A COMPARISON OF RENAL BLOOD FLOW RESULTS OBTAINED IN THE INTACT ANIMAL BY THE NITROUS OXIDE (DERIVED FICK) METHOD AND BY THE PARA-AMINO-HIPPURATE (DIRECT FICK) METHOD [1]

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The nitrous oxide method as originally conceived was applied to the cerebral circulation and found to provide an accurate clinical means of determining cerebral blood flow (1). The use of an inert gas as a test substance which reaches a blood-tissue diffusion equilibrium made possible the application of a derived Fick equation and circumvented the need for direct measurement of the rate of gas uptake by the tissue. Because of this advantage, a technically modified version of the original method was recently advocated (2) as a new approach to the measurement of renal blood flow in the intact subject, human or animal, with a particular application to the anuric state, since other renal flow methods in clinical use then become totally inadequate. In that communication, a comparison of results obtained by the nitrous oxide method with those obtained by the simultaneous use of a bubble flow meter was made and the agreement found under the prevailing conditions was felt to be quite good; approximately ± 15 per cent (95 per cent limits). Following that study an opportunity has arisen during the course of other experiments to test further this method in intact dogs under more varied conditions (excluding anuria) at the same time as para-amino-hippurate (PAH) Fick renal blood flow measurements were being made. Since the PAH Fick method is recognized as the most accurate one clinically available for measuring renal blood flow, and since it too was previously found to show a good agreement with bubble flow meter measurements (3), an evaluation of the agreement obtained between PAH and nitrous oxide determinations seemed worthwhile. Such an evaluation forms the substance of the present report and appears to justify our increasing confidence in the nitrous oxide method under most conditions, providing certain important precautions are observed.

METHOD

Mongrel male dogs weighing from 19 to 32 Kg. were used. Anesthesia was produced by an intravenous injection of Pentobarbital sodium 25 to 30 mg. per Kg. Access to renal venous blood was obtained by passing a number seven venous catheter, under fluoroscopic guidance, into the left renal vein from an external jugular vein. Catheterization of the left renal vein in the dog is considered preferable to the right, because the catheter can be placed more distal to the spermatic vein on the left than to the vena cava on the right. Access to arterial blood was obtained by the introduction of a polyethylene plastic catheter into the femoral artery. Each catheter was then attached to a manifold consisting of five three-way stop-cocks. Thus multiple blood samples could be drawn quickly and consecutively. These samples were drawn into oiled, heparinized 10 ml. syringes. A catheter was placed in the bladder and urine samples were collected through it into 250 ml. volumetric flasks. This catheter was clamped off during the nitrous oxide flows.

The PAH and the nitrous oxide flows were not run in exactly simultaneous fashion. In a few experiments, the five minute nitrous oxide determination was carried out in the middle of a fifteen minute PAH clearance period while in most experiments the nitrous oxide flow was done immediately after the last PAH clearance period.

The details of the technique of the nitrous oxide flows, method of analysis of samples, and the calculation of results have been considered at length in previous reports (1, 2). In brief, a gas mixture containing 15 per cent nitrous oxide was administered *via* tracheal tube for five minutes. Multiple, simultaneous, integrated arterial and renal venous blood samples were withdrawn at intervals over the three to five minute period necessary for the nitrous oxide concentration between the blood and the renal parenchyma to reach equilibrium. The samples were

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analyzed in the Van Slyke-Neill manometric apparatus. The results were calculated from the formula

$$RBF_{N_2O} = \frac{R^{t'} \cdot S}{\int_0^{t'} (A - R)\, dt} \cdot 100,$$

where

RBF = renal blood flow/100 grams of kidney/minute,
R = renal venous concentration of N_2O,
S = partition coefficient between blood and tissue (here assumed to be unity),
A = arterial concentration of N_2O,
t' = time of blood tissue equilibrium.

and reported in ml. per 100 Gm. of renal tissue per min. to the nearest 5 ml.

The details of the technique of PAH whole blood flow used here, the analysis of the samples, and the calculation of the results have also been discussed in previous communications (3, 4). The general outline is as follows: A constant intravenous infusion of PAH sufficient to give whole blood concentrations of 5 to 10 mg. per cent was used. Two, or more commonly, three urine collection periods of fifteen minutes each were run. Arterial and renal venous blood samples were drawn at the beginning and end of each period. The blood samples were analyzed for whole blood PAH and the urine samples for PAH using the Evelyn photoelectric colorimeter. The only important difference in the analytical procedure from that outlined in the Smith modification of the Bratton and Marshall method was the use of trichloracetic acid in a final concentration of 2.5 per cent as the protein precipitating agent rather than cadmium sulfate. The flow results were calculated to the nearest 5 ml. per min. from the Fick equation,

$$RBF_{PAH} = \frac{UV}{A - R},$$

where

RBF = total blood flow for both kidneys per minute,
UV = amount of PAH excreted in the urine per minute,
A = arterial concentration of whole blood PAH,
R = renal venous concentration of whole blood PAH.

Since the results here are expressed in terms of total flow for both kidneys whereas nitrous oxide results are expressed in terms of 100 Gm. of one kidney, it was necessary to sacrifice the animals at the end of the experiment and determine the total weight of the two kidneys so that a common denominator of flow could be reached for comparison (ml. of flow per 100 Gm. of renal tissue per min.).

The renal blood flow comparisons were made under several experimental conditions produced for the purpose of making an evaluation of renal hemodynamic and functional changes brought about by agents known or thought to play an important role in the production of anuria. When the circumstances of the experiment were appropriate to the use of both the Fick (PAH) and the gas diffusion (N_2O) method, a comparison was made. A tabulation of the existing experimental states is as follows:

1) One animal was studied during the intravenous infusion of 1:40,000 epinephrine (U.S.P.) at the rate of 0.1 ml. per min.
2) One animal was given 400 mg. per Kg. of sodium potassium tartrate intramuscularly and studied forty-eight hours later.
3) One animal was subjected to hemorrhage and studied at a time when the mean arterial pressure was 90 mm. Hg.
4) One animal was studied three and one-half hours and one studied forty-eight hours after an intravenous infusion of 7.5 ml. per Kg. of human blood of Type A and O respectively.
5) Three animals were studied two to four hours after 0.6 Gm. per Kg. of human hemoglobin had been given intravenously and two studied forty-eight hours after the same dose, and finally,
6) Three animals were studied three, four, and forty-eight hours, respectively, after 0.7 Gm. per Kg. of human globin solution had been given intravenously.

RESULTS

Thirteen flow comparisons, nitrous oxide *versus* PAH, were made in thirteen dogs under varying experimental conditions. The results of the comparison along with the existing experimental states are summarized in Table I. Both nitrous oxide flows and PAH flows are recorded in milliliters of renal blood flow per 100 grams of renal tissue per minute. The ratio of flows $\frac{RBF_{N_2O}}{RBF_{PAH}}$ is recorded in the last column. The flows as determined by PAH ranged from 90 to 325 ml. per 100 Gm. per min. so that the comparison covered flow values from the normal range down to less than one-third of normal. The ratio of flow varied from 0.82 to 1.22. The difference of this ratio from unity has been analyzed statistically. The mean ratio RBF_{N_2O}/RBF_{PAH} is 1.02. The difference of this mean from unity (+ 0.02) has a S.D. of ± 0.128, a S.E. of the mean of ± 0.036 and a t value of 0.55, indicating that at the commonly accepted level of statistical significance no systematic error is demonstrated.

DISCUSSION

A. Validity of blood flow comparison

The lack of exact simultaneity in the comparative flow determinations may be raised as an objection to the validity of the comparison. However, since the animals were kept essentially stable at a constant depth of anesthesia through the short period involved in making the two measurements there is no reason to suspect more than the usual

TABLE I

*Comparison of renal blood flow by N_2O method and PAH method (last three columns)**

Dogs	Experimental condition	RBF_{N_2O} *ml. per 100 Gm. kid./min.*	RBF_{PAH} *ml. per 100 Gm. kid./min.*	$\frac{RBF_{N_2O}}{RBF_{PAH}}$
1	During (I.V.) U.S.P. epinephrine	170	155	1.10
2	48 hrs. after injection (I.M.) of NaK tartrate	305	315	0.97
3	Hemorrhage with moderate hypotension	220	180	1.22
4	3½ hrs. after human blood infusion	260	260	1.00
5	48 hrs. after human blood infusion	300	285	1.05
6	2 hrs. after human hemoglobin infusion	160	195	0.82
7	2½ hrs. after human hemoglobin infusion	200	180	1.11
8	4 hrs. after human hemoglobin infusion	100	90	1.10
9	48 hrs. after human hemoglobin infusion	325	325	1.00
10	48 hrs. after human hemoglobin infusion	260	295	0.87
11	3 hrs. after human globin infusion	210	240	0.87
12	4 hrs. after human globin infusion	150	165	0.90
13	48 hrs. after human globin infusion	240	200	1.20
			Mean	1.02

* Mean = 1.02; S.D. = ±0.128; S.E. of the mean = ±0.036; t = 0.55.

minor variations in renal blood flow. We, therefore, do not feel that any serious error is introduced by considering the flows as simultaneous. A second objection to the validity of the comparison arises from the fact that the nitrous oxide method relates flow to a representative 100 Gm. of kidney, in this case the left, whereas the PAH method gives results in terms of total blood flow through both kidneys. One must then determine the total kidney weight in order to reduce the two flow results to a common denominator at the same time assuming that all (100 Gm.) parts of each kidney have essentially the same flow. These problems are also thought to offer no more than minor difficulty since the method of weighing the kidneys so as to include all renal blood (capillary) involved in the nitrous oxide equilibrium, but no other, can introduce only a small error; since the abnormalities being produced during the course of the studies involved both kidneys equally; since *post mortem* examination of the kidneys showed no focal vascular abnormalities; and finally, since the present experimental results with one method (PAH) recording total renal flow and another (N_2O) recording flow per unit of renal tissue involved are in good agreement.

B. *Accuracy of the nitrous oxide method*

The ratio of nitrous oxide flow to PAH flow RBF_{N_2O}/RBF_{PAH} and the standard deviation of the difference of the ratio from unity approximate the values which can be calculated from previous studies comparing the nitrous oxide and the PAH methods for renal blood flow with the bubble flow meter (2, 3). The experimentally determined values are 1.02 and 12.8 per cent and the calculated values 1.03 and 9.0 per cent. The accuracy of the nitrous oxide method in the intact animal then appears to have no significant systematic error and a random error of no more than ± 25 per cent (95 per cent limits). Actually it appears to have a random error little more than the ± 15 per cent (95 per cent limits) found when it was compared with the bubble flow meter, most of the remaining variability residing in the PAH determinations.

These statistics give us increased confidence in the ability of the nitrous oxide method to measure satisfactorily renal blood flow in the intact subject under most circumstances, anuria or no anuria, providing renal venous blood may be sampled. The one circumstance under which significantly large inaccuracies may arise, *i.e.*, rapid changes in renal weight, have been alluded to previously (2).

However, if one is to obtain consistently accurate results a rather rigid attention to detail must be observed. This derives from the fact that renal blood-parenchyma equilibrium occurs early, normally in two to three minutes. Therefore, the renal arterio-venous differences in several of the samples will usually be less than one volume per cent. All catheters, syringes, and connections to the manifolds as well as the stop-cock connections themselves must have no gas leaks, there must be no air in the systems, the timing and rate of draw-

ing of blood samples must be very accurate, the gas mixture must be administered in a constant fashion without leakage, the bladder catheter, if in place, perhaps should be clamped off, and finally the analysis of samples must be carefully done by well-trained personnel.

Since the gas diffusion technique described herein appears to give satisfactorily accurate results in the determination of renal blood flow, use of the infra-red gas analyzer and the application of isotopic techniques should make this method even more feasible for use in human or animal investigation, particularly in the anuric subject. These new advances increase the ease, rapidity, and perhaps the accuracy of analysis at the same time reducing to 25 ml. or less the total amount of blood needed for one flow determination.

CONCLUSIONS

1. Renal blood flow as measured by a gas diffusion (nitrous oxide) method was compared with that obtained by the Fick (PAH) method in thirteen intact dogs.

2. A good agreement between the two methods was obtained under the prevailing conditions. The $\frac{RBF_{N_2O}}{RBF_{PAH}}$ has a mean of 1.02 and the difference of this mean from unity has a S. D. of $\pm$ 0.128, a S. E. of the mean of $\pm$ 0.036, and a t value of 0.55.

3. The results indicate that the gas diffusion (nitrous oxide) method should provide a satisfactory clinical measure of renal blood flow under most conditions, provided renal venous blood may be sampled and the details of the method are carefully observed.

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