Coronary Blood Flow Measured by $^{131}$Iodo-antipyrine

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 Coronary blood flow in intact animals is usually measured by the nitrous oxide method of Kety and Schmidt as modified by Eckenhoff et al. The technical and theoretical difficulties involved have stimulated the search for a more convenient method. $^{86}$Rb clearance and $^{131}$I albumin dilution curves require major assumptions and give variable results. Hollander et al. have employed clearance of Na$_{131}$ injected directly into the myocardium and Herd et al. have utilized direct coronary artery injection of K$_{85}$ both applying the principle of Kety for the case in which arterial concentration of indicator is zero. For technical reasons neither of these two methods can be used readily, in present form, to measure coronary blood flow in man.

The inertness and rapid diffusibility into tissue water of antipyrine, 4-amino antipyrine, and the iodinated derivative I$_{131}$ antipyrine, have prompted their use for blood flow determinations. Huckabee applied the Kety principle using 4-amino antipyrine (measured chemically) to determine flow in the heterogeneous tissues of the hind limb and gravid uterus, tissues sometimes not suitable for nitrous oxide equilibration. Sapirstein, however, has stated that antipyrine does not equilibrate rapidly enough with limb water to be used in the application of the Kety method. In further studies in which flow was calculated from proportional extraction ratios, this worker found I$_{131}$ antipyrine to be useful for cerebral blood flow but not coronary flow. His methods for the heart are not applicable to the intact animal.

**Methods**

After preliminary trials to establish technics of timing and sampling, studies were performed on 20 dogs (18-25 kg). They were anesthetized with morphine sulfate (3 mg/kg intramuscularly), followed by approximately 50 ml of chloralose (1.6%) -urethane (16%) intravenously; and in some animals intravenous pentobarbital (20 mg/kg) was used alone. Catheters were placed fluoroscopically in the coronary sinus and femoral artery. Respiration was controlled with a Harvard pump attached to a cuffed endotracheal tube. Circulatory function was allowed to vary spontaneously or was altered by atropine, hemorrhage, hypoxia or surgical shock to achieve different rates of coronary flow. Ten determinations were performed with the chest and pericardium opened widely.

Approximately 20-50 µg of I$_{131}$ antipyrine (I$_{131}$ A.P.) were diluted in saline. After a blood background sample was taken, I$_{131}$ A.P. was injected intravenously over a 2-minute period by a constant infusion pump. Simultaneously, nitrous oxide desaturation was begun according to the method of Goodale and Hackel. After a 3 to 5-second delay to allow venous transit, samples were taken anaerobically in heparinized syringes, making proper time allowance for catheter dead space. Separate arterial and venous samples were drawn smoothly during each minute for four minutes. Timed spot samples were then taken from artery and vein as quickly as possible. Aliquots were taken from each syringe with care to prevent loss of nitrous oxide, and 1 ml was pipetted into uniform test tubes and counted in a well-type scintillation counter. The blood remaining in the syringe was used for manometric determination of nitrous oxide. The coronary flow (CF) was calculated from the Kety formula.

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CORONARY BLOOD FLOW

\[ \text{CF} = \frac{100 \times V_2 \times S}{\Sigma (A-V)t} = 100 \times \frac{\left( \frac{\text{counts/min}}{\text{ml}} \times \frac{\text{counts/min}}{g} \times \frac{\text{counts/min}}{\text{ml}} \right)}{\frac{\text{counts/min}}{\text{ml}} \times \frac{\text{min}}{\text{min}}} \]

\[ = \frac{\text{counts/g}}{\text{counts/ml} \times \text{min}} = \text{ml/100g/min} \]

The coronary arteriovenous difference \( \Sigma (A-V)t \) in counts per minute times minutes, was summed arithmetically. \( V_2 \) represented venous concentration of \(^{131}\)I.A.P. at two minutes in counts per minute per ml. Its value was derived from a curve drawn through the graphically plotted mean venous concentrations from 0-2 minutes (fig. 1). \( S \) = partition coefficient (in counts per minute per gm tissue divided by counts per minute per ml coronary venous blood), equalled 1.00 ml/g. \( V_2 \) is therefore substituted for tissue concentration in counts/g.

To verify the graphic derivation of \( V_2 \), in seven experiments, a single rapid sample was removed at precisely two minutes via a second catheter (in the same position in the coronary sinus), while integrated sampling was continued through the first catheter.

To ascertain the partition coefficient, myocardial biopsies were obtained in 13 open chest dogs. \(^{131}\)I.A.P. was infused as before and integrated minute samples taken for two minutes. At two minutes a coronary venous spot sample was taken and simultaneously a 1-2 g wedge of left ventricle (apex or anterolateral wall) was excised from the beating heart. This tissue was blotted lightly, large vessels or adipose tissue trimmed away, and 0.5 - 1.0 g samples weighed immediately, accurate to 0.01 g. These samples were placed in test tubes, counted, and compared with the two-minute venous blood sample.

Nitrous oxide desaturation was considered adequate after four minutes and constituted the reference method. In 16 determinations comparison was also made between the coronary blood flow calculated from both the two-minute and four-minute periods of desaturation by the method of Goodale and Hackel. \(^{17}\)

Results

Preliminary work demonstrated the following: (a) Serial spot samples may be unreliable particularly during the first minute of infusion when the arterial concentration was changing rapidly. Furthermore, rapid sampling through the venous catheter was not always possible. Use of integrated sampling gave consistent reproducible, smooth curves. (b) The use of two-minute infusion periods was empirically derived to achieve a balance between the very rapid fluctuations of a single injection with possible lag in tissue equilibration on the one hand, and the narrow arteriovenous differences and prolonged sampling time necessary with longer infusions on the other. (c) Simultaneous comparison of \(^{131}\)I.A.P. with nitrous oxide proved more reliable than sequential determinations since, by this means, sampling, timing, and physiological state were identical. (d) "Washout" (or negative arteriovenous difference) occurs after cessation of "uptake" during the 2-minute infusion. The difference between the two or net uptake may be used to calculate flow over the total time period (usually 4 minutes). The flow so derived did not show as high a degree of correlation \((R = 0.79 \pm 0.25)\) with the 4-minute nitrous oxide flow as did the 2-minute flow.

Figure 2 demonstrates the correlation in 21 experiments between the \(^{131}\)I.A.P. method and the simultaneous 4-minute integrated nitrous oxide flow over a range of antipyrine flows from 26 to 280 ml/100 g per minute. The nitrous oxide flow tended to be 10% higher. The graphic estimation of the 2-minute venous concentration of \(^{131}\)I.A.P. was reliable in comparison with simultaneous spot samples (in the experiments with 2 catheters in the coronary sinus) and yielded a ratio of estimated to actual concentration of 0.99 \pm 0.07.

The partition coefficient for \(^{131}\)I.A.P. at two minutes over a range of flows was 1.04 \pm 0.13 as determined by direct left ventricle/blood ratio (table 1). The site of left ventricular sampling was representative of other areas of the left ventricle within approximately 10%. \(^{18,19}\)

Analysis of fractions of the nitrous oxide desaturation curves gave good agreement be-
Typical curve drawn from a 4-minute integrated $I^{131}$ antipyrine flow during a 2-minute infusion. During the third minute, the mean venous concentration of $I^{131}$ antipyrine usually equaled or exceeded the arterial concentration and then declined to reach equilibrium. This represented the usual form of indicator dilution buildup and washout. The final method includes only the first two minutes with a venous spot sample for $I^{131}$ antipyrine concentration taken immediately afterward.

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**Discussion**

The deficiencies of the nitrous oxide method are well known. Practically, the technique is relatively time-consuming (10-20 minutes), requires multiple manometric analyses, does not permit results to be available at the time of study, requires significant phlebotomy, and demands close patient cooperation. Theoretically, it is subject to the vagaries of the steady state, physiological and pathological tissue variations in diffusion rate, and most important, to variable tissue perfusion rates.

The use of antipyrine tends to minimize or obviate some of these problems. The total time required for the determination is only 2 1/4 minutes; simple well-counting (or direct recording) is substituted for manometric analysis of nitrous oxide; total phlebotomy required averages 35 ml (including oxygen analysis); patient cooperation and mouthpiece breathing are not necessary. On the theoretical level, these experiments permitted validation, during the actual conditions of the determination, of the partition coefficient and blood/tissue equilibration, which has not been feasible with nitrous oxide. The partition coefficient for nitrous oxide in myocardium has been determined only in vitro; the value of 1.05 in dogs was reduced for convenience to 1.00. The partition coefficient for $I^{131}$ A.P. was directly determined in vivo. The value of 1.04 left ventricle/blood so obtained suggests free diffusion into total tissue water (theoretical = 0.98). A value of 1.00 was assumed for convenience in the calculations.

The blood-tissue comparison at the 2-minute point in the flow also demonstrated directly the blood-tissue equilibration of $I^{131}$ A.P. With nitrous oxide the criterion for equilibration has been virtual disappearance.

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of the arteriovenous difference (0.3 vol %). Sapirstein\textsuperscript{20} pointed out that the arteriovenous difference for nitrous oxide may lie within limits of measurement, and theoretically, poorly perfused tissue may remain unequilibrated. The present study demonstrated that normal canine left ventricular myocardium is evenly and rapidly perfused so that equilibration of $^{131}$I A.P. occurred at the end of the 2-minute infusion period. It is therefore not necessary that blood arteriovenous difference approach zero before the Kety equation may be applied. The agreement between nitrous oxide and antipyrine flows implies that since antipyrine is equilibrated with tissue at two minutes, therefore, there must be similar equilibration of nitrous oxide with tissue at two or four minutes even though at four minutes the arteriovenous differences for nitrous oxide may not be negligible. This complements previous work\textsuperscript{17} and our present data in animals which demonstrate that fractional portions of the nitrous oxide curve yield values for flow equivalent to those obtained when arteriovenous equilibrium is complete. In man, unpublished observations indicate a similar correspondence between the flows calculated from curves of complete desaturation and from fractional portions thereof. This would suggest that under most circumstances after the second minute of nitrous oxide desaturation, the venous blood is in more or less constant equilibrium with myocardium. Sapirstein\textsuperscript{5,20} criticisms of the general theory of nitrous oxide for organs of differing perfusion rates probably do not apply to the normal myocardium. In pathological conditions with considerable fibrosis, none of the available methods, including $^{131}$I A.P., can distinguish differential perfusion rates or shunting.

The similarity of blood and left ventricular tissue concentrations indicates the dominant influence of left ventricular venous drainage on the composition of coronary sinus blood. Lafontant et al.\textsuperscript{21} have questioned the validity of coronary sinus blood as a measure of left ventricular events, although Bayford et al.\textsuperscript{22} have shown good correspondence.

Loss of radioactive tag from the $^{131}$I A.P. molecule has been reported by Sullivan and Rose\textsuperscript{23} after prolonged in vitro dialysis. In the very brief period of time required for the present measurement of coronary blood flow, loss of tag was not considered significant by these authors.

Tritium oxide probably could be used in similar fashion as $^{131}$I A.P. In two experiments not reported here, $^{131}$I A.P. and $\text{H}_2\text{O}$ were given simultaneously, counted differentially, and resulted in similar arterial and venous concentration curves. Tritium oxide is subject to some loss of tag, requires more time and extensive equipment for analysis, and is not readily adaptable to continuous recording (\textit{vide infra}).

Initial studies in man have shown similar $^{131}$I A.P. curves and a reasonable agreement between nitrous oxide and antipyrine coronary flows. The method was modified to allow for a longer venous transit time (15 sec) before beginning sampling. Use of the appearance time on a previously determined dye dilution cardiac output has proven helpful in this regard. The method permits continuous sampling from arterial and venous catheters through paired scintillation counters. By direct recording of the changing radioactivity, coronary flow may be calculated without delay. Such modifications are in progress. $^{131}$I A.P. is excreted in urine with a biological and effective half life of 18-24 hours. The radiation to the body is thus approximately 9\%
of an equal dose of I\textsuperscript{131} albumin, and is therefore not a significant limiting factor in human subjects. Lugol's solution may be used the day prior to administration to block any thyroid uptake.

**Summary**

The use of I\textsuperscript{131} antipyrine for determining coronary blood flow in intact dogs is described. The correlation with the simultaneous nitrous oxide method was 0.91 \( \pm \) 0.22. Partition coefficient and tissue equilibration were directly determined \( (1.04 \pm 0.13) \). The method as applied requires only two minutes of sampling and is adaptable to direct writing methods.

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**References**

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