

Myocardial ultrastructural changes during extracorporeal circulation with anoxic cardiac arrest and its prevention by coronary perfusion

Experimental study

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Balibrea, J. L., Bullon, A., de la Fuente, A., de la F. Alarcon, A., Fariñas, J., Collantes, P., Gil, M., Gombau, M., Morales, R., and Sanchez, F. (1975). Thorax, 30, 371–381. Myocardial ultrastructural changes during extracorporeal circulation with anoxic cardiac arrest and its prevention by coronary perfusion. Experimental study. This experimental work has been carried out with the aim of studying the ultrastructural myocardial changes caused by prolonged anoxic cardiac arrest during cardiopulmonary bypass, and their prevention by means of two different techniques of coronary perfusion—systemic-pressure continuous and low-pressure intermittent perfusion.

After 30 minutes of cardiac anoxia, the ultrastructural changes of the myocardial cell were reverted to normal by coronary perfusion; when anoxic cardiac arrest was prolonged up to 60 minutes there was severe myocardial damage, with marked mitochondrial changes and dehiscence of intercalated discs, which persisted in spite of restoring coronary flow. These morphological data were in accordance with the fact that no dog which underwent anoxic cardiac arrest for 60 minutes recovered.

Both intermittent and continuous coronary perfusion were effective in preventing anoxic damage; cardiac muscle cells were better preserved by low-pressure intermittent perfusion than by systemic-pressure continuous perfusion, which caused intracellular and intramitochondrial oedema.

In open-heart surgery, when aortic occlusion is used as in surgery of the aortic valve and direct myocardial revascularization, it is very important to determine a safe period in order to avoid irreversible damage to the myocardium, adopting a method for protecting the heart if cross-clamping of the aorta should be prolonged. These data are also useful for cardiac transplantation, allowing evaluation of different methods of heart preservation (Copeland *et al.*, 1973).

On a functional basis, it has been established that contractility of the myocardium is depressed after 20 minutes of anoxia, which is in part compensated by an increase in the end-diastolic

volume (Sanmarco *et al.*, 1969); after 45 minutes of myocardial anoxia the left ventricular function is severely disturbed (Sarin, Hall, and Ross, 1968), and after 60 minutes of anoxic arrest there are deleterious effects on the compliance and contractility of the left ventricle (Enright, Staroscik, and Reis, 1970). According to determinations of myocardial disposability of oxygen, MacGregor *et al.* (1972) have established that, with individual variations, the safe period of anoxia is about 45 minutes. This poor resistance of cardiac muscle to anoxia is due to the very low content in the myocardium of enzymes of the anaerobic metabolic cycle, and hence the myocardium cannot

be supplied with sufficient energy (Scheuer, 1967).

Structural studies with electron microscopy have shown that the most significant effects of anoxia are on the mitochondria: after 15 minutes of anoxia there are no changes, but later one observes a decrease in the amount and size of large mitochondrial granules and condensation of the matrix; when anoxia is longer than 30 minutes, there is in addition to these changes an increase in the amount of intracellular fluid, with dramatic progression of this oedema if anoxia is maintained for 45 minutes (Burdette and Ashford, 1965). After 60 minutes of anoxic arrest, mitochondria show fragmentation of cristae, clearing of the matrix, and even disruption of the outer membranes (Jennings, Baum, and Herdson, 1965).

An attempt to extend myocardial tolerance to anoxia has been made by the use of induced cardiac arrest; nevertheless, the use of ventricular fibrillation (Wilson *et al.*, 1972) or chemical agents which do not stimulate adenosine triphosphate breakdown (Kirsh, Rodewald, and Kalmár, 1972) have not conferred any important advantage. Application of general deep hypothermia for this purpose (Hoelscher, Just, and Merker, 1961) has not found practical use.

Selective coronary perfusion is a recognized technique for avoiding myocardial anoxia during prolonged aortic cross-clamping; nevertheless some complications from manipulation and placement of the cannulae have been reported, and from high perfusion pressure which causes an increase in coronary vascular resistance. These complications are responsible for a considerable percentage of operative deaths in aortic valve surgery (Fishman, Youker, and Roe, 1968). Complications described include intimal trauma with dissecting haematoma (Heilbrunn and Zimmerman, 1965; Ramsey *et al.*, 1967) and coronary ostial obstruction by aortic intimal disruption (Iben, Firpo, and Hurley, 1972). Furthermore, when congenital anatomical variations are present, or if cannulae are introduced too far, there is the risk of perfusing only one of the main branches of the left coronary artery, causing operative infarcts and death (Furlong *et al.*, 1972).

In order to avoid these complications some apparently satisfactory techniques have been described. These include continuous hypothermic coronary perfusion (Hirose and Bailey, 1969); intermittent coronary perfusion with hypothermic blood (Ebert *et al.*, 1962) or with normothermic blood (Benzing *et al.*, 1973); low-pressure coronary perfusion (Brown *et al.*, 1969); and topical cardiac hypothermia (Robicsek *et al.*, 1970), although its

prolonged application may result in myocardial and pericardial damage. In direct myocardial revascularization retrograde perfusion through the coronary sinus has been employed satisfactorily (Hammond, Davies, and Austen, 1967).

This experimental work was carried out with the aim of studying the myocardial ultrastructural changes caused by prolonged anoxic cardiac arrest during cardiopulmonary bypass, its reversibility when coronary flow is restored, and the effect on cardiac muscle morphology of continuous systemic-pressure and intermittent low-pressure coronary perfusion.

MATERIAL AND METHODS

A total of 18 healthy adult mongrel dogs of both sexes, weighing from 20 to 26 kg, were utilized for the experiments. They were anaesthetized with intravenous sodium pentobarbital, 30 mg per kilogram of body weight, intubated with a cuffed endotracheal tube, and ventilation was maintained by a Dragwenport positive pressure respirator supplying oxygen.

The right chest was entered through the fourth intercostal space, and heparin, 3 mg per kilogram of body weight, was administered intravenously. The pericardium was opened widely and its edges were marsupialized to the incision. A catheter for venous return was inserted into the right atrium, the pulmonary artery was looped for later occlusion, and the inflow cannula was placed in the ascending aorta; the left ventricle was also drained for decompression while on bypass. Extracorporeal circulation was maintained by means of a gravity-return Bentley-Temptrol bubble oxygenator (blood was oxygenated with 8 litres per minute of oxygen) and a Sarn roller-pump machine and heat exchanger. The extracorporeal circuit was primed with 500 ml of homologous heparinized blood and 600 ml of Ringer's lactate solution (maximal haemodilution: 30 ml/kg body weight); perfusion requirements were calculated on the basis of 2200 ml blood flow per minute per square metre of body surface area. Animals were maintained normothermic, the temperature being monitored by means of an oesophageal electrode, systemic arterial and central venous pressures were continuously monitored via the femoral artery and femoral vein, respectively, with appropriate catheters connected to Statham strain gauges.

The experiments were divided into four groups, as described below:

GROUP I Control: Five dogs were placed on

cardiopulmonary bypass for 60 minutes without aortic clamping, and the heart was allowed to beat.

GROUP II Anoxia: Five dogs were included in this group. After being placed on cardiopulmonary bypass, the aorta was clamped for 60 minutes. In experiments II-4 and II-5, the aortic clamp was removed after 60 minutes, extracorporeal perfusion continuing during an additional period of 15 minutes.

GROUP III Low-pressure intermittent coronary perfusion: In this group four dogs were treated identically with those in group II except that the aorta was clamped for 30 minutes. Afterwards the heart was perfused by a catheter (self-inflating balloon-tip) inserted into the aortic root proximal to the clamp. The catheter was connected to a coronary perfusion line supplying a flow of 200 ml per minute under a pressure of 50 mmHg. The coronary perfusion was carried out during 30 minutes intermittently, with intervals of 10 minutes, perfusing for three minutes each time. The duration of extracorporeal circulation was 60 minutes.

GROUP IV Continuous coronary perfusion at systemic-pressure: In this group four dogs were treated as those in group II for 30 minutes. Afterwards the heart was perfused continuously for 30 minutes, supplying a flow of 270 ml per minute under a pressure of 110 mmHg through a catheter inserted as described for group III. The dogs were considered to have recovered if they maintained, without drug support, stable haemodynamics after bypass was discontinued, with arterial systolic blood pressure higher than 100 mmHg and central venous pressure lower than 7 mmHg. No attempt was made to obtain long-term survival in the dogs which survived the procedure.

Biopsies were taken in each animal for ultrastructural study immediately before it went on cardiopulmonary bypass, after 30 minutes, and after 60 minutes from the beginning of bypass. In experiments II-4 and II-5 a fourth specimen was taken after the additional 15 minutes' period of perfusion with the aorta unclamped. Biopsies were taken by scalpel from the right ventricle, avoiding the sites of previous biopsies. The specimens were immediately divided into small portions and the fragments were fixed in glutaraldehyde at 4°C for 90 minutes; they were then washed in a phosphate buffer solution and postfixed in 1%

osmic acid solution for 60 minutes; dehydration was carried out in graded concentrations of acetones, and the specimens were embedded in Durcupan (Fluka). The tissues were sectioned by LKB Ultratome III-C, and the sections were stained with uranyl acetate and lead citrate. The observations were made with an electron microscope (Zeiss EM 9 S) at 60 kV.

RESULTS

GROUP I All dogs recovered after the procedure. Electron microscopic observations after 30 minutes on bypass showed normal cardiac muscle (Fig. 1). After 60 minutes the myocardium showed insignificant slight changes: mitochondria increased in size with occasional rounding, and slight clearing, of the matrix; sarcolemma and intercalated discs were unremarkable, and the sarcoplasmic reticulum was enlarged (Fig. 2).

GROUP II The five animals of this group, which included the two dogs which underwent an additional 15 minutes of extracorporeal perfusion after the aortic clamp was removed, failed to come off bypass although resuscitation was carried out with methods usually employed in man. After 30 minutes of anoxia there were the following ultrastructural abnormalities: sarcoplasmic oedema and mitochondria appeared rounded and swollen, with clearing matrix and occasional disorganization of cristae. After 60 minutes of anoxic arrest the following changes were noted: the cardiac muscle cell was markedly *disorganized*, with severe intracellular oedema and dislocation of myofilaments; mitochondria showed swelling and loss of cristae, disruption of the outer membranes, and appearances of lamellar degeneration (Fig. 3). The intercalated discs showed dehiscence, and a prominent finding was the pronounced lymphatic dilatation. Examination of specimens taken after the coronary circulation had been re-established showed that the myofibres were less damaged, and the mitochondria, with dilated cristae, adopted a characteristic tubular appearance with dense granules in the matrix (Fig. 4). In other cells mitochondria were reduced in number and showed lamellar degeneration. A constant feature was the persistent dehiscence of intercalated discs, even in the less damaged myofibres (Fig. 5).

GROUP III The four dogs included in this group recovered. Data from examination of specimens taken after 30 minutes of anoxia were similar to

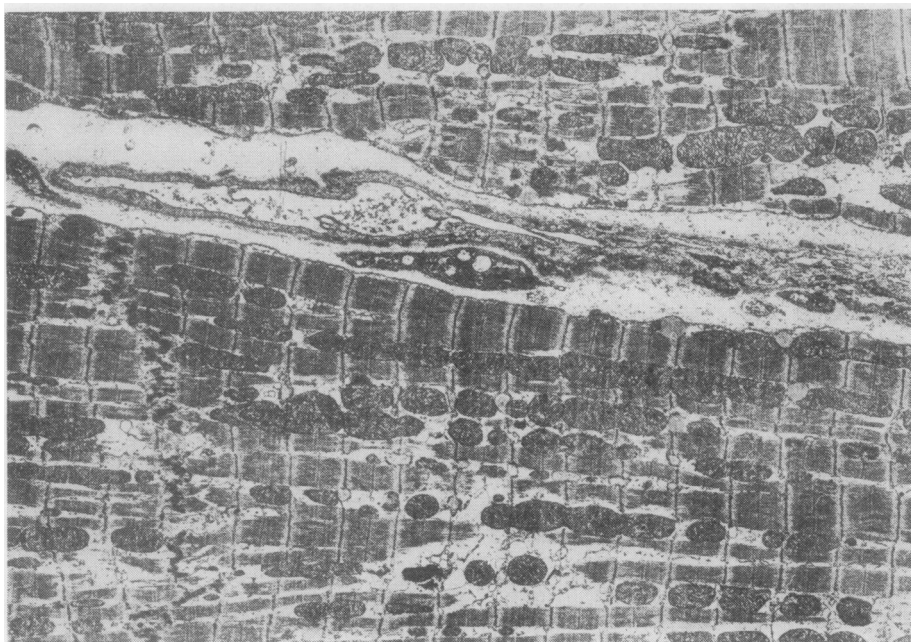


FIG. 1. *Group I. Cardiac muscle morphology after 30 minutes on cardiopulmonary bypass (×13 700).*

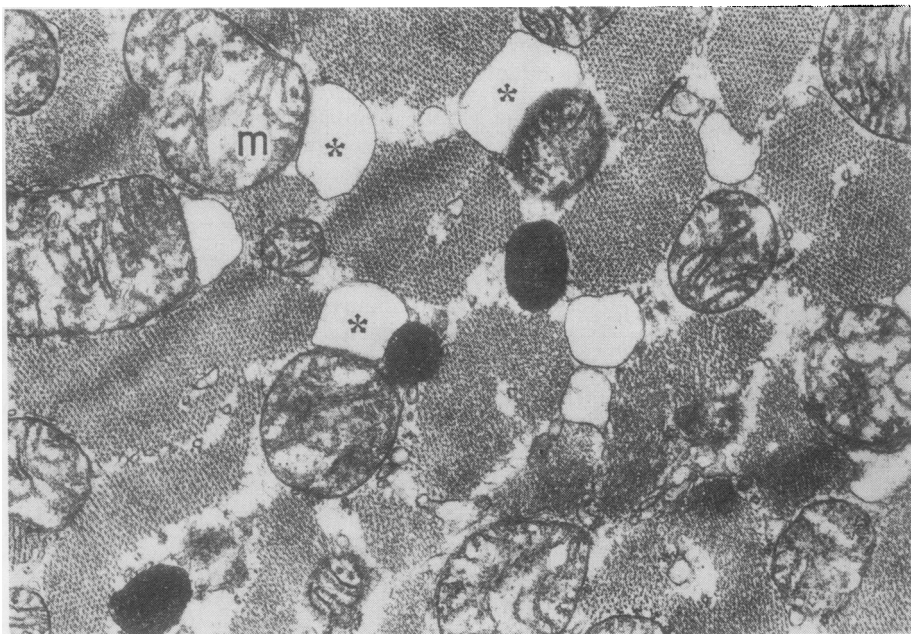


FIG. 2. *Group I. Myocardium after 60 minutes of extracorporeal circulation. Mitochondria (m) with slight changes. Sarcoplasmic reticulum enlarged (asterisks) (×28 800).*

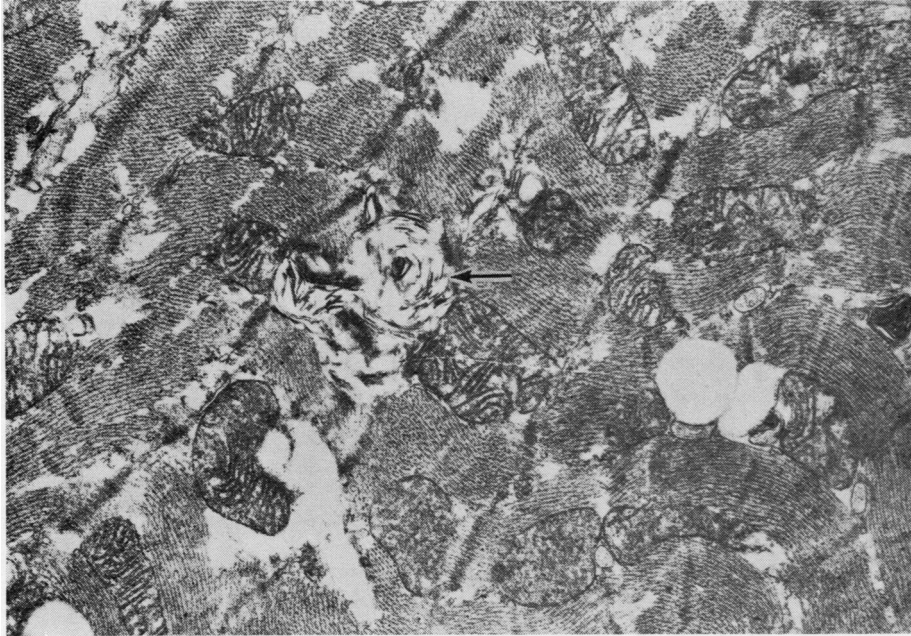


FIG. 3. *Group II. Sixty minutes of cardiac anoxia. Figures of mitochondrial lamellar degeneration (arrow) ($\times 18\ 200$).*

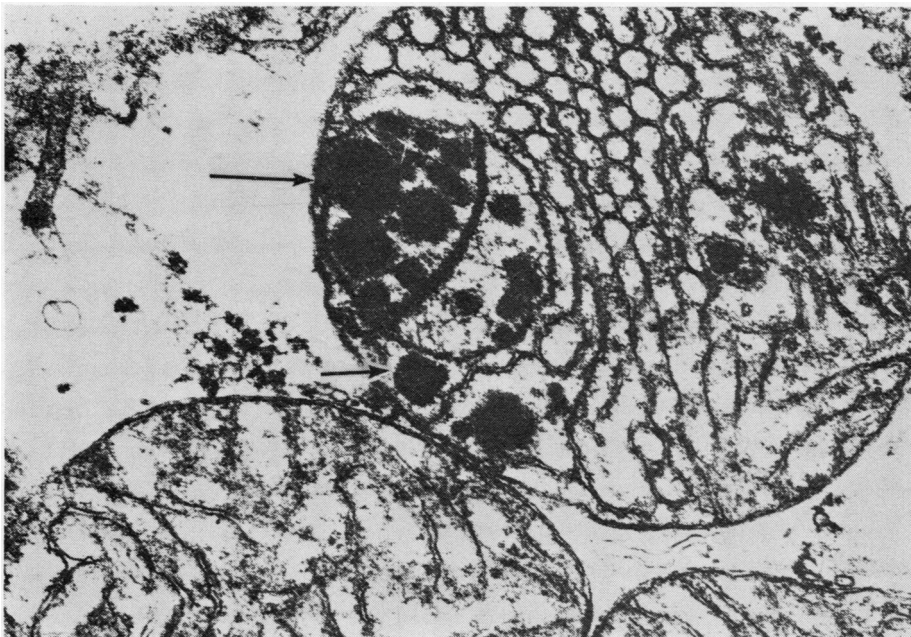


FIG. 4. *Group II. Experiment II-4. Mitochondria with dilated cristae and dense granules (arrows) in the matrix ($\times 53\ 300$).*



FIG. 5. *Same heart as in Fig. 4. Dehiscence along an intercalated disc (between asterisks) ($\times 17\,500$).*

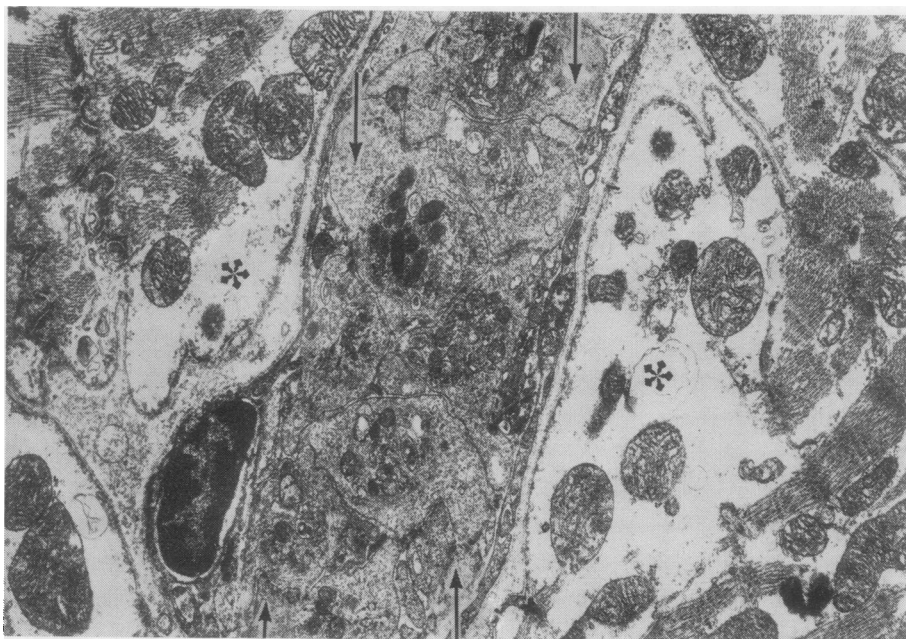


FIG. 6. *Group III. Subsarcolemmic oedema (asterisks). Platelet aggregate (arrows) in capillary blood-vessel ($\times 18\,000$).*

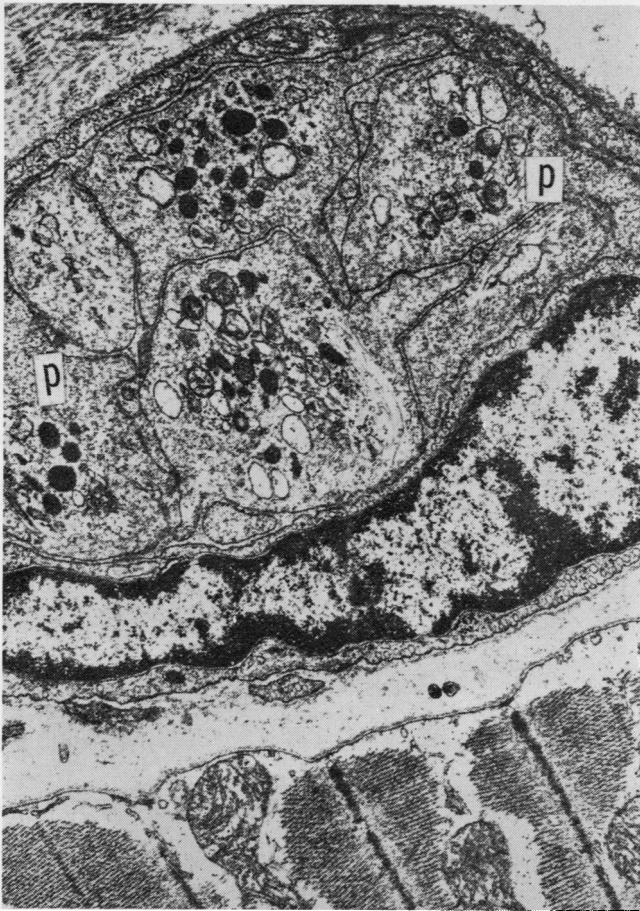


FIG. 7. Group III. Platelet (p) aggregate in capillary bloodvessel; there is neither platelet degranulation nor fibrin between them ($\times 19\ 000$).

FIG. 8. Group IV. Perinuclear oedema with large vesicles (asterisks) ($\times 18\ 000$).

FIG. 7.

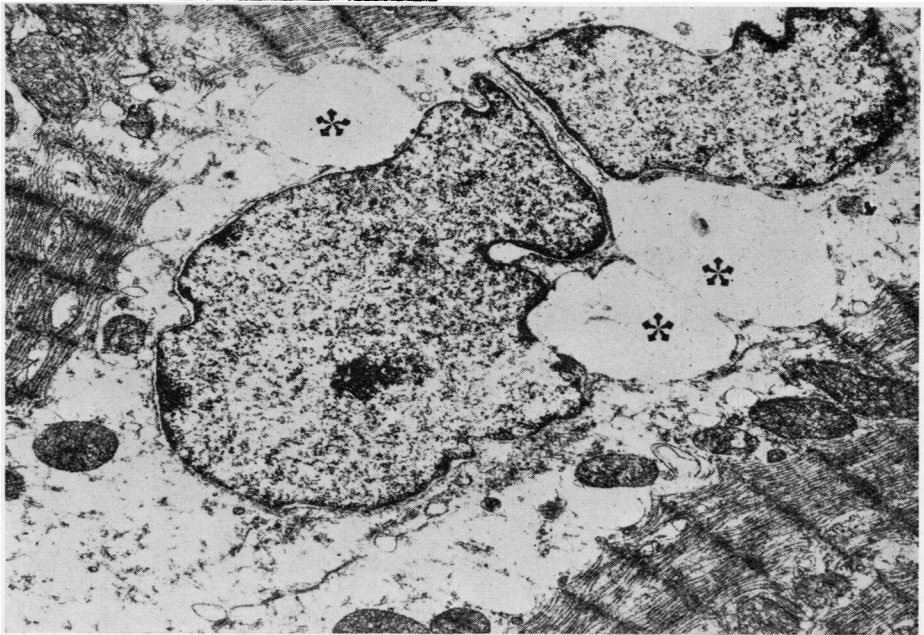


FIG. 8.

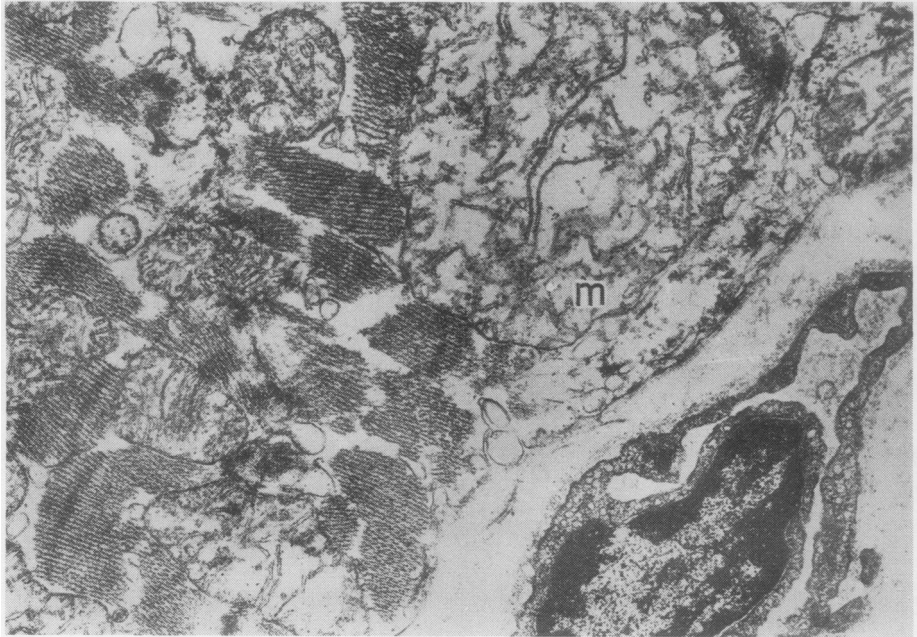


FIG. 9(a). *Group IV. Subsarcolemmic mitochondria (m) with clearing of the matrix and very thin cristae ($\times 18\ 000$).*

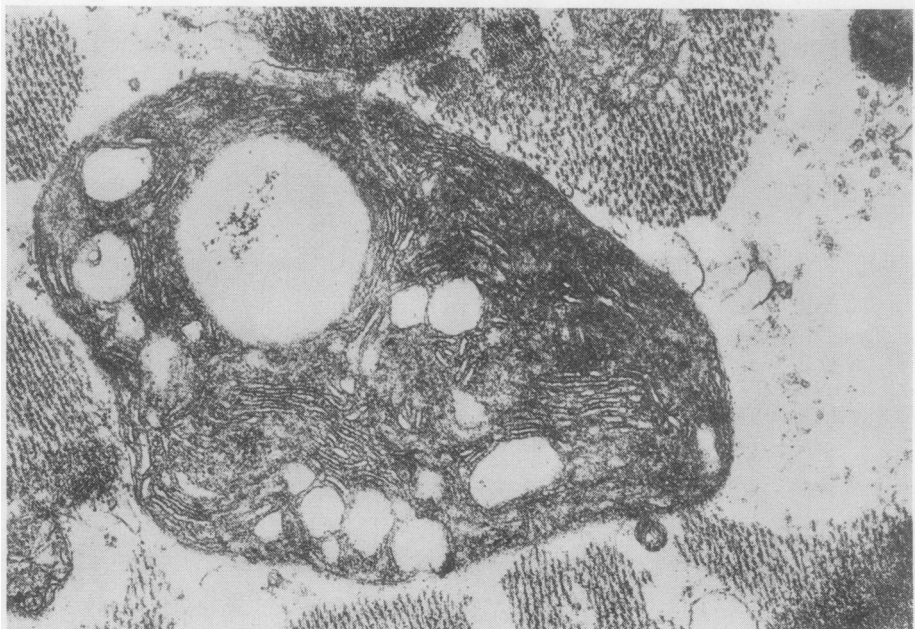


FIG. 9(b). *Group IV. Enlarged mitochondria with dense matrix, parallel cristae, and clear vesicles ($\times 28\ 000$).*

those described for group II at the same time. After 30 minutes of intermittent coronary perfusion, the myocardial cell appeared well preserved, but the following changes were seen: slight intracellular oedema located in subsarcolemmic areas, and the presence in capillary blood vessels of some platelet aggregates. Platelets appeared without degranulation and no fibrin was seen between them nor endothelial cell damage (Figs 6 and 7).

GROUP IV All animals included in this group recovered. Ultrastructural changes after 30 minutes of anoxia were identical with those described in groups II and III at the same time. Specimens taken after 30 minutes of continuous coronary perfusion showed marked perinuclear oedema with vacuolization, which displaced the sarcomeres (Fig. 8). Mitochondria, mainly at subsarcolemmic level, were enlarged with clearing of the matrix and very thin cristae (Fig. 9a), and in some observations there were intramitochondrial vesicles and cristae in parallel disposition (Fig. 9b).

DISCUSSION

It is evident from these results that after 30 minutes of extracorporeal circulation with anoxic cardiac arrest there are ultrastructural changes in the myocardium, but they are slight and reversible. When coronary perfusion is re-established anoxic lesions disappear. On the other hand, if the aortic clamping is maintained for 60 minutes, there are severe mitochondrial changes and intercalated disc dehiscence. From the practical point of view, it is very important to know whether these morphological changes are reversible or not. Lev *et al.* (1965) observed after 45 minutes of myocardial anoxia lesions such as disruption of the outer membranes of the mitochondria and an increased amount of diffuse material adjacent to the intercalated discs, which they considered to be on the borderline between reversible and irreversible damage; but Bloodwell *et al.* (1969), considering myocardial biopsies taken after similar periods of anoxia, believed that these changes were reversible despite severe mitochondrial lesions, based upon their clinical experience. O'Brien *et al.* (1972) agree that myocardial metabolic changes after 60 minutes of anoxia may revert. Nevertheless, Iyengar *et al.* (1972) found that all animals which underwent anoxic cardiac arrest for 60 minutes died with sub-endocardial haemorrhagic necrosis; our results are in agreement with this, since no dog recovered after the same anoxic period. With regard to

longer periods of myocardial anoxia, Kottmeier and Wheat (1966) observed that the morphological changes seen, which were mainly lamellar degeneration and granular degeneration, were irreversible, even after the coronary flow had been re-established for 30 minutes.

We believe that 30 to 45 minutes should be considered a safe period of anoxic cardiac arrest during extracorporeal circulation; a longer period may be hazardous, and after 60 minutes of anoxia, at least from the morphological point of view, dehiscence of intercalated discs and mitochondrial lamellar degeneration observed by us must be considered as evidence of irreversible cell damage. On the other hand, changes such as enlargement of the capillary lymphatics, as a result of diversion of interstitial fluid to them caused by blockade of blood circulation (Bullon and Huth, 1972), do not represent a danger.

It is necessary to discuss one fact: our experimental data were obtained from normal canine myocardium, while in human cardiac surgery the hypertrophied hearts of aortic valve disease or ischaemic heart disease have a different tolerance to anoxia. Iyengar *et al.* (1973), studying dogs with left ventricular hypertrophy produced by sub-coronary aortic stenosis, found that myocardial lesions were much more extensive in these animals than in those with normal hearts. Similar conclusions are drawn from clinical experience of coronary artery surgery with anoxic cardiac arrest (Reul *et al.*, 1971).

Among the several methods devised for myocardial protection, we have studied the effects of continuous and intermittent coronary perfusion, at systemic and low pressure respectively. Based upon recovery and ultrastructural data, both methods are useful in preventing anoxic damage. The morphological appearance of cardiac muscle after low-pressure intermittent perfusion is almost the same as after extracorporeal circulation with unclamped aorta, though some platelet plugs were seen in the capillaries. As degranulation or interplatelet fibrin were not seen, these aggregates must have developed immediately before the specimens were taken. This was done after finishing the last three minutes of intermittent perfusion, and thus this finding is surely related to perfused blood changes. The principal change arising from continuous coronary perfusion at systemic pressure was intracellular and intramitochondrial oedema due to altered hydrostatic pressure. Nevertheless the main criticisms of this technique, namely trauma to the coronary arteries and the risk of perfusing inadequately a coronary

branch, were avoided with the method used in the present experiments. We accept that aortic root perfusion with a balloon-tip catheter is not applicable in the clinical situations when aortic cross-clamping is necessary.

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