Myocardial Blood Flow Measurement with ¹³³Xenon Effect of Glyceryl Trinitrate in Dogs

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Earlier methods for the measurement of blood flow through the coronary arteries and myocardium caused much disturbance of the normal circulation, and it was not until the introduction of the nitrous oxide method (Eckenhoff *et al.*, 1948) that a suitable technique became available for use in the intact animal, and in man. The use of saline solutions of ¹³³Xenon for the measurement of myocardial blood flow has recently been described (Ross *et al.*, 1964), and has some advantages over the nitrous oxide method. The clearance of the isotope from the myocardium is measured with a præcordial scintillation counter and, from this, myocardial blood flow may be obtained in ml./unit weight of muscle.

For each measurement a single injection of ¹³³Xenon in solution is made into the coronary artery. This is carried to the heart and diffuses rapidly from the capillaries throughout its substance. Thereafter, arterial blood containing no ¹³³Xenon removes the isotope from the tissues of the heart, and since it is highly diffusible, its rate of removal is determined by the capillary blood flow. Thus the clearance of ¹³³Xenon from the heart is a direct method for measuring myocardial blood flow.

Although determination of both cardiac output and tissue blood flow depends on indicator dilution, there is a basic difference between the two methods. For the former the indicator remains in the vascular compartment, whereas measurement of myocardial blood flow requires the rapid diffusion of indicator throughout the tissue space and then its gradual removal by progressive re-equilibration with fresh capillary blood. Thus the rate of removal of ¹³³Xenon from the heart, which is the essential measurement, is much slower than that of indicators restricted to the blood stream.

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We have used this method to measure myocardial blood flow in dogs whose coronary circulation is undisturbed, together with the determination of cardiac output, arterial blood pressure, and coronary sinus blood oxygen content. We report our findings with glyceryl trinitrate on the normal heart.

Before dealing with the detailed mathematics, it is important to appreciate the following principles.

The method assumes that the isotope diffuses rapidly throughout the tissue supplied by the left coronary artery in concentrations determined by the partition coefficient (λ) between myocardium and blood,

$$\left(\lambda = \frac{\text{concentration Xe in myocardium}}{\text{concentration Xe in blood}} \right).$$

It is assumed that partition equilibrium occurs within the capillary transit time, that the detector over the heart gives a counting rate proportional to the radioactivity in the cardiac tissues, and that the arterial blood during the measurement contains no ¹³³Xenon.

It is further assumed that we measure a single system in which capillary flow, at the time, is constant, that the indicator is removed only by the blood stream, and that changes in background radioactivity do not affect the measured clearance.

CALCULATION OF MYOCARDIAL BLOOD FLOW

Let Q_x be the mass of myocardial Xenon beneath the detector and V the volume of that myocardium.

Then rate of clearance of Xenon

$$-\frac{dQ_x}{dt} = \text{Flow (F)} \times \text{Concentration of Xenon in venous blood.}$$

Now, concentration of Xenon in venous blood

$$= \frac{\text{concentration of Xenon in tissue}}{\lambda}$$

and, concentration of Xenon in tissue

$$= \frac{Q_x}{V}$$

Therefore,

$$-\frac{dQ_x}{dt} = \frac{FQ_x}{V\lambda} \quad (i)$$

This is a standard differential equation whose solution is of the form

$$Q_x = Q_x(0) e^{-kt} \quad (ii)$$

This is the exponential decay expression in which $Q_x(0)$ is the value of Q_x at the initial part ($t=0$) of the measured decay. For our purposes, we will refer to k as the clearance rate constant.

From this it follows that

$$-\frac{dQ_x}{dt} = kQ_x \quad (iii)$$

Therefore, from equations (i) and (iii)

$$\frac{FQ_x}{V\lambda} = kQ_x$$

$$\text{or } \frac{F}{V} = k\lambda \quad (iv)$$

In practice the factor k in equation (iv) is not measured directly. What is measured is the time required for the exponentially decaying Q_x to fall from any value to half that value. Calling this time $t_{\frac{1}{2}}$ it can be shown to be related to k by

$$k = \frac{\log_e 2}{t_{\frac{1}{2}}} \quad (v)$$

It is customary to express myocardial blood flow (MBF) in ml./min./100g. tissue.

Using equation (iv)

$$\text{MBF} = \frac{k \lambda 100}{\rho} \text{ ml./min./100 g.}$$

where ρ is the density of myocardium (1.05 mg./ml.). λ was found to be 0.72 by Conn (1961) and this figure was confirmed by Ross *et al.* (1964). Using these constants, and the measured clearance rate constant k , MBF is thus derived. Most of the MBF values in this paper have been used in a comparative way, and the conclusions can be reached from the $t_{\frac{1}{2}}$ values, without assuming specific values for ρ and λ . It is an advantage of the ^{133}Xe method, however, that actual flow rates can be obtained when desired.

In practice, counts per second above background are plotted at 5-second intervals. The major part of the curve is exponential and a straight line can readily be drawn on semi-log paper. The $t_{\frac{1}{2}}$ in minutes is measured and the value for MBF obtained. The measured curve occupies $\frac{1}{2}$ to 1 minute.

Determinations can be repeated within 4-5 minutes as soon as the background levels are reached. The appearance of ^{133}Xe in the right heart or lungs does not affect the measured part of the clearance curve. Most of the isotope is cleared during the first transit of the lungs so that recirculation is not a problem. An illustrative ^{133}Xe wash-out is shown in Fig. 2.

METHODS

Mongrel dogs weighing 9-18 kg. were used. They were anaesthetized with intravenous pentobarbitone sodium 30 mg./kg. body weight, intubated, and respired with a modified Starling pump. Anaesthesia was maintained as light as possible with the abolition of spontaneous respiration. Tidal volumes were controlled by repeated arterial Po_2 , Pco_2 , and pH determination. Body temperature was maintained with a heated mattress.

Heparin, 5,000 units, was administered hourly. An electrocardiogram was recorded using needle electrodes; arterial pressure was measured through a polythene cannula introduced into the aorta through the right femoral artery using a Satham P23G Strain Gauge Transducer. Drugs were given through a catheter in the right femoral vein. Coomassie Blue Dye was delivered to the right atrium through a polythene catheter of known capacity via the left femoral vein. Blood for the dye curves was withdrawn from a short wide bore catheter introduced into the lower aorta through the left femoral artery (Fig. 1).

Using an image intensifier, the coronary sinus was cannulated via the external jugular vein with a Cournand No. 5 catheter. This was positioned with its tip well inside the mouth of the coronary sinus, care being taken to avoid obstructing the vessel.

The left coronary artery was next cannulated under radiographic control with a Sones No. 8 catheter, via the left common carotid artery. In the dog, the left coronary artery is large and can be entered with relative ease. The tapered tip of the catheter was positioned just within the artery. At the end of the experiment, the catheter was withdrawn during the inscription of a ^{133}Xe wash-out curve. There was no discontinuity in the curve, confirming that the catheter had caused no obstruction to myocardial blood flow. Usually the cannulation of both coronary artery and sinus was completed within 10 minutes.

Radioxenon (^{133}Xe) was obtained from the Radiochemical Centre, Amersham, in batches of 7-28 mC in saline solution. A dilution was made in normal saline, so that 1-2 ml. of solution injected into the coronary artery gave peak counting rates of 1000-3000/sec. Præcordial radioactivity was detected by an ERD Mark II Universal Scintillation Counter, using a 2 in. crystal with wide angle collimation. The counter was centred over the heart using fluoroscopy and remained in the same position throughout. Wash-out curves were recorded using an Ekco Ratemeter operating with a 2.5 second time constant (1 sec. for rapid curves). Background counts remained at the same low level in all satisfactory preparations, even after many measurements of myocardial blood flow.

Cardiac output was measured by the dye dilution technique. The dye was given as a single bolus into the right atrium, and arterial concentration was measured with a Waters cuvette. A six-point calibration curve was constructed at the start of the experiment and a further sample was withdrawn at the end of each cardiac output determination. It was thus possible to locate each dye curve with respect to the original cali-

FIG. 1.—Experimental plan.

FIG. 2.—Strip chart record of ^{133}Xe clearance curve after left coronary artery injection of isotope solution.

bration curve and to correct for any base-line drift. Samples were extracted by the method of Taylor and Shillingford (1959). The number of dye curves performed in any experiment was limited to 8, and the amplifier gain and balance were unaltered throughout. In preliminary experiments close correlation was found with cardiac output determinations made by the Fick method.

The oxygen content of blood samples was measured by the method of Roughton and Scholander (1943). The capillary tube of the syringe in all cases had been previously calibrated with mercury.

Data was recorded on a multichannel direct writing Sanborn oscillograph, and the dye dilution curves were simultaneously reproduced on a separate chart recorder.

Glyceryl trinitrate 0.01 mg./kg. body weight was given by intravenous injection over periods up to 10 minutes to correspond with its use by man. In pilot studies, myocardial blood flow was measured at varying times, and in later experiments, glyceryl trinitrate was given over 1 minute, and measurements were standardized at $\frac{1}{2}$, 3, 6, and 10 minutes after the start of drug injection. Because such flow cannot be measured again until background is constant, the experiment in each animal was divided into two parts.

Part 1: After combined control determinations of myocardial blood flow and cardiac output, glyceryl trinitrate was given, and further measurements were timed to start at $\frac{1}{2}$ and 6 minutes later. During these, blood samples for oxygen analysis were withdrawn from the coronary sinus and femoral artery.

Part 2: After 30 minutes the whole experiment was repeated, but with measurements at 3 and 10 minutes

after the start of drug injection. All blood samples were replaced by transfusion from a donor animal.

In a further study the effect of glyceryl trinitrate over a wide range of dosage was examined when the maximum fall in blood pressure had occurred. After control measurements, increasing doses of the drug were given by rapid intravenous injection (0.075, 0.15, 0.3, and 0.6 mg.). Myocardial blood flow and cardiac output determination were repeated when the blood pressure reached its lowest value, $1\frac{1}{2}$ to 4 minutes after injection. The next dose was given when blood pressure had returned to control level.

RESULTS

Over-all results are expressed as percentage of control values. Absolute figures are obtained from the following formulæ, but again, the comparative results in this paper may be derived simply from the basic data.

Symbols

Measured data

$\dot{Q}_s$	= Cardiac output	l./min.
$\dot{Q}_o$	= Myocardial blood flow	ml./min./100 g.
$\bar{P}_a$	= Mean systemic arterial pressure	mm. Hg.
Ca_{O_2}	= Arterial oxygen content	ml./100 ml.
Ccs_{O_2}	= Coronary sinus oxygen content	ml./100 ml.
m	= Left ventricular mass	
	= 0.00369 body weight (kg.)	g.
	(Herrmann, 1925)	

Derived data

$\dot{W}_{LV}$	= Left ventricular power	
	= $1.36 \bar{P}_a \dot{Q}_s 10^{-2}$	kgf. m./min.
R_s	= Systemic vascular resistance	
	= $\frac{\bar{P}_a}{\dot{Q}_s}$	units*
R_o	= Coronary vascular resistance	
	= $\frac{10^5 \bar{P}_a}{m \dot{Q}_o}$	units*
$\dot{Q}_{O_2}$	= Myocardial oxygen consumption	
	= $(\text{Ca}_{O_2} - \text{Ccs}_{O_2}) \dot{Q}_o m 10^{-4}$	ml./min.
$\dot{L}_{LV}$	= Theoretical left ventricular power available	
	= $2.057 \dot{Q}_{O_2}$	kgf. m./min.
E_{LV}	= Left ventricular efficiency	
	= $\frac{100 \dot{W}_{LV}}{\dot{L}_{LV}} \%$	

The energy equivalent of 1 ml. of oxygen is assumed to be 2.057 kgf. m. with a respiratory quotient of 0.82 (Macleod, 1926; Bing *et al.*, 1949).

*The resistance units employed are mm. Hg divided by litres per minute.

Controls. During the control period readings were stable, though levels varied from animal to animal depending to some extent on resting arterial blood pressure. Myocardial blood flow was 60–120 (mean 97) ml./min./100 g.

Glyceryl Trinitrate. The pilot studies showed a biphasic response to the injection of glyceryl trinitrate, whether given quickly or slowly. An initial rise in flow is followed by a fall.

Results are shown in Fig. 3–6, as percentage variation from control. Each comparison is represented by a bar showing the time over which the clearance was determined. Results from repeated experiments in several animals are shown in each figure.

When glyceryl trinitrate was given by a single rapid intravenous injection, there was only a brief rise in tissue flow (Fig. 3). When the drug was given gradually over one minute the biphasic response was more obvious (Fig. 4). An immediate rise occurred in flow, which was greatest at the end of the injection, fell away at 3 minutes, reached a

FIG. 3.—Changes in myocardial blood flow (MBF) for 10 minutes after rapid injection of glyceryl trinitrate. Composite graph from three dogs. Each bar represents percentage change in flow compared with control.

FIG. 4.—Changes in myocardial blood flow (MBF) for 10 minutes after glyceryl trinitrate injection over 1 minute. Composite graph from 6 dogs.

minimum at 6 minutes, and then returned towards control. When the same dose was injected more slowly over 3 minutes (Fig. 5) the results were similar. The increase in myocardial blood flow was again greatest at one minute and then fell off

FIG. 5.—Changes in myocardial blood flow (MBF) after glyceryl trinitrate injection over 3 minutes. Composite graph from 2 dogs.

FIG. 6.—Changes in myocardial blood flow (MBF) after glyceryl trinitrate injection over 10 minutes. Composite graph from 3 dogs.

even though the injection continued. Finally, slow injection of the same amount over ten minutes is shown in Fig. 6. The increase in flow was slight but was again early when only one-tenth of the dose had been given. The effect persisted longer but, again, was over before the injection was completed, suggesting that the action of the drug had been blocked.

Mean results of the main experiment in 10 dogs are shown in Fig. 7. At $\frac{1}{2}$ minute, the cardiac output rose sharply, blood pressure fell, and there were increases both in myocardial flow and in left ventricular power, or rate of work. Coronary and systemic vascular resistances were equally reduced. The O_2 content of coronary sinus samples doubled, and there was a fall in O_2 consumption, despite the increase in cardiac work rate. Thus, external cardiac efficiency, a measure of work done per unit O_2 consumed, was increased. At 3 minutes, the effects were very different. The blood pressure had risen but cardiac output, tissue flow, and myocardial rate of work had all fallen. Vascular resistance was back in the normal range, and oxygen consumption was further reduced, with a return to control level of external cardiac efficiency. The results at 6 and 10 minutes were similar and varied only in degree.

The results of the further group of experiments in 8 dogs, when varying doses of glyceryl trinitrate were given rapidly, are shown in Fig. 8. The changes resemble those at 3 minutes in the main experiment except for the greater changes in blood pressure and left ventricular work.

TIME	½ min.	3 mins.	6 mins.	10 mins.
Cardiac output	160	91	91	100
Mean Blood Pressure	76	91	89	92
Myocardial Blood Flow	146	83	82	87
Left Ventricular Power	126	82	88	93
Coronary Resistance	54	105	114	106
Systemic Resistance	51	99	92	96
Oxygen Consumption	93	82	85	91
Efficiency	132	97	103	100

FIG. 7.—Over-all response at ½, 3, 6, and 10 minutes after glyceryl trinitrate. Mean percentage change from control values. Each block is the mean of 10 experiments. Composite graph from 10 dogs.

DISCUSSION

This study was planned to interfere as little as possible with the heart and circulation, and it was shown that myocardial blood flow was not affected by the method used to measure it.

Previous workers with glyceryl trinitrate have given the drug by single rapid intravenous injection, and when we did this, we found the initial increase in tissue flow to be very transient, and only detected where measurements were started immediately. It was our aim, however, to imitate the therapeutic use of the drug, and we therefore gave it over similar times to those required for sub-lingual uptake, using the intravenous route for convenience and accuracy in timing.

DOSE mg.	0.075	0.15	0.3	0.6
Cardiac output	85	80	85	80
Mean Blood Pressure	76	77	61	57
Myocardial Blood Flow	84	78	69	63
Left Ventricular Power	65	61	52	44

Coronary Resistance	91	101	88	110
Systemic Resistance	90	98	72	76

FIG. 8.—Over-all response to glyceryl trinitrate at maximum fall in blood pressure using different doses. Each block is the mean of 8 experiments.

When this was done, the biphasic effect of glyceryl trinitrate on myocardial blood flow was obvious, showing that the timing of measurements is particularly important in deciding the effects of this drug.

The normal heart responds to glyceryl trinitrate with a substantial rise in flow lasting a few minutes, and followed by a lesser but more prolonged reduction. The fluctuations in coronary vascular resistance (Fig. 7) suggest that the activity of the drug on the coronary vessels is brief, and restricted to the first phase. The slight rise in coronary resistance noted subsequently might be an auto-regulatory adjustment to the reduced myocardial oxygen requirement at this stage. Otherwise, the effect of the drug on systemic and coronary vascular resistance is very similar, and the biphasic variation in myocardial blood flow is reflected in the changes in cardiac output.

The finding of increased left ventricular efficiency with glyceryl trinitrate has not previously been reported, and it may be important for its therapeutic effect that myocardial oxygen consumption falls despite an increase in the rate of work. This useful effect is probably the result of reducing arterial blood pressure and need not be attributed to a metabolic effect at tissue level.

It is known that changes in arterial blood pressure

cause much greater fluctuations in myocardial oxygen requirement than do changes in cardiac output of similar proportion. Braunwald *et al.* (1958) showed, in the isolated supported heart preparation, that an increase in cardiac rate of work could be achieved with a reduction in oxygen consumption if the arterial blood pressure was reduced while the cardiac output rose. It seems likely that glyceryl trinitrate reproduced the conditions of this experiment in our intact animals.

If these results can be applied to man we might expect an early increase in myocardial flow subsiding at about 3 minutes. However, in the few studies that have been done in patients, flow measurements have not been started until this time, when a substantial fall in blood pressure had occurred. Moreover the nitrous oxide method is not suitable for detecting the swings in flow that might occur with this drug, because of the long time needed for each determination. It remains a possibility, therefore, that glyceryl trinitrate might still be shown to increase myocardial blood flow in patients with ischaemia, despite recent reports to the contrary. In fact, studies on such flow in man using the nitrous oxide method have given variable results. Gorlin *et al.* (1959) found a reduction in coronary flow following glyceryl trinitrate in patients with ischaemic heart disease, whereas the same group (Brachfeld, Bozer, and Gorlin, 1959) found an increase in normal subjects. In a small number of patients with both normal and abnormal coronary arteriograms, Ross *et al.* (1964) found variable changes in myocardial blood flow after glyceryl trinitrate.

Our studies do not support the suggestion that the drug is of value because of reduction in cardiac rate of work. The over-all reduction in myocardial oxygen requirement and the initial rise in external cardiac efficiency appear more significant. The lack of correlation between cardiac rate of work and oxygen consumption is unfortunate for studies in man, as the former is easily measured, whereas the latter is very difficult to determine, involving measurement of both myocardial blood flow and the myocardial oxygen extraction fraction.

The results with glyceryl trinitrate show two effects of potential therapeutic value: an initial increase in blood flow through the myocardium, and a prolonged reduction in its need for oxygen. The applicability of the former, in the presence of ischaemic disease, is debatable and perhaps varies between patients, and from time to time in a particular patient. The value of the latter effect is obvious. It remains to be shown what happens in animals

with obstructed coronary arteries, and in patients with angina.

SUMMARY

The use of saline solutions of $^{133}\text{Xenon}$ for the measurement of myocardial blood flow in intact dogs is described.

Injection of glyceryl trinitrate, 0.01 mg./kg. body weight over 1 minute, caused an increase in flow up to 3 minutes, followed by a reduction in flow up to 10 minutes.

Measurements of myocardial blood flow, cardiac output, and myocardial oxygen extraction were made at standardized times after giving the drug. At $\frac{1}{2}$ minute myocardial blood flow and left ventricular rate of work were both increased. Myocardial oxygen consumption was reduced and coronary sinus oxygen content rose by 4.4 vol. per cent. At 3 minutes the vasodilator effect was over. There was reduced myocardial blood flow and rate of work; myocardial oxygen consumption was reduced further. Measurements at 6 and 10 minutes differed from those at 3 minutes only in degree, with a gradual return to control values.

It is suggested that the beneficial effects of glyceryl trinitrate are (1) initial increase in myocardial blood flow, (2) initial increase in external cardiac efficiency, and (3) prolonged reduction in oxygen consumption. These possibilities need to be studied in the ischaemic heart.

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REFERENCES

- Bing, R. J., Hammond, M. M., Handelsman, J. C., Powers, S. R., Spencer, F. C., Eckenhoff, J. E., Goodale, W. T., Hafkenschiel, J. H., and Kety, S. S. (1949). The measurement of coronary blood flow, oxygen consumption, and efficiency of the left ventricle in man. *Amer. Heart J.*, **38**, 1.
- Brachfeld, N., Bozer, J., and Gorlin, R. (1959). Action of nitroglycerin on the coronary circulation in normal and in mild cardiac subjects. *Circulation*, **19**, 697.
- Braunwald, E., Sarnoff, S. J., Case, R. B., Stainsby, W. N., and Welch, G. H., Jr. (1958). Hemodynamic determinants of coronary flow: Effect of changes in aortic pressure and cardiac output on the relationship between myocardial oxygen consumption and coronary flow. *Amer. J. Physiol.*, **192**, 157.
- Conn, H. L. (1961). Equilibrium distribution of radio-xenon in tissue: xenon-hemoglobin association curve. *J. appl. Physiol.*, **16**, 1065.

- Eckenhoff, J. E., Hafkenschiel, J. H., Harmel, M. H., Goodale, W. T., Lubin, M., Bing, R. J., and Kety, S. S. (1948). Measurement of coronary blood flow by the nitrous oxygen method. *Amer. J. Physiol.*, **152**, 356.
- Gorlin, R., Brachfeld, N., MacLeod, C., and Bopp, P. (1959). Effect of nitroglycerin on the coronary circulation in patients with coronary artery disease or increased left ventricular work. *Circulation*, **19**, 705.
- Herrmann, C. R. (1925). Experimental heart disease. 1. Methods of dividing hearts; with sectional and proportional weights and ratios for 200 normal dogs' hearts. *Amer. Heart J.*, **1**, 213.
- Macleod, J. J. R. (1926). *Physiology and Biochemistry in Modern Medicine*, 5th ed., p. 780, table V. C. V. Mosby, St. Louis.
- Ross, R. S., Ueda, K., Lichtlen, P. R., and Rees, J. Russell (1964). Measurement of myocardial blood flow in animals and man by selective injection of radio-active inert gas into the coronary arteries. *Circulat. Res.*, **15**, 28.
- Roughton, F. J. W., and Scholander, P. F. (1943). Micro gasometric estimation of the blood gases. 1. Oxygen. *J. biol. Chem.*, **148**, 541.
- Taylor, S. H., and Shillingford, J. P. (1959). Clinical applications of coomassie blue. *Brit. Heart J.*, **21**, 497.