THE ADAPTATION OF THE NITROUS OXIDE METHOD TO THE DETERMINATION OF RENAL BLOOD FLOW AND *IN VIVO* RENAL WEIGHT IN MAN [1]

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(Submitted for publication May 28, 1956; accepted August 7, 1956)

Since 1945 sodium para-aminohippurate (PAH) (1) has been widely used for the measurement of renal blood flow (2). Since the urinary output of PAH must be measured in this procedure, the errors inherent in urine collection contribute to the error in the determination of renal blood flow. Furthermore, the need to obtain values of urinary concentrations in the calculation of renal blood flow precludes the use of PAH, and therefore of this method, in the study of patients with oliguria or anuria. Recently, Conn, Anderson, and Arena (3) adapted the nitrous oxide method of Kety and Schmidt (4) to the determination of renal blood flow in dogs. The following is a description of the adaptation of this method to the estimation of renal blood flow in man as well as of the utilization of this method for the calculation of renal weight *in vivo*.

METHODS

Five normotensive fasting subjects without clinical evidence of renal disease and seven patients from the medical wards of the University Hospitals were studied. As noted in Table II, the latter presented a great variation in renal functional impairment.

In the five normotensive subjects renal hemodynamics and metabolism were studied by means of standard clearance techniques and right renal vein catheterization (2, 5). In these individuals three fifteen-minute control clearance periods were followed by the determination of renal blood flow by the nitrous oxide method (see below).

In the seven patients demonstrating a variety of renal disease states as well as different degrees of renal functional impairment the method of study was similar to that described for the normotensive subjects with the exception that following the first nitrous oxide determination the study was continued for three additional fifteen-minute periods (45 minutes), at which time a second nitrous oxide renal flow study was performed. This part of the study was designed to determine the reproducibility of the technique.

Renal blood flow by the nitrous oxide method. Renal blood flow was determined by the nitrous oxide method using a modification of the method of Kety and Schmidt (4). A mixture containing approximately 15 per cent nitrous oxide, 20 per cent oxygen and 65 per cent nitrogen was delivered into a 150-liter bag which in turn was attached to a large-bore corrugated rubber tubing through a three-way valve for respiratory gases. The valve was arranged so that the investigators could change the patient's inspiratory gases from room air to the nitrous oxide mixture without the patient's knowledge. Delivery was made to the patient through a rubber mouthpiece attached to the corrugated tubing. The nose was clamped to avoid all leaks. Arterial blood was sampled from the femoral artery through a Cournand needle attached to a plastic tubing which in turn was connected to a manifold consisting of four three-way stopcocks and an end exhaust syringe containing 2 to 5 ml. of heparin. Renal venous blood was obtained from the right renal vein through a cardiac catheter connected directly to a manifold similar to that described for the arterial collection. Since the arterial-venous differences of nitrous oxide in the determination of renal blood flow were very small, it was of great importance to have the dead space on both sides of the blood collection systems as equal as possible, and to have maximal accuracy in the timing and collection of samples as well as in the analysis of specimens of N_2O. The mean difference between duplicate samples of the same blood analyzed for N_2O in this laboratory was .02 vol. per cent (S.D.[4] .02 vol. per cent).

Specimens of blood were collected in oiled, heparinized syringes. Syringes were mounted consecutively on the arterial and venous manifolds with the first specimen most distal to the patient. Prior to each renal blood flow determination, arterial and renal venous blanks were drawn. These should be identical in value as a check of the N_2O analyses.

[1] This investigation was supported in part by grants from the Wisconsin Heart Association, Wisconsin Alumni Research Foundation, and National Heart Institute of the National Institutes of Health (H-1495-C), Public Health Service.

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[4] S.D. = Standard Deviation.

At the time of sampling, blood was drawn into the exhaust syringes at the distal ends of each manifold at a steady rate of one ml. per 5 seconds. The nitrous oxide mixture was turned on after 2½ ml. of blood had been drawn into the exhaust syringes. This arbitrarily assumed volume was equal to 2.5 times the total dead space (one ml.). This volume was assumed to remove from the dead space heparinized blood which was present between determinations or samples. Following the beginning of the administration of the gas mixture, sampling into the exhaust syringes was continued until a total volume of 4 ml. had been obtained. The difference in the volumes of blood in the exhaust syringes between the initial 2½ ml. and the final 4 ml., namely 1½ ml., was estimated to be equal to the time necessary for the nitrous oxide to reach the lung, be absorbed, and be carried in the blood to the orifice of the first sample syringe. These arbitrarily assumed values were not believed to carry particular weight in the determination.

After 4 ml. had been withdrawn into the exhaust syringes, sampling was begun into the first sample syringe at a rate of one ml. per 5 seconds. During the first 1½ minutes of the flow determination three consecutive 6-ml. specimens were obtained at this rate of withdrawal. The fourth 6-ml. specimen was collected from 2 minutes and 45 seconds to 3 minutes and 15 seconds, whereas the fifth specimen consisting of 8 ml. was withdrawn from 4 minutes and 40 seconds to 5 minutes and 20 seconds after the onset of gas administration. At the completion of specimens 3, 4 and 5 the blood in the exhaust syringes was reinjected to clear the system and prevent clotting. Prior to the collection of specimens 4 and 5, the sampling systems were again cleared by drawing blood back into the exhaust syringes at a rate of one ml. per 5 seconds to a total volume of 4 ml. as had been done prior to the first specimen. It is important that the collection into the exhaust syringes proceeds at the same rate as the collection of the specimens in the sample syringes, since the volume of the dead space has already been filled when sampling has begun and this blood constitutes part of the sample submitted for analysis.

The arterial and venous concentrations of N_2O were plotted at the midpoint of the sampling times. The scales used were such that the base line (time) exceeded by two times in length the vertical scale (arterial and renal venous N_2O concentrations). This purposeful distortion of the curves was produced in an effort to gain more accurate readings of the arterial-venous nitrous oxide differences at half-minute intervals instead of minute intervals. The obtaining of half-minute $A\text{-}V_{N_2O}$ differences seemed preferable since the flow rate per unit weight of kidney was rapid and the area between the curves small. Furthermore, since equilibrium by this method is reached in the kidney in 5 minutes, in contrast to a 10-minute period for the brain (4) and myocardium (6), this manner of calculation for renal blood flow provided the same number of $A\text{-}V_{N_2O}$ differences as it is customary to utilize in calculating cerebral or myocardial blood flows. However, this distortion of the curves in the renal method necessitates the multiplication of the final calculated results by two. In these calculations the partition coefficient was assumed to be unity. Such an assumption was based on the findings in the dog kidney where the validity of the N_2O method utilizing this coefficient for measurement of renal blood flow has been demonstrated by comparison with the bubble flow meter (3). More justification for its use may be derived from the similarity of partition coefficients for dog and human brain (4) and myocardium (6).

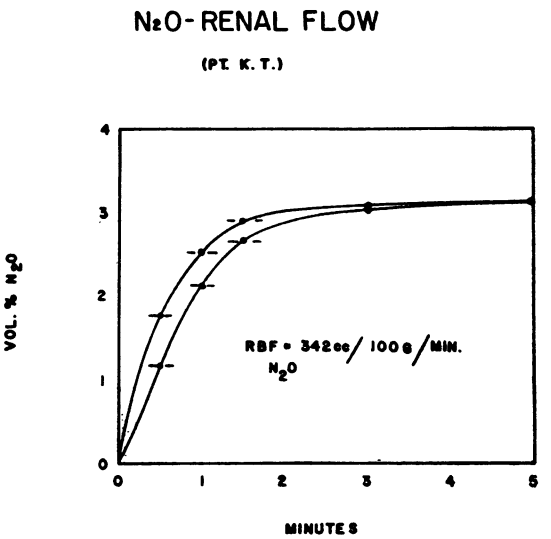

FIG. 1. REPRESENTATIVE NITROUS OXIDE FLOW CURVE

The upper and lower curves represent the arterial and venous, and the nitrous oxide concentrations, respectively. The short horizontal lines denote the 30-second periods of time when the individual samples were obtained during the first 1½ minutes and from which the midpoints were selected (see Methods).

RESULTS

A typical nitrous oxide flow curve is shown in Figure 1.

The data on the five normotensive subjects are presented in Table I. The values for renal blood flow (PAH) ($\bar{x}$ [5] = 1200, S.D. 96 ml. per minute), arterial-renal venous oxygen difference ($\bar{x}$ = 1.3, S.D. 0.2 vol. per cent), and renal oxygen consumption ($RBF_{PAH} \times A\text{-}R_{O_2}$) ($\bar{x}$ = 15.2, S.D. 2.0 ml. per 2 kidneys per minute) are in accord with previous publications (7).

Renal blood flow as determined by the nitrous oxide adaptation equaled $\bar{x}$ = 322, S.D. 59 ml. per 100 G. per minute, whereas renal oxygen consumption, utilizing this method, was $\bar{x}$ = 4.1, S.D. 0.5 ml. per 100 G. per minute.

[5] $\bar{x}$ = mean.

TABLE I

Renal weight in vivo (normal male) *

Pt.†	Age *yrs.*	TRBF *ml./min.*	RBF (N_2O) *ml./100 G./min.*	Renal weight *grams*	A-Ro_2 *vols. %*	Qo_2 *ml./100 G./min.*	Qo_2 *ml./2 Kg./min.*
1	21	1,205	328	367	1.0	3.3	12.1
2	26	1,300	342	380	1.2	4.1	15.1
3	34	1,050	260	404	1.5	3.9	15.8
4	37	1,260	406	310	1.2	4.9	15.1
5	34	1,175	272	432	1.5	4.1	17.8
$\bar{x}$‡	30	1,200	322	379	1.3	4.1	15.2
σ§		(±96)	(±59)	(±46)	(±0.2)	(±0.5)	(±2.0)

* TRBF = total renal blood flow = $\frac{UV_{PAH}}{A_{PAH} - R_{PAH}}$.
RBF (N_2O) = renal blood flow nitrous oxide technique.
Renal weight = $\frac{TRBF}{RBF\ (N_2O)} \times 100$.
A-Ro_2 = arterial-renal venous oxygen difference.
Qo_2 = RBF (N_2O) × A-Ro_2 = ml. oxygen/100 G./min.
Qo_2 = ml. oxygen/2 kidneys/minute = TRBF × A-Ro_2.
† Patient—all were free of organic disease and on psychosomatic service.
‡ $\bar{x}$ = mean.
§ σ = standard deviation.

Renal weight, calculated as the quotient of total renal blood flow (PAH), expressed as ml. per minute, and renal blood flow (N_2O), expressed as ml. per 100 G. per minute, multiplied by 100, was $\bar{x} = 379$, S.D. 46 grams. This figure compares favorably with the kidney weights obtained at autopsy by Wald of $\bar{x} = 323$, S.D. 57 grams (8), and with the mean value of 388 grams given by Lee and Thomas (9) for normotensive males of this age group.

Table II shows initial (control) values as well as values determined 45 minutes later for renal blood flow, determined by the nitrous oxide technique, and for renal weights in seven patients with a variety of renal disease states. The means of the individual ratios relating the initial (control) observations to those obtained 45 minutes later were 1.02 (S.D. 0.11) and 1.02 (S.D. 0.13) for renal blood flow and renal weight, respectively.

DISCUSSION

The assumptions upon which the adaptation of this method to studies of renal blood flow are based have been discussed by Conn, Anderson, and Arena (3) and Conn, Wood, and Schmidt (10) and insofar as possible have been tested in these experiments. In addition it was determined that, even allowing for clearance of the urinary tract "dead space," the urinary loss of nitrous oxide during the five minutes of inhalation was negligible ($< .002$ ml. N_2O). Obviously it has not been possible to check the estimated renal blood flow by the nitrous oxide technique against a direct method, as was done by previous investigators in the dog (3), but the good agreement of the calculated renal weights reported in this paper with those obtained at autopsy (8, 9) supports the validity of the data.

Furthermore, in the calculation of renal weight, particularly in disease states, it must be assumed that: 1) The blood flow per unit weight of kidney (*i.e.*, the N_2O flow) is uniform throughout both kidneys. This might not be true in conditions such as polycystic disease of the kidneys, renal tumors, unilateral renal disease, *etc.* 2) The measurement of renal blood flow by the Fick PAH method represents the *total* renal blood flow through both kidneys. An example for which such an assumption would not be valid is complete unilateral ureteral obstruction. In such an instance the total renal blood flow, and therefore the renal weight, might be in error by 50 per cent of the actual renal weight. 3) The partition coefficient of N_2O remains unity. In these studies this has been assumed to be true. In experiments on dogs (10) in which bilateral renal disease was purposely induced by a variety of methods this coefficient remained unchanged. However, further studies should be performed to evaluate this point either directly, as in patients with renal

TABLE II *

Reproducibility of renal blood flow (N_2O) and renal weight

Pt. Diagnosis	Control			45 minutes			Ratio $\frac{C}{45\ min.}$	
	TRBF *ml./2 Kg./ min.*	RBF (N_2O) *ml./100 G./ min.*	Renal weight *grams*	TRBF *ml./2 Kg./ min.*	RBF (N_2O) *ml./100 G./ min.*	Renal weight *grams*	RBF (N_2O)	Renal weight
P. S. Chronic pyelonephritis	390	157	248	380	167	228	0.94	1.09
D. S. Diabetic nephropathy	2,030	398	510	1,790	406	440	0.98	1.16
S. R. Essential hypertension	630	336	186	630	348	182	0.97	1.02
L. A. Essential hypertension	1,215	398	307	1,285	394	327	1.01	0.94
K. L. Essential hypertension	1,070	303	353	1,060	324	328	0.94	1.08
M. H. Chronic glomerulonephritis	675	307	220	675	246	274	1.27	0.80
G. G. Chronic glomerulonephritis		377			367		1.03	
$\bar{x}$ =							1.02	1.02
σ =							±0.11	±0.13

* Symbols as in Table I.

disease who are brought to operation for other reasons, or indirectly by comparing *in vivo* and *in vitro* renal weights in such patients as may come to autopsy.

This method presents the following advantages in man over the conventional PAH method: 1) It obviates the necessity for urine collection and its associated error. 2) It may permit the determination of renal blood flow in oliguric states. 3) The entire procedure for the collection of the specimens for subsequent nitrous oxide analyses can be performed in five minutes, thereby allowing a more "instantaneous" measurement of flow than other methods. Such a factor becomes important in calculations of renal oxygen consumption. 4) The technique and materials quantitatively require no greater skills or costs than the PAH method. 5) It provides a method for the determination of renal oxygen consumption in terms of ml. per 100 G. per minute *in vivo* which should lend itself to comparison with renal oxygen consumption *in vitro*.

The disadvantages are those which have been found to hold for the adaptation of this method to the study of cerebral (4) and coronary (11) blood flows.

SUMMARY AND CONCLUSIONS

1. The adaptation of the nitrous oxide technique to the determination of renal blood flow in man is presented.

2. In five healthy male subjects renal blood flow (N_2O) was found to be $\bar{x}$ = 322, S.D. 59 ml. per 100 G. per minute.

3. A comparison of calculated renal weights, utilizing this method and total renal blood flow ($\bar{x}$ = 379, S.D. 46 grams), with those obtained by others at autopsy ($\bar{x}$ = 323, S.D. 57 grams and 388 grams) (8, 9) was favorable and adds validity to the method.

ACKNOWLEDGMENTS

The authors wish to thank Mrs. Phyllis Fosshage, Mrs. Audrey Peterson, Mrs. R. Gilis, Miss T. Wuebben, Miss V. Putna, and Miss M. Dahl for their technical assistance in this work.

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