Measurement of Coronary Blood Flow in Dogs and Man from Rate of Myocardial Nitrous Oxide Desaturation

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A technic of measuring left ventricular coronary blood flow in dogs and man from the rate of desaturation of nitrous oxide from the myocardium is presented. The desaturation technic appears valid in comparison with the previously described saturation technic, and affords definite technical advantages. By combining the two technics, flows may be measured within much less time than by repeating either method alone.

STUDIES in metabolism of the intact heart were given a new impetus with the development of (1) a technic of sampling coronary venous blood from the left ventricular myocardium under essentially normal physiologic conditions,1 and (2) application of this technic in conjunction with the nitrous oxide blood flow method of Kety and Schmidt to measure coronary blood flow.2 The method was found to give results comparable to simultaneous measurements with the bubble flow meter by Eckenhoff and his associates3 and was then used in collaboration with Bing and his co-workers to measure coronary blood flow in man.4 Up to this time a great deal of information had been obtained by many investigators from in vitro, heart-lung, isolated heart and open chest preparations, and in morphinized dogs with a brass cannula inserted into the coronary sinus. The method described in the present series of reports permits these previous observations to be checked, modified, or extended under conditions which, for the first time, produce a minimum of physiologic abnormality. Gregg's excellent monograph5 and the recent review of Olson and Schwartz6 have already emphasized the necessity of repeatedly correlating observations obtained by coronary venous catheterization with controlled in vitro experiments in order to permit anything approaching a reasonable over-all interpretation.

This paper will present the technic and advantages of a modified nitrous oxide coronary blood flow method which has been used throughout these studies. Soon after the first validation of the nitrous oxide method for coronary blood flow by Eckenhoff and associates,3 it was found to be more advantageous first to saturate the subject with 15 per cent nitrous oxide and then to measure coronary blood flow from the rate of desaturation, or elimination of nitrous oxide from the myocardium, following a return to breathing room air. The desaturation technic has already been applied to several completed reports,4,7,8 which deferred detailed description and validation of the method to the present report. In addition, evidence will be presented concerning the inherent reproducibility of the nitrous oxide method.

METHODS AND CALCULATIONS

Coronary blood flow was measured in five dogs and five patients by the saturation technic2 immediately followed by a second measurement by the desaturation technic. This was done in order to determine the inherent reproducibility of results by...
the method, as well as the validity of the desaturation in comparison with the saturation technic. In addition, a series of 12 normal nembutalized dogs (20–25 mg. per kilogram intravenously) was studied by the desaturation technic alone, after achieving the characteristic steady state described by Corcoran and Page. The conditions included a slightly elevated mean arterial blood pressure (mean: 145 mm. Hg), a rapid regular pulse rate (mean: 158 per minute), normal respiratory exchange and oxygenation, intact pupillary and corneal reflexes, absence of shivering, and a normal or slightly elevated rectal temperature. In addition, a dog to be considered normal had to show normal arterial blood levels of glucose, lactate and pyruvate.

Of the five patients studied, three had apparently normal cardiovascular systems, one (B. C.) showed a mild labile essential hypertension without signs of cardiac or renal abnormality, and one (F. B.) had hypertension with left ventricular hypertrophy associated with a coarctation of the aorta, but no signs or symptoms of cardiac or renal insufficiency. All patients were studied in the postabsorptive state, approximately two to three hours after a light breakfast of fruit juice, cold cereal, toast and jam, and coffee, and were lying supine on the fluoroscopy table, made comfortable by a relatively radiolucent rubber mattress. Sedation was limited to 50 to 75 mg. of Demerol intramuscularly given before observations were made.

Coronary venous and pulmonary arterial catheterizations were performed by the maneuvers previously described in detail in dogs and man. Femoral arterial samples were obtained in dogs through inlying polyethylene tubing and brachial or radial arterial samples were obtained in man through a short bevel no. 20 gage inlying needle connected to stiffened polyvinyl tubing. The latter has the advantage of being stiff enough to permit accurate arterial pulse pressure recordings. A length of tubing was selected which would make the volume of the arterial sampling system equal to that of the coronary venous catheter in order to avoid errors from unequal deadspace volumes.

Mean arterial pressures were measured in the dog by a direct mercury manometer and in man by electrical integration with a Sunborn electro-manometer.

Expired air samples were collected in a Douglas bag through a minimal dead space flutter valve system using a packed endotracheal tube in dogs, and in man a standard metabolism mouthpiece and nose clip. A Blodgett dog mask may be used in unanesthetized dogs. Volumes were measured in a standard wet test meter. Analyses for oxygen and carbon dioxide were performed on 1 instead of 2 cc. samples used by Kety and Schmidt. The water after deaeration is returned to the 4 cc. instead of the 5 cc. mark in the cup above the extraction chamber, leaving the total reacting volume for the final reading exactly the same as when a 2 cc. blood sample is used. The conversion factor, $P_{N_2O}$, then becomes double the factor reported for a 2 cc. sample, and varies linearly with temperature from 0.2912 at 20 C. to 0.2766 at 30 C.

Duplicate analyses are performed and are required to agree within 0.1 volume per cent. Particular care must be taken with the temperature correction for water vapor pressure, especially if the room temperature changes much during the analyses.

The calculation of coronary blood flow in cubic centimeters/100 Gm. of left ventricular muscle per minute is illustrated in figure 1 and table 1. This process is made easier by the practice of drawing multiple, self-integrating one-minute samples consecutively.

A complete protocol of the saturation-desaturation technic will be furnished with the authors' reprints if requested.

**Nitrous Oxide Saturation-Desaturation Technic for Measuring Coronary Blood Flow**

Duplicate observations of coronary blood flow within the shortest possible time, not only enabled us to compare and validate the desaturation with the standard saturation technique, but also to determine the inherent reproducibility of the nitrous oxide method. If all assumptions upon which the method is based are correct, then the shape of the arterial and venous uptake and elimination curves (myocardial saturation and desaturation) should be similar, except for the possible influence of loss of nitrous oxide through the pericardium as suggested by Gregg. The flow values thus obtained should nearly duplicate each other. To provide the greatest possible accuracy in sampling, a series of one minute integrated samples is drawn continuously and simultaneously from arterial and coronary venous catheters throughout the critical first 4–5 minutes of saturation or desaturation when nitrous oxide levels are changing rapidly and the coronary arteriovenous nitrous oxide differences are greatest. If partial coronary venous obstruction by the catheter has been avoided, and a smooth sampling through the catheter obtained, the arterial and venous levels are sufficiently close to equilibrium after four minutes in dogs and after five minutes in man so that further extension of the period of observation is unnecessary. Only a final spot check sample is taken at 8–10 minutes to confirm the shape of the curves and their validity by observing their final approach toward equilibrium.

The blood samples are analyzed for nitrous oxide by the method of Kety and Schmidt with the following exceptions: analyses in dogs are performed on 1 instead of 2 cc. samples used by Kety and Schmidt. The water after deaeration is returned to the 4 cc. instead of the 5 cc. mark in the cup above the extraction chamber, leaving the total reacting volume for the final reading exactly the same as when a 2 cc. blood sample is used. The conversion factor, $P_{N_2O}$, then becomes double the factor reported for a 2 cc. sample, and varies linearly with temperature from 0.2912 at 20 C. to 0.2766 at 30 C.
continuously throughout the critical initial four to five minutes of observation, for the nitrous oxide A-V differences may be calculated by simple subtraction of each integrated venous level from the simultaneous arterial level (or arterial from venous in the case of the desaturation curve). The plotting of the venous curve is necessary to estimate the venous level at the end of each minute, which determines the numerator of the Fick equation. The present technic is preferable to the practice of drawing only one continuous arterial and venous sample throughout the entire period of observation with a final spot venous level as advocated by Scheinberg and Stead for the following reason: much of the accuracy of the nitrous oxide method depends upon ability to evaluate critically the pattern of the arterial and venous nitrous oxide curves. Errors in sampling, analysis, or technic of nitrous oxide administration are often obvious from an irregularity in the shape of one or both of the curves. Evaluation of such errors, which are bound to occur at times, is impossible if only one long integrated arterial and venous sample is drawn throughout the critical period of change in nitrous oxide concentration.

The following procedure was used to measure left ventricular weight at autopsy: the auricles were carefully removed down to the ventricular myocardial rim. All epicardial fat was removed. The right ventricle was cut away, including the trabeculae carneae and papillary projections jutting from the septum. Since most of the work of the septum is concerned with the work of the left ventricle, it was included in the left ventricular weight. The ratio of left ventricular weight to total body weight averaged 0.0049 (standard error of mean = .0002) in 30 dogs by the present technic, compared with 0.0037 by Herrman's method. This 22 per cent difference in mean ratio is readily explained by the fact that Herrman divided the septum along the medial raphe.

Individual variations in left ventricle-body weight ratio were so great that it did not appear practical to use any such ratio to estimate left ventricular weight indirectly from body weight alone in any given experiment. The coefficient of variation of the ratio left ventricle-body weight was 22.0. Hellerstein and Santiago-Stevenson have further emphasized the variability of heart weight-body weight ratio and the gross inaccuracy of predicting one from the other by any such formula as suggested by Herrman for dogs or by Smith for man. Coefficient of correlation of left ventricular weight with total body weight was significant (r = 0.650) but far from close, with a nearly identical correlation coefficient of 0.656 with surface area. However, a left ventricle-body weight factor may be used to advantage as an approximation when comparing changes in myocardial efficiency in a single subject over a short period of time. In the present observations comparing different animals, only those whose hearts were actually weighed at postmortem examination were used in calculations involving left ventricular weight. In addition, only those autopsies were considered valid which took place immediately after the experiment, or in which the weight and general condition of the animal were unchanged.

Cardiac output in liters per minute was measured by the Fick principle by dividing the total body oxygen consumption, in cubic centimeters per minute, by the arteriovenous oxygen difference between systemic and pulmonary arteries in cubic centimeters per liter. This was expressed as the cardiac index by dividing by body surface area.

Total body oxygen consumption was obtained by collecting expired air in a Douglas bag during the latter five minutes of the desaturation coronary blood flow measurement. The systemic arteriovenous oxygen difference was determined from blood samples drawn simultaneously over a one-minute period during the collection of the expired air sample. Peripheral arteriolar resistance (PAR) was measured in arbitrary units calculated by dividing the mean pressure gradient in millimeters Hg (mean arterial pressure minus 25 mm. assumed capillary pressure) by the cardiac output in cubic centimeters per second.

Coronary vascular resistance (CVR) was measured by dividing mean arterial blood pressure (mm. Hg)
by coronary blood flow in cubic centimeters per 100 Gm. per second. CVR was expressed in this way rather than as coronary resistance of the total left ventricular myocardium since it would provide a closer basis for comparison in a homogeneous tissue where total left ventricular weight may vary greatly or would not be known, as in studies in man.

Left ventricular work in kilogram meters per minute was determined as the product of cardiac output in kilograms of blood per minute and mean arterial blood pressure in meters of blood.

Several objections to the derivation of both terms in calculating efficiency may be raised. First, the peripheral work of the left ventricle (mean arterial blood pressure times cardiac output) may be 5 to 30 per cent less than the central mechanical work developed in cardiac ejection, even with a normal aortic valve, and fails to account for velocity and variations in the shape of the ejection curve.\textsuperscript{14} Second, the nitrous oxide method gives values for coronary blood flow and myocardial oxygen consumption only per 100 Gm. of left ventricle, since

\begin{table}
\centering
\caption{Nitrous Oxide Flow Calculations From Figure 1, Patient B. C.}
\begin{tabular}{|c|c|c|c|c|}
\hline
Time Mins. & $\int_{T_1}^{T_2} \frac{A}{dt}$ & $\int_{T_1}^{T_2} \frac{V}{dt}$ & $\int_{T_1}^{T_2} (A-V) \frac{dt}{S}$ & $A\frac{V}{S} \cdot 100 + \int_{0}^{T_2} \frac{(A-V) \cdot dt}{S}$ = Flow \\
\hline
0-1 & 1.76 & 1.26 & 1.12 & 98 \\
1-2 & 3.50 & 3.86 & 2.50 & 97 \\
2-3 & 3.79 & 3.65 & 3.29 & 96 \\
3-4 & 3.91 & 3.71 & 3.71 & 95 \\
4-5 & 4.02 & 3.92 & 4.18 & 94 \\
5-8 & 4.20 & 4.26 & 4.63 & 93 \\
\hline
\end{tabular}
\caption*{Calculated Flow/100 Gm. L. Ventricle/min. = 101 cc.}
\end{table}

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Time Mins. & $\int_{T_1}^{T_2} \frac{A}{dt}$ & $\int_{T_1}^{T_2} \frac{V}{dt}$ & $\int_{T_1}^{T_2} (V-A) \frac{dt}{S}$ & $A\frac{V}{S} \cdot 100 + \int_{0}^{T_2} \frac{(V-A) \cdot dt}{S}$ = Flow \\
\hline
0-1 & 2.23 & 1.76 & 1.41 & 88 \\
1-2 & 1.17 & 1.39 & 2.41 & 96 \\
2-3 & 1.53 & 1.68 & 2.91 & 94 \\
3-4 & 1.10 & 1.45 & 3.26 & 92 \\
4-5 & 0.88 & 1.42 & 3.32 & 90 \\
5-8 & 0.70 & 1.31 & 3.70 & 88 \\
\hline
\end{tabular}
\caption*{Calculated Flow/100 Gm. L. Ventricle/min. = 92 cc.}
\end{table}

Abbreviations: $\int_{T_1}^{T_2} \frac{A}{dt}$ = integrated values for arterial (A) and coronary venous (V) nitrous oxide levels and A-V differences obtained by continuous sampling at indicated times. $S$ = Nitrous oxide partition coefficient = solubility of nitrous oxide in human heart muscle + solubility in blood.\textsuperscript{4}

Left ventricular efficiency was expressed as the mean peripheral work of the left ventricle in kilogram-meters per minute, divided by the total left ventricular oxygen consumption converted to equivalent kilogram meters per minute. The latter was obtained from oxygen consumption in cubic centimeters per minute multiplied by a conversion factor which varies linearly with the myocardial respiratory quotient between a value of 2.00 at a respiratory quotient of 0.70 and 2.10 at a respiratory quotient of 1.00.
values whose significance and accuracy cannot be assessed from present knowledge.

**RESULTS AND DISCUSSION**

The calculation of the coronary blood flow in patient B. C. by both saturation and desaturation technics is demonstrated in figure 1, and table 1. Equally close correlation existed for the values obtained by the two methods in most of the other nine double determinations (table 2). There was a rather marked variation in the rate of coronary blood flow from one dog to another, with extremes from 110 to 260 cc. per 100 Gm. per minute (excluding dog 63), in contrast to the generally slower and more consistent values in man. This variation is undoubtedly due to the fact that dogs under Nembutal anesthesia are not in a true basal condition, and the increased cardiovascular activity is similar to that occurring in mild activity. The human subjects reported here, however, had no medication other than mild sedatives and were in an essentially basal condition.

This same variation in Nembutalized dogs is apparent in the larger series of observations presented in table 3. For example, there is a rather high mean coronary blood flow of 147 cc. per 100 Gm. per minute. The values for arterial blood pressure, cardiac index, left ventricular work, pulse rate, and total body oxygen consumption are also high. These cardiovascular effects of Nembutal anesthesia need not be further elaborated here, since they have been discussed by Foltz and colleagues and comparisons have been made of the results in anesthetized and unanesthetized dogs and humans by Spencer and co-workers. The use of morphine and a mixture of Nembutal and di lurethane, as recommended by Foltz, results in a cardiac status more closely resembling the normal as far as pulse rate and blood pressures are concerned.

The close correspondence of the values for saturation and desaturation coronary blood flows gives support to the validity of the desaturation as compared with the saturation technic. The main reason for the preference for the desaturation method is that it avoids the possible danger of leaks in the respiratory system during saturation. This problem is
especially troublesome in patients in whom a mouthpiece is used rather than an endotracheal tube. The beginning of desaturation is more instantaneous and less subject to contamination due to deadspace than is true with the saturation technic. Furthermore, measurement of coronary blood flow during desaturation enables the simultaneous measurement of cardiac output, for expired air can be collected and blood analyzed for oxygen and carbon dioxide during the desaturation period. During saturation the excess flow of inhaled gas mixture and high nitrous oxide concentration render collection and analysis of expired air impractical.

One of the errors inherent in the nitrous oxide method has been brought out by Gregg and co-workers, who found that nitrous oxide may diffuse outward through the pericardium at a rate which becomes particularly significant when the myocardium is nearly saturated. This may explain persistent small A-V nitrous oxide differences in some cases even after prolonged saturation, which in turn adds an error to calculations of flow over prolonged periods of high nitrous oxide concentration. In the desaturation method, however, when the myocardial concentration is high, the coronary A-V difference is wide and the external loss is relatively less significant with correspondingly less error in calculation. Some support for Gregg's external nitrous oxide loss phenomenon is obtained from our observation that arterial and venous nitrous oxide levels often approximate one another more quickly during desaturation than during saturation. The period of observation necessary to obtain arteriovenous equilibrium is thus advantageously shortened.

The average normal coronary blood flow was 96 cc. per 100 Gm. of left ventricular muscle per minute in eight determinations in our four patients, ranging from 91 to 103 cc. This was higher than the average of 65 cc. reported in our initial study, for three possible reasons:

1. The original normal values were all calculated from saturation curves in which the error due to loss of nitrous oxide into epicardial fat and pericardium may be greater than with the desaturation technic.
2. No allowance was made at that time for the greater solubility of nitrous oxide in human, compared with canine, heart muscle. Utilization of the solubility coefficient of 1.1 reported for the human heart would raise this previously reported average from 65 to 77 cc. per 100 Gm. per minute.
3. The residual discrepancy between 72 cc. and our present mean value of 96 cc. is probably attributable to a difference in interpretation of the nitrous oxide curves after five minutes. Between three and five minutes the arterial curve becomes nearly a straight line and the arteriovenous difference soon approaches the total estimated sampling error of 0.2 to 0.4 volumes per cent. Attempts to calculate the Fick equation much beyond this point only lead to a cumulative error, usually in the direction of a lower value for observed coronary flow. Fortunately, an almost instantaneous equilibration between coronary blood and myocardial nitrous oxide concentrations occurs, as suggested by the fact that flow values calculated at two or three minutes are usually not much greater than at four or five minutes. Thus, unlike cerebral blood flows measured by the nitrous oxide method, it may be unnecessary and perhaps inaccurate to carry out the calculation of coronary blood flow to the point of complete arteriovenous nitrous oxide equilibration, particularly in a saturation curve. It is important, however, to calculate the flow from the curves to a point where the arterial level is relatively stable, and the venous nitrous oxide level can be estimated accurately enough to obtain a valid numerator for the Fick equation.

The use of the saturation method followed immediately by desaturation has afforded an opportunity to do repeated coronary blood flows within a shorter period of time than has been possible previously. The close correspondence of the values thus obtained demonstrates the inherent reproducibility of the nitrous oxide method for measuring coronary blood flow.

**SUMMARY AND CONCLUSIONS**

1. A technic of measuring left ventricular coronary blood flow in dogs and man from the rate of myocardial nitrous oxide desaturation...
has been presented. The values obtained are nearly identical with values obtained by an immediately preceding observation of the rate of saturation. The desaturation technic is therefore considered valid in comparison with the saturation technic and affords definite technical advantages which are discussed.

2. The technic and calculations in dogs and man are similar except for the necessity of correcting for the greater solubility of nitrous oxide in human heart muscle. The coronary blood flow in resting normal man averaged 96 cc. per 100 Gm. of left ventricle per minute.

3. Utilization of the desaturation technic is illustrated by a series of hemodynamic changes on normal Nembutalized dogs.

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