Animal models of myocardial ischaemia

N. WOOLF

From the Bland-Sutton Institute of Pathology, The Middlesex Hospital Medical School, London

The objective of any satisfactory model of myocardial ischaemia in experimental animals must be to reproduce, as accurately as possible, the spectrum of ischaemic heart disease as seen in man. Since we are still, to a very considerable extent, ignorant of the pathophysiology of angina pectoris and of sudden death causally associated with coronary atherosclerosis and thrombosis, it is, in my view, impossible to construct valid models for these important clinical expressions of ischaemic heart disease. This brief review is, therefore, exclusively concerned with myocardial necrosis.

In human disease, the various patterns of ischaemic myocardial necrosis (Davies, 1977) occur for the most part against the background of a coronary circulation widely compromised by stenosing atherosclerosis. In regional infarction arterial occlusion, either by thrombus or by a mixture of thrombus and atheromatous debris, is present in the majority of cases (Davies *et al*, 1976) and the occlusions are usually related to splits or tears in the connective tissue caps of atherosclerotic plaques. These pathogenetic factors should certainly be subsumed in any experimental model which attempts to mimic the human disease. At present, it must be admitted that we cannot economically devise models which meet these requirements.

Nevertheless our attitude, though critical, need not be totally nihilistic. Although the models we have are inherently unsatisfactory, they can provide some useful information on a variety of matters such as mapping infarcts, metabolic and structural changes in underperfused regions of the myocardium and the effects of various forms of intervention on these changes.

Regional myocardial infarction

This is not the place to discuss the role of occlusive coronary artery thrombi in the pathogenesis of myocardial infarction. One thing, however, is certain: that it is not possible to produce transmural, regional infarction without complete or near complete prior interruption of coronary arterial flow to the affected region of the myocardium. In this section, therefore, we shall be concerned with the

various methods which have been devised for this purpose and their respective advantages and limitations.

Existing techniques for producing an interruption in coronary artery flow may be classified essentially in two ways: first in relation to the time taken to produce arterial underperfusion and hence myocardial ischaemia and secondly according to whether thoracotomy is required or not.

OPEN-CHEST METHODS

In most acute open-chest procedures a major branch of the coronary arteries is ligated. This model, though clearly very far from being a homologue of acute human aortic myocardial ischaemia, has a number of real advantages. Under direct vision it is not difficult to locate the favoured site for occlusion with a fair degree of precision and this in turn makes for a high degree of reproducibility. In larger animals such as the dog, primates or pigs where, thanks to modern anaesthetic techniques the chest can be kept open for fairly long periods, one can 'map' the ischaemic areas by means of multi-positioning of a surface electrode (Kjekshus and Mjøs, 1973) and quantify some aspects of the ischaemic damage by measuring average ST segment elevation obtained under different circumstances. Some of the metabolic changes in the ischaemic myocardium can be monitored by taking blood samples from local surface veins and it is, of course, not difficult to sample tissue from the ischaemic areas with a view to studying the sequential changes occurring in myocardial fibres deprived of normal arterial perfusion (Jennings and Ganote, 1972).

The advantages in studying some aspects of acute interruption in coronary flow which are inherent in the open chest situation are counterbalanced by some crippling limitations. Thoracotomy, on its own, is associated with considerable haemodynamic changes and the fact that this procedure must be carried out under anaesthetic still further militates against its being regarded even as a distant homologue of the human situation. With coronary ligation there is acute interruption of previously normal flow which is very unlike the postulated natural history of human occlusive coronary artery thrombosis occurring in an

already stenosed segment of the coronary artery. The thrombotic element so often found in human disease is absent in this situation—a double disadvantage since a mass of aggregated platelets almost certainly exerts powerful local metabolic effects apart from its more obvious role as an occlusive 'plug'.

Nevertheless for those chiefly interested in the morphological and histochemical consequences of acute coronary underperfusion the open chest model has something to recommend it. Almost by convention most experiments using this model have been carried out on the dog with variable results. By comparison, the rat has been rather neglected. Selye and his associates (1960) have described a fairly simple technique for ligating the coronary arteries in the rat which my colleagues, Mr R Langford and Mr P Rowles in the Bland-Sutton Institute, have recently been applying. This model has the great advantage of comparative cheapness and ligation a few millimetres below the atrio-ventricular groove appears regularly to produce classical transmural infarction involving most of the circumference of the lower one-third of the left ventricle including part of the interventricular septum (fig 1). The anatomical distribution is probably due to the fact that the rat has no true circumflex artery. From 24 hours after ligation the characteristic histological features of infarction are present in abundance (fig 2), though loss of glycogen, phosphorylase and oxidative enzymes very rapidly becomes apparent (Bajusz and Jasmin, 1964). The success of the ligation can be assessed *in vivo* by injecting the animal with a small dose of technetium-labelled sodium diphosphonate and imaging it with a gamma camera. The technetium localizes preferentially in the necrotic myocardium, and hence the infarcts are easily demonstrable (fig 3).

CLOSED-CHEST METHODS

In general, closed-chest methods for producing interruptions in coronary flow offer several advantages which are not present in the open chest situation, in particular the minimal surgical trauma required to produce occlusion and the fact that the local autonomic supply to the heart is not interfered with, something that is almost inevitable in open-chest preparations. Basically, myocardial ischaemia in a closed-chest preparation can be brought about in three ways: (1) the production of thrombus either by passing a current via an intracoronary catheter or simply by leaving a helical copper wire in the lumen of the coronary artery; (2) occlusion of the lumen by a balloon catheter; (3) embolization, eg, by radioopaque micro-spheres of different sizes or by intracoronary injection of mercury.

The first group of methods which, in my opinion,

Fig 1 Transverse sections through rat heart. Left coronary artery ligated 24 hours before. Section nearer apex shows pale zone of ischaemic necrosis extending round ventricular cavity. More cephalad section shows transmural anterolateral infarction.

show the most promise, derive from the studies of Sawyer and Pate (1953) who suggested that one of the sets of circumstances under which arterial thrombosis could occur was when there was a loss or reduction of normal electrical negativity of the arterial endothelium in relation to the adventitia. Using this as a starting premise, Salazar (1961) devised a method in which a Teflon-coated stainless steel electrode, exposed at its tip for approximately 3 mm, is advanced into a coronary artery through a modified West coronary catheter. Once the electrode is correctly positioned, it is connected to the positive side of a circuit which is completed by a negative electrode in the chest wall. A direct current from a 3-volt dry cell battery is then passed through the current, the intensity of the current being regulated by

Fig 2

Fig 3

Fig 2 Rat left ventricle: phosphotungstic acid haematoxylin $\times 20$.

Note sharply demarcated zone of transmurular necrosis involving papillary muscles. Left coronary artery ligated 30 hours before death.

Fig 3 Gamma camera picture of rat injected with technetium-labelled sodium diphosphonate 25 hours after ligation of left coronary artery. The isotope is normally demonstrable in the kidneys and bladder. In this animal the isotope is also localized in the region of the infarct and thus an image of part of the heart is obtained.

means of a small potentiometer. In all the animals in Salazar's study, complete coronary artery occlusion was induced in periods ranging between 18 and 93 minutes of current flow. The time required for occlusion was related to the intensity of the current employed and the diameter of the artery: when a current in excess of 600 microamperes was used the vessel wall underlying the thrombus was the seat of marked intimal and subintimal injury but this can be avoided if currents of less than 200 microamperes are used, though thrombosis still occurs regularly. Postoperative mortality is low in this model, there being a much lower incidence of ventricular fibrillation than in the acute open-chest preparation. It is obviously less well suited to acute metabolic studies than the open-chest preparation but might have a valuable role in assessing the efficacy *in vivo* of pharmacological agents which modify platelet behaviour *in ex-vivo* systems.

While the Salazar technique has undeniable attractions, the fact that the animal has to remain more or less immobile during the flow of current is a disadvantage. A model system which overcomes this has been devised by Kordenat and his colleagues (1972). Like the Salazar system this is a model for subacute thrombotic occlusion and depends on the insertion of a wire helix consisting either of copper or a magnesium alloy into the anterior descending branch of the left coronary artery or into the circumflex artery. Thrombus forms in relation to the wire and occludes the artery. This process may take as short a time as one hour or may be prolonged for several days. The variation in time is related in part to the position of the wire helix in the coronary tree, occlusion taking place more rapidly when the helix is impacted in the proximal part of the artery. The number of loops into which a standard length of wire is twisted also appears to play a part in determining the speed of occlusion. When copper wire helices 8-12 mm in length and with four to six loops are used, complete occlusion usually takes place within an hour. The degree of infarction produced by such a device is of the order of 25 per cent of the left ventricular muscle. This method works well in a variety of animals including the mini-pig. It is now being used in a number of centres, and I am grateful to Dr Peter Walton and his associates at the Alderley Edge laboratories of Imperial Chemical Industries for allowing me to see it in operation.

Yet another variation on the theme was presented by Nakhjavan and his colleagues in 1968. These workers inserted siliconized stainless steel cylinders into the coronary artery using a coronary catheter and a guide wire. Unless the animal is heparinized occlusion takes place rapidly. However, in the heparinized animal the cylinder produces a segment

of narrowing through which flow takes place for a few hours. During this stage it should be possible to carry out studies of coronary blood flow and relate the diminution of flow through the lumen of the cylinder to electrocardiographic and biochemical changes.

COMBINATION OF OPEN- AND CLOSED-CHEST METHODS

Several attempts have been made to devise methods which combine the anatomical accuracy of open-chest methods and yet avoid the disadvantages already described. Chimoskey and his associates (1967) devised an interesting system in which a short length of the circumflex artery was dissected free after a thoracotomy and a silastic balloon with a non-distensible nylon backing placed round the vessel. The balloon can be inflated and deflated via a length of tubing which passes out through the chest wall. Occlusion of the vessel can be produced rapidly or slowly as dictated by the needs of a particular study and this part of the procedure is carried out in a conscious animal. The reversibility of the occlusion is, of course, another advantage. A somewhat more sophisticated version of this model has been developed in which inflation of the balloon is produced by use of a micro-infusion pump (Hood *et al*, 1970). The mortality rate with this procedure is low and in a group of eight dogs in which the balloon cuff was placed round the LAD, all developed well defined anterior infarction with loss of muscle ranging from 22 to 49 per cent (mean 34 per cent). Both this and the previous study of myocardial ischaemia in conscious dogs are noteworthy in that, as judged by the behaviour of the animals, pain was not a characteristic feature of the coronary occlusion.

Gradual occlusion of coronary arteries by using hygroscopic 'cuffs' was first described in 1957 by Litvak *et al*. The material used is a plastic derived from casein and called Ameroid which gradually swells. An Ameroid cuff of known diameter is placed round one of the coronary arteries in a dog or pig and enclosed in a stainless steel sleeve. A dry cuff placed in saline halves its lumen in 16 days but this period can be prolonged by pretreating the Ameroid with petrolatum jelly. Several studies have been carried out with this model (Vineberg *et al*, 1960; Lumb *et al*, 1962; Peter *et al*, 1966). Mortality is quite high (Peter *et al*, 1966) and the degree of myocardial necrosis produced varies considerably.

Subendocardial necrosis

The pathophysiology of subendocardial necrosis (laminar infarction) in both humans and experimental animals differs considerably from that of

regional infarction, and this pathological picture may be seen in patients in whom coronary arteries show no significant stenosis. In a variety of situations the subendocardial muscle is much more likely to develop ischaemic necrosis than is the subepicardial muscle, this presumably being due to the differences of intramyocardial compressive forces which in the subendocardial region are equal to or greater than intraventricular pressures, while in the subepicardial region they fall to near atmospheric pressure levels (Kirk and Honig, 1964; Brandi and McGregor, 1969; Baird *et al*, 1970). The effect of this increase in intramural pressure relative to distance from the epicardium has the effect of making the subendocardial region totally dependent on diastolic perfusion. This perfusion might be expected to be compromised in circumstances where the coronary perfusion pressure is reduced, where there is a rise in left ventricular end-diastolic pressure or where the time available for diastolic perfusion is reduced as in tachycardias of various types. Thus we see subendocardial necrosis where there is severe stenosing atherosclerosis of the major coronary branches ('triple vessel disease') in patients who have had a stormy postoperative period following cardiac bypass and in some cases of aortic valve disease (Davies, 1977).

Neither the pathological picture of subendocardial necrosis nor the mechanisms producing it are easy to reproduce in experimental situations. Subendocardial ischaemia has been produced in dogs with normal coronary arteries by Buckberg and his associates (1972) by a variety of manoeuvres which included the opening of arteriovenous fistulae to lower aortic diastolic pressure, constriction of the ascending aorta to raise left ventricular diastolic pressure and electrical pacing to shorten diastole. All these were associated with markedly reduced subendocardial perfusion.

The morphological picture of subendocardial necrosis can also be produced by the administration of large doses of isoprenaline to the rat (Woolf *et al*, 1976). A ring-like loss of subendocardial muscle fibres occurs which is perhaps most effectively demonstrated by staining sections to show succinic dehydrogenase. The pathogenesis of these lesions is a complex question since a number of different factors may play a part. The heart rate in these animals increases sharply with a consequent drop in diastolic perfusion time; the myocardial fibres are overloaded with calcium leading to a deficiency of high-energy phosphate and possibly to a decrease in diastolic compliance of the fibres (Fleckenstein, 1971) and the increase in plasma free fatty acids which follows the administration of sympathomimetic amines (Oliver, 1972; Oliver *et al*, 1972).

It is possible to obtain some morphological

appreciation of the distribution of intramyocardial blood flow by injecting a small dose of the fluorescent dye Thioflavine S approximately 30 sec before killing the animal and removing its heart. This dye binds non-specifically to endothelium and its presence in small vascular channels indicates the recent passage of blood through these vessels. The application of this method to our own studies of isoprenaline cardiotoxicity has shown patchy loss of staining in the subendocardial region within 10 to 15 minutes of the amine having been administered. This suggests that the effects of this powerful inotrope when given in large doses are associated with at least temporary underperfusion of the subendocardial region.

This work was supported by a grant from the Medical Research Council. The experiments described would not have been possible without the help of my colleagues Miss E. Steen and Messrs P. Rowles and R. Langford. I am also grateful to Messrs P. Turner and A. Aylott for valuable technical assistance and to Dr P. Ell of the Department of Nuclear Medicine, The Middlesex Hospital, for the imaging of the infarcts in the rat.

References

- Baird, R. J., Manktelow, R. T., Shah, P. A., and Ameli, F. M. (1970). Intramyocardial pressure: study of its regional variations and its relationship to intraventricular pressure. *Journal of Thoracic and Cardiovascular Surgery*, **59**, 810-823.
- Bajusz, E., and Jasmin, G. (1964). Histochemical studies on the myocardium following experimental interference with coronary circulation in the rat. 1. Occlusion of coronary artery. *Acta Histochemica (Jena)*, **17**, 222-237.
- Brandi, G., and McGregor, M. (1969). Intramural pressure in the left ventricle of the dog. *Cardiovascular Research*, **3**, 472-475.
- Buckberg, G. D., Fixler, D. E., Archie, J. P., and Hoffman, J. I. E. (1972). Experimental subendocardial ischaemia in dogs with normal coronary arteries. *Circulation Research*, **30**, 67-79.
- Chimoskey, J. E., Szentivanyi, M., Zakheim, R., and Barger, A. C. (1967). Temporary coronary occlusion in conscious dogs: collateral flow and electrocardiogram. *American Journal of Physiology*, **212**, 1025-1032.
- Davies, M. J. (1977). The pathology of myocardial ischaemia. *Journal of Clinical Pathology* (Royal College of Pathologists), **30**, Suppl. 11, 45-52.
- Davies, M. J., Woolf, N., and Robertson, W. B. (1976). Pathology of acute myocardial infarction with particular reference to occlusive coronary thrombi. *British Heart Journal*, **38**, 659-664.
- Fleckenstein, A. (1971). Specific inhibitors and promoters of calcium action in the excitation-contraction coupling of heart muscle and their role in the prevention of production of myocardial lesions. In *Calcium and the Heart*, edited by P. Harris and L. H. Opie. Academic Press, London.
- Hood, W. B., Jr., Joison, J., Kumar, R., Katayama, I., Neiman, R. S., and Norman, J. C. (1970). Experimental myocardial infarction. 1. Production of left ventricular failure by gradual coronary occlusion in intact conscious dogs. *Cardiovascular Research*, **4**, 73-83.

- Jennings, R. B., and Ganote, C. E. (1972). Ultrastructural changes in acute myocardial ischaemia. In *Effect of Acute Ischaemia on Myocardial Function*, edited by M. F. Oliver, D. G. Julian, and K. W. Donald, pp. 50-74. Churchill Livingstone, Edinburgh and London.
- Kirk, E. S., and Honig, C.R. (1964). An experimental and theoretical analysis of myocardial tissue pressure. *American Journal of Physiology*, **207**, 361-367.
- Kjekshus, J. K., and Mjøs, O. D. (1973). Effect of inhibition of lipolysis on infarct size after experimental coronary artery occlusion. *Journal of Clinical Investigation*, **52**, 1770-1778.
- Kordenat, R. K., Kezdi, P., and Stanley, E. L. (1972). A new catheter technique for producing experimental coronary thrombosis and selective coronary visualization. *American Heart Journal*, **83**, 360-364.
- Litvak, J., Siderides, L. E., and Vineberg, A. M. (1957). The experimental production of coronary artery insufficiency and occlusion. *American Heart Journal*, **53**, 505-518.
- Lumb, G., Singletary, H. P., and Hardy, L. B. (1962). Collateral circulation following experimental gradual narrowing of the coronary arteries. *Angiology*, **13**, 463-465.
- Nakhjavan, F. K., Shedrovlzky, H., and Goldberg, H. (1968). Experimental myocardial infarction in dogs. *Circulation*, **38**, 777-782.
- Oliver, M. F. (1972). Methods of inducing myocardial ischaemia. In *Effect of Acute Ischaemia on Myocardial Function*, edited by M. F. Oliver, D. G. Julian, and K. W. Donald, pp. 11-18. Churchill Livingstone, Edinburgh and London.
- Oliver, M. F., Rowe, M. J., and Vetter, N. (1972). Metabolic intervention. In *Effect of Acute Ischaemia on Myocardial Function*, edited by M. F. Oliver, D. G. Julian, and K. W. Donald, pp. 354-366. Churchill Livingstone, Edinburgh and London.
- Peter, R. H., Rau, R. W., Whalen, R. E., Entman, M. L., and McIntosh, H. D. (1966). *Circulation Research*, **18**, 89-96.
- Salazar, A. E. (1961). Experimental myocardial infarction: induction of coronary thrombosis in the intact closed chest dog. *Circulation Research*, **9**, 1351-1356.
- Sawyer, P. N., and Pate, J. W. (1953). Bio-electric phenomena as an etiologic factor in intravascular thrombosis. *American Journal of Physiology*, **175**, 103-107.
- Selye, H., Bajusz, E., Grasso, S., and Mendell, P. (1960). Simple technique for the surgical occlusion of coronary vessels in the rat. *Angiology*, **11**, 398-407.
- Vineberg, A., Mahanti, B., and Litvak, J. (1960). Experimental gradual coronary artery constriction by ameroid constrictors. *Surgery*, **47**, 765-771.
- Woolf, N., Davies, M. J., Shaw, M. J., and Trickey, R. J. (1976). Experiences with isoprenaline induced myocardial necrosis in the rat. *Journal of Pathology*, **120**, 65-73.