

Elective Cardiac Arrest: *

Its Effect on Myocardial Structure and Function

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INDUCED CARDIAC ARREST during open-heart operations has made the repair of certain lesions more precise and has diminished the required operating time. Potassium citrate, used according to the Melrose technic,²⁶ was accepted as an arresting agent after early reports. Further clinical use, however, has shown that potassium citrate arrest may be followed by irreversible changes in cardiac rhythm, structure and function; for this reason this method of arrest has been discontinued by most surgeons. Other preparations and methods have been employed, principally intermittent anoxia produced by aortic clamping with or without hypothermia, and coronary perfusion. The period of arrest which may be safely employed without the subsequent development of irreversible myocardial effects, using anoxia or potassium, has been estimated to be about 30 minutes¹⁴ but this time limit has not been clearly defined. Bentall¹ has stated that there is no safe period of cardiac arrest. Recent papers have emphasized changes in myocardial metabolism and function which follow various methods of cardiac arrest.

Glycogen loss from the myocardium and changes in cellular morphology have been demonstrated within a few minutes following coronary ligation in the rabbit, by Caulfield and Klionsky,⁷ by employing histo-

chemical technics and electron microscopy. These methods appeared to have important application in the study of the deleterious effects of various methods of induced cardiac arrest upon the myocardium.

The present study was undertaken to determine: 1) Whether early ultrastructural changes and the histochemical disappearance of glycogen are associated with various forms of elective cardiac arrest; 2) if change could be demonstrated, the length of time of arrest required to produce these changes; and 3) to correlate the development of changes with the myocardial function following arrest, as measured by the force of myocardial contraction, blood pressure, and the electrocardiogram.

Method

Adult mongrel dogs weighing from 10.4 to 16.8 kilograms were used for these experiments.

A. Coronary Ligation Group (25 Dogs). A positive control group was established by experimental coronary ligation. A method similar to that reported by Jennings,²¹ in which uniform left ventricular myocardial infarcts may be produced, was used. The animal was anesthetized with 2.5 per cent pentothal sodium and the lungs inflated intermittently with oxygen by a positive pressure respirator. The left chest was entered through the fourth intercostal space. The circumflex branch of the left coronary artery was isolated and a ligature passed beneath it but not tied. A small plastic catheter was inserted into the left subclavian

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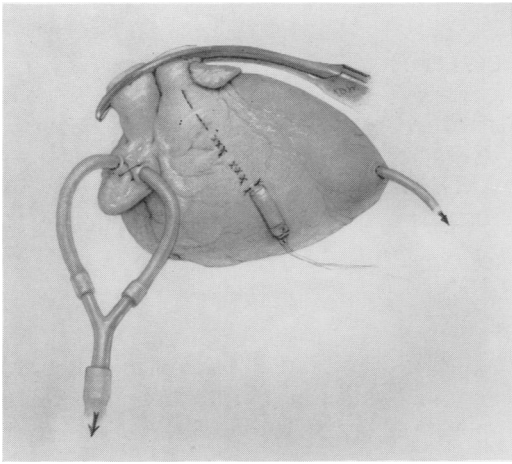


FIG. 1. Heart preparation showing location of Walton-Brodie strain gauge arch, biopsy sites, left ventricular vent and caval catheters.

artery, threaded into the proximal aortic arch, and tied in place. Mean arterial blood pressure and lead II electrocardiogram were monitored throughout the experiment. The coronary artery was ligated and the heart then was arrested, after the elapse of one to 60 minutes, by injection of potassium citrate (Melrose solution) into the proximal aorta as the brachio-cephalic artery and the aortic arch were occluded distally. The heart was removed quickly and sections taken from the ischemic and the nonischemic areas for the histochemical determination of glycogen and study by electron microscopy. Tissue was fixed within 30 seconds in 1.0 per cent osmium tetroxide buffered to pH 7.3–7.5, and sucrose added.^{8, 29} After rapid dehydration in graded alcohols the tissues were imbedded in a 10–90 mixture of methyl and n-butyl methacrylate. Sections were cut on a Porter-Blum microtome, then stained in 2.0 per cent uranyl acetate for one to two hours and studied by one of us (P. R.) using an RCA EMU-3 electron microscope.

Portions of biopsy specimens were frozen in liquid propane and prepared for freeze substitution in a variety of fixatives, including alcohol, acetone, and alcohol with mercuric chloride, according to the method of

Feder and Sidman.¹³ Fragments of fresh tissue were frozen in liquid nitrogen and then cut in a cryostat. If additional material was available, as usually was the case at the final time of sacrifice of the animals, in perfusion experiments, additional blocks of tissue were fixed in neutral buffered formalin and in absolute alcohol. In each case, sections were stained with an alcoholic periodic acid Schiff technic for glycogen, and studied by one of us (B. K.).

The frequent development of ventricular fibrillation before the intended time interval between coronary ligation and sacrifice necessitated moving the site of coronary ligation more distally along the circumflex branch in subsequent experiments.

B. Perfusion Group. In the perfusion group, four series of experiments were done:

1. *Controls (6 Dogs).* These dogs were subjected to total cardiopulmonary bypass using the Kay-Cross oxygenator and Sigma-motor TM1 pump for a period of one hour at flow rates of 52–98 cc./Kg./min., which maintained systolic blood pressure of 50–140 mm. Hg. No form of cardiac arrest was used. Myocardial biopsies of right or left ventricle were taken at intervals throughout the one hour perfusion and studied for the presence of glycogen and for electron microscopic changes (Fig. 1). Myocardial contractile force was monitored using the Walton-Brodie strain gauge arch sewn to the right ventricle.⁴ The electrocardiogram, arterial blood pressure and pulse also were monitored and recorded on a multiple channel recorder. Samples for blood pH, CO₂ content and O₂ content were drawn at the beginning and at the termination of the perfusion in most dogs. Blood volume was regulated in the post-perfusion period by direct measurement replacement or by regulation of central venous pressure. Animals were observed for varying periods extending to 24 hours postoperatively.

2. *Anoxic Arrest (8 Dogs).* The method was similar to that used in the control

group. After a control myocardial biopsy had been obtained and cardiopulmonary bypass had been established, the aorta and pulmonary artery were cross-clamped. The left ventricle was vented by a cannula placed through the apex and sutured in place by a pursestring suture. Right ventricular distention was prevented by loosening the caval tapes when necessary. Right ventricular chamber temperature was measured by a thermistor inserted through the right ventricular wall. Rectal temperature also was measured. The period of cardiac arrest was one to two hours. Myocardial biopsies, from four to seven in number, were taken at intervals varying from five to 120 minutes during the bypass. The volume of blood flowing through the left ventricular vent was measured intermittently. After the arrest period, extracorporeal circulation was maintained while resuscitation of the heart and establishment of circulatory homeostasis were attempted. The left ventricle was vented during this period. The intermittent use of vasopressor (metaraminol bitartrate 10 mg./500 ml., or phenylephrine hydrochloride 5 mg./500 ml.) as an intravenous drip often was necessary to maintain a satisfactory arterial blood pressure.

3. Potassium Citrate Arrest (7 Dogs). This series of experiments was similar to the anoxic arrest group except that potassium citrate (Melrose technic), in an amount necessary to effect cardiac arrest, was injected into the left ventricular chamber after the aorta and the pulmonary artery had been clamped. Ten to 20 ml. of Melrose solution usually was required. The remainder of the experiment was similar to that described for series 2.

4. Anoxia and Hypothermia (6 Dogs). In this series, selective cooling of the heart was accomplished by irrigation of the pericardial sac with cold saline solution, lowering the intracardiac temperature to approximately 30° C. Cardiac normothermia was reestablished rapidly at the termination of the arrest period by releasing the aortic

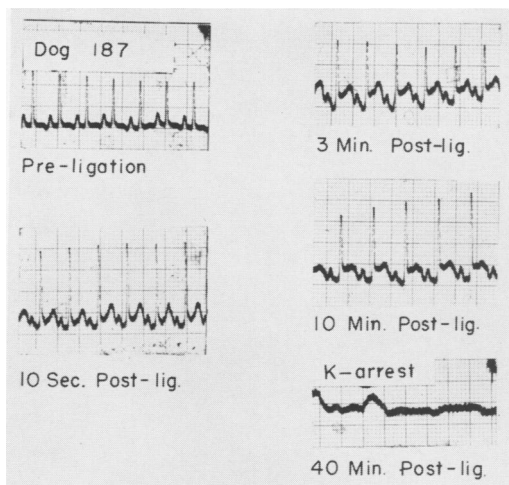


FIG. 2. Electrocardiograms showing early changes observed after coronary ligation in a control animal.

clamp. The remainder of the experiment was similar to that for the other arrest groups.

Results

A. Coronary Ligation Group. The control group with only ligation of a coronary artery was included to establish for this study a base line with which to evaluate intracellular hypoxic changes in subsequent arrest groups and to confirm previous findings in the rat and rabbit.^{6,7} In this group, a mild drop in mean blood pressure occurred in 12 animals after the ligation and in nine animals no change was noted. An area of purplish-blue discoloration appeared on the posterior lateral wall of the left ventricle in the distribution of the circumflex branch of the left coronary artery in many animals; however, this area was not always inspected, in order to disturb the heart as little as possible. Eleven animals developed ventricular fibrillation spontaneously within 20 minutes after the ligation.

Electrocardiographic changes, usually consisting of ST segment elevation and peaking of T-waves, were observed as early as six seconds after ligation, usually within 30 seconds. In two animals changes ap-

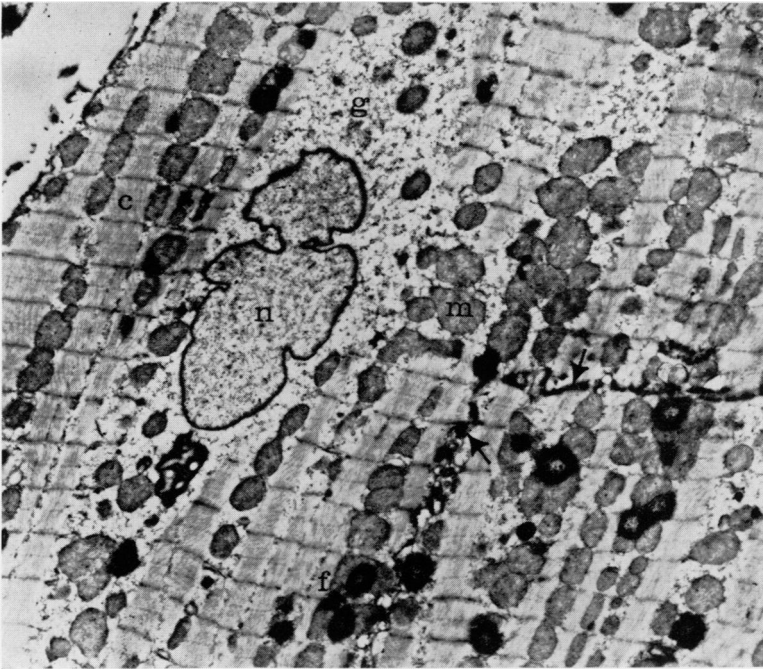


FIG. 3. Electron micrograph of a myocardial cell of a control animal. The homogeneous nucleus is at (n). The area of the cell corresponding to the major perinuclear histochemical localization of glycogen is seen at (g). A mitochondrion is indicated at (m). Fatty degeneration adjacent and often contiguous to mitochondria is seen at (f). Arrows indicate a membrane separating two cells. The contractile elements are labeled as (c). (Approx. $\times 6,000$.)

peared within five minutes and 60 minutes, respectively. In the latter animal, in which slight ST segment depression was delayed for 60 minutes, discoloration suggesting infarction was grossly visible shortly after

the ligation. In this animal, microscopic changes were localized to the papillary muscle. The observation of ventricular tachycardia or numerous premature ventricular contractions usually was soon fol-

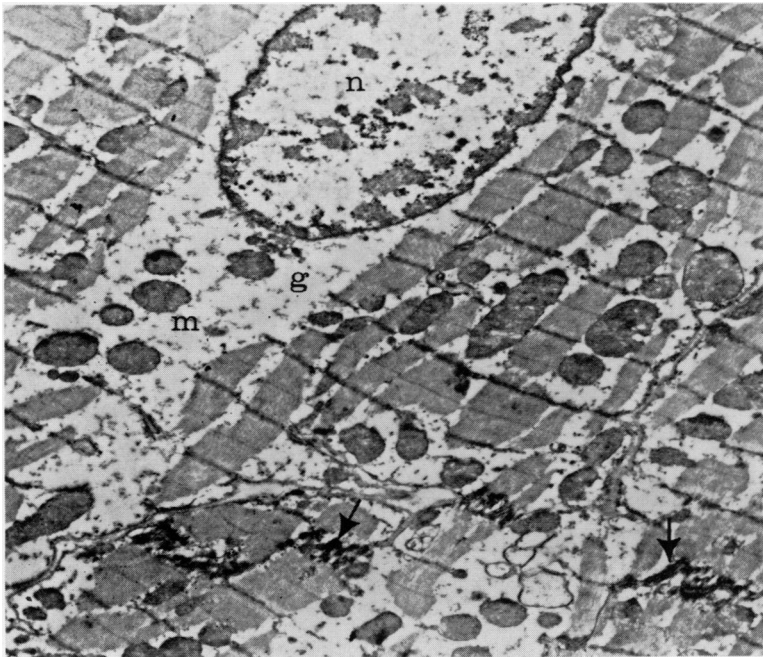


FIG. 4. A myocardial cell after 25 minutes of ischemia due to occlusion of the corresponding nutrient coronary artery. Extensive rarefaction of the nucleoplasm (n) has occurred with severe clumping of the chromatin and margination of it at the nuclear membrane. Rarefaction of the glycogen spaces (g) also has occurred, as well as separation of cytoplasmic elements. Cell membranes and intercalated discs are indicated by arrows. A mitochondrion is at (m). These cell changes are manifest in a few cells by 5 to 10 minutes of ischemia. By 20 to 25 minutes almost all cells in the distribution of the occluded artery are so altered. (Approx. $\times 6,300$.)

lowed by ventricular fibrillation. Electrocardiographic changes usually were progressive over the period of observation; in one instance, the changes appeared to revert toward normal within five minutes. Other isolated changes included ST segment depression, ventricular premature beats, and inversion of T-waves. A representative series of lead II electrocardiograms is shown in Figure 2.

Those hearts which fibrillated were considered unsatisfactory for study by electron microscopy and were eliminated, since previous observations⁷ have suggested that this rhythm, in a short period, may effect changes in cellular structure which could be superimposed upon the changes of the ligation alone.

Figure 3 illustrates the normal intracellular morphology of canine heart muscle.

After 15 to 20 minutes of coronary occlusion myocardial cells in ischemic areas exhibited moderate rarefaction of nucleoplasm and some margination of nucleoplasm toward the nuclear membrane (Fig. 4). "I" bands were not prominent at this time, however. At one hour of ischemia, swelling had become manifest in almost all mitochondria.

The results of histochemical determinations for glycogen closely paralleled those previously observed in the study of myocardial ischemia and early infarction in the rabbit.⁷ There was dramatic and almost total loss of glycogen in the distribution of the occluded vessel within five minutes. The few attempts made to study animals with ischemia for shorter periods of time showed somewhat equivocal patterns of subtotal glycogen loss. Figure 5 shows a section after 30 minutes of coronary ligation. The use of the fresh frozen cryostat sections prevented the flight of glycogen which occurs in formalin and alcohol fixatives. The pattern of distribution obtained was almost identical with that seen in frozen substituted sections.

The hearts that did fibrillate after 30 minutes were studied in most cases for their glycogen content. The pattern of glycogen loss was compatible with the length of the antecedent ischemia.

B. Perfusion Group.

1. *Controls.* Data obtained from the control perfusion group and arrest groups are summarized in Table 1. Myocardial force is plotted in Figure 6. In the four animals

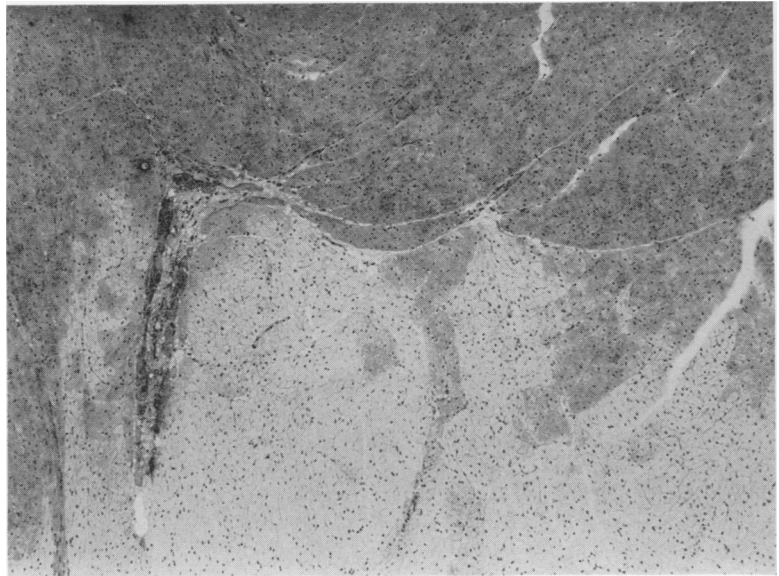


FIG. 5. Following 30 minutes of coronary ligation the pale glycogen-depleted areas stand in sharp contrast to the normal muscle. Cryostat section. (PAS-Hematoxylin, green filter, from $\times 55$.)

TABLE 1

No. Dogs	Flow Rate (ml./kg.)	Duration Arrest or Control Perfusion (min.)	Perfusion Arterial Pressure (mm.Hg)	R. Ventric. Temp. at Termination of Arrest or Perfusion	Early Post-Arrest EKG Changes	Postarrest Arterial blood pressure
Controls						
6	75 (52-98)	60	100 (50-140)	35.1 (31.5-38)	Normal 4 dogs ST seg. depr. 1 dog Peaked T waves 1 dog	Normal 4 dogs Depressed 2 dogs
Anoxia arrest						
8	74 (45-99)	60 (6 dogs) 120 (2 dogs)	104 (80-160)	34 (30-37)	Persistent fibrillation (3) Wide QRS (4) ST elevation or depression (3) Low EMF (1) Peaked T waves (3)	Normal (1) Severely depressed (7)
Potassium citrate arrest						
7	70 (50-86)	60 (5 dogs) 120 (2 dogs)	94 (80-120)	32.4 (26.5-35)	Wide QRS (4) Elevated ST segment (3) Fibrillation (1) Peaked T waves (2)	Normal (3) Depressed or absent (4)
Anoxia with hypothermia arrest						
6	71 (50-86)	60	93 (80-120)	29.5 (29-30)	Wide QRS (2) Low EMF (2) ST segment changes (3) Deep S waves (2)	Normal (1) Depressed (5)

* Value in a single animal.

in which myocardial force was obtained the force of contraction during the perfusion was as great as 150 per cent of that recorded as the base line reading in two animals. The contractile force did not fall below 41 per cent of the control level during perfusion in these animals. Early postperfusion readings were 80-100 per cent of the control value. Occasional variation in contractile force was observed with different phases of respiration, heart position, placement of caval catheters, and arrhythmias. The values chosen as representative were those taken when the waves were most stable, or the smallest of the waves when the height in the same tracing was variable. After the strain gauge arch had been in place three to four hours, the wave forms

often became choppy and for this reason were considered unreliable. This probably was caused by loosening of the sutures which held the arch on the myocardium. All animals survived the immediate post-perfusion period and only two showed hypotension, which responded well to vasopressors during this period of time. Myocardial contractile force was not measured in those two animals. Mild electrocardiographic changes were observed in two animals. At the end of perfusion, arterial and venous O_2 content, pCO_2 and pH in dogs tested showed no significant variation from normal.

2. *Anoxia.* As shown in Table 1 and Figure 7, dogs subjected to periods of cardiac arrest by aortic clamping for one to two

TABLE 1.—(Continued)

Prearrest			Postarrest		
Arterial O ₂ Saturation (%)	Venous O ₂ Saturation (%)	Arterial pH	Arterial O ₂ Saturation (%)	Venous O ₂ Saturation (%)	Arterial pH
93 (82-103)	61 (47-65)	7.39 (7.32-7.43)	97 (93-100)	59 (40-66)	7.365 (7.26-7.43)
95 (85-100)	50 (26-61)	7.39 (7.34-7.48)	95 (87-112)	53 (31-80)	7.29 (7.27-7.33)
96	60	7.36	94	59	7.28*
88 (80-100)	54 (52-56)	7.37 (7.33-7.41)	90 (73-100)	54 (46-60)	7.30 (7.13-7.44)

hours showed severe changes in cardiac function, the single exception being Dog 71, which will be discussed in more detail.

After the aorta was cross-clamped, some contractions were visible from seven to 15 minutes, except in heart of Dog 71 which continued to beat throughout the period the aorta was cross-clamped. Myocardial force diminished rapidly after aortic cross-clamping (except in Dog 71), falling to zero within six minutes.

In all animals, arterial perfusion pressure was adequate. The two hearts arrested for two hours became indurated with rigor mortis after approximately 90 minutes of arrest, and, upon release of the aortic clamp, focal refractory fibrillary movement was the only activity observed. Further

pump support for an additional period of 13 minutes did not effect resuscitation in either instance.

All hearts (except Dog 71) fibrillated when the aortic clamp was released to establish coronary flow. The fibrillation was converted easily to a regular rhythm by one or two electric shocks.

Some electrocardiographic changes were observed in all animals. The usual findings were widening of the QRS complexes and ST segment changes. In Dog 66 these changes had almost disappeared four hours postperfusion, although bradycardia and poor contractile force persisted. The arterial blood pressure was decreased or absent in the postperfusion period in all animals except Dog 71 and, although three animals

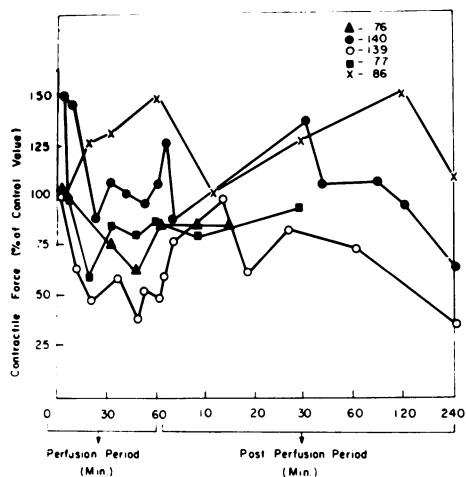


Fig. 6 Control Perfusion

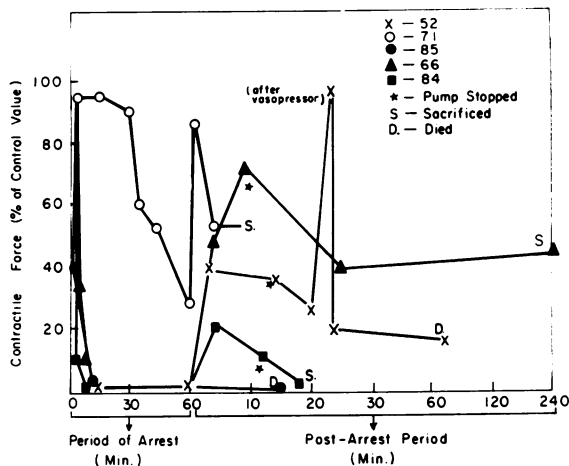


Fig. 7 Anoxia

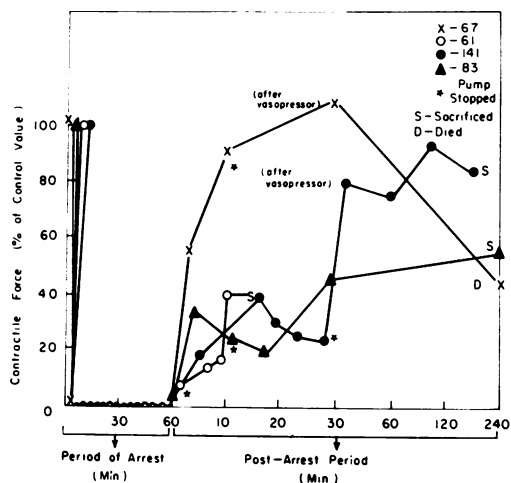


Fig. 8 Potassium Citrate

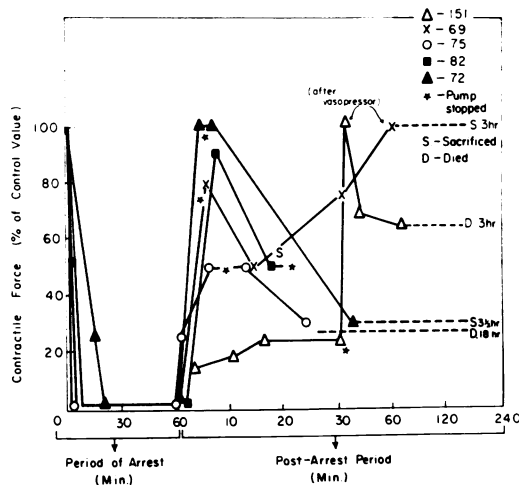


Fig. 9 Anoxia & Hypothermia

FIG. 6. Myocardial contractile force in 5 perfusion control animals. FIG. 7. Myocardial contractile force recorded during and after arrest induced by anoxia. Early postarrest depression is observed in all animals except Dog 71, in which the bronchial flow was increased. FIG. 8. Contractile force observed in 4 animals after potassium citrate arrest. FIG. 9. Myocardial force observed in 5 animals after arrest by anoxia with mild hypothermia.

survived longer than two hours postperfusion, all were severely hypotensive, requiring vasopressor during that time. Although after release of the aortic clamp the myocardial force rebounded moderately while the pump still was supporting the circulation, the contractile force fell rapidly after the pump was stopped.

A vent was placed into the left ventricular chamber through the apex in order to

prevent distention of the left side of the heart during and following the arrest period. Measured quantities of blood vented from the left ventricle were 5.0 to 12 cc./min. The vent was left unclamped until the myocardium appeared to contract with sufficient strength to discontinue pump support. Two exceptions were Dogs 71 and 85. In Dog 85, both pulmonary hili were ligated and all bronchial artery drainage from the

pulmonary veins occluded; consequently there was no blood obtained from the vent. This heart became indurated, as with rigor mortis, suggesting that in other dogs even with the vented left ventricle some small amount of blood provides cardiac nourishment, either through the Thebesian vessels or the coronary arteries.

Dog 71 is quite interesting and unique in that the left ventricle was vented but the vent kept occluded and, despite complete aortic clamping distal to the coronary ostia, the heart continued to beat and maintain a relatively normal electrocardiogram and myocardial force. After 45 minutes the vent was unclamped, draining the bronchial venous flow entering the left ventricle. Cardiac function immediately deteriorated, with decreased pulse rate and myocardial contractile force and electrocardiographic changes of peaked T-waves and ST-segment elevation. The measured vent flow was 44 cc./min. Upon sacrifice, this animal was found to have heart worms and a few granulomas of the lung, apparently responsible for the increased bronchial flow.

3. *Potassium Citrate.* The group in which hearts were arrested by potassium citrate for one to two hours showed diminished contractile force in the postarrest period but this was not as severe as that in the anoxia group. Postperfusion blood pressure was normal and sustained in three of the seven dogs but was depressed or absent in the remaining four (Table 1). One heart showed consistency suggesting rigor mortis after 90 minutes of arrest and could not be resuscitated. Each of these four required vasopressor to maintain the blood pressure postoperatively. Three animals died and four were sacrificed three to four hours postperfusion. All hearts showed electrocardiographic changes early after the arrest period but these changes were returning toward normal at the time of sacrifice.

The myocardial contractile force (Fig. 8) in four animals in which this parameter was obtained showed early postperfusion de-

pression in three animals. The fourth had resumed the control contractile force at 30 minutes but vasopressor was utilized during this period. In three animals, at two to four hours postperfusion, 45, 55 and 84 per cent of the control contractile force was recorded, respectively.

Pump support was required for two to 34 minutes after release of the aortic clamp in this group before independent circulation was reestablished. All hearts fibrillated upon release of the aortic clamp and all except one, which required six stimuli, defibrillated easily with electrical stimulation. Left ventricular vent flow was measured at 2.0 to 28 cc./min.

4. *Hypothermia and Anoxia.* Dogs in which hypothermic hearts were subjected to anoxia for a period of one hour likewise did not maintain blood pressure well in the postoperative period. Two animals died during the night after operation. One of these had a right ventricular infarct in addition to bilateral pneumothorax. Two of the six animals had relatively normal blood pressure after the arrest period. Ventricular fibrillation developed in four animals upon release of the aortic clamp after the arrest period but defibrillation was accomplished easily by means of electric shock. Myocardial contractions ceased visibly in eight to 26 minutes after the aorta had been clamped. The volume of left ventricular vent flow was measured to be 3.0 to 8.0 ml./min.

The force of myocardial contractions decreased rapidly to zero within a few minutes after aortic clamping (Fig. 9). After release of the aortic clamp contractile force resumed fairly quickly but in most animals it soon declined after the pump support or vasopressor had been terminated. Electrocardiographic changes observed after termination of the arrest period consisted of widening of the QRS complexes, ST segment elevation and decreased electromotive force.

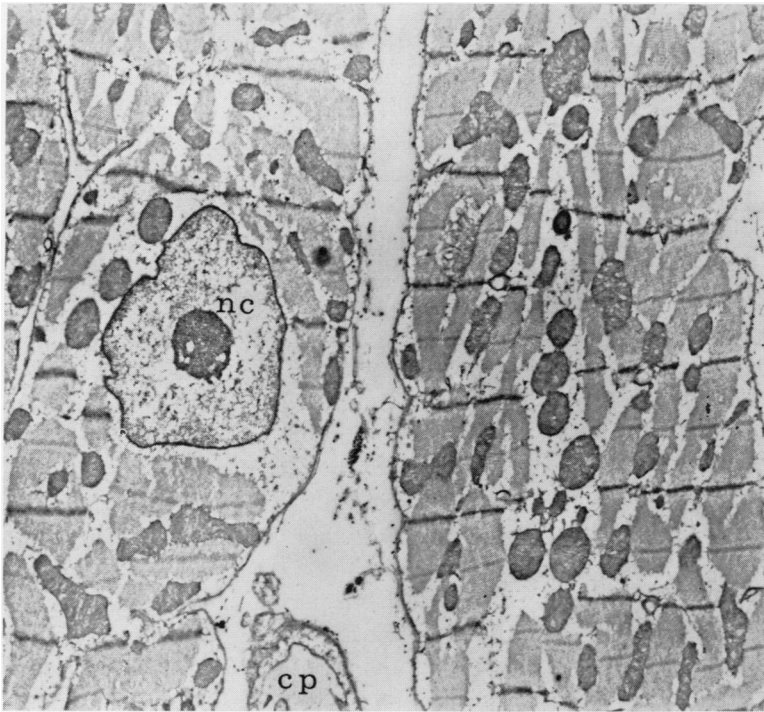


FIG. 10. Two myocardial cells are seen after 30 minutes of anoxic arrest. A prominent nucleolus is labeled (nc). Nucleoplasm is only minimally rarefied and not at all margined. Some separation of cytoplasmic elements is noted, however. A portion of a normal capillary is seen at (cp). (Approx. $\times 7,000$.)

Electron Microscopic Findings

No cellular changes were evident by electron microscopy in the *perfusion control* group even after one hour of perfusion, the appearance being similar to that in Figure 3. In addition to the normal morphology, fatty metamorphosis frequently was observed in "normal" hearts even before perfusion. Care must be taken then in using this parameter as a criterion for anoxic change without suitable control biopsy sections prior to the test procedure.

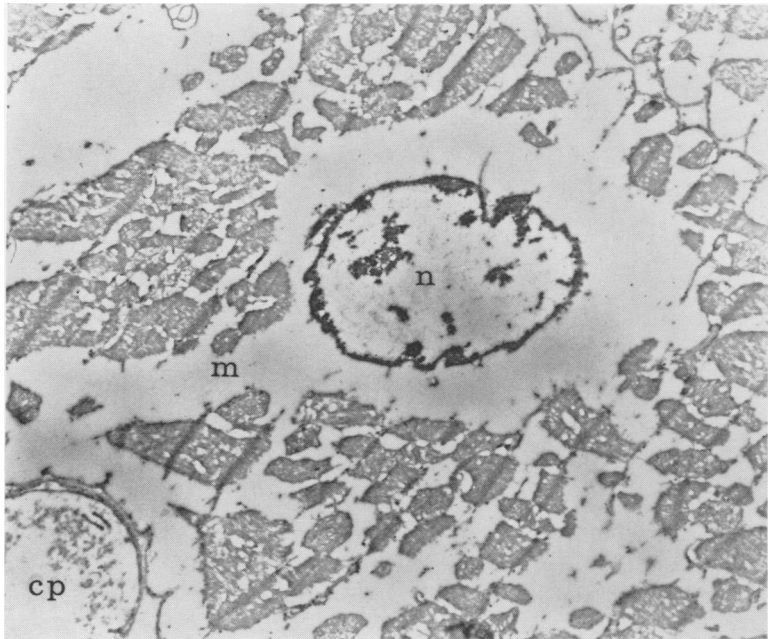
After 30 minutes of *anoxic arrest* (Fig. 10), nuclear morphology usually was only slightly abnormal as compared to the coronary ligation controls, where in 15 to 20 minutes moderate nuclear changes already had taken place. This abnormality consisted of slight rarefaction of the nucleoplasm. This preservation of morphology was not universal, however. Occasionally the myocardium showed changes almost comparable to 15 to 20 minutes of ischemia due to coronary occlusion. At one hour (Fig. 11), more severe changes, to the extent of

varying degrees of nuclear degeneration with margination of chromatin at the nuclear border, ensued. Nucleoli remained. In the cytoplasm, edema with separation of organelles, including myofibrils and mitochondria, was manifest. At one hour mitochondria generally were well preserved although some mitochondria at this state showed evidence of separation of cristae. I-bands were prominent at this stage as well. After two hours of anoxic arrest nuclei were similar to those in hearts arrested for one hour.

In Dog 71, during the period that the aortic clamp was in place and the left ventricular vent clamped, normal morphology was present in biopsy specimens.

Considerable variability was noted in foci throughout the ventricular walls. At times the myocardium maintained normal morphology for a thickness of two to four cells beneath the epicardium, even after an arrest period of one to two hours. Some foci of myocardium were better preserved than others. In this regard, epicardial myo-

FIG. 11. After one hour of anoxic arrest, extreme margination of chromatin has occurred. More extensive separation of cytoplasmic elements also is noted. Mitochondria are seen adjacent to (m). The disruption of the contractile elements may indicate irreversibility. (Approx. $\times 6,000$.)



cardium was less changed than was deeper myocardium. Myocardial and subepicardial areas of the left and right ventricles, in the few instances in which the left and right ventricles were biopsied, tended to show similar morphology with the two to four

cell thickness directly beneath the epicardium. Preservation of morphology and glycogen was better than deeper in the myocardium.

In the group in which local *mild hypothermia in addition to arrest by anoxia* was

FIG. 12. A myocardial cell is shown after one hour of arrest by potassium citrate by the Melrose technique. No disturbance of myocardial morphology is evident. A well preserved capillary is seen at upper right. (Approx. $\times 6,500$.)



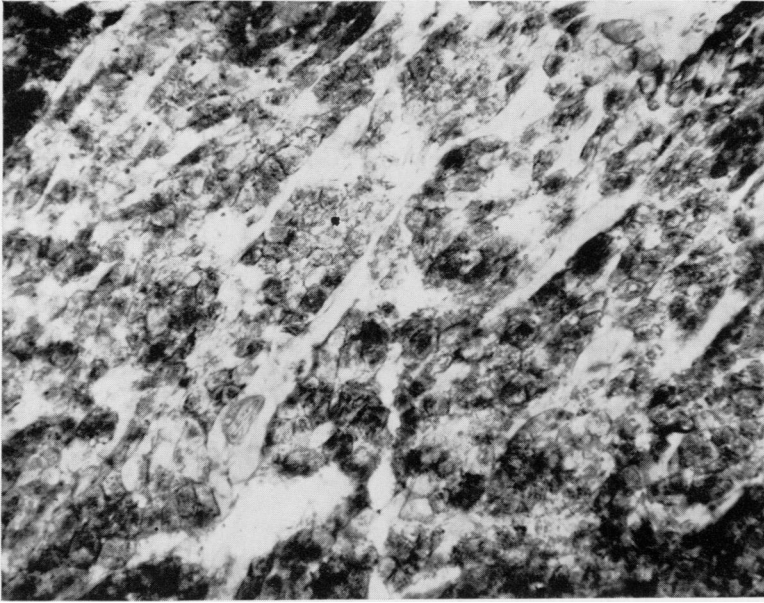


FIG. 13. The non-arrested heart contains maximal normal glycogen stores even following one hour of pump oxygenator support. Freeze-substituted section. (PAS-Hematoxylin, green filter, from $\times 200$.)

used, preservation of ultrastructure was variably improved. Thus at times, by one hour, only minimal anoxic change was evident. This is comparable to the changes that ensued after 30 minutes of arrest with anoxia at more normal temperatures.

Of interest were the ultrastructural changes which accompanied *potassium citrate arrest*. Here, even after one hour, morphology was perfectly preserved (Fig. 12). Indeed, in one dog, after two hours of arrest using potassium citrate, morphology was comparable to that of normal myocardium. Other work, reported by Helmsworth *et al.*^{19, 20} has indicated that extensive necrosis supervenes after one to two days of recovery from potassium arrest. Preliminary experiments here, with 24 to 48 hours of recovery following potassium arrest, corroborate their observations.

Myocardial Glycogen

The hearts of the *perfusion control* group showed a normal pattern and quantity of glycogen histochemically (Fig. 13). The results in the dogs with cardiac arrest were somewhat variable, although definite trends could be detected.

The animals subjected to *anoxic arrest* showed much preservation of glycogen. After 30 minutes and 60 minutes there seemed to be some loss of glycogen but in no case did the loss, even at the end of 60 minutes, reach the degree of severity obtained within five minutes following ligation of the coronary artery in the contracting heart. It became apparent that there were regional differences in the amounts of glycogen depletion or preservation. The areas beneath the epicardium and the endocardium and following major branches of epicardial vessels as they penetrated into the myocardial retained more glycogen than did muscle in the mid myocardium (Fig. 14). It is of striking interest that Dog 71, in which the heart did not stop following aortic-pulmonary clamping, had almost no myocardial glycogen even though the heart continued to contract.

No significant differences were noted between those hearts arrested with anoxia at normal temperatures and those with *anoxia and mild hypothermia*.

The best morphologic preservation of glycogen occurred in those hearts subjected to *potassium citrate arrest*. In every in-

stance, at the end of one hour, there were large quantities of glycogen remaining with definite perinuclear localization (Fig. 15). The two animals which were studied after potassium arrest for two hours revealed almost total depletion at the end of this period of time (Fig. 16).

Discussion

The rapidity and complexity of the processes during the normal contraction of heart muscle make the study of the deliberate interruption of this amazing cycle extremely difficult. The formation and splitting of actomyosin, adenosine-tri-phosphate (and other high energy phosphates), oxidative phosphorylation, membrane permeability, and ionization are a few of the factors involved.³⁸ The exact manner in which this cycle is broken down in artificial arrest is not known but Redo³⁰ has suggested an explanation on the basis that potassium accumulates outside the cell membrane in drug-induced potassium arrest as well as in other forms of arrest.

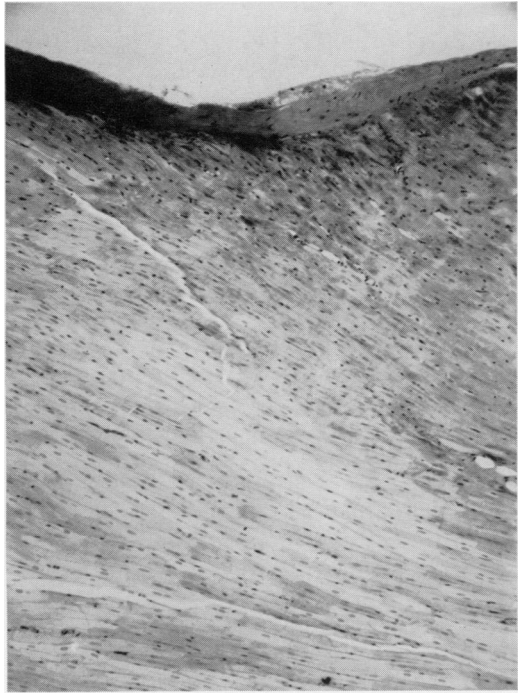
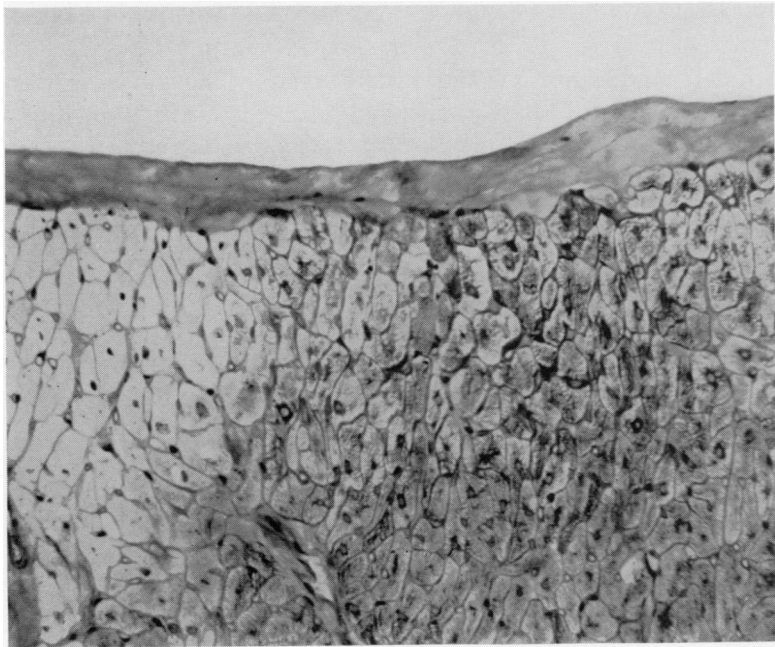


FIG. 14. This illustrates focal preservation of glycogen in myocardial fibers beneath the epicardium and about superficial vessels. Two hours after anoxic arrest. Freeze-substituted section. (PAS-Hematoxylin, green filter, from $\times 100$.)

FIG. 15. Following one hour of potassium arrest the myocardium shows some slight generalized degree of glycogen depletion. Most fibers still retain appreciable glycogen quantities with predominant perinuclear localization. A few patchy areas of total depletion as illustrated on the left become apparent. (Freeze-substituted sections, PAS-Hematoxylin, green filter, from $\times 250$.)



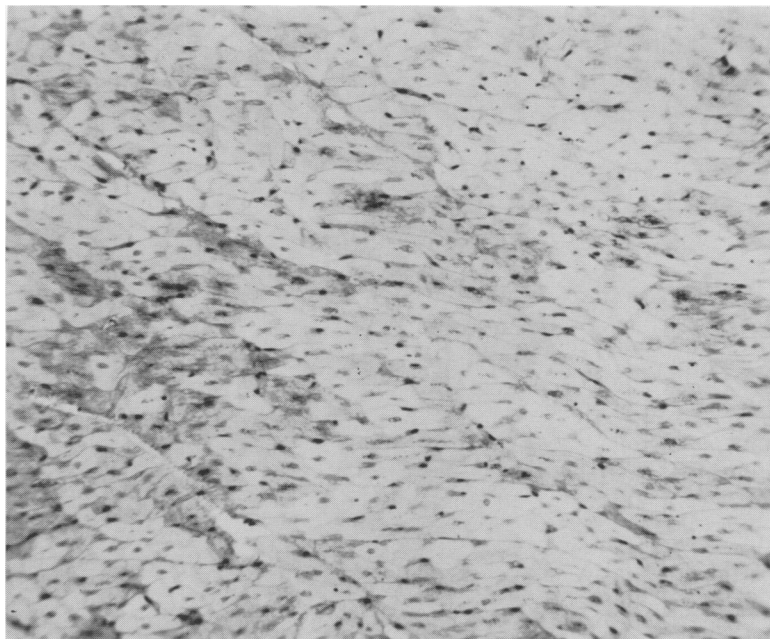


FIG. 16. Two hours after potassium arrest pale areas indicative of patchy glycogen loss are more prominent. Many fibers still retain small quantities of glycogen. (Freeze-substituted section, PAS-Hematoxylin, green filter, from $\times 160$.)

While it is surprising that the heart can be stopped by various means for prolonged periods and be resuscitated, it is not surprising that these periods of arrest have been found to have deleterious effects upon the structure and function of the heart subsequently. Reports of such effects following potassium-induced arrest have been numerous.^{5, 16, 19, 20, 23, 27, 34, 42} Others, in comparative studies on isolated rabbit¹ and guinea pig³⁰ hearts, have considered potassium to be a superior method of arrest. That early functional impairment of the myocardium results from this form of arrest was borne out by this study. The ventricular biopsies may have contributed to postarrest cardiac dysfunction in these animals, as has been reported previously, associated with ventriculotomy;³⁷ however, data from control animals, the hearts of which also were biopsied, would tend to eliminate the importance of this factor.

The early preservation of ultrastructural morphology following potassium arrest, when compared to other forms of arrest, was unexpected and possibly represents a local fixative effect of potassium upon the

myocardium. Another possible explanation for this preservation of morphology is the abrupt cessation of myocardial contraction at the time of potassium arrest, which prevents further loss of glycogen and breakdown of enzyme systems.

While the clinical use of elective arrest by anoxia alone appeared to have less depressing effect upon function in the immediate postarrest period than that by potassium, the time limit that the heart would tolerate anoxia without residual impairment was not known but was estimated to be 20 to 30 minutes. Experimental studies by Blumgart³ and Jennings²² and their co-workers, in the dog, wherein coronary arteries were ligated temporarily, have demonstrated that some myocardial cells are irreversibly injured after 20 to 25 minutes of ischemia. After 60 minutes of occlusion the size of the infarct is almost the same as the one resulting from permanent vessel occlusion. One heart with the coronary vessel occluded for 20 minutes showed tiny fibrotic scars 34 days later when the animal was sacrificed. That damage comparable to this occurs clinically with induced arrest is

suggested by the patient (reported by Lillehei²⁴) who previously had a 31.5-minute period of ischemia during potassium citrate arrest and was found to have diffuse myocardial fibrosis at the time of late death.

The direct comparison of the heart following coronary ligation and the heart following induced anoxic arrest probably is not valid. The results of coronary ligation indicate that glycogen disappears very rapidly when oxygen is eliminated and when oxidative phosphorylation becomes impossible. Contraction probably continues until high energy phosphate bonds of adenosine triphosphate and creatine phosphate are available no longer. The anaerobic breakdown of glycogen can produce some high energy phosphate bonds. In the animal with induced anoxic arrest there still are fairly abundant supplies of glycogen at the time of cessation of cardiac contraction. This would indicate that the mechanism of arrest in this situation may be different from that in the dog in which a coronary vessel is ligated. The preservation of some portion of glycogen may indicate further that the source of aerobic metabolism is not eliminated completely. This is further suggested by the better preservation of glycogen and of ultrastructural morphology in areas beneath the epicardium and about penetrating vessels. Other factors which may be of importance in the subepicardial preservation of morphology are lower surface temperature, with lower activity of glycolytic enzymes, or the diffusion of atmospheric oxygen into these tissues.

Electron microscopic changes in the nucleus usually were demonstrated in this study after only 30 minutes of anoxic arrest and were comparable to the changes produced after five to 15 minutes of coronary ligation. That these structural changes may be reversible is suggested in the few animals in which recovery for three or four hours had taken place.

The intimate structural relationships between mitochondria and myofibrils has sug-

gested to Harman and Figelson¹⁸ that the mitochondria play an active role in the contractile process, perhaps in the transfer of high energy phosphate bonds. Their studies of the rabbit heart³⁹ indicate a close relationship between structure and metabolic function of mitochondria, and that their oxidation capacity is intimately affected by form. Swollen mitochondria had a diminished capacity to metabolize.

Although no electron microscopic morphologic criteria of irreversibility have been established, Bryant has suggested that mitochondrial alterations may reflect these conditions.⁶ In his electron microscopic study of experimental myocardial infarction, mitochondrial alterations were the most prominent and were indicative of fluid imbibition. Swelling of the sarcoplasmic reticulum was another prominent feature. Although mitochondria were involved by morphologic changes in the current study, their appearance followed the earlier nuclear changes. Bing² believed the main factor for irreversible cessation of cardiac activity after arrest was neither loss of contractility of proteins nor death of respiratory enzymes but change in the muscle fiber membrane with loss of conductivity and rhythmicity.

The salutary effect of hypothermia, local or generalized, upon post-arrest cardiac recovery which has been reported^{9, 10, 15, 35, 36, 39, 40} was observed also in the electron microscopic sections, in comparison to the anoxic group without hypothermia. This difference probably would have been greater had there not been a tendency for the heart temperature in the anoxic group to assume room temperature during the period of aortic clamping. In some instances this approximated the level in the hypothermia group at the termination of the arrest period. Mild hypothermia did not significantly improve myocardial function over the anoxic group when such a prolonged arrest period was used.

The disappearance of glycogen from the heart during arrest was evaluated by Gott

and associates.¹⁵ Glycogen, the main anaerobic source of high energy phosphate, was not depleted as rapidly in hypothermia as in normothermia potassium arrest. TP and phospho-creatine levels also fell far less rapidly in the hypothermia arrest hearts than in potassium arrest.

In Dog 71 there was almost no myocardial glycogen even though the heart continued to beat. This illustrates two important principles: 1) The quantity of glycogen present in the muscle can by no means be taken as an indication of the viability or lack thereof in the tissues; and 2) In an animal with intact mechanisms of oxidative phosphorylation, the loss of glycogen following brief periods of ischemia is a fully reversible process.

Profound hypothermia, at levels of 15° C., appears to have additional protective influence upon cellular morphology, as observed in early experiments in a continuing study to determine the reversibility of these structural changes. The establishment of a regular rhythm in all hearts arrested for one hour, regardless of the manner of arrest, was surprising. Failure to resume a regular rhythm was observed only in those hearts which developed a state of rigor mortis, in each case in approximately 90 minutes. Ventricular fibrillation, almost a routine occurrence upon release of the aortic clamp, was easily stopped by electric shock except in those hearts showing rigor mortis.

Darby and coworkers¹¹ studied contractile force after relatively short periods of potassium citrate arrest with ventriculotomy in dogs, and found that with adequate total body perfusion (35–50 cc./kg.) there was little impairment of myocardial function following recovery. Studies in patients after arrest showed a contractile force reduction of as much as 40 per cent after correction of the defects. They believed these findings could have resulted from a decreased heart size or metabolic acidosis during bypass, since lactic acid decreases myocardial contractility. This latter factor could be a contributing factor in the results

herein reported, since postarrest pH decreases were observed in all arrest groups.

Various substrates and oxygen have been given relative importance as requirements of the myocardium during the arrest period which will influence the postarrest contractile function. Oxygen probably is the most important single factor to consider. Isolated dog hearts have continued to beat for several hours with only gaseous oxygen perfusion of the coronaries.³³ Experiments in guinea pigs, by Redo and Porter,³¹ to determine the relative importance of oxygen and glucose upon reversibility of function after arrest showed oxygen to be more important than glucose in perfused hearts. Hearts arrested without coronary perfusion of oxygen or glucose showed even better postarrest contractile force.

In these studies, hearts arrested without perfusion responded more favorably in the postarrest period than did hearts which had been perfused but deprived of oxygen or substrate. On the basis of these results, Redo believed arrest produced by accumulation of endogenous potassium was reversible, while that produced by lack of oxygen, substrate, or both was not reversible.

Dodrill¹² showed that the addition of ATP to the potassium arrest mixture enabled hearts to utilize oxygen better in the postarrest period. Glucose and insulin have been utilized by Mavor²⁵ in resuscitation of hearts arrested by potassium, during hypothermia, in order to replenish properly the myocardial potassium, which enters muscle in the presence of glucose. Some work¹² indicates that the heart in the postarrest state is unable to utilize oxygen properly. Wallace⁴¹ believed that the enzyme systems involved in the utilization of oxygen and lactate appear to be most sensitive to anoxia and probably are damaged irreversibly by anoxia. The importance of the prevention of distention of the cardiac chambers with change in diastolic fibre length during arrest has been shown by Ross *et al.*³²

It is apparent from our study and from

the literature that various methods of elective arrest have different effects upon the myocardium, some of which may be irreversible.

Summary and Conclusions

1. Dogs were subjected to extracorporeal circulation and induced cardiac arrest using potassium citrate, anoxia, and anoxia with mild hypothermia. Serial myocardial biopsies were examined by electron microscopy and by histochemical methods for glycogen, to detect early changes and to compare with hearts in which a coronary artery had been ligated.

2. Following coronary ligation, changes consisting of nuclear rarefaction and almost total loss of glycogen occurred within five to 20 minutes.

3. Biopsies of hearts from dogs subjected only to total cardiopulmonary bypass for one hour demonstrated essentially normal morphologic and histochemical appearance. Postperfusion myocardial contractile force was 80–100 per cent of the control value.

4. In the anoxia arrest group, severe functional impairment of myocardium was observed in the postarrest period. Abnormal nuclear morphology was observed after 30 minutes of anoxic cardiac arrest and was comparable to that observed in hearts in which the coronary artery had been ligated for 5–15 minutes. More severe changes were evident at one hour.

5. In the group in which local mild hypothermia was used, in addition to arrest by anoxia, preservation of the ultrastructure was variably improved. Postperfusion impairment in cardiac function again was observed.

6. The changes in heart function following potassium citrate arrest, although severe, were somewhat less than those observed in other groups. Electron microscopy showed normal preservation of architecture even after one hour of arrest. Preliminary recovery experiments following potassium arrest revealed extensive necrosis of heart muscle.

7. Arrested hearts in no instance showed the degree of histochemical glycogen loss observed after five minutes of coronary artery ligation. Glycogen was preserved better in hearts arrested by potassium than in those arrested by anoxia with or without local hypothermia.

References

1. Bentall, H. H.: Cardiac Metabolism in Induced Arrest. *Extracorporeal Circulation*. Springfield, Charles C Thomas. Page 395.
2. Bing, R. S.: Myocardial Metabolism. *Extracorporeal Circulation*. Springfield, Charles C Thomas. Page 361.
3. Blumgart, H. L., D. R. Gilligan and M. J. Schlesinger: Experimental Studies on the Effect of Temporary Occlusion of Coronary Arteries. II. The Production of Myocardial Infarction. *Am. Heart J.*, 22:374, 1941.
4. Boniface, K. J., O. J. Brodie and R. P. Walton: Resistance Strain Gauge Arches for Direct Measurement of Heart Contractile Force in Animals. *Proc. Soc. Exper. Biol. & Med.*, 84:263, 1953.
5. Brockman, S. K. and E. Fonkalsrud: Experimental Open Heart Surgery Employing Hypothermia, Mecholyt Arrest, and Carotid Perfusion. *Surgery*, 43:820, 1958.
6. Bryant, R. E., W. A. Thomas and R. M. O'Neal: An Electron Microscopic Study of Myocardial Ischemia in the Rat. *Circulation Res.*, 6:699, 1958.
7. Caulfield, J. and B. Klionsky: Myocardial Ischemia and Early Infarction: An Electron Microscopic Study. *Am. J. Path.*, 35:489, 1959.
8. Caulfield, J. B.: Effects of Varying the Vehicle for OsO_4 in Tissue Fixation. *J. Biophysical & Biochemical Cytology*, 3:827, 1957.
9. Cooper, T., V. L. Willman, P. Zafracopoulos and C. R. Hanlon: Myocardial Function After Elective Cardiac Arrest During Hypothermia. *Surg., Gynec. & Obst.*, 109:422, 1959.
10. Cross, F. S., R. D. Jones and R. M. Berne: Localized Cardiac Hypothermia as an Adjunct to Elective Cardiac Arrest. *Surgical Forum*, p. 355, 1957.
11. Darby, T. D., E. F. Parker, W. H. Lee, Jr. and J. D. Ashmore: The Influence of Cardiopulmonary Bypass on Cardiac Arrest and Right Ventriculotomy on Myocardial Contractile Force. *Ann. Surg.*, 147:596, 1958.
12. Dodrill, F. D. and S. Takagi: The Use of Anaerobic Energy in Elective Cardiac Arrest. *Surgery*, 47:314, 1960.
13. Feder, N. and R. L. Sidman: Histochemical

- Fixation by a Modified Freeze-Substitution Method. *J. Biophysical and Biochemical Cytology*, 4:593, 1958.
14. Gerbode, F.: Discussion of paper by Sabiston, D. C., Jr., J. L. Talbert, L. H. Riley, Jr. and Alfred Blalock. *Ann. Surg.*, 150:361, 1959.
 15. Gott, V. L., M. Bartlett, J. A. Johnson, D. M. Long and C. W. Lillehei: High Energy Phosphate Levels in the Human Heart During Potassium Citrate Arrest and Selective Hypothermia Arrest. *Surgical Forum*, page 544, 1959.
 16. Greenberg, J. J., L. H. Edmunds and R. B. Brown: Myocardial Metabolism and Post Arrest Function in the Cold and Chemically Arrested Heart. *Surgery*, 48:31, 1960.
 17. Harmon, J. W. and Feigelson, M.: Studies on Mitochondria: III. The Relationship of Structure and Function of Mitochondria from Heart Muscle. *Exper. Cell Research*, 3:47, 1952.
 18. Harmon, J. W. and Feigelson, M.: The Cytological Localization of Mitochondria in Heart Muscle. *Exper. Cell Research*, 3:58, 1952.
 19. Helmsworth, J. A., S. Kaplan, T. C. Clark, Jr., A. J. McAdams, E. C. Matthews and F. K. Edwards: Myocardial Injury Associated with Asystole Induced with Potassium Citrate. *Ann. Surg.*, 149:200, 1959.
 20. Helmsworth, J. A., Shabetai, R. W., J. E. Albers and P. J. Wozencraft: Local Effect of Potassium Citrate Solution in Atrial Pouches of Dogs. *J. Thoracic Surg.*, 36:220, 1958.
 21. Jennings, R. B. and W. Wartman: Production of an Area of Homogeneous Myocardial Infarction in the Dog. *A. M. A. Arch. Path.*, 63:580, 1957.
 22. Jennings, R. B., H. M. Sommers, G. A. Smith, H. A. Flack and H. Linn: Myocardial Necrosis Induced by Temporary Occlusion of a Coronary Artery in the Dog. *Arch. Path.*, 70:68, 1960.
 23. Lam, C. R., T. Gahagan, C. Mota and E. Green: Induced Cardiac Arrest (Cardioplegia) in Open Heart Surgical Procedures. *Surgery*, 43:7, 1958.
 24. Lillehei, C. W.: Discussion of paper by Greenberg *et al.* (No. 16 above). *Surgery*, 48:31, 1960.
 25. Mavor, G. E.: Elective Cardiac Arrest with Potassium During Hypothermia. *J. Roy. Coll. Surgeons (Edinburgh)*, 3:1, 1957.
 26. Melrose, D. G., B. Dreyer, H. H. Bentall and J. E. Baker: Elective Cardiac Arrest. *Lancet*, 6879:21, 1955.
 27. Mendelsohn, D. Jr., T. N. Mackrell, D. W. Macdonald, C. Nogueira, L. R. Head, Jr. and E. B. Kay: Management of the Patient During Open Heart Surgery. *Surgery*, 45:949, 1959.
 28. Nunn, D. D., C. A. Belisle, W. H. Lee, Jr. and E. F. Parker: Comparative Study of Aortic Occlusion Alone and of Potassium Citrate Arrest During Cardiopulmonary Bypass. *Surgery*, 45:848, 1959.
 29. Palade, G. E.: A Study of Fixation for Electron Microscopy. *J. Exper. Med.*, 95:285, 1952.
 30. Redo, S. F.: An Evaluation of Various Cardioplegia Methods Utilizing Isolated Perfused Guinea Pig Hearts. *Surg., Gynec. & Obst.*, 108:211, 1959.
 31. Redo, S. F. and B. Y. Porter: The Role of the Lack of Oxygen in Irreversible Cardiac Arrest. *Surg., Gynec. & Obst.*, 109:431, 1959.
 32. Ross, J. J., J. W. Gilbert, E. H. Sharp and A. G. Morrow: Elective Cardiac Arrest During Total Body Perfusion. *J. Thoracic Surg.*, 36:534, 1958.
 33. Sabiston, D. C., Jr., J. L. Talbert, L. H. Riley, Jr. and A. Blalock: Maintenance of Heart Beat by Perfusion of the Coronary Circulation with Gaseous Oxygen. *Ann. Surg.*, 150:361, 1959.
 34. Schramel, R. J., E. Ross, R. D. Morton and O. Creech: Observations on Controlled Cardiac Asystole in Intact Dogs. *Surgical Forum*, page 348, 1959.
 35. Sealy, W. C., I. W. Brown, Jr., W. G. Young, W. W. Smith and A. M. Lesage: Hypothermia and Extracorporeal Circulation for Open Heart Surgery. *Ann. Surg.*, 150:627, 1959.
 36. Shumway, N. E. and R. R. Lower: Topical Cardiac Hypothermia for Extended Periods of Anoxic Arrest. *Surgical Forum*, page 563, 1959.
 37. Stirling, G. R., P. H. Stanley and C. W. Lillehei: The Effects of Cardiac Bypass and Ventriculotomy upon Right Ventricular Function. *Surgical Forum*, page 433, 1957.
 38. Szent-Gyorgi, A.: Contraction of the Heart Muscle Fiber. *Bull. New York Acad. Med.*, 28:3, 1952.
 39. Urschel, H. C., Jr., J. J. Greenberg and C. A. Hufnagle: Elective Cardioplegia by Local Hypothermia. *New England J. Med.*, 261:1330, 1959.
 40. Urschel, H. C., Jr. and J. J. Greenberg: Differential Hypothermic Cardioplegia. *Surgical Forum*, page 506, 1959.
 41. Wallace, H. W.: Cardiac Metabolism. *New England J. Med.*, 261:1322, 1959.
 42. Willman, V. L., T. Cooper, P. Zafiracopoulos and C. R. Hanlon: Depression of Ventricular Function Following Elective Cardiac Arrest with Potassium Citrate. *Surgery*, 46:792, 1959.