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Tissue Reaction to Extracorporeal Circulation and Elective Cardiac Arrest*

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Summary. Tissue reaction to extracorporeal circulation and elective cardiac arrest by cross clamping of the aorta was studied in dogs with normal hearts by a combination of pathological methods (including histopathology and histochemistry of frozen and dried serial biopsies) as well as physiological and biochemical methods. Thirty dogs had biopsies of the left ventricle wall and the liver prior to extracorporeal circulation, after resuscitation from extracorporeal circulation with and without elective arrest and after a 6 hour recovery period. At the end of the experiments a complete autopsy was done. After 30 minutes arrest at 37° C, the hearts could be easily resuscitated. In spite of focal glycogen losses, they were able to support the circulation during the recovery period. After 45 minutes arrest at 37° C, the response was unpredictable. Greater amounts of glycogen lost from the myocardium indicated eventual failure of the heart. The failure of the resuscitated heart to support the circulation was attributed to a lack of substrate (glycogen). It was not due to structural alteration or changes in the local enzyme capacity as measured histochemically. The correlative patterns which appeared for all three modes of evaluation form the baseline for the application of the developed systemic analysis to experiments with longer recovery periods and to dogs with abnormal hearts.

Introduction

For ages cardiac arrest meant death, usually sudden and often unexpected. Resuscitation attempts had been few and were largely tried when the arrest occurred in the operating room. When the problems of cardiopulmonary arrest were investigated and a better understanding achieved, the previous fatalistic approach was given up for more active intervention (Jude, 1964). With the introduction of cardiac bypass procedures, elective cardiac arrest became feasible in cardiac surgery. The type of cardiac arrest preferred by the surgeon and the safe length of time for a given type of arrest were largely based on personal experience and are not necessarily agreed upon. The same is true for type and time of extracorporeal circulation alone.

For these reasons the study of the effects of extracorporeal circulation with and without cardiac arrest by cross clamping of the aorta was undertaken. It was hoped that by combining our newly developed physiological, biochemical and pathological methods, we could get an assay of the functional state of the heart during arrest, after resuscitation, and after a 6 hour recovery period. By strict control of the variables in the experiment (including avoidance of drugs other than

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anesthetics and heparin) the true effects could be analyzed if the conventionally introduced artifacts of the pathological procedure were avoided. (Barka and Anderson, 1965; Eckner *et al.*, 1968, 1969).

Materials and Methods

I. Animals

The animal experiments were carried out on heparinized anesthetized dogs as follows:

All animals were anesthetized with an initial modest dose of thiamylal sodium (Table 2). Throughout the procedure and the recovery period with cannulae in place and thorax loosely approximated small doses of thiamylal sodium (Table 2) were added well between periods of dynamic studies and biopsies to maintain adequate anesthesia with minimal depression. Heparinization was maintained with a pre- bypass dose of 3000 units sodium heparin per kg body weight intravenously. The perfusate and all blood additions contained heparin (10000 units/500 ml). No pressor or inotropic agents were used. As in any prolonged heparinized and bypass (and post bypass) state blood transfusions were given to maintain a satisfactory filling pressure. If, in the presence of adequate blood volume and normal filling pressure, a drastic fall in systemic pressure and/or cardiac output occurred, this was accepted as an indication of failure and the experiment was terminated. Such a state was easily definable as all parameters would deteriorate, left ventricular end diastolic pressure would rise, cardiac output would fall and the left ventricular pressure derivative loop would become abnormal.

1. Baseline Series: Introduction of tubing with or without biopsies, sacrifice of dog, formalin fixation before and after rigor mortis[1] 14
2. a) Anesthesia, heparin and blood were given in the same time format as would be required for the 2 hour extracorporeal circulation with or without arrest as used in the later series 5
   b) Addition of extracorporeal circulation (2 hours) 5
   c) Addition to (b) at 37° of 30 minutes arrest by cross clamping of the aorta 9
   d) Addition to (b) at 37° of 45 minutes arrest 5
   e) Addition to (b) at 30° of 30 minutes arrest 6
   f) Addition to (b) at 37° of 30 minutes arrest and distention of chambers during resuscitation 5

The exclusion of distention in the preparations (a) to (e) had been virtually a fetish because of the clinical impression of the deleterious and generally unrecognized extent of the distention. The distention is not related to excessive perfusion. It is due to the accumulation of coronary (venous and thebesian) blood in the cardiac chambers, especially in the left ventricle, when these chambers are not yet able to empty under their own power.

The pressure derivative loop technique had been specifically developed to allow prevention of distention in preferance to early detection (Moulder *et al.*, 1968). The distention in group (f) refers to intentional grossly visible enlargement of the heart to 2–3 times normal size.

Catheters for sampling and transduction were placed as follows:
double lumen aortic catheter for pressure and flow (by differential);
abdominal aortic and inferior vena cava for indicator dilution curves and blood sampling;
left ventricular for pressure and derivatives;
pulmonary artery for pressure and blood sampling;
coronary sinus (special cage tipped catheter developed for non-fail withdrawal with sufficient alacrity to forestall static changes in e.g. lactate and pyruvate measurements).

For extracorporeal circulation, a Travenol disposable bubble oxygenator was used with a Brown-Harrison heat exchanger and Sigmamotor finger peristaltic pump. The perfusion was carried out at 80 ml/kg body weight/min. Details of the surgical procedures and physiologic and biochemical studies are presented elsewhere (Blackstone *et al.*, 1971; Moulder *et al.*, 1971).

1 Numbers refer to numbers of dogs or experiments.

II. Pathological Examination

1. Myocardial Biopsy

a) Repeated punch biopsies were taken from the left and right cardiac ventricle. The tool was a modified cork borer with inside cutting edge, 0.5 cm interior diameter, which slid over a metal tube connected to a syringe by a plastic tube. Details of the biopsy tool and the innovative system for obtaining a non-artifactual adequate specimen have been described (Eckner *et al.*, 1967).

For taking the biopsy, gentle suction was applied to hold the tube in place on the epicardium. The cutting tool was rotated and pushed through the ventricle wall to give a full thickness biopsy of the ventricle. A previously placed mattress suture away from the biopsy site was then drawn up and the opening closed. The repeated biopsies were taken as follows:

1. After the pericardium was opened.

2. After extracorporeal circulation or extracorporeal circulation and anoxic arrest. After arrest the biopsy was taken at a time when the heart beat had been re-established.

3. 6 hours after biopsy No. 2.

b) Liver biopsies were taken at the same time as the myocardial biopsies. Specimens of lung, kidney and jejunum were taken at the end of the procedure. All tissues were frozen in Isopentane cooled in liquid nitrogen to $-165°$ C.

2. Histological-Histochemical Method

All methods given used freeze drying, paraffin embedding, deparaffinization in petroleum ether (or xylene) (Eckner *et al.*, 1967, 1968, 1969) or freezing followed by vacuum drying at $-30°$ C of cryostat sections. If the sections were denatured after deparaffinization, the mode of denaturizing is given ahead of the name or description of method.

a) For General Survey: Alcohol, *hematoxylin and eosin.* Alcohol, *Elastic tissue-Weigert-modified van Gieson* (Sirius red F3 BA) (Puchtler and Sweat, 1964a; Sweat *et al.*, 1964a).

b) Connective Tissue: Alcohol, *Resorcin fuchsin* after periodic acid oxidation (Puchtler, 1964b). Alcohol, *PAS-Sirius Supra Blue GL* in picric acid (Sweat *et al.*, 1964b). Alcohol, Amylase-digestion, *Silver stain* for reticulum fibers (Nassar and Shanklin, 1961).

c) Glycogen and Glycoproteins: Alcohol, PAS with/without amylase digestion and buffer control.

d) Reactivity of Proteins: Alcohol, DNFB (Zerlotti and Engel, 1962). Alcohol, SH-groups with mercury orange (Engel and Zerlotti, 1964). Alcohol, SH-groups with DDD-reaction (Bahr, 1957).

e) Frozen and Dried Cryostat Sections. Plasmal reaction, Fatred 7B.

f) Enzyme Procedures: (local enzyme capacity).

α) Cytochrome oxidase (Burstone) (Barka and Anderson, 1965; Eckner *et al.*, 1968).

β) Succinic dehydrogenase (Nitro-BT) (Barka and Anderson, 1965; Eckner *et al.*, 1968).

γ) Acid phosphatase, alkaline phosphatase [Naphthol AS-MX (Sigma)].

δ) Phosphorylase and branching enzyme reactions (Eckner, 1968a, 1969a).

g) Fluorescence-microscopy: Primary fluorescence. Secondary fluorescence with acridine orange and coriphosphine at pH 4.0 (Korb and Hecht, 1962).

h) Polarization Microscopy: Polarization microscopy was performed on a Leitz-polarizing microscope SM-POL with a Brace-Köhler compensator of 1/20 wavelength.

The quantitative phenol test was carried out according to H. P. Missmahl (1964).

3. Autopsy

After termination of the experiments and removal of tubing and surgical instruments the heart was fixed with formalin under controlled pressure (Eckner *et al.*, 1969b). In some cases the heart was fixed immediately in situ before rigor mortis, in a few cases the heart was fixed after rigor mortis had set in. The remainder of the organs were inspected, further blocks were taken and fixed in 10% buffered formalin for comparisons.

Results

I. Time of 2nd Biopsy (after Extracorporeal Circulation with or without Cardiac Arrest)

Gross: With the exception of small focal subepicardial hemorrhages at the biopsy sites and cannulation sites, the external appearance of the beating hearts was normal. The color was pink and the muscle tone was good. Occasional focal atelectases were found in the otherwise well aerated lungs. The livers and kidneys were without gross pathological changes. The small bowel was free of external hemorrhages. (These organs were seen because the incision extended into the abdomen).

Microscopic

Heart: The architecture of the myocardial fibers was undisturbed with the exception of the outer zone of alteration due to cutting into the beating myocardium (Eckner *et al.*, 1967). No unusual numbers of leukocytes and mast cells were present in the myocardium of all groups.

Fat stains were consistently negative. The primary fluorescence of myocardial fibers was gray-white. Secondary fluorescence after coriphosphine or acridine-orange was light green for muscle fibers (fixed or unfixed), yellow for nuclei with a few orange granules in endothelial cells. This remained the same when compared to the first biopsy. The plasmal reaction remained unchanged.

The visible amounts of stainable glycogen (PAS) remained unchanged in the baseline and in the groups on extracorporeal circulation only. Small foci of muscle fibers showed loss of glycogen after extracorporeal circulation and 30 minute arrest. Larger areas of glycogen loss were apparent after 45 minutes of 37° arrest (Fig. 2B, 3B, 4B). Hypothermia (30°) and 45 minute arrest were somewhat in between. The extent of glycogen loss was predictable in the 30 minute arrest group, but variable with the longest arrest. The glycogen loss in the group with intentional overdistention was in proportion to the length of the arrest (Series 2f).

The enzyme reactions were unaltered when compared with the initial biopsies. The phosphorylase and branching enzyme reactions had been carried out in the conventional manner. They showed a close correlation between the pre-existing stainable glycogen and the presumed enzyme activity. At no time and in no place did we find "enzyme activity" unless there had been previously demonstrable glycogen. In evaluation of the existing methods we found that one could not prove new synthesis of polysaccharides in tissue sections (Eckner *et al.*, 1969a). The methods therefore were unsuitable and constituted nothing other than a

Fig. 1A–C. M82, extracorporeal circulation and 30 min arrest at + 37° C, left ventricular wall biopsies frozen and dried, paraffin sections, 7 μ, PAS, Magn. × 100. A, Control after entering, the pericardial sac. B, After resuscitation from arrest. C, 6 hrs recovery period post resuscitation: focal loss of glycogen

Fig. 2A–C. M85, extracorporeal circulation and 45 min arrest at + 37° C, left ventricular wall biopsies frozen and dried, paraffin sections, 7 μ, PAS, Magn. × 100. A, Control. B, After resuscitation: focal loss of glycogen similar to 1C. C, After 6 hrs recovery period: glycogen loss more diffuse than in 2B

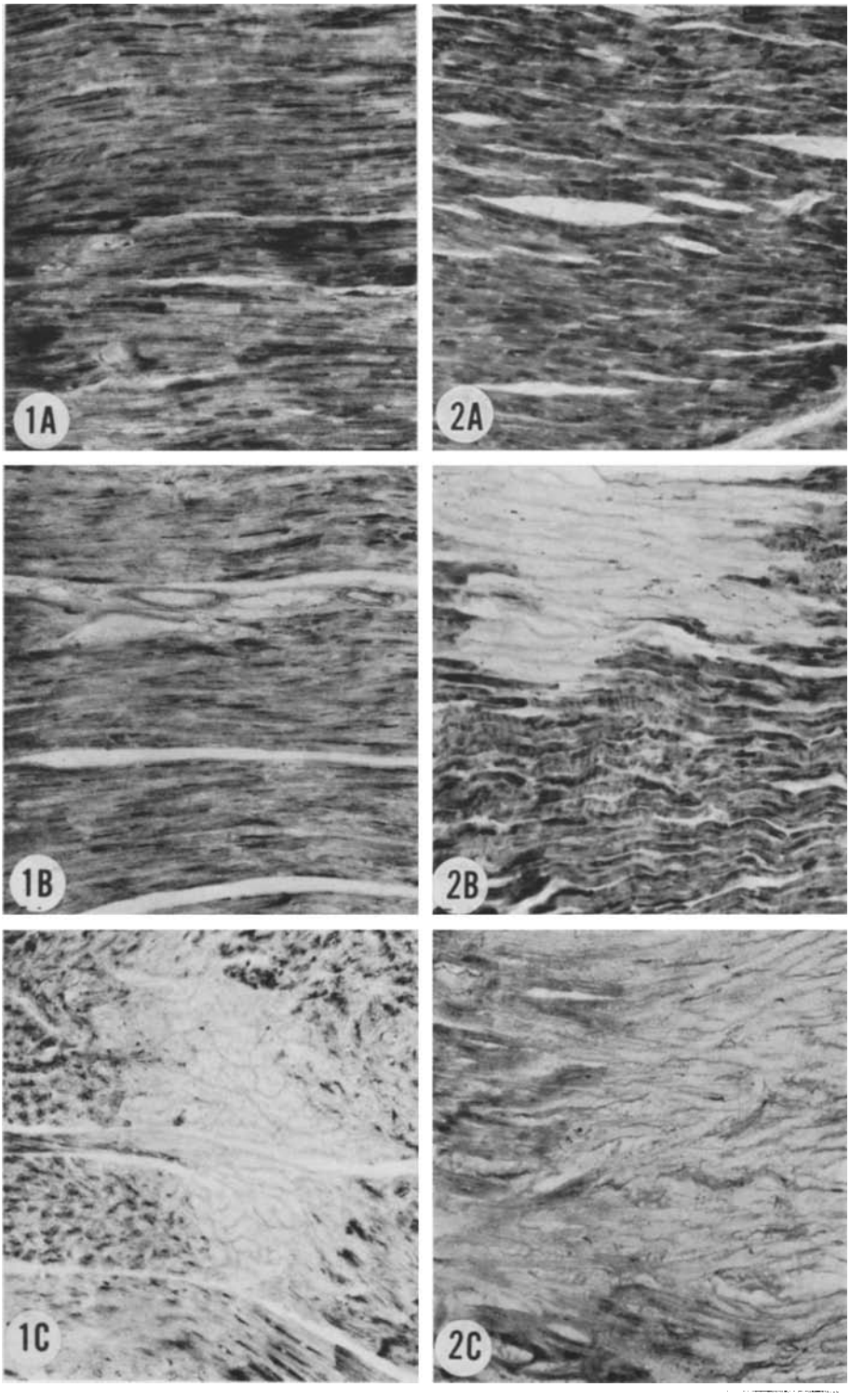

Fig. 1A–C and Fig. 2A–C

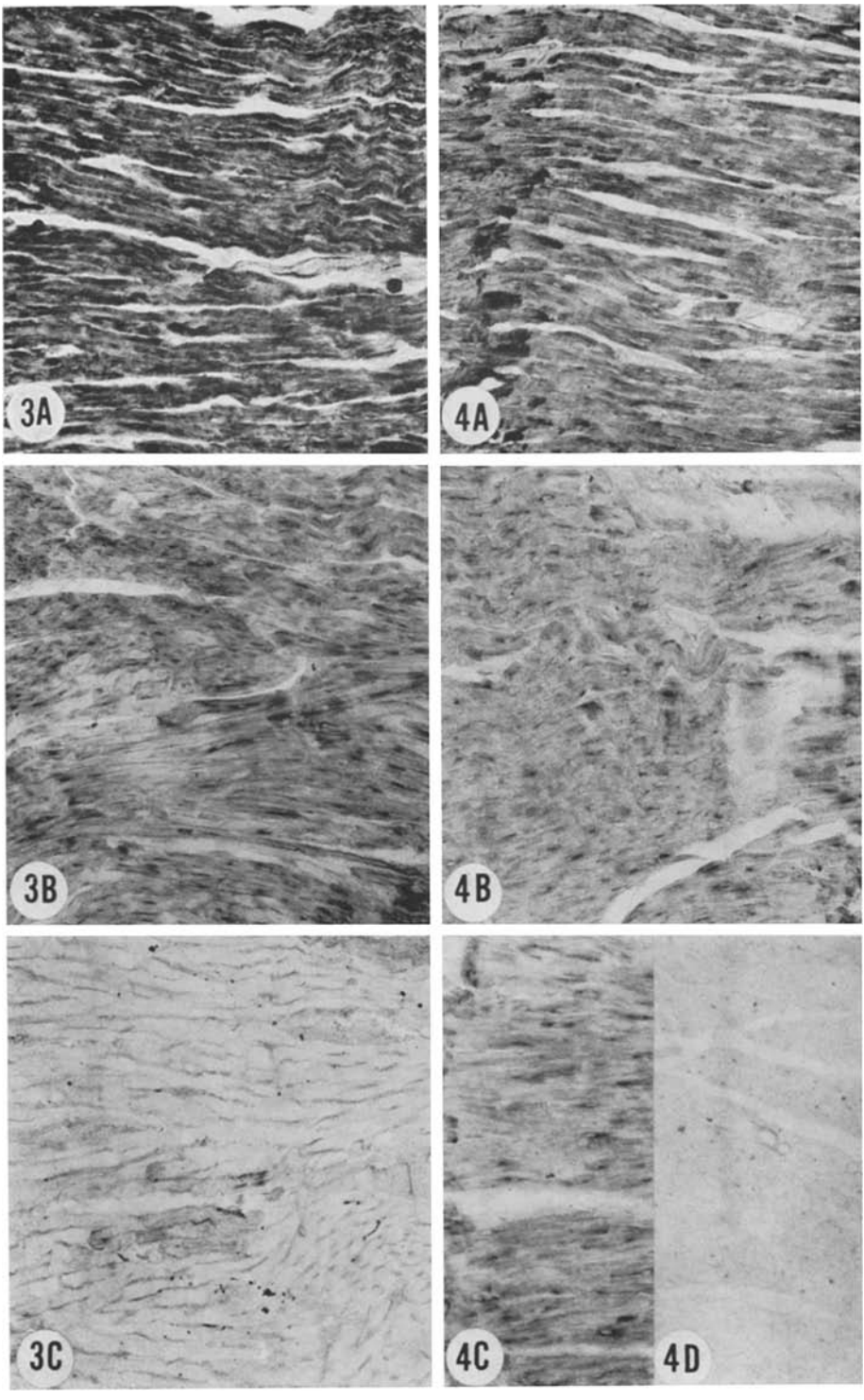

Fig. 3A–C and Fig. 4A–D

Table 1. *Quantitative phenol reaction of collagen fibers*

	Control biopsies		Biopsies after 2 hours		Biopsies at end of experiment	
	$\frac{a+b}{a} \pm$ S.D.	F	$\frac{a+b}{a} \pm$ S.D.	F	$\frac{a+b}{a} \pm$ S.D.	F
A, Time series			1.519 ± 0.055	0.65	1.526 ± 0.045	0.35
B, addition of extracorporeal circulation	1.551 ± 0.071	1.69*	1.511 ± 0.077	0.84	1.367 ± 0.032	26.54
C, addition to B of 30 min arrest at 37° C			1.429 ± 0.045	8.47**	1.326 ± 0.055	33.49
D, addition to B of 45 min arrest at 37° C			1.386 ± 0.032	16.35	1.336 ± 0.104	22.85
E, addition to B of 45 min arrest at 30° C			1.413 ± 0.063	13.33	1.252 ± 0.077	60.42

a = phase difference of positive birefringence in H_2O. b = phase difference of negative birefringence in 80% Phenol. Analysis of variance: * F of 7.94 is significant at 0.001-level. ** F of 7.81 is significant at 0.01-level. All others: F of 14.91 is significant at 0.001-level.

manipulation of pre-existing glycogen. No acid phosphatase was demonstrated in the myocardial cells. The alkaline phosphatase reaction was confined to the blood vessels and did not change throughout the experiments.

The phenol test for collagen fibers gave lower values in all arrest groups. Since the intrinsic birefringence of the collagen fibers was essentially unaltered, the change in the phenol test value is believed to reflect an altered relationship of ground substance and collagen fibers (Table 1).

Silver impregnation of the reticulum fibers was somewhat erratic in the initial experiments when the tissue was frozen and dried. Therefore alcohol denatured sections were used. The glycogen which was also stained in the procedure inhibited the silver impregnation of the reticulum fibers. When the glycogen was removed by amylase digestion the silver reaction was uniformly successful (Fig. 13), and did not change under the experimental conditions of the various groups. The

Fig. 3A–C. M60, extracorporeal circulation and 45 min arrest at +37° C, left ventricular wall biopsies frozen and dried, paraffin sections, 7 μ, PAS, Magn. × 100. A, Control. B, After resuscitation: diffuse and focal glycogen loss. C, Termination of experiment after 2 hrs of recovery: nearly complete loss of glycogen

Fig. 4A–D. M77, extracorporeal circulation and 45 min arrest at +37° C, left ventricular wall biopsies, frozen and dried, paraffin sections 7 μ, PAS, Magn. × 100. A, Control: left margin with histologic evidence of heart beat. B, After resuscitation: loss of glycogen similar to 3B. C, After 6 hrs recovery period: glycogen at the level of 4B, but no histologic evidence of heart beat, outer third of biopsy. D, Inner third of same biopsy: evidence of heart beat, complete loss of glycogen

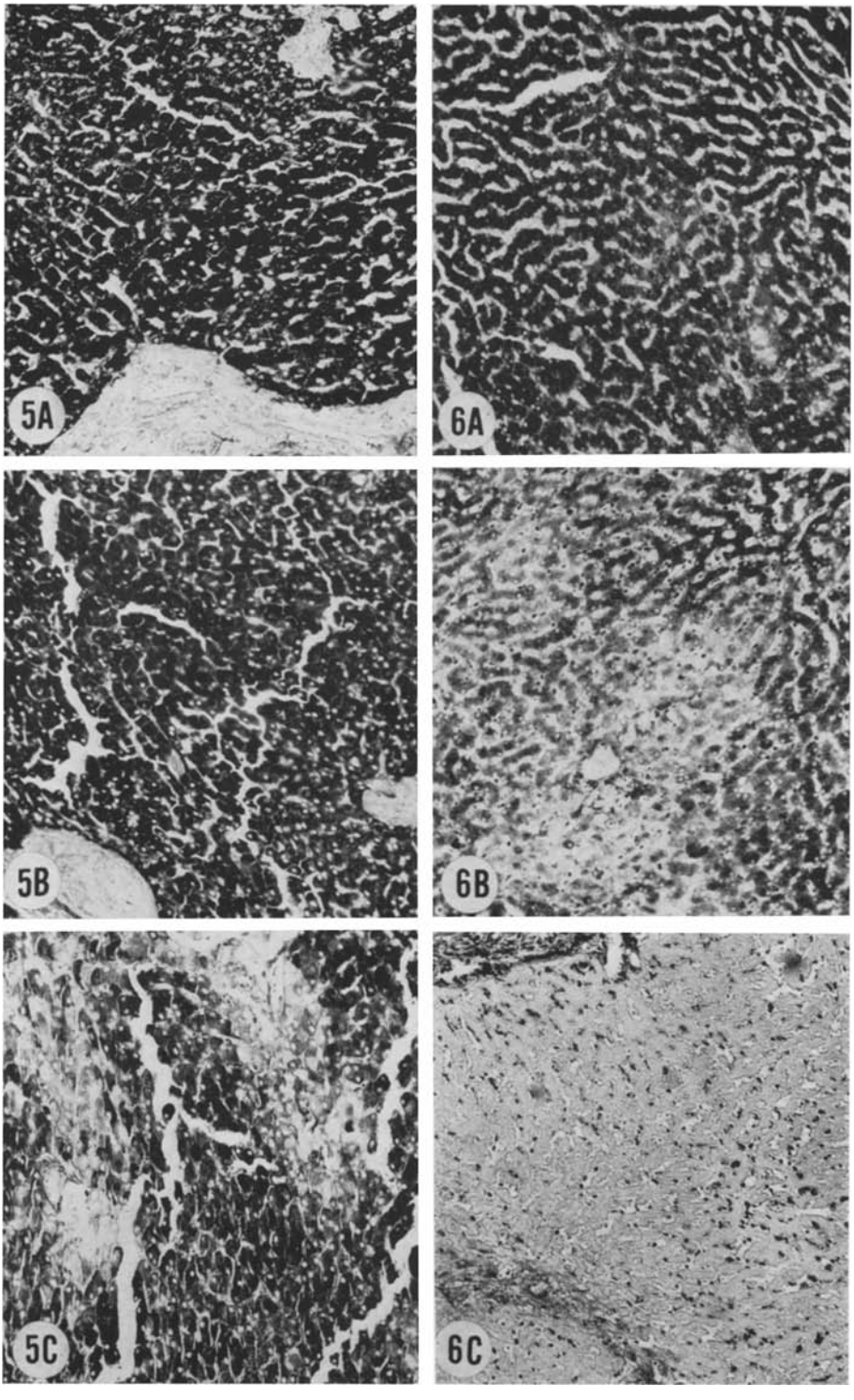

Fig. 5A–C and Fig. 6A–C

DNFB-reaction and the reaction for SH-groups were not visibly altered in the various experimental groups.

Lungs: No lung biopsies were taken at this time.

Liver: The liver architecture was well preserved in all groups (Figs. 5–7), but there was focal loss of glycogen from the periportal areas after extracorporeal circulation with or without arrest of any duration. More leukocytes were present in vessels and sinusoids than in the previous control biopsies. Spaces of Disse were not seen in any of the biopsies. After extracorporeal circulation and arrest the alkaline phosphatase reaction which had been confined to larger bile canaliculi became more pronounced. Nuclei remained unstained (Fig. 8).

Kidneys: No biopsies were taken at this time.

Jejunum: No biopsies were taken at this time.

II. Time of the 3rd Biopsy (Termination of Recovery Period)

Gross: Baseline, extracorporeal circulation and 30 minute arrest groups with or without distention were essentially unchanged after the 6 hour period of recovery. Some of the 45 minute arrest hearts would not support the circulation over the full period and went into intractable fibrillation so that the experiments had to be terminated earlier. In one case the outside of the left ventricle had stopped beating when there was evidence of contraction on the inside. This experiment was also terminated before the planned time.

Microscopic

Heart: The architecture of the muscle fibers remained unaltered in each of the groups. DNFB-reactivity of proteins and SH-groups reaction remained unaltered. The vessels were without blood clots. Leukocytes were slightly increased in number over the previous two biopsies and mast cells were possibly increased in numbers. Fluorescence was unchanged but more orange granules appeared in endothelial cells. Fat stains remained negative. The previously noted glycogen losses were, in general, more widespread. But they remained focal in the 30 minute arrest group with or without distention.

With the longer period of arrest the variability of the glycogen loss was again noted. Complete loss of stainable glycogen was found in the myocardial biopsies of an experiment which had to be terminated before the planned time (Figs. 2–4). The loss was complete only in the biopsies which showed evidence that they had been cut out of the beating heart. Some glycogen, but less than in the previous

Fig. 5A–C. M86, tissue series (biopsies only), liver biopsies frozen and dried, paraffin sections 7 μ, PAS-Sirius Supra Blue GL, Magn. × 100. A, Control. B, After 2 hrs C, 6 hrs after B: small loss of stainable glycogen

Fig. 6A–C. M85, extracorporeal circulation and 45 min arrest at + 37° C, liver biopsies frozen and dried, paraffin sections, 7 μ, PAS-Sirius Supra-Blue GL, Magn. × 100 (corresponding myocardium in Figs. 2A–2C). A, Control. B, After resuscitation: centrilobular loss of glycogen. C, After 6 hrs recovery period: liver glycogen largely depleted, large numbers of leukocytes in B and C

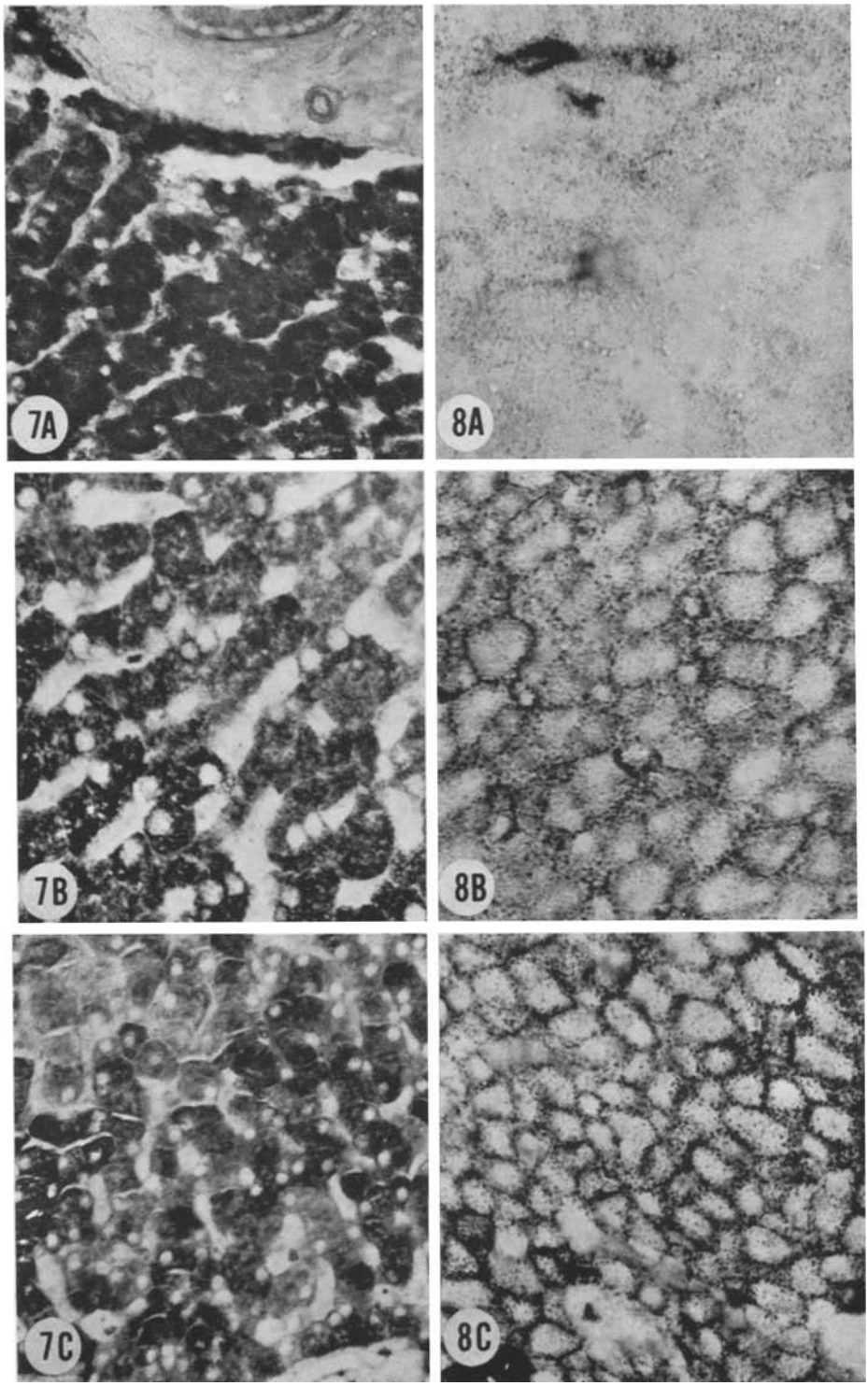

Fig. 7A–C and Fig. 8A–C

normal controls, was present in areas where there was no evidence of beating at the time the biopsy was taken (Fig. 4C).

Again enzyme reactions were unchanged within as well as between groups. The comments that have already been made with regard to the phosphorylase reactions apply here. Phosphatases were unaltered in their respective absence or presence. The same is true for the silver reaction for reticulum fibers (after glycogen digestion).

The phenol test values for collagen fibers were lower than normal and they were lower than in first and second biopsy after extracorporeal circulation with arrest of any length of time.

Lungs: The lung architecture was intact. The focal atelectases have been described grossly. All blood vessels contain large numbers of leukocytes (the glycogen of which is readily and completely digested by amylase). No PAS stainable material was found in leukocytes after digestion. Morphologic changes in leukocytes were not found. Mast cells were present in fair numbers. Alveoli were empty, even when collapsed. The bronchi were not remarkable. No thrombi were found (Figs. 9A and B).

Liver: The architecture was preserved. Vessels and sinusoids contained large numbers of leukocytes.

Kupffer cells were not remarkable, and spaces of Disse were not evident. Stainable liver glycogen was largely absent after extracorporeal circulation with or without arrest (Fig. 6). The alkaline phosphatase reaction was very pronounced. Bile canaliculi and all liver cells had reaction products as previously described. The other enzyme reactions were unaltered, but the phosphorylase and transglycosidase reactions corresponded to the presence of stainable glycogen. DNFB and SH-group reactions were without visible alteration.

Kidney: The kidney sections of all groups showed the normal kidney architecture (Griffith *et al.*, 1967) after cutting off the blood supply, that is, absence of tubular lumina. Glomeruli were of normal cellularity. There was no thickening of basement membranes. Primary fluorescence of unstained, unfixed frozen dried sections was gray-white with numerous bright yellow granules in tubular cells (tubuli contorti I). (These granules disappeared during the manipulation of ssection such as staining, fixation, alcoholic or water cover-slipping). Fat stains were negative. No stainable glycogen was present in kidney cells. Large numbers of mature leukocytes were present in the blood vessels which were without evidence of thrombosis.

Jejunum: The tissue which contracted further during cutting prior to freeze-drying sections was without histopathologic changes; there were no hemorrhages or edema.

Fig. 7A–C. M88, extracorporeal circulation and 45 min arrest at $+37°$C, liver biopsies frozen and dried, paraffin sections 7 μ, PAS, Magn. $\times$ 360. A, Control. B, After resuscitation. C, Termination of experiment after 2 hrs recovery period

Fig. 8A–C. M88, as in Fig. 7, liver biopsies, frozen and dried, paraffin sections, 7 μ, alkaline phosphatase reaction (Naphthol AS-MX), Magn. $\times$ 360. A, B, and C. Corresponding to 7A, B and C

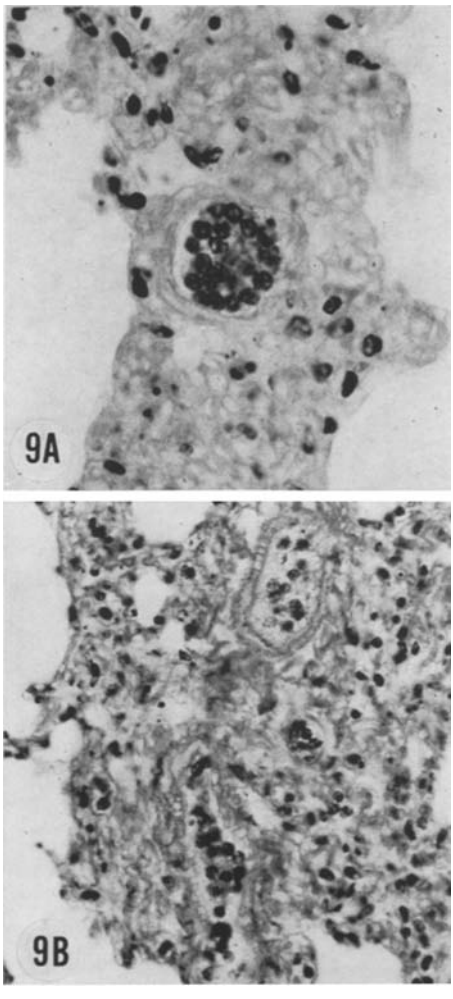

Fig. 9A and B. M68, extracorporeal circulation and 45 min arrest at +30°C, lung biopsy at end of experiment, frozen dried, paraffin sections 7 μ. A, PAS, Magn. × 360. B, PAS-Sirius Blue GL, Magn. × 100

III. Autopsy

Gross

General Remarks: Hearts fixed prior to rigor mortis contracted at the time when fixative filled the coronaries. The contraction could be seen by inspection of the surfaces. It was also verified by the increased perfusion pressure after the onset of perfusion. Still the hearts remained larger than comparable hearts which were allowed to go into rigor mortis prior to fixation. Compared with the hearts contracted in rigor mortis the fresh fixed hearts had a straight septum.

Heart: The specimen showed the hemorrhages seen previously. Some hearts had also subendocardial hemorrhages especially in the right ventricular outflow

tract (anterior and septal wall). Interstitial hemorrhages in the region of the biopsies were largely related to the sutures of the hole in the ventricle wall, since they were absent from the site of biopsies which were not sutured but had had the hole plugged by finger pressure on the epicardium. They were also absent from biopsies during which the biopsy, after penetration of the free ventricle wall, had inadvertently entered the septum (Fig. 10B). No diffuse interstitial hemorrhages were found in hearts which could not support the circulation for the planned period of time. There were no gross signs of ischemic necrosis; the coronaries were patent throughout. No distinction between the groups was possible by gross examination (Figs. 10–11). As a matter of fact, some of the grossly visible damage at the time of autopsy appeared to be related to the surgical handling of that tissue after the termination of the experiment and was due to the fact the muscle fibers were not arrested simultaneously. Hemorrhages did not involve the conduction system grossly.

Lung, Liver, Kidneys: These were not abnormal.

Bowel: No hemorrhages were found throughout the small intestine and large intestine.

Spleen: After being cut from the circulation the spleens contracted. One case had numerous intrasplenic spleniculi.

Microscopic

Heart: The gross alterations in the specimens of all groups were documented microscopically. In addition, there was focal distortion of the architecture of myocardial cells, especially in the anterior papillary muscle of the right ventricle, but also in the left ventricular papillary muscle and in areas away from the biopsy sites. Microscopic examination of the biopsy sites (in serial sections) revealed hypercontraction bands and "vacuolization of fibers" which we previously were able to relate to cutting into beating myocardium. No interstitial hemorrhages were present around unsutured biopsy sites. Even in areas of interstitial hemorrhages the muscle fibers were intact, though separated from each other by hemorrhage. The conduction system appeared to be intact and was without hemorrhages in it or adjacent to it. In the majority of cases thick walled arteries were found in the right anterior papillary muscle of the right ventricle (Fig. 12). These are probably a variation of the normal anatomy and unrelated to the experimental study.

The focal areas of myocardial fiber alteration were related to the fact that the injected fixative was unable to immobilize all the muscle fibers instantaneously and simultaneously. The changes (which might be erroneously interpreted as "early necrosis" or the like were not found in frozen dried biopsy material, but were consistently present in biopsy material fixed by immersion in a fixative solution. Light microscopic examination of fixed tissue therefore proved to be of limited value (as already emphasized by Meessen, 1964).

Lung, Liver, Kidneys, Jejunum: The microscopic examination of formalin fixed autopsy material was not very contributory, even with the very short post mortem interval. The striking difference between the frozen dried and formalin fixed material with respect to cell architecture and chemistry was obvious (Eckner

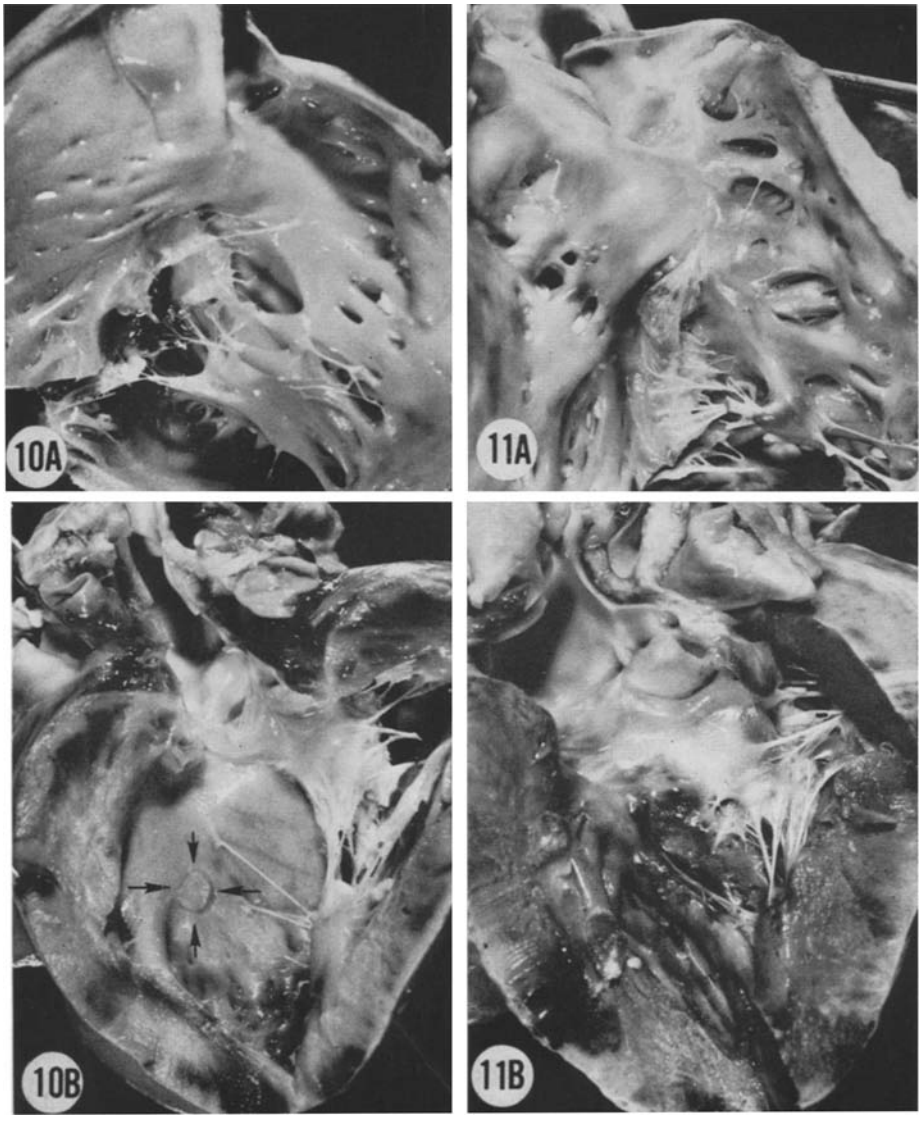

Fig. 10A and B and Fig. 11A and B

Fig. 10A and B. M59, extracorporeal circulation and 45 min arrest at + 37° C, heart: formalin fixed by controlled pressure method at end of experiment. A, Right ventricular outflow tract. B, Left ventricle: arrows marked area where biopsy tool accidently entered septum

Fig. 11A and B. M60, as in Fig. 10 (Histopathology Figs. 3A–C). A, Right ventricular outflow tract. B, Left ventricle

et al., 1967). The PAS reaction should not be performed on formalin fixed tissue, since the formaldehyde groups in the tissue fixed in this manner will, of course, give a positive reaction with Schiff's reagent, and are never completely removable.

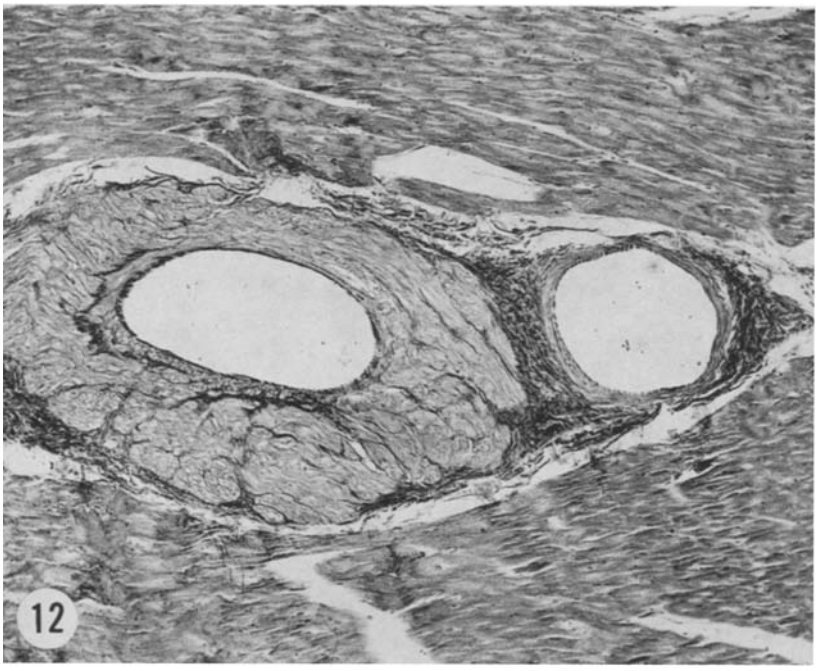

Fig. 12. M47, Time series, heart: formalin fixed by controlled pressure method, septum serially sectioned at 10 μ, Weigert-van Gieson-elastic tissue. Block AV_2, section 24, Magn. $\times 100$

Correlation of Pathologic Findings with Physiological, Metabolic, and Structural Chemical Studies

The physiologic, metabolic, and structural biochemical studies will be presented in detail elsewhere, but the relationship to the pathological findings will be summarized here. Pursuant to this the description of the character of the studies is as follows:

The physiological studies consisted of simultaneous measurement of aortic, left ventricular, and pulmonary artery pressures, indicator dilution curves for cardiac output, deriving from these left ventricular end diastolic pressure, and left ventricular maximum dP/dt.

In addition calculations of various work parameters on a direct and on a body surface area basis were performed. The pressure derivative loop although used primarily for visual assessment of the left ventricular dynamics was also reproduced in the digital computation using a summary of digits of 10 seconds of pressure curves superimposing the various loops (Fig. 14). At the same intervals a mini-Starling curve was produced by performing a set of observations, adding a volume load, usually blood, and then repeating the observation so that 2 points on a LVED to output (stroke volume) could be derived. Summaries of cardiac function developed below are therefore a summary of these findings with relation to the physiological parameters.

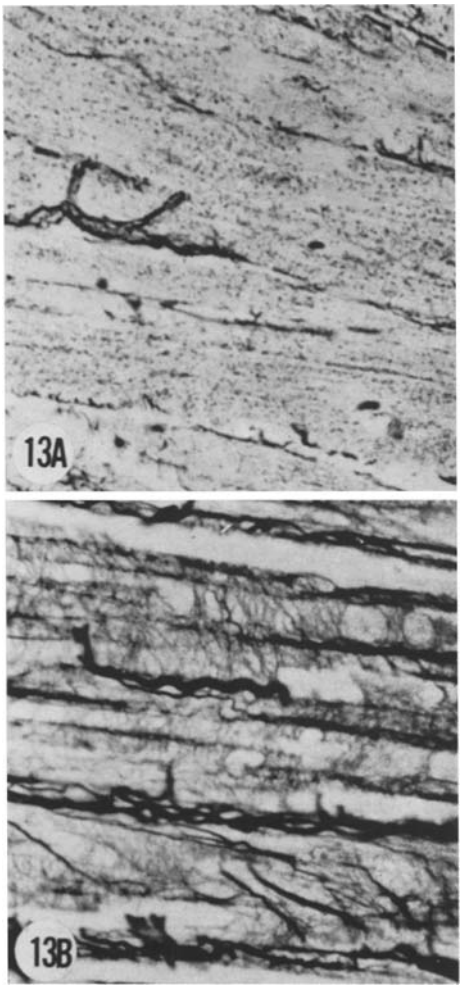

Fig. 13 A and B. M93, Control biopsy: frozen dried paraffin section, 7 μ. A, Silver impregnation for reticulum fibers. B, Silver impregnation after removal of glycogen by α-amylase digestion, Magn. $\times$ 450

For the metabolic studies again performed at the stated intervals bloods were drawn from the systemic, pulmonary artery, and mixed venous on the pump system for determination of hemoglobin hematocrit; whole blood pH, P_{CO_2}, P_{O_2}; and whole blood lactate and pyruvate. The unique set of studies other than the established points of all comparable studies was the period after immediate reinfusion of the heart when a rapid series of coronary sinus samples was taken as blood coursed through the coronary vascular system at the immediate time of resuscitation so that upwards of 8 samples were obtained in 2 minute periods, and after this other samples were repeated at 5, 10, 15 minute or thereabout to obtain as many points in the immediate coronary reinfusion state. The P_{CO_2} and

pH were useful for indicating metabolic and respiratory acid state; and the latter for an indication of the aerobic and anaerobic metabolic states. The computational program used in analysis derived the various buffer base, standard bicarbonate and so forth indicators of the acid-alkaline metabolic states. From the oxygen, lactate and pyruvate data; Huckabee's excess lactate, lactate pyruvate ratio and an indicator of aerobic metabolic rate (AMR) and Bing's electromotive force (EMF) were calculated. The computational programs calculated cross bed differences using the systemic to coronary sinus and systemic to pulmonary artery (mixed venous) for this purpose to show, for example, delta buffer base and delta excess lactate. The data discussed below on metabolism is in reference to this and in particular the reference to the immediate post resuscitation metabolic state of the coronary sinus effluent proved to be the most significant factor to follow. The normal situation was to have the metabolic rate to become calculated as anaerobic with an immediate swing to a high aerobic state and then readjusting back to the normal level within a relatively short time. Abnormalities were primarily related to no "swing" to a high aerobic state, slow return to normal, and very erratic swing to anaerobic status. Fig. 15 shows examples of the normal response and one of the abnormal curves. The structural biochemical examinations were performed as described in a series of normal canine hearts (Moulder *et al.*, 1966). The various partitions of intracellular and extracellular space and major cation concentrations calculated in this manner represent the structural integrity referred to below.

Biopsy procedures, anesthesia and extracorporeal circulation with oxygenated blood at the rate of 80 ml/kg/min were of little import in all parameters studied. The pathological examination seemed to bear this out. The loss of liver glycogen during perfusion and in the recovery phase was correlated with a moderate rise in blood sugar (to about 200 mg-%). The function of the hearts was excellent. There were no metabolic abnormalities. The structural water electrolyte studies were normal.

After 30 minutes arrest at 37°, the hearts were easily resuscitated and were able to support the circulation for the 6 hour observation periods after the arrest, even though the focal glycogen losses became somewhat more widespread in that period. The liver glycogen was largely depleted during the recovery period with the moderate rise in blood sugar (as noted above). The structural integrity of the myocardial cells was borne out by their normal histological appearance, unaltered DNFB and SH-group tests (which would be increased as more groups became available in damaged tissue), unaltered plasmal reaction, unaltered primary and secondary fluorescence, and lastly by the normal structural ion analysis results (Blackstone *et al.*, 1971). At the same time, the initially anaerobic metabolic rate (after occlusion) overshot into an aerobic rate and predictably returned to a normal aerobic rate during the recovery, while the histochemical enzyme capacity was unaltered.

After 45 minutes arrest, the changes were less predictable. The hearts were resuscitable, but in some cases unable to sustain the circulation for the planned period. Some of these hearts in addition to being unable to sustain the circulation were also subject to rhythm abnormalities and on occasion this would lead to rhythms requiring return to the extracorporeal circulation and discontinuance of the experiment.

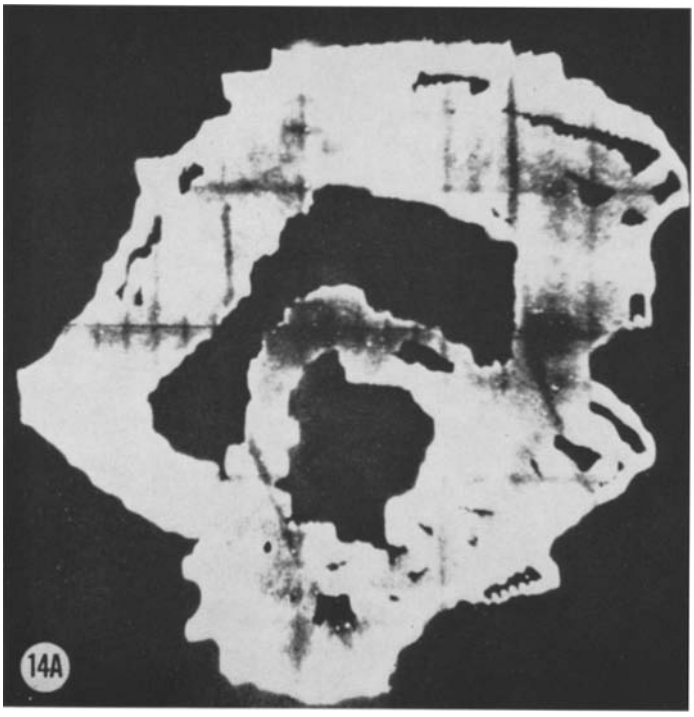

Fig. 14 A–C. Pressure Derivative Loop. A, A composite series of analog loops is displayed on a memory cathode ray oscilloscope with the heart in a bigeminal rhythm. The two different ventricular pressure derivative loops are seen readily with their different pressure but comparable contractility characteristics. Note that end diastolic pressure is elevated for both sites of ventricular contraction. B, Similar representation of a bigeminal rhythm in the digitized form. This computer developed display is usually developed on microfilm (from which this figure was made) and shows a series of loops in a specific situation. This is a useful indicator to recall and check the analog findings from the acute situation. C, This digitized format loop is of the type obtained when the heart is not ready for coming off cardiac bypass. The pressure is high but the derivative indicates poor contractility. This heart would go on to distention if allowed to continue accepting blood. This loop characteristic appears at a time many seconds before any visible or other indicator would demonstrate the heart to be in difficulty

As above, the structural integrity of the myocardium was preserved by all the criteria applied, but the glycogen losses in heart and in liver were more pronounced and widespread. The metabolic responses were unpredictable but correlated in retrospect with the amounts of stainable glycogen present. Oscillations between aerobic and anaerobic metabolism were frequent.

Hypothermia at 30° in the small number of animal studies converted the 45 minute arrest to a group roughly similar to the 30 minute arrest group. Therefore it seemed to be quite beneficial. Reviewing the physiological, metabolic and pathological studies overdistention was not found as delecterious to the myocardium as had been anticipated.

Since all histological, histochemical (protein and phospholipid-reactions, local enzyme capacities) and structural chemicals results were unchanged when com-

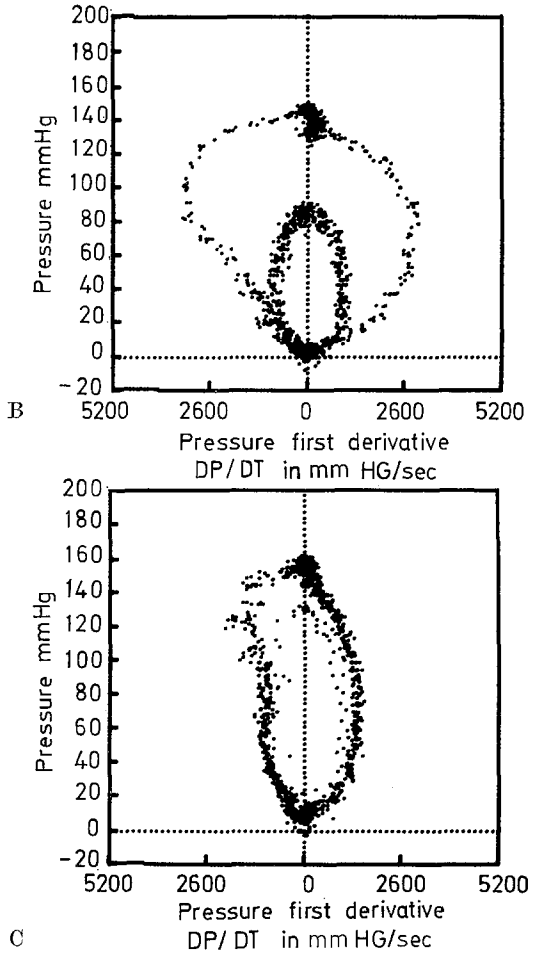

Fig. 14B and C

pared with the controls, the degree of glycogen loss was a measure of the metabolic and physiologic responses to be encountered. The failure to have a resuscitated heart support the circulation was due to the lack of the metabolic substrate (glycogen) and not to a structural alteration or a change in local enzyme capacity (as measured histochemically). Nor can changes in metabolic intermediates be considered in isolation.

All findings are summarized in Table 2.

Discussion

Due to the complexity of the problems in elective cardiac arrest and extracorporeal circulation, studies until now have tried only to answer single and seemingly simple questions. We are confronted by a vast literature dealing with the biochemical tissue analysis of cardiac metabolism during cardiac arrest in

Table 2. *Hemodynamic, metabolic, struc-*

Experimental preparation	Arrest after control biopsies	Resuscitation
1. 10 hours anesthesia, heparin and blood transfusion (AHB)		
2a) 10 hours AHB 2 hours extracorporeal circulation (EC)		
2b) 10 hours AHB, 2 hours EC 37°–30 min without coronary blood flow 2e) 30°–45 min without coronary blood flow	HD: loss of contractions and strength of contractions leading into arrest, occasional intervening ventricular fibrillation	HD: good recovery in 5–10 min with careful loop-controlled refilling of the heart M: perfused blood in coronary sinus initially in severe anaerobic state, swinging to high value aerobic state and rapid return to normal (Fig. 15a)

* Some animals did not survive the full ten hours. A few animals required more than
** All animals had respiratory alkalosis from the respirator.

C.O. = Cardiac output. PLVED = left ventricular end diastolic pressure. HD = Hemo-
PA = Pathologic-anatomical evaluation of serial biopsies.

Anesthesia: 1 = initial dose: Thiamylal I.V. 20 mg/kg body weight. 2 = Prior to place-
physiol. and metabolic study): Thiamylal I.V. 5 mg/kg body weight.

tural-chemical and pathologic summary

Status post EC and resuscitation	Status 2 hours post EC and resuscitation	Terminal status
HD: normal M: normal PA: *heart and liver:* glycogen, enzyme capacities and phenoltest unaltered	HD: normal M: normal	HD: near normal dynamics M: respiratory alkalosis** SC: normal water and electrolyte structure PA: *heart*: glycogen unaltered from first two biopsies, enzyme capacities and quantitative phenoltest unaltered *liver*: no abnormalities *lung*: focal atelectases kidneys and jejunum: normal
HD: normal, except for occasional atrial or nodal rhythm M: normal PA: *heart*: as above in 1 *liver*: focal loss of glycogen (periportal), leukocytosis	HD: normal rhythm and dynamics M: occasional mild systemic acidosis (metabolic), respiratory alkalosis**	HD: normal, occasional need for blood M: some had mild systemic acidosis SC: occasional slight increase in extracellular water PA: *heart*: glycogen and enzyme capacities unaltered, lower phenoltest value *liver*: glycogen largely absent, alk. phosphatase reaction as in second biopsy lung, kidneys and jejunum: as above leukocytosis
HD: often nodal rhythm with premature ventr. contractions, good gross appearance, good C.O., normal PLVED, normal Starling response to blood load M: myocard. effluent acidotic, systemic normal or alkalotic (respiratory) PA: *heart*: focal glycogen loss, normal enzyme capacities, lower phenoltest value *liver*: as above in 2a, increased alk. phosphatase reaction	HD: near normal with good Starling response M: occasional moderate systemic acidosis	HD: normal, occasional need for blood M: mild acidosis SC: mild increase in extracellular water PA: *heart*: glycogen loss more widespread, but focal, enzyme capacities unaltered, phenoltest value lower than in second biopsy and than above. *liver*: as above in 2a, leukocytosis *lung, kidneys and jejunum:* as above in 2a

2 hours of extracorporeal circulation because of slow resuscitation.

dynamic evaluation. M = Metabolic evaluation. SC = Structural chemical evaluation.

ment on by-pass: Thiamylal I.V. 10 mg/kg body weight. 3 = about every 2 hours (after

Table 2

Experimental preparation	Arrest after control biopsies	Resuscitation
2d) 10 hours AHB*, 2 hours EC* 37°–45 min. without coronary blood flow	HD: same as in 2b and 2e	HD: few similar to 2b/e, most delayed in resuscitation, remainder not capable of resuscitation M: some without metabolic swing to aerobic and normal state (Fig. 15b)
2f) 10 hours AHB*, 2 hours EC*, Distension arrest without coronary blood flow	HD: rapid arrest in bloated glassy surfaced state	HD: wide variation in resuscitability, most resuscitated M: similar metabolic variability

various forms and during recovery from cardiac arrest (Gott *et al.*, 1959; Günther and Hölscher, 1963; Helbig, 1961; Hölscher and Günther, 1962; Isselhard, 1967, 1968, 1965, 1964; Kübler, 1964; Kübler *et al.*, 1968; Maurer *et al.*, 1969; Moulder *et al.*, 1957, 1961; Müller, 1962; Scheuer, 1967; Sebening and Trautschold, 1962; Sickinger *et al.*, 1961). The biochemical findings have been reviewed and conclusions have been drawn with regard to possible therapeutic intervention. Measures have been proposed for improved recovery after arrest (Brettschneider, 1964; Isselhard *et al.*, 1964; Kübler, 1964; Kübler *et al.*, 1968; Maurer *et al.*, 1969). Various metabolites have been implicated in limiting the metabolism in arrest and in recovery after arrest (ATP, phosphocreatine). But due to the methods used, focal changes would remain undetected (Reichelt, 1968). One should add that the biochemical studies have had little or no physiological evaluation and no histologic controls. No comparable studies of extracorporeal circulation are known. While all these studies related to high energy phosphates and their breakdown products have provided interesting details, two basic questions remained unanswered:

1. How long can extracorporeal circulation safely be employed?
2. How long can the heart be safely arrested and then resuscitated?

(Continued)

Status post EC and resuscitation	Status 2 hours post EC and resuscitation	Terminal status
HD: irregular rhythm, commonly electrical and mechanical alternans, C.O. normal or low with elevated PLVED, near normal M: myocardial effluent acidotic, often remaining anaerobic PA: *heart*: focal glycogen loss more widespread, otherwise as above in 2b/e above *liver*: as above in 2b/e	HD: myocardial function depressed with elavated PLVED, low C.O., initial good Starling response to blood load, but this was not maintained. Around that time or just before some hearts went into intractable ventricular fibrillation and termination of experiment M: mild acidosis	HD: Survivors with normal or near normal dynamics, the latter with slight elevation of PVLED and slight decrease in C.O. M: mild acidosis SC: increased extracellular water, decreased cellular K and Mg, inconsistent changes in cell related Ca PA: *heart*: glycogen loss, focal or complete,depending on functional state, enzyme capacities unaltered, lower phenoltest value, no histologic structural abnormalities, *liver*: glycogen absent, otherwise as above in 2b and 2e *lung, kidneys and jejunum*: as above
HD/M: as in groups 2b/e and 2d, depending on the length of the arrest PA: as above depending on the arrest time	HD: as in 2b and 2e or in 2d M: as in 2b and 2e or in 2d	HD: as above depending on the length of arrest M: as above SC: as above in 2b/e or 2d PA: as above in 2b/e or 2d

As Isselhard (1968) put it in a discussion during the Oslo Conference on Resuscitation: "We excluded the heart from circulation for 20 minutes at normal body temperature. In 50% of the animals, there was sufficient return for permanent survival. We do not know whether the deaths in the remaining 50% were due to impaired myocardial metabolism or to extracorporeal circulation." It is surprising that the pathological evaluation of extracorporeal circulation and cardiac arrest has hardly been attempted and that the majority of modern textbooks do not mention the problem at all. Hudson's chapter on extracorporeal circulation (1965) dealt with the machinery involved but since it gave no pathologic information, one can safely presume that it did not exist. The next chapter on cardiac arrest referred to work on potassium citrate arrest by Helmsworth *et al.*, (1959) and McFarland *et al.* (1960). Hudson emphasized the value of histochemistry in the study of myocardial dysfunctions (Niles *et al.*, 1964). Gould's book (1968) did not mention any pathological changes in the heart due to arrest and extracorporeal circulation and only Lam in his chapter on cardiac surgery remarked that anoxic damage to the myocardium results when an arrest is extended beyond 15 minutes. Meessen and Poche (1963) discussed findings attributed to anoxic

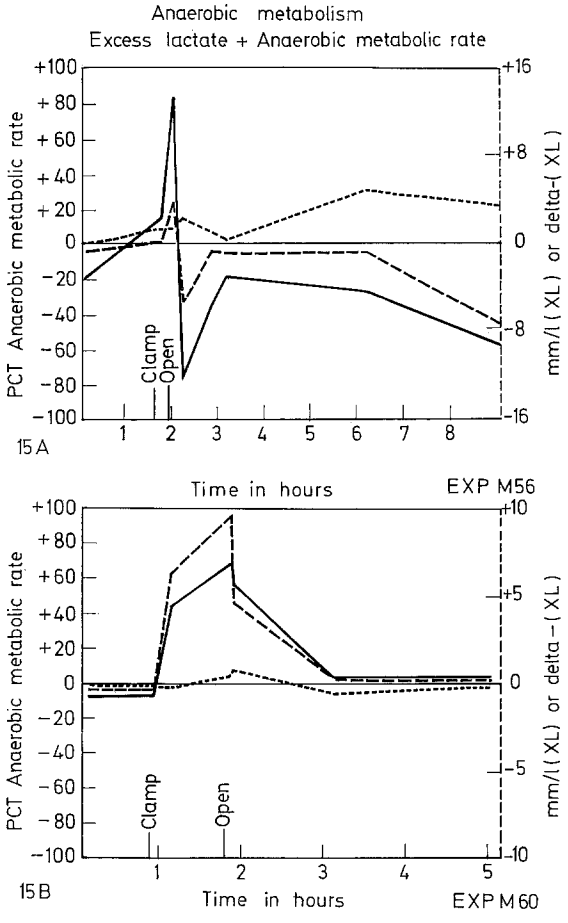

Fig. 15A and B. This figure presents two facets of this general study: First, this is the response of cardiac metabolism in the 30 minute arrest at 37°C, a pattern generally seen in those animals surviving with near normal dynamics and histochemical patterns: second, the use of a computer derived summary of the experiment without either time consuming calculations or collection of stacks of paper filled with computer output. A, M56, the "percent AMR venous" represents Huckabee's anaerobic metabolic rate for transcardiac metabolism which is classified "aerobic" in the baseline studies because of lactate destruction. The initial post arrest reaction is to severe anaerobiosis followed by a marked overshoot to the aerobic side. The cross bed excess lactate (delta XL) follows a similar pattern. The mixed venous excess lactate representing the whole body is little affected. B, Notations as in those for Fig. A, M60, 45 min of arrest at 37°C. This is the pattern found in general in the nonsurviving and/or poorly resuscitatable type of animal preparation in which the anaerobic phase can be prolonged and strikingly there is no post disocclusion aerobic phase. ——— PCT AMR Venous; ····· Mixed Venous XL; – – – – Delta XL Venous

arrest which had been reported by Ohm and Poche (1963). The study included animal experiments as well as autopsy material. In the opinion of the authors, the changes described were largely reversible. Meessen (1964) cautioned against overoptimistic interpretation of the histological findings in a given case. Due to

the complexity of the problem, a definite decision with respect to nature of the lesion, its pathogenesis and reversibility, seemed to be impossible. Further autopsy studies did not help in the analysis of the problem at hand, since no distinction could be made between the effects of arrest, the extracorporeal circulation, resuscitation attempts, and handling of the tissue by the surgeon and the pathologist (Albert *et al.*, 1961; Köhn and Richter, 1960; Morales *et al.*, 1967; Henson *et al.*, 1969). The same can be said for experimental studies, mostly involving ultrastructure (Hölscher 1962a, 1962b, 1965; Lev *et al.*, 1965; Löhr *et al.*, 1968; Miller *et al.*, 1961; Poche *et al.*, 1967).

Our study was initiated by the clinical problems related to evaluation of the heart in its post-operative stages as to the effects of the anesthesia, the heart-lung machine and the use of elective cardiac arrest during the repair procedures. The study was programmed to become as broad-based as possible, but in view of the great numbers of variables to be relatively restrictive with respect to the variables to be modified. Therefore, four major groups were planned:

1) a series in which time of anesthesia and biopsies alone were studied;

2) added to this was a standard time of extracorporeal circulation (2 hours) followed by a 6 hours resuscitative period;

3) a prime experimental group which would include as near a separation of lethal and sublethal cardiac arrest as could be defined;

4) added to this group with distention of the chambers during resuscitation.

We recognized that limited studies (including those using isolated hearts) would not approximate the clinical situation, and that their value was open to question. Removal of the heart from the body with sampling at various intervals thereafter or cardiac arrest by cutting the aorta with exsanguination and sampling seemed hardly applicable models for the study of cardiac arrest as used in cardiac surgery. These exercises represented to us some form of controlled or uncontrolled autolysis rather than a meaningful evaluation of elective arrest, especially since the all important event of resuscitation could never be investigated with such a preparation. Our own experience with perfusion of isolated hearts showed that hypoxic perfusions would bring about cessation of cardiac function within a short time (Blackstone *et al.*, 1968a, 1968b). Profound metabolic changes had occurred within that time, but it was impossible to pinpoint any one factor which might be responsible for the catastrophe. Similar metabolic changes were found in total body hypoxic perfusions, but these could be carried out over longer periods (up to $2^1/_2$ hours) without collapse of the cardiac function. In either case no conventional pathological changes were found, but the amount of stainable glycogen seemed to provide an indication of the cardiac function at that time. In the total body hypoxic perfusions a remarkable correlation existed between the myocardial glycogen and the liver glycogen. In the course of the experiment the liver glycogen disappeared, first in the periportal areas, then around the central veins. When only minute amounts were stainable in the intermediate zone of the liver lobules, the myocardial glycogen also disappeared and cardiac function ceased. The reversibility of the process was not tested in the previous experiments, but these are now under way. In comparing the hypoxic perfusion studies with the experiments in this communication one could certainly conclude that a "bad" perfusion was definitely worse than no perfusion at all.

For proper pathological evaluation, a number of questions had to be answered. We had to develop a biopsy technique, histopathological and histochemical methods which were free of the artifacts that in the past have led to grave errors in interpretation of histological appearances. We had to be concerned not only with the reproducibility of the methods, but much more so with their authenticity. In order to prove authenticity we had to use methods which would give "the best morphological preservation and least chemical alteration" (Barka and Anderson, 1965). Therefore we abandoned the use of fixative solutions which regardless of mode of application (immersion or perfusion of the specimen)—were unable to immobilize all cells instantaneously. The problems of fixing viable muscle had been recognized for a long time. Thulin (1914) pointed out that hypercontraction bands in fixed muscle fibers present artifacts of fixation. They are absent in native preparations, and their formation could actually be observed when fixation was controlled microscopically. Similar warnings were issued by Schaffer (1918) and Heidenhain (1918). Heidenhain emphasized that one should not fix muscle immediately after removal from the living body, but let it lie before immersion in fixatives until no reaction could be elicited. This drawback of fixative solutions (failure of instant immobilization) had also been demonstrated for smooth muscle (Grupp and Goerttler, 1958) and even for amoebae (Wohlfarth-Bottermann and Komnick, 1966).

Proof of authenticity of "lesions" could only be provided by independent methods, and not by repetition of the same fixation procedures. Conventional cryostat sectioning (Martin and Hackel, 1963) and its artifacts have been previously discussed (Eckner *et al.*, 1967).

The old concept of Nissl that "equivalent pictures" could be compared after identical pretreatments had often been violated by alterations in the pretreatments (Zeiger, 1938). In accepting this concept of "equivalent pictures" one also makes several assumptions:

1) the effect on tissues of fixative solutions, alcohols, xylene, etc. as used in conventional tissue preparation for pathological examination is the same at any given time, 2) the effects mentioned are the same for normal as well as for experimentally altered tissues. These assumptions, however, cannot be made as was shown in the case of glycogen by Leske and v. Mayersbach (1969). There is little reason to believe that the situations are different with respect to other substances and structures.

The chemical effects of solutions on tissues are: 1) extraction of material, 2) translocation and redistribution of material [e.g., "glycogen flight", redistribution of nuclear material (Schaffer, 1918)], 3) artificial binding of material (e.g., fixative, extraneous material) to the tissue. The result of these conventional treatments are readily apparent in pictures of scanning electron and can only be described as "cell ruins" (Haggis, 1970). While these conventional pathological methods gave reproducible results they were not acceptable as representative of the microscopic structure during life.

Tissue preparation by freezing and drying was employed in the present study and gave an authentic and reproducible preservation of chemistry and morphology of cells and tissues at oil immersion resolution (Eckner *et al.*, 1967, 1968a, 1969).

Our biopsy technique had several advantages: a) repeat biopsies from the same animal allowed each animal to serve as its own control or baseline. Our findings emphasized the importance of trauma by surgeons and pathologists (Henson *et al.*, 1969) in the evaluation of morphological appearances both macroscopic and microscopic.

The basic questions of safe time of extracorporeal circulation and of elective cardiac arrest could be answered for the normal dog heart as follows: two hours of careful extracorporeal circulation were tolerated under the given circumstances. No histochemical and histological changes were evident after the period, but changes in the relation of ground substances and collagen were found after the recovery period. Cardiac arrest at 37° was well tolerated for 30 minutes. After 45 minutes the response to resuscitation became unpredictable: all the hearts could be resuscitated in the sense that they resumed a normal beat, but some were unable to support the circulation for any length of time. The amount of stainable glycogen present at a given time seemed to be an indicator of existing and future function. Even though it is believed that glycogen does not play an important role in cardiac aerobic metabolism, it is the primary reserve energy source in the myocardium. It is unclear what role its absence would play for the recovery from insult (Isselhard, 1970), particularly if aerobic phases would alternate with anaerobic phases during that important period. Hyperglycemia has been described in the clinical situation (Moffitt *et al.*, 1970). No ready explanation for this explanation for this phenomenon was available, when "pump priming" was ruled out as a causative factor. There is also no ready explanation for the apparent inability of the myocardium to use the available glucose, unless one assumes that the glucose has to be polymerized into glycogen in order to be usable (Davis, 1965).

In the absence of conventional histological abnormalities we concluded that the cells in the failing hearts are intact. The local enzyme capacities which were studied appeared to be unaltered. In other words, the machinery of the cells seemed to be intact, but substrate or fuel was lacking for proper function, contraction and relaxation. The reversibility of the process is being studied now in long term experiments. The isolated finding that connective tissue collagen exhibited an altered phenol test, (i.e., a change in birefringence and presumably in organization) seems to be a short term effect. In a series of 44 operated cases of tetralogy of Fallot, we found lower values for a period of about 24 hours postoperatively. The experiments described here provide a baseline study for the evaluation of responses in pathologically altered hearts. We hope that the combined application of modern physiological, biochemical and pathological methods will establish objective criteria for extracorporeal circulation and elective cardiac arrest leading to better management of the diseased heart.

Addendum. After this paper was submitted for publication, L. M. Buja *et al.* reported "Acute and Chronic Effects of Normothermic Anoxia on Canine Hearts" (Circulation **43**, Suppl. I: 44–50, 1971). In the absence of a detailed description of the surgical procedure, comparison with our study becomes difficult. The pathological evaluation of these authors relied heavily on postmortem examination and estimations of glycogen contents of heart muscle were based only on electron-microscopic preparations. Nevertheless, Buja *et al.* confirmed our observation that the dog tolerated 30 min of elective cardiac arrest at 37°C by cross clamping of the aorta. If the arrest was extended to 45 min, response to resuscitation became unpredictable (Eckner *et al.*, 1968b).

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