods(1,5,6). They seem to confirm that in acute anuria the renal blood flow is not as markedly reduced as was formerly believed. In the 2 cases with shock anuria, the time of appearance of the dye was normal or perhaps slightly increased. The renal arteriovenous oxygen differences were normal, so that oxygen consumption was reduced to about the same extent as was blood flow. In the 2 cases with mercury poisoning, the time of appearance of the dye was extremely short (<1 sec). The arteriovenous oxygen difference was 0 in one case and 0.3 vol. % in the other, so that oxygen consumption was very low.

Summary. A dye dilution method of determining renal blood flow in man is described. This technic requires catheterization of the renal artery and the renal vein but no sampling of urine. Data on 11 patients with measurable urine flow, PAH clearance and PAH extraction ratio, as well as on 4 anuric subjects, are reported. Comparison with the PAH data indicates that the results of the dye method are probably valid. Possible causes of error are discussed.

1. Conn, H. L., Wilds, L., Helwig, J., *J. Clin. Invest.*, 1954, v33, 732.

2. Gibbs, F. A., Maxwell, H., Gibbs, E. L., *Arch. neurol. and psychiat.* 1947, v57, 137.

3. Hamilton, W. F., Riley, R. L., Attyah, A. M.,

Cournand, A., *et al., Am. J. Physiol.,* 1948, v153, 309.

4. Kramer, K., Thurau, K., Deetjen, P., *Pflüger's Arch.,* 1960, v270, 251.

5. Mériel, P., Galinier, F., Suc, J. M., *Proc. 1st Internat. Congress of Nephrology,* Karger & Basle, New York, 1961, p224.

6. Munck, O., *Renal circulation in acute renal failure,* C. C Thomas, Springfield, Ill., 1958.

7. Oedman, P., *Acta radiol.,* 1956, v45, 1.

8. Smith, H. W., *The kidney in health and disease,* Oxford Univ. Press, New York, 1950.

9. Sparling, C. M., *Recording and quantitative interpretation of dye dilution curves obtained by reflectometry in red or infrared light.* Van Gorcum & Co., Groningen, 1961.

10. Stewart, G. N., *Am. J. Physiol.,* 1921, v57, 27.

11. Warren, J. V., Brannon, E. S., Merrill, A. J., *Science,* 1944, v100, 108.

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Passive Acute Glomerulonephritis Induced by Antigen-Antibody Complexes Solubilized in Hapten Excess.* (27915)

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The studies of Germuth(1) and of Dixon *et al.*(2) have established that the glomerular lesions of serum sickness occur during the phase of immune elimination of the antigen, when antigen-antibody complexes circulate in the blood, and that antigen can be detected in the lesions by the fluorescent antibody technic. Recent experiments from this laboratory have shown that the lesions of serum sickness, especially acute glomerulonephritis, can be induced passively in mice by intravenous injection of adequate amounts of antigen-antibody complexes prepared from rabbit(3) or mouse antibody(4) and solubilized in antigen excess. The fate and distribution of the complexes have been studied by the fluorescent antibody technic(5). These findings have been confirmed and extended by Mellors and Brzosko(6), who also observed the localization of the injected complexes in the glomerular lesions by immunofluorescence. The significance of this observation was weakened, however, by their finding that normal rabbit gamma globulin conjugated with fluorescein, when injected intravenously, also localized to some extent in normal mouse glomeruli(6). The possibility that this property of the gamma globulin was the result of conjugation with fluorescein was not investigated.

To provoke glomerulonephritis by intravenous injection of soluble antigen-antibody complexes, large amounts of antigen have to be used in order to maintain the complexes soluble. While control experiments have shown that similar injections of foreign proteins do not induce the lesions of glomerulonephritis by themselves(3), it seemed desirable to modify the technic to eliminate this objection. In experiments reported here, acute glomerulonephritis has been produced in mice with rabbit anti-2,4-dinitrophenyl bovine gamma globulin (DNP BγG) antibodies precipitated at equivalence with 2,4-dinitrophenyl bovine serum albumin (DNP-BSA) and dissolved in excess hapten, ϵNH_2 DNP lysine. The rapid diffusion of the small molecular hapten allows formation of the larger immune aggregates, which have been shown by the fluorescent antibody technic to localize characteristically in the reticuloendothelial system(7) and in glomeruli(5). Control experiments carried out with equal doses of purified rabbit anti-DNP antibody alone showed absence of similar localization.

Materials and methods. Antigen. Bovine gamma globulin (BγG) and bovine serum albumin (BSA) (Armour Pharmaceutical Co., Illinois) were conjugated with 2,4-dinitrofluorobenzene as described previously(8).

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