Arch Jpn Chir 52(4), 435～466, Juli, 1983

原　　著

An Experimental Study of Myocardial Protection with Special Reference to Cold Blood Potassium Cardioplegia: I. Morphological and Biochemical Studies

YOSHISADA SHIRAISHI

The 2nd Department of Surgery, Faculty of Medicine, Kyoto University
(Director: Prof. Dr. YORINORI HIKASA)
Received for Publication, May 9, 1983.

Introduction

Recently a number of intracardiac and coronary artery operations have been carried out throughout the world. Most cardiac surgical procedures can now be performed with minimal operative morbidity and mortality[6,57,62,75,92]. Intraoperative myocardial infarction and low cardiac output syndrome were common in the past[58,89]. These complications, however, are far less frequently encountered now[11,12]. This improved prognosis is attributed greatly to the evolution of more effective methods of intraoperative myocardial protection[12,69,79] as well as the progress in both preoperative diagnostic techniques and pre and postoperative managements of pateints.

The search for a more ideal cardioprotective method is being conducted all over the world. However, the best method for myocardial protection is controversial. At present the method most commonly used for the myocardial protection is termed hypothermic potassium-induced cardioplegia[1,19,81]. The method provides a surgeon with excellent operative conditions; a flaccid heart and a bloodless operative field. The rationale for this procedure is that by reducing myocardial wall tension as a result of arresting the heart in diastole, and by maintaining the heart at a profound low temperature, it should be possible to lower the basal metabolic rate of the myocardium and thereby to suppress the consumption of high-energy phosphates such as creatine phosphate (CP) and adenosine triphosphate (ATP)[43,64,98]. Unfortunately, it has proved impossible to alleviate ischemic damage to the myocardium simply by reducing the consumption of high-energy phosphates[26,33,88]. Accordingly, attempts have been made, not merely to conserve, but also to replenish the energy sources. Such attempts have sought to promote the production of ATP either by administering glucose[83], a method which involves anaerobic

Key word: Cold blood potassium cardioplegia, Glucose-insulin-potassium cardioplegia, Anaerobic metabolism, Aerobic metabolism, Electron microscopy.
索引語：心筋保護，好気性代謝，嫌気性代謝，電顕，エネルギー代謝
Present address: The 2nd Department of Surgery, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto, 606, Japan.

glycolysis, or by administering some metabolites in the tricarbonic acid (TCA) cycle such as glutamic acid etc.[27,63], a method which depends on aerobic metabolism. In 1978 FOLLTTE and associates[34] introduced a new method for the myocardial protection during ischemic arrest. Ths method is called cold blood potassium cardioplegia (CBKC) which, while adopting some elements of the cold potassium cardioplegia (CKC) mentioned above, depends primary on blood to supply oxygen to the ischemic heart. This technique is based on the theory that ischemic myocardum under aortic cross-clamping can be delivered both oxygen and energy substrates by intermittent reinfusion of blood resulting in synthesis of ATP via the oxidative phosphorylation pathway. These investigators[35] presented both experimental and clinical data which strongly suggested that the use of CBKC offered excellent preservation of both ventricular function and energy substrate levels during a two-hour period of aortic clamping.

The author's attention has been drawn to this excellent method for the myocardial protection during ischemic cardiac arrest. The present study was undertaken to examine the efficacy of cold sanguineous cardioplegia (CBKC) in comparison with cold asanguineous cardioplegia (represented by glucose-insulin-potassium cardioplegia (GIKC)) morphologically and biochemically.

Materials and methods

Adult mongrel dogs of either sex, weighing between 8 to 17 kilograms, were anesthetized with 25 mg per kilogram of sodium pentobarbital intravenously. A cuffed endotracheal tube was inserted and ventilation was maintained by a Harvard respirator. The thoracic cavity was opened by a bilateral incision in the fourth intercostal space. The heart was exposed and suspended in the pericardial cradle. After systemic heparinization (3 mg per kilogram), cannulation for removing blood from the body and for returning blood to the body was carried out into the right atrium and the right common carotid artery respectively, and cardiopulmonary bypass was started. The pump was primed with 500 ml of Ringer's lactate, 40 ml of 7% (W/V) sodium bicarbonate, 10 ml of 8.5% calcium gluconate, 50 ml of 20% mannitol, and 25 mg of heparin. The hematocrit value during perfusion was approximately 22%. Blood was reinfused to the body at a rate of 80 ml/kg/min by means of a roller pump (Sarns Inc.) after oxygenation of blood using bubble oxygenators (Bentley Laboratories or Japan Medical Supply Cp., Ltd.) When the esophageal temperature reached 32°C during perfusion cooling, the ascending aorta was cross-clamped and 20–30 ml of diluted potassium chloride solution (150–200 mEq/l), cooled to 4°C, was injected manually via a fourteen gauge metal cannul with a three way stop cock into the aortic root in order to arrest the heart in diastole. Topical cardiac hypothermia was employed simultaneously using ice sluch which was made from physiological saline, thus the temperature of the myocardium was maintained below 15°C throughout the ischemic periods. At this point, cardiopulmonary bypass was discontinued and the blood was drawn off into the oxygenator so that it could be used in the CBK solution. Immediately after achieving diastolic cardiac arrest, the first specimen of the myocardium was excised from the apex of the left ventricle. Subsequently, 10 ml per kilogram of the cardioplegic solution, either GIK or CBK solution, the composition of which is shown in Table 1, was infused every 30 minutes by gravity from a height of about

Table 1. Composition of cardioplegic solutions.

	Solution I	Solution II
Substrate	5% Glucose 500 m*l*	Oxygenated heparinized blood 500 m*l*
Regular insulin	10 units	10 units
Potassium chloride	10 m*l*	10 m*l*
7% sodium bicarbonate	10 m*l*	10 m*l*
Na (mEq/*l*)	18	125±8
K (mEq/*l*)	18	24±2
Ca (mEq/*l*)	0	3.7±0.6
Mg (mEq/*l*)	0	1.4±0.2
pH	7.9	7.6±0.1
PO_2 (mmHg)	195	316±98
PCO_2 (mmHg)	15	32±7
Osmorality (mOsm/*l*)	362	347±14

100 cm. During this procedure, specimens of myocardial tissue were taken every 30 minutes until the period of ischemia reached 180 minutes.

I. Morphological study

1) Light microscopic study

Specimens were fixed for two days with 10% neutral formalin. After fixation the specimens were cut into several pieces about 5 cubic millimeter and washed in water. The blocks were dehydrated with increasing concentrations of ethanol, and after removing the ethanol with xylene, they were embedded in paraffin. Thin sections were stained with hematoxylin and esoin.

2) Electron microscopic study

Specimens obtained were divided into three layers; outer, middle, and inner. They were minced into 1 to 2 mm pieces in cold 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2 and were fixed with this fixative for two hours at 4°C After washing with several changes of the cold buffer supplemented with 0.1 M succharose, the tissues were post-fixed with cold 1% osmium tetroxide in Milonig's phosphate buffer, pH 7.2 for one hour, and rinsing was carried out in the same buffer containing 0.1 M saccharose. The blocks were dehydrated in grade series of ethanol and propylene oxide and routinely embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead acetate, and examined with a Hitachi HU-11D-S electron microscope.

II. Biochemical study

1) Measurement of high energy phosphate compounds in the myocardium

Immediately after excision, specimens were frozen in liquid nitrogen. Although results of an experimental study by Lowe and associates[68] showed that ATP depletion is most prominent in the subendocardial layer, division of the tissue into subendocardial and subepicardial parts was not carried out in the present experiments because any larger time interval before freezing might result in a breakdown of metabolities. The frozen tissue specimens were crushed, and 3 ml of perchloric acid solution was added to 1 gram of the crushed tissue. The sample was

centrifuged at 10,000 rpm for 15 minutes, and the supernatant was assayed for ATP and CP by the method of LAMPRECHT and associates[61], and for ADP and AMP by the method of JAWOREK and associates[48]. The energy charge (EC) which was proposed by ATKINSON[8,9] was calculated from the values of ATP, ADP, and AMP as follows.

$$EC=\frac{ATP+1/2\ ADP}{ATP+ADP+AMP}$$

2) Measurement of lactate, pH, and carbon dioxide tension in blood

Immediately after achieving diastolic cardiac arrest, the right atrium was opened by a 3-cm transverse incision. A balloon catheter was directly introduced into the great cardiac vein through the orifice of the coronary sinus. The first 3 ml of blood, which flow out through the catheter when infusing the cardioplegic solution into the aortic root, was withdrawn. Blood gas analysis was performed by a ABL II gas analyzer (Radiometer Laboratory). Lactate was measured by the method of GATMANN and WAHLEFELD[42].

Results

1) Light microscope

Figures 1 to 6 illustrate the structure of the myocardium seen in sections taken at 180 minutes after the onset of global ischemia when treated by means of CBKC. Swelling and clearing of the myocardial cell and the nucleus, intercellular edema, and formation of contraction band, are the major findings seen in these figures. These structural disintegrations, if present, appeared mild in the middle third of the myocardium (Fig. 2). On the other hand, severely damaged myocardium was found in the inner third (Figs. 3, 4, 5, 6). In the outer third, the myocardium shows almost normal cellular structure except for the subepicardial region where slight swelling of the cell and nucleus, and contraction band are noted (Fig. 1).

2) Electron microscope

Figures 7–10 illustrate the normal structure of the myocardium seen in sections taken prior to induction of global ischemia

The sarcolemma (SL), which appeared to be the morphological outer limit of the cell,

Figs. 1 to 6. Sections of the left ventricular myocardium taken at the end of 3 hours of global ischemia. Throughout the period, the myocardium was protected by means of CBKC.

Fig. 1. A longitudinal section of the outer third. Cross-striation is not clear in the area beneath the pericardium. Mild degrees of contraction band formation is apparent in the lower region. (×200)

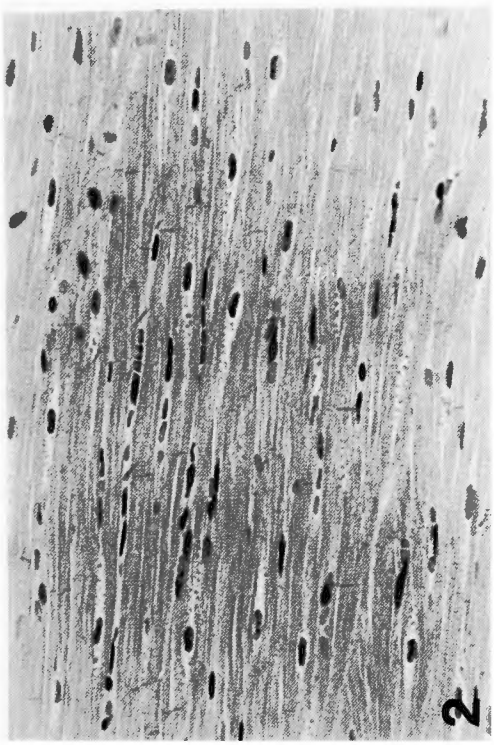
Fig. 2. A longitudinal section of the middle third. No abnormal finding is noted. (×400)

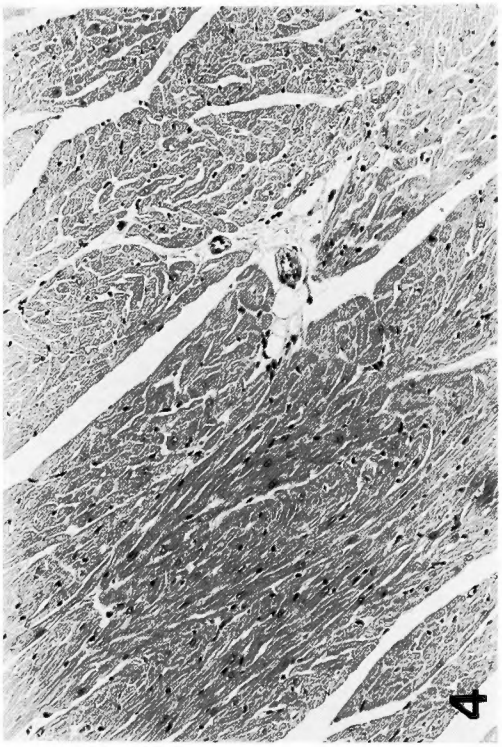
Fig. 3. A longitudinal section of the inner third. Contraction band formation and swelling of the nucleus are conspicuous. (×400)

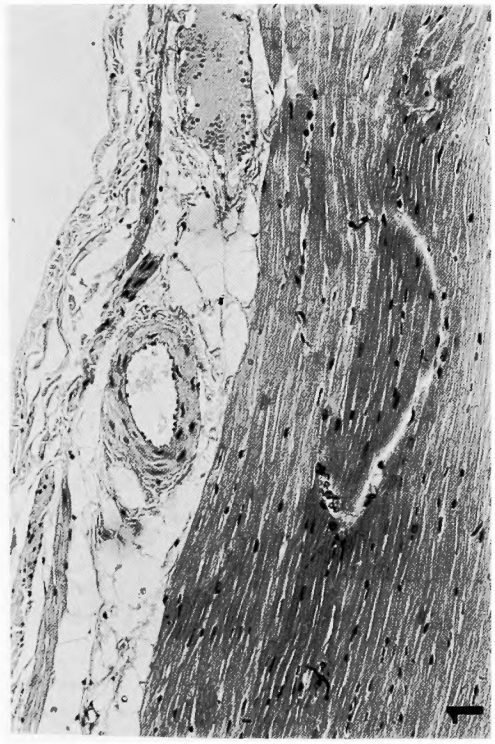
Fig. 4. Low power view of a transverse section of the inner third. Interstitial edema and perivascular edema are noted. (×100)

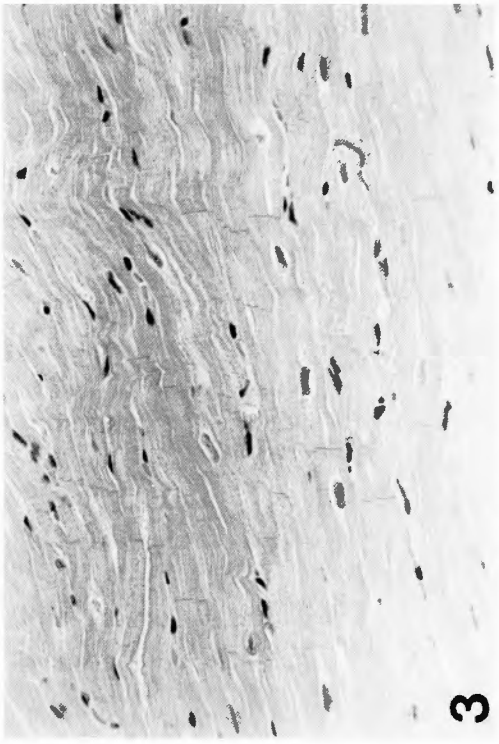
Fig. 5. A transverse section of the inner third. Marked subendocardial edema is found. There appears to be edema around the pyknotic nucleus. Disappearance of the nuclei is also observed. (×200)

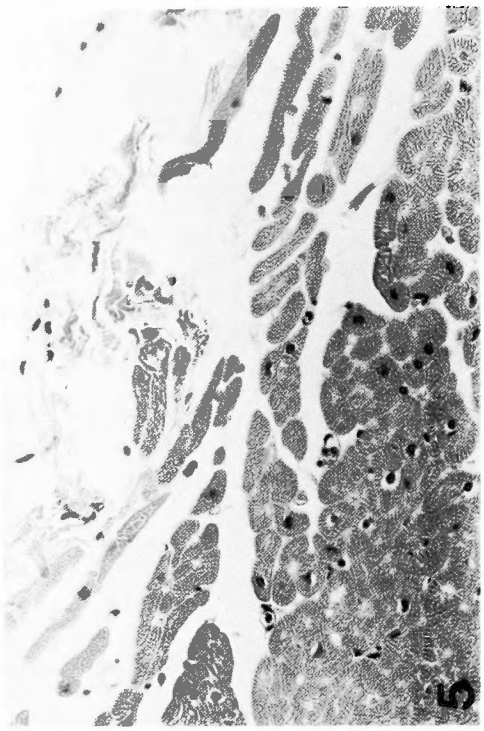
Fig. 6. A transverse section of the inner third. Swelling of the nucleus, perinuclear edema, extracellur edema, and lysis of the myocardial fiber are the major findings in this photograph. (×400)

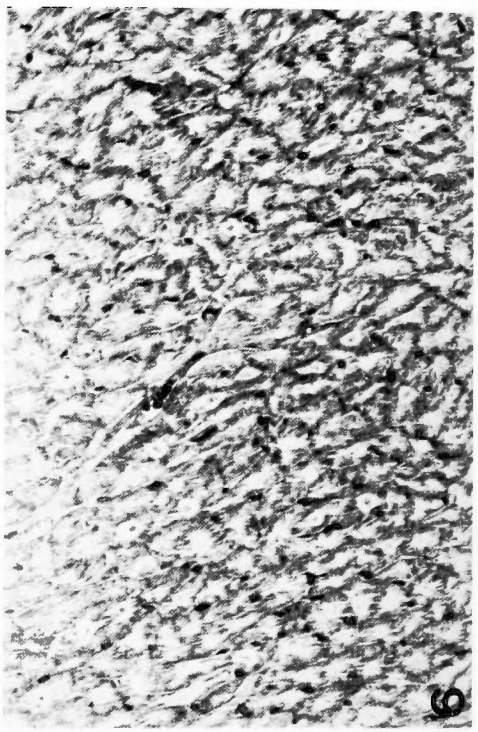

2


4


1


3





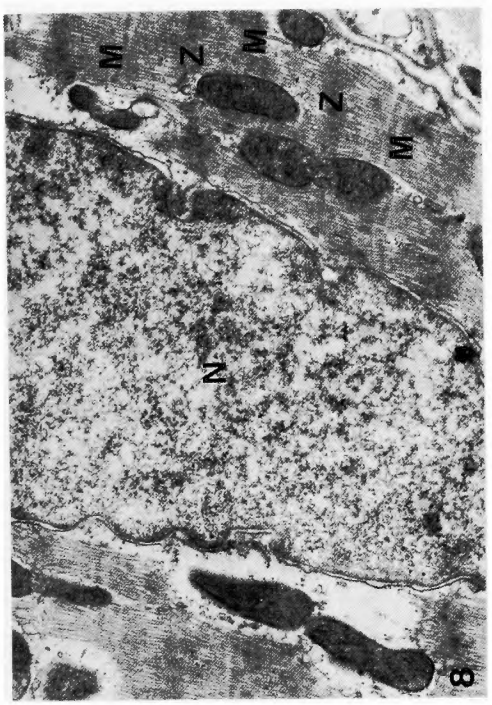
demonstrates a 'unit membrane' structure: on the external aspect, the plasma membrane was invested by a moderately dense basement membrane of fairly uniform width. Along the lateral margins, SL was regularly indented in register with the Z line. This gave a scalloped appearance to the working myocardial cell. SL had occasional small invaginations resembling the so-called

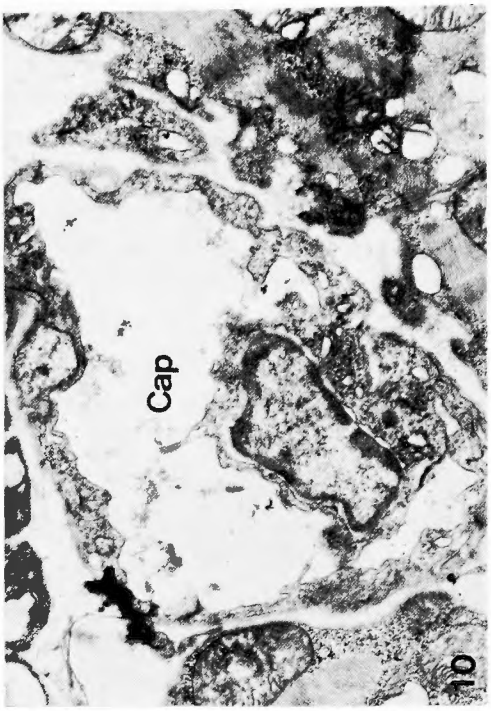
Fig. 7. Electron micrograph of parts of four cardiac muscle fibers in transverse section. The right upper two cells are joined side to side by a typical steplike intercalated disc (ID). Close apposition of the sarcolemmal membrane (SL) forms so-called nexus (Nx). Glycogen (G) is abundant, especially in the subsarcolemmal space. Numerous mitochondria (MT) are diffusely distributed throughout the cell. They seems to vary in size and shape. Groups of cristae run parallel, but their orientation is not consistent and, as a consequence of their curving course, they are tangential or parallel to the plane of section in some areas. Dense matrix granules are abundant in the mitochondria. A variety of round shaped or narrow elongated sarcoplasmic reticulum tubules (SR) are located adjacent to or between mitochondria. Extracellular space (Ex) shows a relatively high electron density. (Original magnification ×5600)

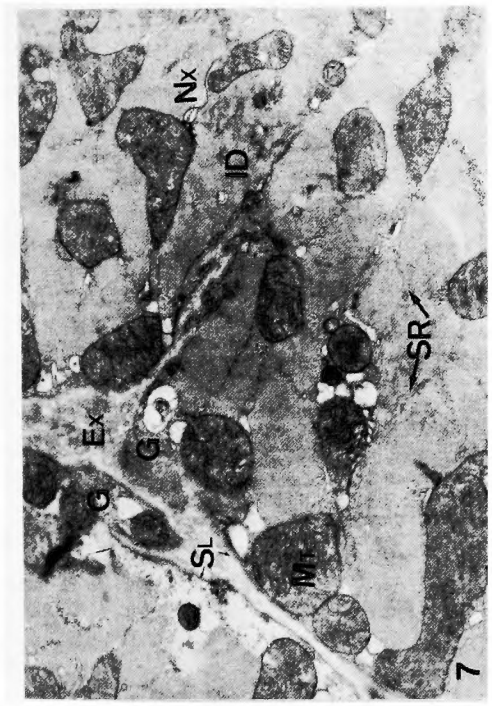
Fig. 8. Longitudinal section of the left ventricular myocardium illustrating portion of the nucleus (N). It consists of two membraneous layers which circumscribe evenly distributed electron-dense granules. Z and M indicate Z line and M line in the sarcomere respectively. (Original magnification ×8200)

Fig. 9. A micrograph of longitudinal section of the myocardial fiber. In the upper half of the figure, electron dense and undulating intercalated disc (ID), which runs transversely along Z line of the sarcomere, is well defined. In segment of the junction between the insertion of bundles of myofilaments, typical desmosomes (Ds) are found. A transverse tubule (T) of the sarcolemma and two associated terminal cisternae of the sarcoplamic reticulum (SR) comprise triad. (Original magnification ×11000)

Fig. 10. A micrograph showing the capillary located in the extracellular space. Capillary wall is made up of a endothelial layer, in which abundant pinocytotic vesicles are seen. (Original magnification ×8200)







micropinocytosis vesicles that are seen in great numbers at the surface of capillary endothelial cells.

The single greatly elongated, fusiform nucleus was generally found in the center of a myo-

cardial fiber. In the nucleus was located a nucleolus. The moderately dense, granular, rather uniformly distributed nuclear chromatin was circumscribed by the nuclear envelope which was traversed by a small number of nuclear pores.

Numerous mitochondria was seen throughout the cell, but were particularly abundant around the nucleus, beneath the SL, and in the myofiber in close proximity to the myofibrils and segments of SR. Mitochondria appeared to vary in size and shape. Mitochondria are delimited by a double membrane and were traversed by numerous cristae. The matrix of mitochondria was moderately dense and contained conspicuous dense granules.

The sarcotubular system formed an extensive complicated network of intracellular tubules, vesicles, and cisternae. It was composed of two principal components. The first was formed by the periodic invagination of SL, and appeared as a double-layered, transversely oriented tubular system known as the T system. The T system regularly invaded the myocaridal cell at the level of Z line in the sarcomere. The second component of the sarcotubular system consisted of a series of thin-layered interconnecting tubules longitudinally oriented parallel to and surrounding the myofibril, and is called sarcoplasmic reticulum (SR). At the level of the Z line, the vesicular dilatation of the two components formed characteristic bi- and tricircular structures called diades and triades. The material within these components appeared to be more dense than the surrounding sarcoplasm.

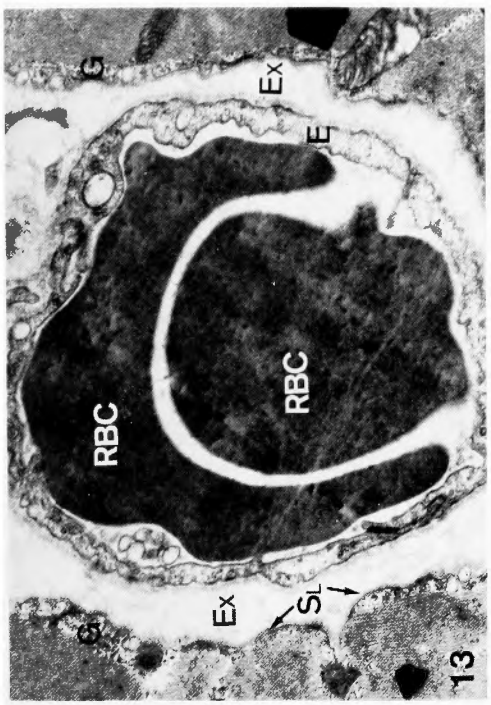
The intercalated disc (ID), which is defined as a structurally distinctive complex consisting of two apposed plasma membrane and an interposed intracellular space, is believed to be modified cell boundaries of adjacent myocardial cells. ID usually pursues an undulating course, oriented transversely in relation to the long axis of the myofibril. The plasma membrane of ID appeared to be more electron-dense than those on the lateral surface of the cell. At the transverse portion of the cell interface, the paired plasma membranes remained separated at a fixed sapce, but a number of points of sarcoplasmic deposits of moderate density were accumulated, thus giving

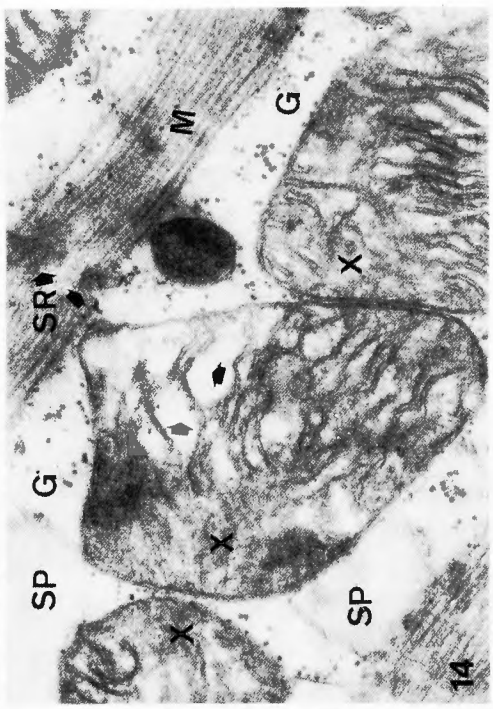
Fig. 11. A micrograph of longitudinal section of the inner myocardial layer taken at 30 minutes after onset of ischemia. The elongated nucleus (N) illustrated shows normal structure. One mitochondria in the right lower region is disrupted. The sarcoplasmic reticulum (SR) in various sizes are aligned parallel to the nucleus. At Z line, it is rather larger. (CBKC, Original magnification ×8200)

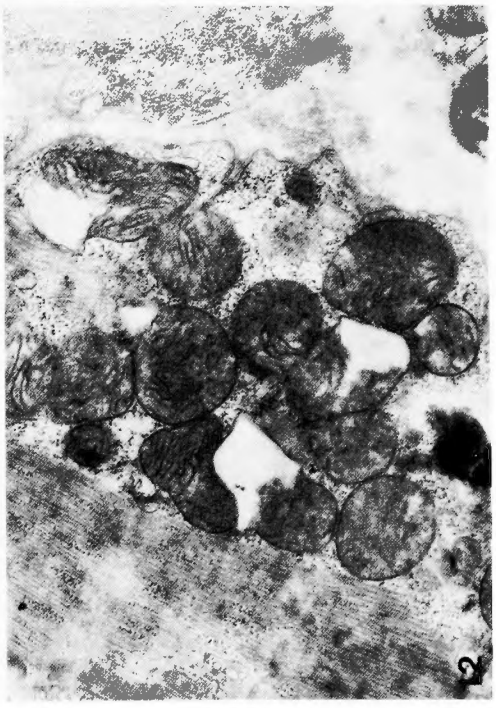
Fig. 12. A cluster of the mitochondria beneath the sarcolemma are shown. Some of the mitochondria are destroyed. Matrix density in the mitochondria is still dense. Numerous glycogen granules are located both in the subsarcolemmal space and in the myocardial fiber. This specimen was taken at 60 minutes after the onset of global ischemia. (CBKC ,Original magnification ×11000)

Fig. 13. A micrograph of transverse section of the subendocardial myocardium taken at the point of 120 minutes after beginning of ischemia. In the center of the figure, the capillary containing two red blood cells (RBC). The endothelium (E) is not swollen. A moderate sized vesicle is seen in the right upper portion of the endothelium. The basement membrane of the sarcolemma is invested by a moderately dense basement membrane of fairly uniform width. (CBKC. Original magnification ×9300)

Fig. 14. A micrograph of longitudinal section of the inner myocardial layer taken at 120 minutes after induction of global ischemia. Vesiculation of the matrix in the mitochondria is visible. Disruption of crista is not apparent. X indicates areas where cristae run tangential to the plane of section though they appear to be absent. The number of glycogen particles is reduced. Intermyofibrillar edema is obvious. (CBKC, Original magnification ×13000)







the dark appearance to ID. When these deposits are short along the line of plasma membrane, they are termed desmosomes. Longer membrane appositions without fusion and without adjacent electron-dense accumulations have been named fascia occludence. Another type of

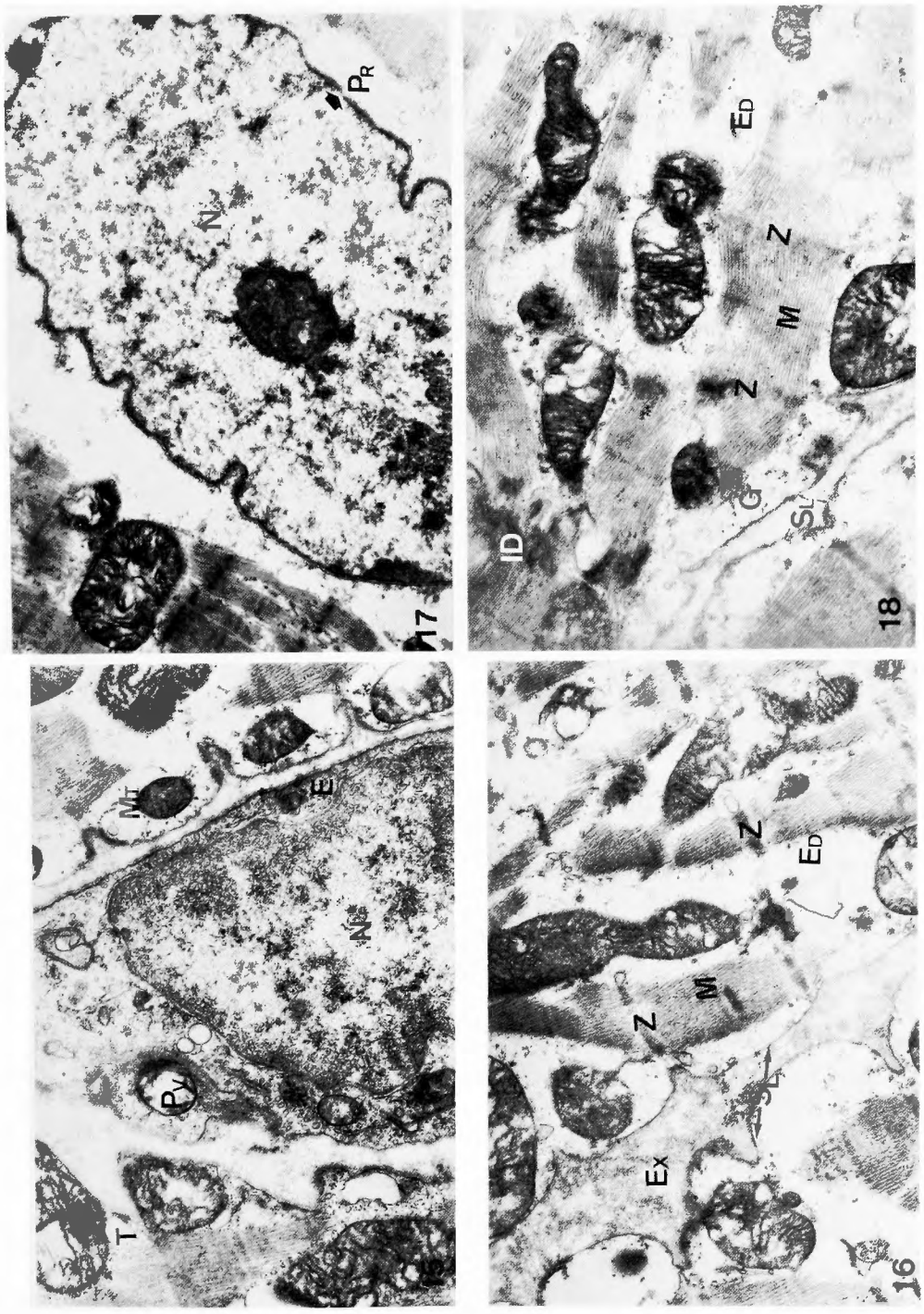
ID, which occurs almost exclusively along the lateral margins of the protrusions from one cell into the other, is formed by actual fusion of the plasma membranes with obliteration of the intercellular space, and is called nexus.

The myofibrils are longitudinally divided into sarcomeres, showing the usual bands of striated muscle. The Z line is distinguished by its relatively marked density. The longitudinal distance between two adjacent Z line defines the limit of the sarcomere. I band, composed of thin filaments, extends toward the center of the sarcomere from each Z line with a longitudinal orientation. The central portion of the sarcomere is subdivided into lateral A bands with a central H band, which in turn is bisected by a central M line. The A band is composed of alternating thick filaments (myosin) and thin filaments (actin). The H band is traversed only by the myosin filaments in an orderly arrangement. The actin filaments appear to end at the junction of A band and H band. The M line in the center of the sarcomere is formed by a zone of segmental thickening of the myosin filaments. Cross-section of the myocardial cell makes it clear that the actin filaments are located at trigonal points in the hexagonal lattice of the myosin filaments (Fig. 1).

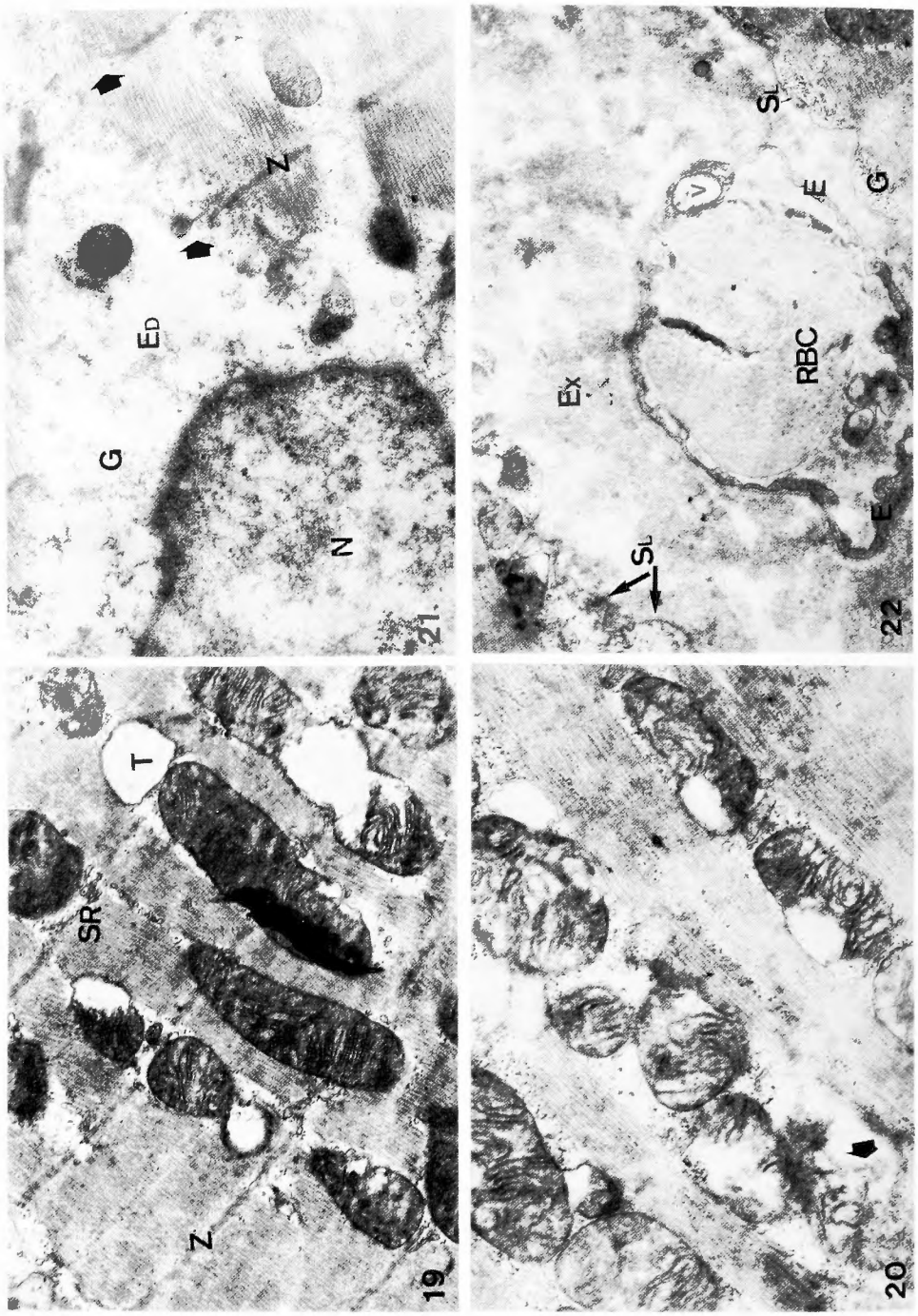
The sarcoplasm, cytoplasm of the myocardial fibers, consists of a slightly dense background, within which lipid droplets and glycogen granules are observed. The lipid droplets are usually found between the ends of the successive mitochondria aligned in clefts within the myofilament mass, and are located at the I band. Glycogen occurs as individual particles rather than in the form of the larger aggregates. The bulk of the glycogen is found in the cones of sarcoplasm at the pole of the nucleus and in the intermyofibrillar clefts in the contractile substance occupied by mitochondria and SR. It is also present in small amounts among the myofilament, in which it is preferentially located as single or as particles aligned in short rows between thin filaments of the I band.

Figures 11 to 18 illustrate the fine structure of the inner myocardial layer of the left ventricle. Myocardial protection was afforded by means of CBKC. Minor changes such as loss of detail in the cristae and occasional vacuolization of the mitochondria were visible, but the structure were well within normal limits during first 60 minutes after the onset of ischemia (Figs. 11, 12). During the next 60 minutes of ischemia, vacuolization of the mitochondria became pronounced. At this time moderate degree of disappearance of glycogen granules, and intermyofibrillar edema were observed (Fig. 14). The capillary still showed normal structure (Fig. 13). However, distinct abnormalities of the fine structure could be seen when periods of ischemia reached 150 minutes.

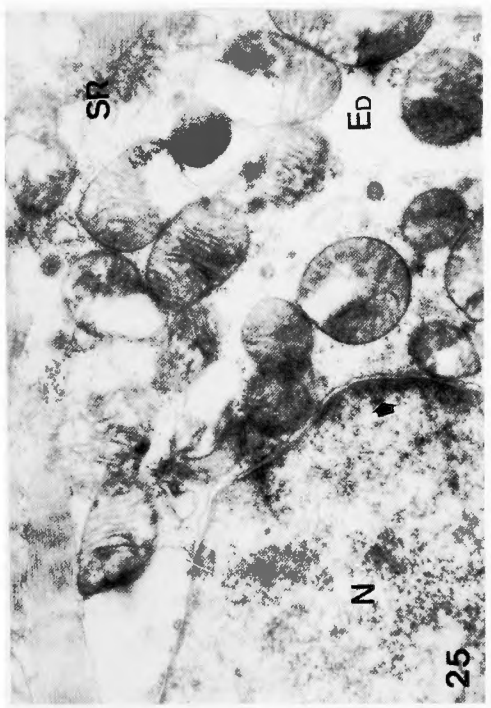
Figs. 15 to 18. Longitudinal sections of the inner layer of the left ventricular myocardium taken at 150 minutes after onset of ischemia. Slight swelling of the capillary endothelium (E) is shown in Fig. 15. The number of pinocytotic vesicles (Pv) in various sizes present in the mitochondria (MT) are disrupted. Vesiculation of the mitochondrial matrix and decrease in matrix density are clearly demonstrated in all of the figures. Marked decrease in the number of glycogen granules (G) in the subsarcolemmal space is noticeable (Figs. 15, 16, 18). Intracellular edema (ED) and myofibrillysis are also found in Figs. 15, 16, 18. Perinuclear edema is visible in Fig. 17. Chromatin in the nucleus slightly aggregates and also marginates. A nucleolus is located in the center of the nucleus. PR indicates nucleus pore. The intercalated disc (ID) does not seem to be widened. (CBKC, Original magnification ×11000)

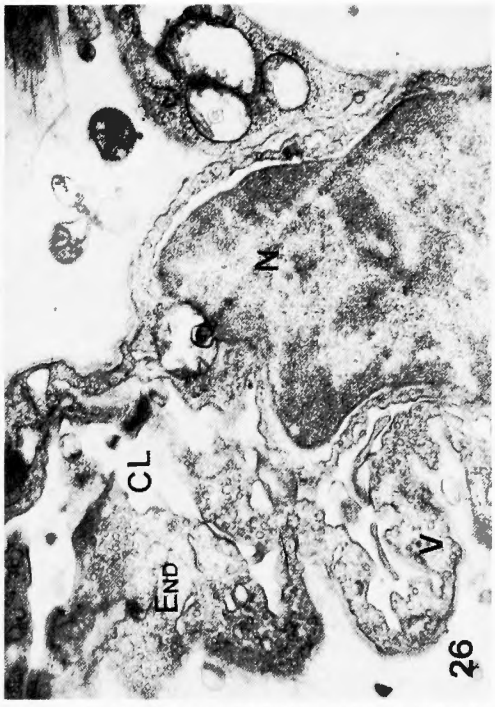


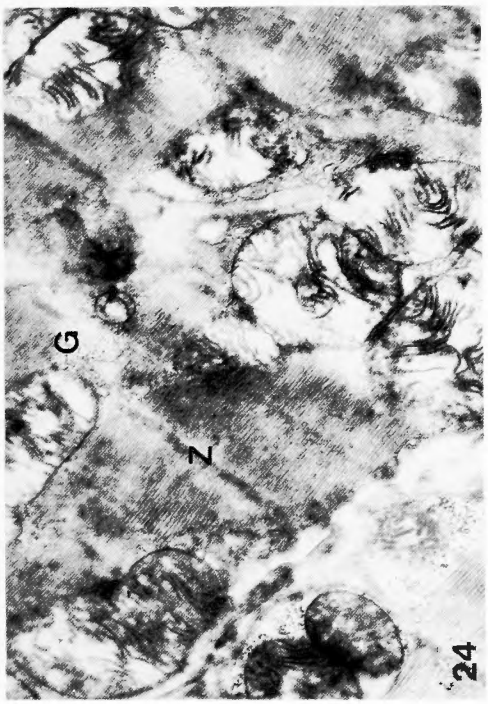
These include disruption of the cristae and decrease in matrix density of the mitochondria, marked decrease in the number of glycogen granules, perinuclear edema, myofibrillysis, aggregation and margination of nuclear chromatin, and swelling of the capillary endothelium with increased



pinocytotic vesicles. (Figs. 15 to 18). Figures 19 to 22 represent ultramicroscopic views of the outer myocardial layer of the left ventricle at 180 minutes after induction of ischemia. Even at this time the degree of severity of disintegration of the cellular structure in the outer layer was







same as or milder than that of the inner layer at 150 minutes of ischemia (Figs. 19 to 22). As illustrated in Figs. 23 to 26 the severe changes occurred in the fine structure of the inner myocardial layer as a result of prolonged ischemia. There was considerable variation in the size and

shape of the mitochondria with destruction of some and vacuolization of others. Clumping and margination of the nuclear chromatin are apparant in Fig. 25. There appeared marked intracellular edema with loss of alignment of the myofibrills. Few glycogen granules were present. Swelling of the endotheliaum of the capillary with numerous pinocytotic vesicles is readily apparent (Fig. 26). Higher magnification views (Figs. 27 to 30) confirm these observations mentioned above. Figures 31 to 36 illustrate ultramicroscopic views of the left ventricular myocardium after 120 minutes arrest, during which myocardial protection was afforded by means of GIKC and 20 minutes reperfusion with oxygenated blood. The fine structure of the outer third layer as well as the middle third were within normal range except for a few of mitochondria whose cristae are disrupted (Fig. 31 to 35). On the other hand, a micrograph in the inner third (Fig. 36) shows not only these mitochondrial changes but also contraction bands, dilatation of sarcotubular system, and mild grade loss of glycogen granules. Figure 37 illustrates a section of the myocardium taken at 180 minutes after the onset of ischemia. In this case the heart was intermittently reperfused with GIK solution throughout the periods of ischemia, but the stone heart developed. There was marked intracellular edema with loss of alignment of the myofibrills and distortion and fragmentation of the sarcomeres. Particularly significant was the observation that glycogen granules were depleted to the point of exhaustion. Swelling, clearing, vacuolization, and destruction of the mitochondria are readily visible. Moreover, the nucleus with clustering and margination of chromatin is observed in the lower center of the figure.

These ultrastructural changes of the myocardium in time course are summarized in Table 2.

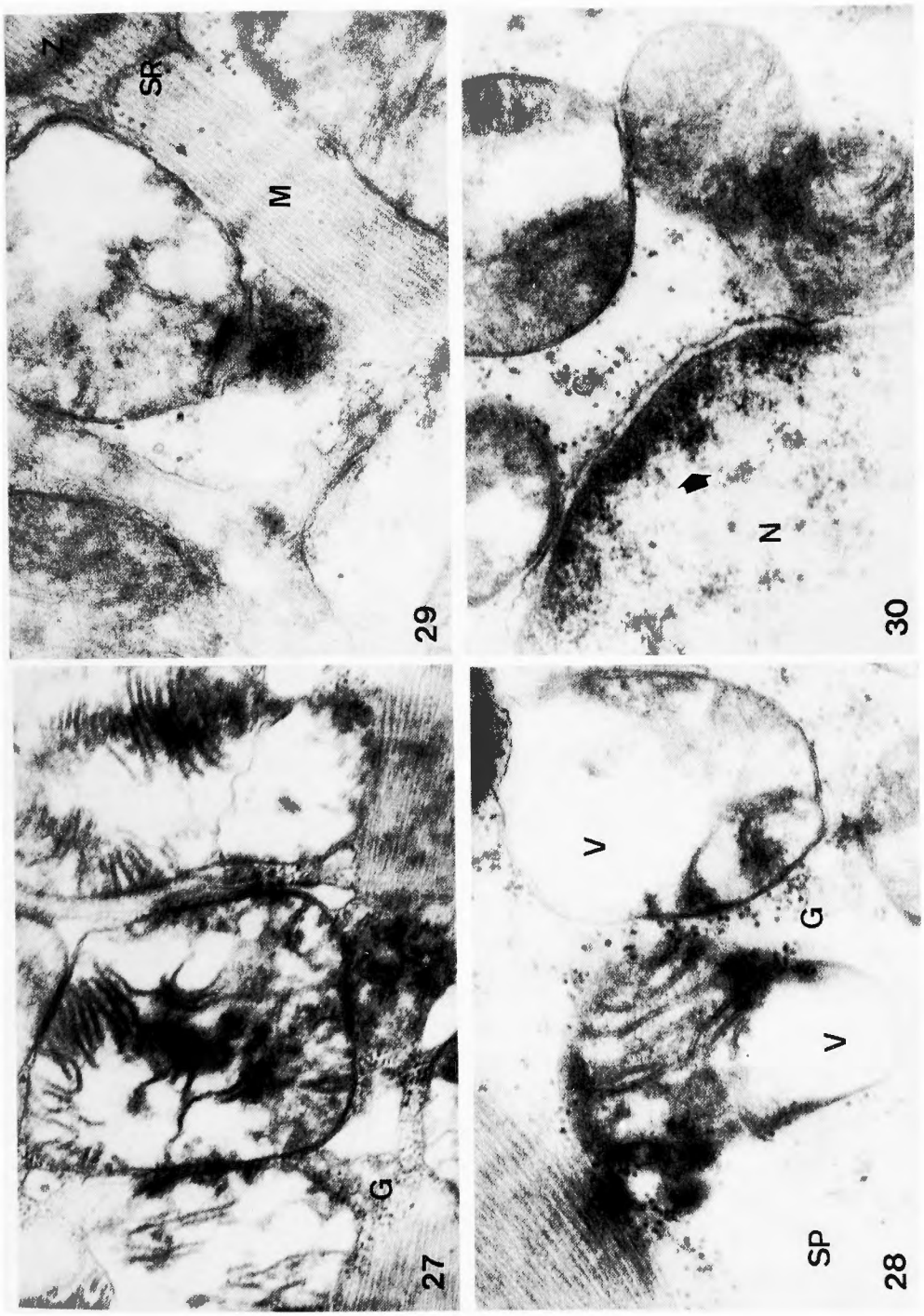
3) Variations in the level of ATP, ADP, AMP and adenylate energy charge in the myocardium (Table 3)

ATP, ADP, and AMP were found to be 3.78 ± 0.07, 0.89 ± 0.05, 0.13 ± 0.01 μmoles/g wet

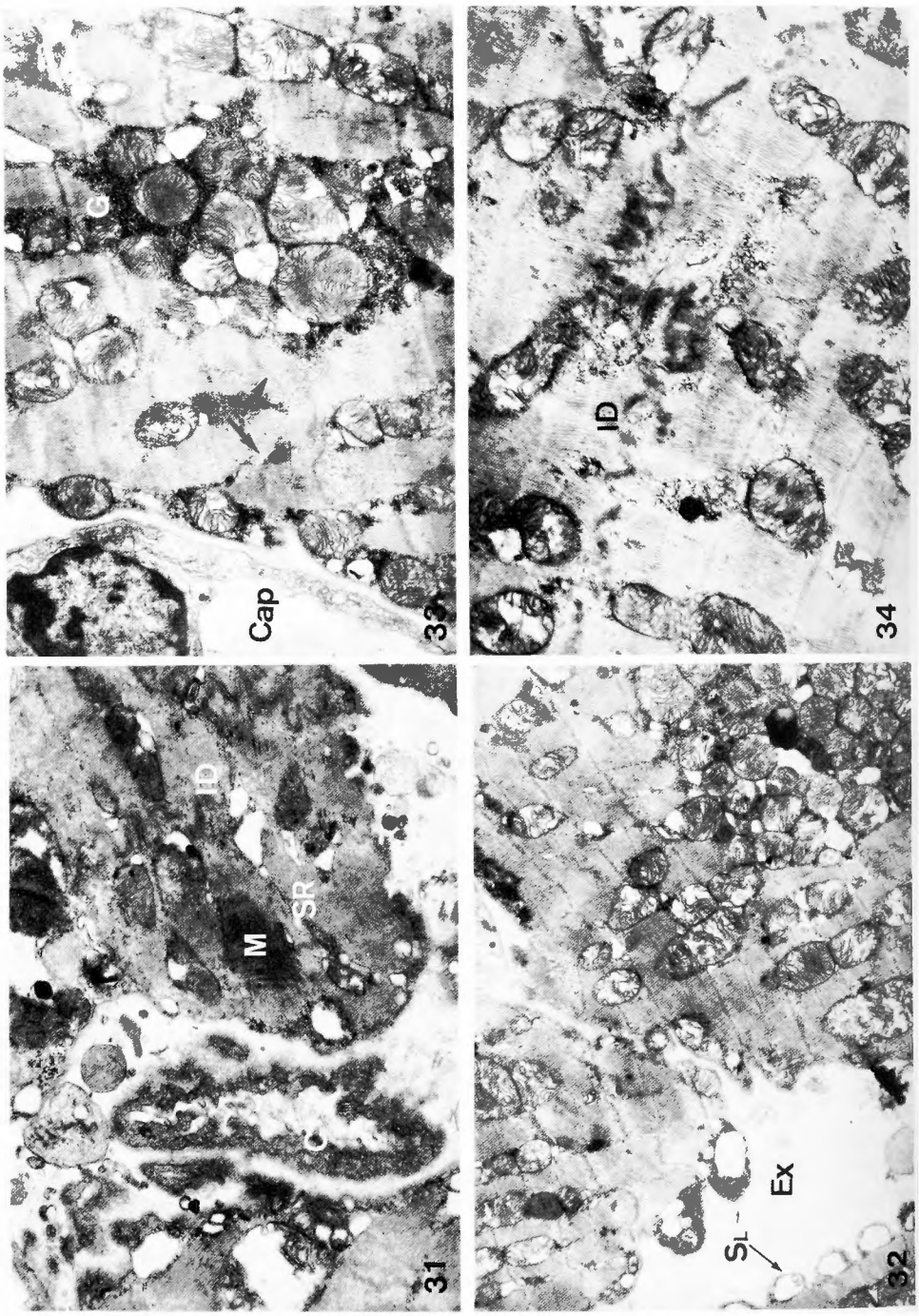
Figs. 19 to 22. Electron micrographs in longitudinal sections of the outer layer of the left ventricular wall taken at 180 minutes after onset of ischemia. A few of destructed mitochondria are visible in Fig. 19 and Fig. 20. The mitochondrion seen in the lower left area in Fig. 20 is completely destroyed, losing its original configuration. A slight to moderate degree of swelling and reduction in matrix density of the mitochondria are noticed. A moderately dilated T tubule is visible in Fig. 19. No, mild, and moderate intracellular edema are seen in Fig. 19, 20, 21 respectively. Glycogen particles markedly disappear both from the perinuclear region and from the intermyofibrillar space. Disruption of the myocardial fiber is evident in Fig. 21. Clustering and margination of nuclear chromatin is noticeable, but electron density in the nucleus does not appear to be reduced. The structure of the capillary shown in Fig. 22 appears normal. (CBKC, Original magnification ×8000 in Fig. 22, ×11000 in Figs. 19, 21, ×13000 in Fig. 20)

Figs. 23 to 26. Structure of the subendocardial myocardium seen in longitudinal sections taken at 3 hours after onset of global ischemia. Destruction of the mitochondria is prominent. Degree of intracellular edema, depletion of glycogen granules, margination of nuclear chromatin (arrow in Fig. 25), thickening and swelling of the endothelium (END) of the capillary within which a number of pinocytotic vescles in various sizes are visible, and possible dilatation of the sarcotubular system (SR) become pronounced. (CBKC, Original magnification ×6300 in Fig. 23, ×8200 in Fig. 26, ×11000 in Figs. 24, 25)

Figs. 27 to 30. Higher magnification views of the same sections seen in Figs. 23 to 26. Disruption of cristae and vesiculation of the mitochondrial matrix are well defined. Margination of nuclear chromatin is prominent. Glycogen granules disappear from the sarcoplasm. (CBKC, Original magnification ×30000)



weight respectively (for the following figures the units of measurement will be omited) just after the aorta had been clamped off. As the period of ischemia continued, ATP value decreased significantly. Similar changes in ADP and AMP levels were seen throughout ischemia; they



declined significantly within 60 minutes after the onset of ischemia, and increased to or near the preischemic levels thereafter. Values of adenylate energy charge (EC) at the onset of ischemia, and then during ischemia reached 60- and 120-minutes marks, were 0.93 ± 0.01, 0.91 ± 0.01,

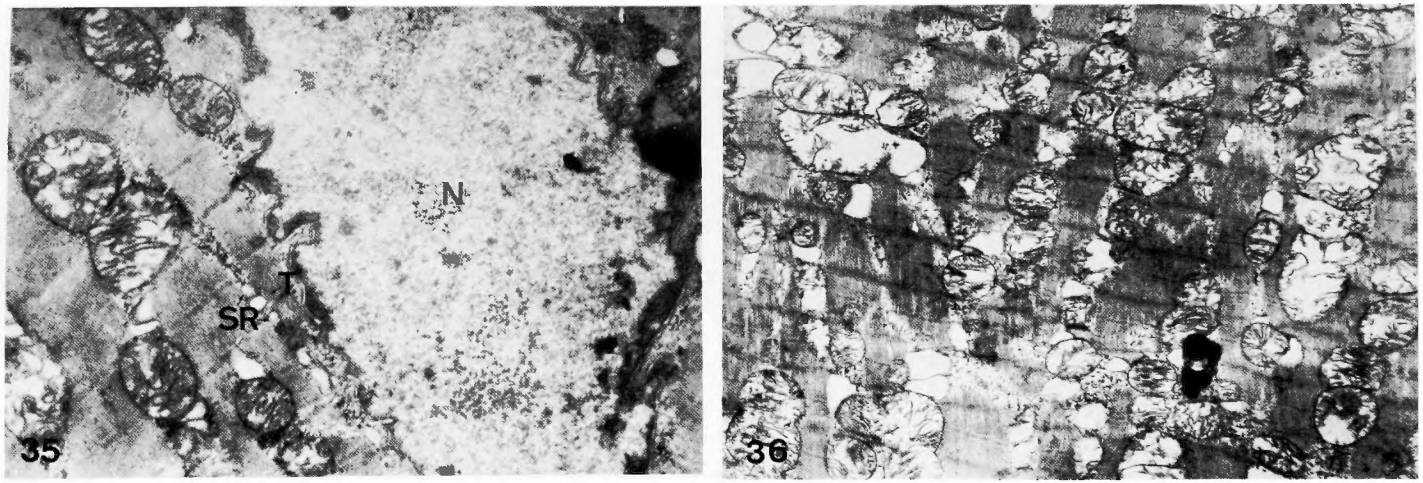


Table 2. Ultrastructural changes of the left ventricular myo-cardium proteted by either cold blood potassium cardioplegia or glucose-insulin-potassium cardioplegia.

Intracellular Structure	Ischemic Change	Cold Blood Potassium cardioplegia									Glucose - Insulin - Potassium cardioplegia								
		Duration of Ischemia (minutes)																	
		0~60			90~120			150~180			0~60			90~120			150~180		
		Epi	Mid	Endo	Epi	Mid	Endo	Epi	Mid	Endo	Epi	Mid	Endo	Epi	Mid	Endo	Epi	Mid	Endo
Mitochondria	Swelling, distortion, vacuolization disruption of cristae decrease in matrix density	−	−	±	±	±	++	++	+	+++	±	±	+	+	+	++	++	++	+++
Glycogen Granule	depletion	−	−	±	−	−	±	++	+	++	−	−	±	+	+	++	++	++	+++
Nucleus	Swelling, decrease in matrix density clustering and margination of chromatin	−	−	−	−	−	+	+	±	++	−	−	−	±	±	+	+	+	++
Sarcotubular System	dilatation	−	−	−	−	−	±	+	±	++	−	−	±	+	+	++	+	+	++
Myofibrill	distortion, fragmentation myofibrillysis intermyofibrillar edema	−	−	−	±	±	++	++	+	+++	−	−	+	+	+	++	++	++	+++
Intercalated Disc	Separation	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

Legend: Epi: Outer myocardial layer; Mid: middle myocardial layer; Endo: inner myocardial layer (−): no ischemic damage; (±): no or slight, if present, damage; (+): mild damage; (++): moderate damage; (+++): severe damage

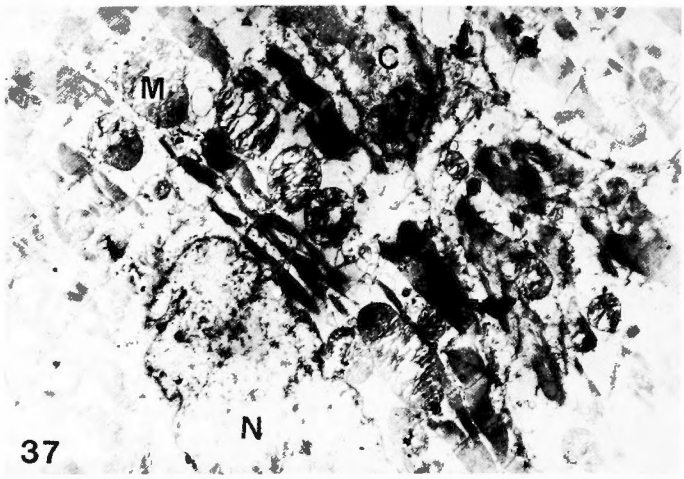


Fig. 37. A micrograph of longitudinal section of the inner layer of the left ventricular myocardium taken at 3 hours after onset of global ischemia. The heart in this case showed signs of 'stone heart' despite myocardial protection afforded by means of GIKC. The cellular structure is severely disintegrated. The mitochondria are swollen. Distruption of cristae is prominent. Matrix density in the mitochondria is reduced. Nuclear chromatin is also reduced. Glycogen granules completely disappear. Intracellular edema and myofibrillysis are remarkable. (GIKC, Original magnification ×3200)

and 0.87±0.01, respectively. After 180 minutes of ischemia, EC significantly declined (Fig. 38)

4) Variations in PO_2, PCO_2, pH, Base Excess, and lactate level in coronary sinus effluent (Table 4)

PO_2, PCO_2, pH, base excess (BE), and lactate level in coronary sinus blood during partial bypass are shown in Table 4. Dogs were in an alkaline state as shown in these data. Values of PO_2, PCO_2, and lactate significantly increased within 30 minutes after the onset of ischemia. pH and BE, on the other hand, showed significant decrease at that time. However, there was little change in all of these parameters thereafter.

Discussion

In cardiac surgery, most corrective procedures as well as myocardial revascularization require a period of cardiac arrests. In the early days of cardiac surgery, the concept of being able to stop the heart and then restart it after the operation seemed the optimal method for

Fig. 31, Figs. 32 to 35, and Fig. 36. Electron micrographs of longitudinal or oblique sections of the outer, middle, and inner myocardial layers of the left ventricle respectively taken after 2 hour ischemic arrest and 20 minutes reperfusion. Throughout the ischemic period myocardial protection was performed by means of GIKC. The ultrstructure both in the outer and in the middle layers is well preserved except for damaged mitochondria. A cluster of the mitochondria and glycogen granules are clearly observed in Figs. 32, 33. In the inner layer, on the other hand, the degree of cellular disintegration caused by ischemia and reperfusion seems to be severe. Numerous mitochondria are swollen. Matrix density in the mitochondria is markedly reduced. The number of glycogen granules has not recovered to the preischemic level. Contraction band is visible in Fig. 36. (GIKC. Original magnification ×3200 in Fig. 36, ×4500 in Fig. 32, ×5600 in Fig. 33, ×8200 in Figs. 31, 34, 35)

Table 3. Changes in the level of ATP, ADP, AMP, and EC of the left ventricular myocardium protected by means of cold blood potassium cardioplegia.

		Duration of ischemia (minutes)			
		0	60	120	180
Adenosine phosphate	ATP	3.78 ± 0.07†	2.55 ± 0.10*	2.16 ± 0.12*	1.79 ± 0.11**
	ADP	0.89 ± 0.05	0.35 ± 0.02*	0.60 ± 0.06	0.60 ± 0.05
	AMP	0.13 ± 0.01	0.06 ± 0.01*	0.14 ± 0.03	0.15 ± 0.02
EC		0.93 ± 0.01	0.91 ± 0.01	0.87 ± 0.01	0.82 ± 0.01*

† μmoles/g wet weight
Legend: ATP adenosine triphosphate
ADP adenosine diphosphate
AMP adenosine monophosphate
EC adenylate energy charge
Data are expressed as mean±standard error of mean
*P<0.01, **P<0.001, n=9

providing ideal surgical conditions and still protect the myocardium from ischemic insult by decreasing oxygen demand. Since myocardial oxygen consumption is rate- and rhythm-dependent[17], chemically induced cardiac arrest should result in minimal oxygen consumption and superb myocardial protection. Potassium-induced cardioplegia is one method that appears to be beneficial in this setting.

Melrose and associates[76] employed cardioplegia induced by potassium citrate in 1955. They did three sets of experiments using this agent to arrest the heart. In the initial experi-

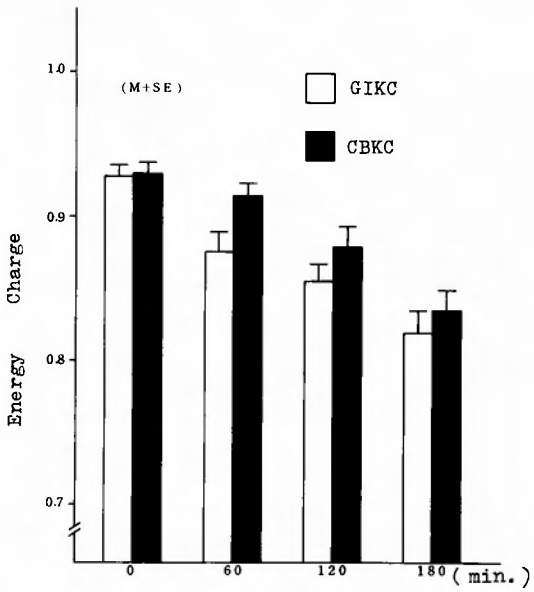


Fig. 38. Changes in myocardial adenylate energy charge. The energy charges of CBKC are slightly higher than those of GIKC throughout ischemic period, but no significant difference between both methods is found.

Table 4. Chages in PO_2, PCO_2, pH, BE, and lactate level in the coronary sinus effluent.

		PO_2 (mmHg)	PCO_2 (mmHg)	pH	Bace Excess	Lactate (mg/dl)
During Bypass		33 ± 3	32 ± 3	7.61 ± 0.03†	10.3 ± 0.1	26 ± 6
During Ischemia (minutes)	30	40 ± 4**	52 ± 6***	7.26 ± 0.06†	−5.6 ± 1.7†	47 ± 6†
	60	41 ± 1*	63 ± 10**	7.22 ± 0.06†	−3.9 ± 1.0†	54 ± 6†
	90	41 ± 4***	71 ± 10***	7.20 ± 0.05†	−3.1 ± 0.7†	52 ± 5†
	120	40 ± 1**	70 ± 9***	7.23 ± 0.05†	−1.6 ± 1.4†	50 ± 4†
	150	39 ± 2**	72 ± 8***	7.21 ± 0.04†	−2.3 ± 1.5†	45 ± 4†
	180	37 ± 2NS.	69 ± 7***	7.21 ± 0.03†	−3.3 ± 1.8†	52 ± 4†

$n=6$, *P<0.05, **P<0.01, ***P<0.005, †P<0.001 (M±SE)

ments, they found 25 to 100 mg of potassium citrate (potassium, about 250 to 1000 mEq/l) resulted in persistent ventricular fibrillation and poor ventricular function. In the second set of experiments, they used 1 to 5 mg of potassium citrate (potassium, 10 to 50 mEq/l) and achieved consistent recovery of electrical activity and force. In the third set of experiments, they used 2 ml of 25% potassium citrate diluted to 20 ml with blood (potassium, 250 mEq/l). In spite of the high concentration of potassium, identical to that in the first set of experiments, they found good electrical recovery in the third set of experiments. These experiments showed that 10 to 50 mEq of potassium chloride could induce temporary cardiac arrest, which was almost completely reversible even after 45 minutes. This result promised a method of achieving excellent surgical conditions, as well as superb myocardial protection. Unfortunately, the results were interpreted as suggesting that 250 mEq of potassium or more be used, and in a variety of experiments and clinical trials following up their works[59,74,96], persistent ventricular fibrillation, depressed ventricular function and focal inflammatory lesions in the myocardium were seen with potassium-induced arrest. As a result, Melrose solution fell into disrepute and the technique was abandoned thereafter. However, interest in potassium-induced cardioplegia was revived in the early 1970s. Gay and Ebert[38] reintroduced this method with good results in 1973. They used an isoosmotic solution containing 12 to 25 mEq/l of potassium, and found experimentally that left venitrcular function was hardly depressed in dogs undergoing 60 minutes of potassium-induced arrest even with normothermia. They examined microscopic changes of the arrested heart with potassium chloride and noted only mild interstitial edema. Tyers and associates[91] pointed out that the reason for the deleterious effect of Melrose solution was due to the excessively high concentration of potassium (more than 250 mEq) and the increased osmolarity (greater than 400 mOs). They found that both of these two factors increased the microscopic damage to the myocardium and resulted in depressed left ventricular function, and concluded that 10 to 40 mEq potassium was the safe range for cardioplegia. Since that time, a variety of experimental[28,45,80] and clinical[2,23,29] experiences concerning potassium-induced cardioplegia (with hypothermia) have been reported with good results up to today.

However, there is not a single best method for myocardial protection. In recent years there has been general agreement concerning the fundamental principles of myocardial protection.

These include immediate arrest of the heart in diastole, uniform cooling of the heart, and prevention of resumption of electromechanical activity during an ischemic period. All of these conditions can be achieved by cardioplegia in conjunction with hypothermia. The theoretical basis is that cardioplegic solutions result in prompt arrest preventing expenditure of ATP stores in unproductive electromechanical work, and further addition of hypothermia results in lowering the rate of myocardial metabolism and thereby reduces the demand of ATP expenditure during the period of ischemia. It is believed that basal metabolism of the noncontracting heart accounts for 15 to 20 percent of energy generated by the heart; the energy required for electrical activity is less than 1 percent; that for contractile work is approximately 80 percent of total cardiac energy consumption. WRIGHT and associates[99] show that substantial ATP stores can be expended during the biref period of electromechanical activity before pharacologic cardioplegia is produced by asanguineous solutions. Accordingly, diastolic arrest of the heart would be achieved as rapid as possible immediately after aortic cross-clamping.

Nevertheless, depletion of ATP levels in the ischemic myocardium cannot be prevented unless some interventions promoting production of ATP are carried out, because aerobic and anaerobic metabolism persists even when metabolic demands in the myocardium can be reduced by means of hypothermic potassium cardioplegia.

Glucose-insulin-potassium (GIK) cardioplegia affords myocardial protection not only by reducing expenditure of ATP, but also by replenishing ATP stores in the myocardium via anaerobic metabolism during ischemia. LOLLEY and associates[66] demonstrated that the ischemically arrested heart utilizing glycogen had improved function. Enhanced glycogen stores have been shown to result in greater production of ATP during anoxia.[67,84] HEWITT and associates[46] noticed an increase in cardiac glycogen levels in dogs fed on all-fat diet for three days and subsequently demonstrated improved postischemic left ventricular function in comparison with control animals. AUSTEN and associates[10], studying anoxic arrest in dogs, also demonstrated improved postischemic left ventricular performance after injecting 200 ml of solution containing glucose and oxygenated blood into the aorta immediately after cross-clamp, giving a perfusate glucose level of 2,000 to 3,000 mg/dl. The amount of ATP produced by glycolysis, however, was too small to maintain good function and structural integrity of the myocardium with prolonged periods of ischemia.

Cold blood potassium cardioplegia (CBKC), on the other hand, is a means for myocardial protection by which blood constituents and cardioplegic media are given intermittently to the ischemic myocardium. In 1978, FOLLETTE and associates[34] introduced this method with good results as an alternative to asanguineous cardioplegia for the first time. Administration of blood during cardioplegic perfusion can prevent depletion and promote the production of ATP during ischemic arrest, thus theoretically affording a greater degree of protection to the myocardium during the arrest interval. They point out several advantages of CBKC. These include 1) the heart is arrested in an oxygenated environment so that there is no loss of high-energy phosphate stores during the short period of electromechanical activity preceding asystole, 2) the heart is intermittently reoxygenated when the blood cardioplegic solution is replenished at

20 minutes interval, 3) intermittent reoxygenation reduces or avoids the need to add extra glucose and insulin to provide substrates for prolonged anaerobic metabolism, 4) blood allows the onconicity which must otherwise be added as plasma protein, dextran, or mannitol, 5) need for pharmacholgic preparation and storage are reduced, because the solution can easily be made up by adding the necessary components to the cardioplegic reservoir at the beginning of extracorporeal circulation. Since that time a number of experimal[13,30,49,60] and clinical[14,24,31,86] results showing the superiority of sanguineous to asanguineous cardioplegia have been reported. The present study also demonstrated the superiority of CBKC to GIKC when assessed morphologically and biochemically.

However, despite the attempts to prevent the myocardium from ischemic insult, the present experimental study confirmed that the occurrence of depletion of high-energy phosphates, production of lactate, proton, and CO_2 in the myocardium, and disintegration of the cellular structure could not be avoided when the periods of ischemia were extended. These unfavorable results suggest that the ischemic myocardium, even protected by means of CBKC, still carries on anaerobic metabolism. The reason for it is not clear, but a possible explanation was postulated by DIGERNESS and associates[25] who show that profound myocardial hypothermia inhibits oxygen delivery with sanguineous media. They measured repayment of oxygen debt by periodic reperfusion of the ischemic myocardium from the view point of the oxygen deliverability of the infusate, and concluded that oxygenated crystalloid media could deliver as much oxygen as the sanguineous media at 10 to 20°C Similar results were reported by MAGOVERN and associates[72]. On the contrary, ENGELMAN and associates[30] found that blood cardioplegia carried nearly 6 vol% of oxygen to the heart and proved superior to either nonoxygenated (0.5 vol%) or oxygenated crystalloid perfusate (3 vol%) in retaining the high-energy phosphates during a 3-hour arrest. For the purpose of delivering more oxygen to the ischemic myocardium, KANTER[52] and ROUSOU[82] with respective co-workers proposed the use of fluorocarbon (fluorcarbon cardioplegia) which carries and supplies oxygen to the tissue at large quantities than blood even at a profound temperature.

It is interesting that the electron microscopic study in the present experiments show that the destruction of the mitochondrial structure and depletion of glycogen granules already occurred in the earlier period of ischemia and became more prominent in proportion to the prolongation of the ischemic periods, especially in the inner layer of the left ventricular myocardium. This result suggests that there is a great difference in susceptibility and metabolism between the myocardial layers. Although there is no transmural ATP gradient in non-ischemic myocardium, it is clearly observed in the ischemic heart in vivo[3,22,41]. Perhaps the enzymatic constitution of the inner layer differs from the outer layers in such a way that accelerated ATP depletion occurs in the inner zone. Either a temperature or a pressure gradient could explain the transmural ATP gradient.

Next we will turn out attention to the metabolic processes taking place in the interior of the cell which bring about such metabolic and morphological deteriorations.

The cardiac muscle cell is known to be rich in the mitochondria, which occupy nearly 40 to

50% of the myocardium. The mitochondria consist of two membraneous layers, an outer and inner layer, and a matrix. The inner membrane infolds toward the matrix and forms a crista. According to Hagihara, the chemical composition of the outer membrane includes such enzymes as cytochrome b_5, monoamine oxidase, and acyl-CoA synthetase, while the inner membrane contains enzymes and enzyme systems. These include the electron transport system, β-hydroxybutylate dehydrogenase, and ATP/ADP translocase. In the matrix are located enzymes involved in the TCA cycle and also enzymes involved in β-oxidation of free fatty acids. Therefore it is thought that the basic function of the mitochondria is to metabolize nutrients such as carbohydrates, fats, and proteins in the course of the TCA cycle and β-oxidation of free fatty acids (FFA), and also to synthesize ATP by means of oxidative phosphorylation linked with electron transport. Thus, as mentioned above, substrate such as glutamic acid, and citric acid can pass electrons and protons to bound nicotine adenine dinucleotide (NAD), after which there is a sequential passage of electrons and protons down the electron transport chain to the ultimate electron acceptor, oxygen. During this process, specific amounts of ATP are synthesized (Fig. 39). This sequential metabolic process, which is normally carried out in the myocardium, is called aerobic metabolism. However, when the coronary circulation is interrupted, the myocardium can no longer perform this function. Consequently, there occurs a marked decrease in oxygen tension in the myocardium once it undergoes ischemia. The mitochondria lack adequate amounts of oxygen for acceptance of hydrogen product of the process. As a result, the electron transport system becomes reduced. Soon cytoplasm becomes reduced as well. This activates glycolysis and glycogenolysis in the cytoplasm through the Embden-Meyerhof cycle in order to enhance ATP formation (3mols per hexose from glycogen, 2 mols from glucose). With increas-

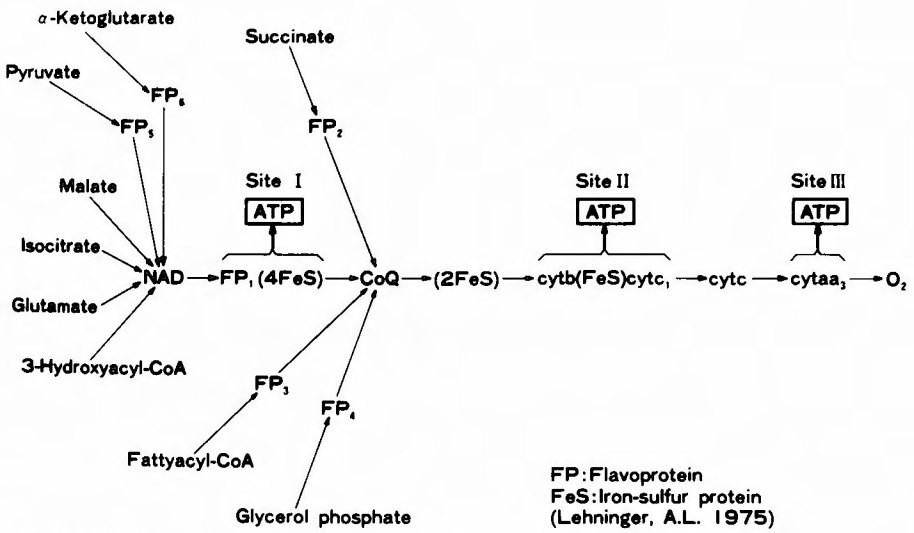


Fig. 39. Electron transport system in mitochondria. Substates such as α-ketoglutarate, glutamate, and malate can pass protons and electrons to bound NAD, after which thefe is a sequential passage of electrons down the electron transport system to the ultimate electron acceptor, oxygen. During this process, ATP is synthesized.

ing glycolysis, lactate is formed rapidly and accumulates in the cytoplasm. NADH, a reduced form of nicotine adenine dinucleotide, also accumulates both in the cytoplasm and in the mitochondria, because it cannot be oxidised to NAD^+ by the oxidative pathways. This is confirmed by CHIBA, a co-worker in the present experiments, who studied a redox state of NAD within the myocardium by means of microfluorometry. His microfluorometric study showed that the intensity of the emitted fluorescence (NADH fraction) increased promptly as soon as the heart was placed in global ischemia. Thus, pyruvate in the cytoplasm becomes, like oxygen, the hydrogen acceptor. Lactate is generated as an end-product of anaerobic metabolism, and when its concentration rises, may overflow into the tissue space and ultimately diffuses into the capillaries and thence to coronary venous blood flow. The production of lactate in the cytoplasm is one of the causes of reduced pH in the cell (lactic acidosis). Since acidosis inhibits limiting enzymes of glycolytic pathway such as phosphofructokinase[73,93] and glyceraldehyde 3-phosphate dehydrogenase[65,95], glycolysis soon ceaces. This is followed by cessation of production of ATP, because the myocardium has neither de novo nor salvage pathways to synthesize ATP. Considering the fact that the process of contraction and relaxation of the cardiac muscle, active transport of some kinds of ions and nutrients across the cell membrane, and when necessary, protein synthesis are all require tremendous chemical energy released when ATP hydrolyzes, it can easily be understood that the maintenance of mechanical work and membrane stability becomes difficult when the amount of ATP in the myocardium falls off to such level as 2 to 3 μmoles/g dry weight[50]. It is well known that excessive depletion of high-energy phosphates ultimately results in ischemic contracture of the heart. The term 'rigor mortis'[40,68] or 'stone heart'[7,15,20,100] is also used to express this state. GAARSH and associates[36] found an increase in wall thickness at the onest of contracture. The contracture is implicated as part of a mechanism of decreased ventricular compliance or increased stiffness following ischemia.[4,90] KATZ and TADA[53,54] suggest that ischemic contracture might be secondary to loss of energy stores in the region of the cardiac cell occupied by the myofilament. According to HEARSE and associates[44] ischemic contracture represents a state of severe metabolic deterioration that results in rigor bond formation between actin and myosin filaments when intracellular ATP stores decrease below a critical level. In the present experiments although the level of ATP in the myocardium, when protected by means of CBKC, markedly decreased to 1.79 microgram per gram wet weight (not expressed as dry weight) after 180 minutes of aortic cross-clamping, none of the hearts showed signs of the ischemic contracture. According to JONES and associates[51] metabolic deterioration during global ischemia is not related merely to time or temperature. Despite the specific degrees of metabolic deterioration associated with the events of contracture, initiation was variable, ranging from 29 to 72 minutes for initiation and 60 to 101 minutes for completion. Ischemic contracture of the heart can be reduced by propranolol and/or hypothermia[21,70,101]. Intermittent myocardial stretch during ischemic arrest also can prevent a decrease in diastric compliance without decreasing recovery of contractile function[5,77].

In addition, GAZITT and associates[39] suggest one potential molecular mechanism through which ATP depletion can lead to the disruption of the plasma membrane of ischemic cells.

Their data indicate that defective phosphorylation of membrane proteins by ATP is the cause of increased susceptibility. Thus, the absence of ATP for phosphorylation of membrane proteins may indirectly lead to disruption of the sarcolemma. The Na^+-K^+-activated, Mg^{++}-dependent ATPase of the sarcolemma is essential for maintenance of cell volume. HOFFMAN[47] shows that this ATPase is phosphorylated in the cell membrane during the course of transport of sodium and potassium across the sarcolemma, and that this phosphorylation requires intracellular ATP: Na^+/K^+ exchange does not occur without this step. Accordingly, depletion of ATP near the sarcolemma would result in loss of cell volume control followed by increase in water content in the myocardium. Similar results have been reported by many investigators.

Evidence also indicates changes in myocardial pH during ischemia. The study of BENZING and associates[16] who measured directly hydrogen ion activity in the interstitial space with pH-sensitive microelectrodes showed that arterial occlusion produced a rapidly reversible decrease in interstitial pH. They found that the interstitial pH decreased by 0.24 unit after 3 minutes and by 0.6 unit or more after 15–30 minutes. WALTERS and associates[94] have developed their own electrode system to measure myocardial interstitial pH as an index of myocardial metabolism during cardiac surgery. Their data suggest that intramyocardial pH measurements reflect intracellular metabolism during elective arrest of the heart. WILSON and associates[97], using an intramyocardial pH needle probe inserted about 10 mm into the left ventricular free wall, studied the effect of potassium cardioplegia on myocardial metabolism at both moderate (27°C) and deep (17°C) hypothermia. Their results show that pH in the myocardium, regardless of the myocardial temperature, declines markedly in proportion to the duration of ischemia and, moreover, potassium cardioplegia does little to further reduce the rate of anaerobic metabolism by the measurement of intramyocardial pH, under conditions of deep hypothermia. Although direct measurement of intramyocardial pH was not carried out in the present study, pH measured in the coronary sinus effluent revealed marked decrease from 7.55 to 7.20 after 30 minutes of ischemia. However, little change in pH in the coronary sinus blood was observed beyond that time.

It is well recognized that carbon dioxide also accumulates as a result of anaerobic metabolism[32,37,55,71,78]. The rise in the intramyocardial carbon dioxide tension ($Pmco_2$) during prolonged anoxia is probably caused by a shift in the bicarbonate buffer system in the myocardium as a result of lactic acid production, with the subsequent production of free carbon dioxide as shown by SCHEUER[85], and SHEA and associates[87]. MACGREGOR and associates[71] continuously monitored $Pmco_2$ by means of mass spectrometry to examine the safe period of anoxic arrest of the heart. They found that an nitial period during which the $Pmco_2$ increased in a linear fashon was followed by a period during which the rate of rise gradually decreased until a plateau was reached. Their findings that all the hearts could be resucitated if the arrest was terminated at the transitional point between these two periods, but none of the hearts could be resucitated if the arrest was terminated when the $Pmco_2$ curve had reached plateau defines a point at which anoxic arrest of the heart can be safely terminated. This point can be significantly extended by reducing metabolic activity of the hearts by hypothermia[70]. KHURI and associates[56] evaluated the

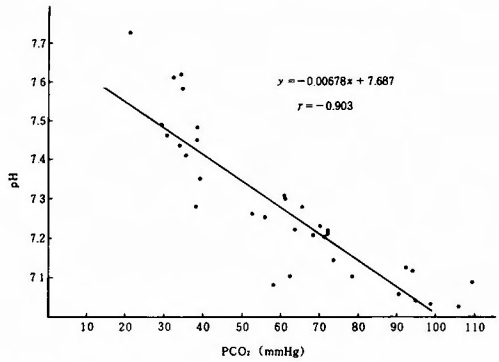


Fig. 40. Relationship between PCO_2 and pH in the coronary sinus effluent. A close correlation is noted between both parameters.

usefulness of changes in Pmo_2 and $Pmco_2$ shortly after coronary artery occlusion as indices of the severity of myocardial ischemic injury, and concluded that the decline in $Pmco_2$ during the 60-minutes occlusion bore no relationship either to the severity of ischemic injury as assessed by histological examination, or to the reduction of regional myocardial blood flow. In contrast, the magnitude of rise in $Pmco_2$ during the 60 minutes of occlusion corresponded closely to both the severity of injury assessed histologically and the reduction of regional myocardial blood flow. CASE and associates[18] measured extracellular Pco_2 with a micro-Pco_2 electrode and concluded that extracellular and myocardial Pco_2 was essentially equal. The data presented in this paper showed that Pco_2 measured in the coronary sinus effluent abruptly increased approximately 200% after aortic-clamping for 60 minutes, but there was little further increase thereafter. A possible explanation for this is that, with multidose chemical cardioplegia, carbon dioxide was washed out not only from the intracellular space but also from the extracellular space. According to MCGREGOR and associates[70,71] the point where the $Pmco_2$ curve formed a plateau was indicated as that of the onset of ischemic contracture of the arrested heart. In the present study, however, none of the arrested hearts, except for the one that was protected by GIKC, showed signs of the stone heart even at 3 hours of ischemia at all. This implies that the plateau formation in a $Pcsco_2$ curve may not always indicate the onset of contracture of the ischemic heart.

The finding obtained in the present experiments that pH and Pco_2 closely correlates when measured in the coronary sinus effluent (Fig. 40) is similar to that of WALTERS and associates[94]. Surprisingly, however, there was almost no correlation between pH and the lactate level in the coronary sinus effluent (Fig. 41). This result suggests that the influence of Pco_2 on pH is a higher than that of the lactate level.

In summary, when global ischemia of the myocardium was induced by cross-clamping of the aorta, and myocardial protection was carried out by means of either cold blood potassium cardioplegia (CBKC) or glucose-insulin-potassium cardioplegia (GIKC), the following observations were made:

1. Ischmic contracture of the myocardium (stone heart) did not occur except in the one that was protected by means of GIKC.

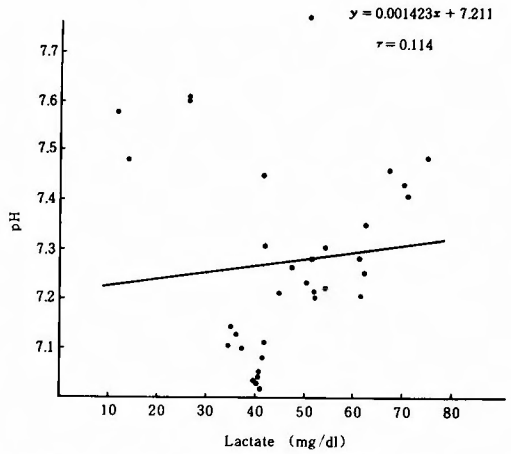


Fig. 41. Relationship between lactate level and pH in the coronary sinus effluent. There is no correlaton between these parameters.

2. Destruction of the ultrastructure of the myocardium was prominent in the inner myocardial layer, moderate in the outer, but mild in the middle. Destruction of mitochondria and loss of glycogen granules were the first ultrastructural changes. The ultrastructure of the myocardium was relatively well preserved for 2 hours after onset of ischemia, especially in the case of CBKC However, the degrees of severity of these structural changes became greater beyond that time.
3. A decrease with time was recognized in levels of adenylate energy charge in the myocardium. This decrease was larger, but not significant, in the case of GIKC than in CBKC.
4. Marked increase in levels of lactate and carbon dioxide tension, but a decrease, on the other hand, in pH values in coronary sinus effiuent were found during the first 30 minutes of ischemia. However, there was little change in these parameters thereafter. This suggests that the myocardium still carries on anaerobic metabolism despite intermittent reoxygenation of the arrested heart by means of CBKC.

It is concluded from these results that CBKC is superior to GIKC for myocardial protection during global ischemia. However, even with CBKC, the occurrence of metabolic and morphological deteriorations could not be prevented. This demonstrates the limitation of CBKC. Therefore, further research to establish the best cardioplegic method that can prevent the myocardium from ischemic injury is required.

Acknowledgement

The author is grateful to Professor YORINORI HIKASA for overall instruction, and Assistant professor NORIKAZU TATSUTA for his helpful guidance.

The author also thanks to Dr. YUKIO CHIBA for his constant collaboration and presentation of data and helpful discussion, and to Miss SACHIKO YUASA for typing this manuscript.

References

1) Adams Px, Cunningham JN Jr, et al: Clinical experience using potassium-induced cardioplegia with hypothermia in aortic valve replacement. J Thorac Cardiovasc Surg **75**: 564–568, 1978.
2) Akins CW, Buckley MJ, et al: Myocardial protection with hypothermia and potassium cardioplegia during operation for ascending aortic aneurysms. J Thorac Cardiovasc Surg **79**: 700–704, 1980.
3) Allison TR, Ramey CA, et al: Transmural gradients of left ventricular tissue metabolite after circum flex artery ligation in dogs. J Moll Cell Cardiol **9**: 837–852, 1977.
4) Apstein CS, Mueller M, et al: Ventricular contracture and compliance changes with global ischemia and reperfusion, and their effect on coronary resistance in the rat. Circ Res **41**: 206–217, 1977.
5) Apstein CS, Ogilby JD: Effects of paradoxical systolic fiber strech on ischemic myocardial contracture, compliance, and contrcatility in the rabbit. Circ Res **46**: 745–754, 1980.
6) Arciniegas E, Farooki Z, et al: Early and late results of total correction of Fallot. J Thorac Cardiovasc Surg **80**: 770–778, 1980.
7) Armstrong RG, Stanford W, et al: The stone heart. Ann Thorac Surg **16**: 480–491, 1973.
8) Atkinson DE: The energy charge of the adenylate pool as a regulatory parameter interaction with feed back modifiers. Biochemistry **7**: 4030–4034, 1968.
9) Atkinson DE: Cellular energy metabolism and its regulation, New York, Academic Press, 1977.
10) Austen WG, Greenberg JJ, et al: Myocardial function and contractile force affected by glucose loading of the heart during anoxia. Surgery **57**: 839–845, 1965.
11) Balderman SC, Bhayana JN, et al: Perioperative myocardial infarction: a diagnostic dilemma. Ann Thorac Surg **30**: 370–377, 1980.
12) Balderman SC, Bhayana JN, et al: Perioperative protection of the myocardium in patients with impaired ventricular function. Ann Thorac Surg **33**: 445–452, 1982.
13) Barner HB, Lacks H, et al: Cold blood as the vehicle for potassium cardioplegia. Ann Thorac Surg **28**: 509–521, 1979.
14) Barner HB, Kaiser GC, et al: Clinical experience with cold blood as the vehicle for hypothermic potassium cardioplegia. Ann Thorac Surg **29**: 224–227, 1980.
15) Baroldi G, Milam JC, et al: Myocardial cell damage in "stone heart". J Moll Cell Cardiol **6**: 395–399, 1974.
16) Benzing H, Gebert G, et al: Extracellular acid-base changes in dog myocardium during hypoxia and local ischemia, measured by means of glass microelectrodes. Cardiology **56**: 85–88, 1971/1972.
17) Braunwald E: Control of myocardial oxygen consumption. Physiologic and clinical considerations. Am J Cardiol **27**: 416–432, 1971.
18) Case RB, Felix A, et al: Measurement of myocardial Pco_2 with a microelectrode: its relation to coronary sinus Pco_2. Am J Physiol **236**(1): H29–H34, 1979.
19) Conti VR, Bertranou EG, et al: Cold cardioplegia versus hypothermia for myocardial protection: randomized clinical study. J Thorac Cardiovasc Surg **76**: 577–589, 1978.
20) Cooley DA, Reul GJ, et al: Ischemic contracture of the heart. Stone heart. Am J Cardiol **29**: 575–577, 1972.
21) Cooley DA, Wukasch DC, et al: Stone heart: prevention by induced myocardial hypothermia. Chest **64**: 390–396, 1973.
22) Crass MF III, Holsinger JR Jr, et al: Transmural gradients in the ischemic dog left ventricle: metabolism of endogenous triglyceride and glycogen. In Recent Advances in Studies of Cardiac Structure and Metabolism Vol 7, edited by Harris P, Bing RJ, and Fleckenstein A, Baltimore, University Park Press, 1976, p. 225–230.
23) Craver JM, Sams AB, et al: Potassium-induced cardioplegia: additive protection against ischemic myocardial injury during coronary artery revascularization. J Thorac Cardiovasc Surg **76**: 24–27, 1978.
24) Cunningham JN, Adams PX, et al: Preservation of ATP, ultrastructure, and ventricular function after aortic cross-clamping and reperfusion: clinical use of blood potassium cardioplegia. J Thorac Cardiovasc Surg **78**: 708–720, 1979.
25) Digerness SB, Vanini V, et al: In vitro comparison of oxygen availability from asanguinous and sanguinous cardioplegic media. Circulation **64** (Suppl II): II-80-II-83, 1981.
26) Ellis RJ, Gertz EW, et al: Mild ventricular dysfunction following cold potassium cardioplegia. Circulation **60** (Suppl I): I-170-I-173, 1979.

27) Engelman RM, Dobbs WA, et al: Myocardial high-energy phosphate replenishment during ischemic arrest: aerobic versus anaerobic metabolism. Ann Thorac Surg **33**: 453–458, 1982.
28) Engelman RM, Rousou JH, et al: A comparison of intermittent and continuous arrest for prolonged hypothermic cardioplegia. Ann Thorac Surg **29**: 217–223, 1980.
29) Engelman RM, Rousou JH, et al: Safety of prolonged ischemic arrest using hypothermic cardioplegia. J Thorac Cardiovasc Surg **79**: 705–712, 1980.
30) Engelman RA, Rousou JH, et al: The superiority of blood cardioplegia in myocardial preservation. Circulation **62** (Suppl I): I-62-I-66, 1980.
31) Engelman RA, Rousou JH, et al: The metabolic consequences of blood and crystalloid cardioplegia. Circulation **64** (Suppl II): II-67-II-74, 1981.
32) Flaherty J, O'Riordan J, et al: Transmural gradients in myocardial gas tensions in regionally ischemic canine left ventricle. In Recent Advances in Studies on Cardiac Structure and Metabolism, vol 12, Cardiac Adaptation edited by Kobayashi T, Ito Y and Rona G, Baltimore, University Park Press, 1978, p. 219–225.
33) Foglia RP, Steed DL, et al: Iatrogenic myocardial edema with potassium cardioplegia. J Thorac Cardiovasc Surg **78**: 217–222, 1979.
34) Follette DM, Steed DL, et al: Advantages of intermittent blood cardioplegia over intermittent ischemia during prolonged hypothermic aortic clamping. Circulation **58** (Suppl I): I-200-I-209, 1978.
35) Follette DM, Mulder DG, et al: Adventages of blood cardioplegia over continuous coronary perfusion on intermittent ischemia. Experimental and clinical study. J Thorac Cardiovasc Surg **76**: 604–619, 1978.
36) Gaasch WH, Bing OHL, et al: Myocardial contracture during prolonged ischemic arrest and reperfusion. Am J Physiol **235**: H619–H627, 1978.
37) Gardner TJ, Brantigan JW, et al: Intramyocardial gas tensions in the human heart during coronary artery-saphenous vein bypass. J Thorac Cardiovasc Surg **62**: 844–850, 1971.
38) Gay WA Jr, Ebert PA: Functional, metabolic, and morphologic effects of potassium-induced cardioplegia. Surgery **74**: 284–290, 1973.
39) Gazitt Y, Ohad I, et al: Phosphorylation and dephosphorylation of membrane proteins as a possible mechanism for ultrastructural rearrangement of membrane components. Biochim Biophys Acta **436**: 1–14, 1976.
40) Gott VL, Dutton RC, et al: Myocardial rigor mortis as an indicator of cardial metabolic function. Surg Forum **13**: 172–174, 1962.
41) Griggs DM Jr, Tchokoev VV, et al: Transmural differencies in ventricular tissue substrate level due to coronary artery constriction. Am J Phyiol **222**: 705–709, 1972.
42) Gutmann I, Wahlefeld AW: L-(+)-Lactate: determination with lactate dehydrogenase and NAD. In Methods in Enzymology, New York, Academic Press, 1957, p. 1464–1472.
43) Hearse DJ, Stewart DA, et al: Recovery from cardiac bypass and elective cardiac arrest: the metabolic consequences of various cardioplegic procedures in the isolated rat heart. Circ Res **35**: 448–457, 1974.
44) Hearse DJ, Garlick PB, et al: Ischemic contracture of the myocardium. Mechanism and prevention. Am J Cardiol **39**: 986–993, 1977.
45) Hess ML, Krause SM, et al: Assessment of hypothermic cardioplegic protection of the global ischemic canine myocardium. J Thorac Cardiovasc Surg **80**: 293–301, 1980.
46) Hewitt RL, Lolley DM, et al: Protective effect of glycogen and glucose on the anoxic arrested heart. Surgery **75**: 1–10, 1974.
47) Hoffman JF: Ionic transport across plasma membrane. In cell membranes: Biochemistry, Cell Biology and Pathology edited by Weissmann G, Claiborne R, New York, Hospital Practice, 1975, p. 95–103.
48) Jaworek D, Gruber W, et al: Adenosine-5'-diphosphate and Adenosine-5'-monophosphate. In Methods in Enzymology, New York, Academic Press, 1957, p. 2127–2131.
49) Jellinek M, Standeven JW, et al: Cold blood potassium cardioplegia: effects of increasing concentrations of potassium. J Thorac Cardiovasc Surg **82**: 26–37, 1981.
50) Jennings RB, Hawkins HK, et al: Relation between high energy phosphate and lethal injury in myocardial ischemia in the dog. Am J Path **92**: 187–214, 1978.
51) Jones RN, Attarian DE, et al: Metabolic deterioration during global ischemia as a function of time in the intact normal dog heart. J Thorac CMrdiovasc Surg **81**: 264–273, 1981.
52) Kanter KR, Jaffin JH, et al: Superiority of perfluorocarbon cardioplegia over blood or crystalloid cardioplegia. Circulation **64** (Suppl II): II-75-II-80, 1981.
53) Katz AM, Tada M: The "stone heart" and other challenges to the biochemist. Am J Cardiol **39**: 1073–1077, 1977.

54) Katz AM, Tada M: The "stone heart" A challenge to the biochemist. Am J Cardiol **29**: 578–580, 1972.
55) Khuri SF, O'Riordan J, et al: Mass spectrometry for the measurement of intramyocardial gas tensions. Methodology and application to the study of myocardial ischemia. In Recent Advences in Studies on Cardiac Structure and Metabolism, vol 10, The Metabolism of Contraction edited by Roy PE and Rone G, Baltimore, University Park Press, 1975, p. 539–550.
56) Khuri SF, Kloner RA, et al: Intramyocardial P_{CO_2}: a reliable index of the severity of myocardial ischemic injury. Am J Physiol **237**(2): H253–H259, 1979.
57) Kirklin JW, Kouchoukos NT, et al: Research related to surgical treatment of coronary artery disease. Circulation **60**: 1613–1618, 1979.
58) Kirklin JW, Rastelli GC: Low cardiac output after open intracardiac operations. Progr Cardiovasc Dis **10**: 117–122, 1967.
59) Kusumoki T, Cheng HC, et al: Myocardial dysfunction after cardioplegia. J Thorac Cardiovasc Surg **40**: 813–822, 1960.
60) Lacks H, Barner HB, et al: Myocardial protection by intermittent perfusion with cardioplegic solution versus intermittent coronary perfusion with cold blood. J Thorac Cardiovasc Surg **76**: 158–172, 1978.
61) Lamprecht W, Transchold I: Adenosine-5'-triphosphate determination with hexokinase and glucose-6-phosphate dehydrogenase. In Methods in Enzymology, New York, Academic Press, 1957, p. 2097–2110.
62) Lawrie GM, Morris GC, et al: Improved survival after 5 years in 1, 144 patients after coronary bypass surgery. Am J Cardiol **42**: 709–715, 1978.
63) Lazar HL, Buckberg GD, et al: Myocardial energy replenishment and reversal of ischemic damage by substrate enhancement of secondary blood cardiplegia with amino acids during reperfusion. J Thorac Cardiovasc Surg **80**: 350–359, 1980.
64) Levitsky S, Feinberg H: Biochemical changes of ischemia. Ann Thorac Surg **20**: 21–29, 1975.
65) Liedtke AJ, Hughes HC, et al: Effects of excess glucose and insulin on glycolytic metabolism during experimental myocardial ischemia. Am J Cardiol **38**: 17–27, 1976.
66) Lolley Dm, Hewitt RL, et al: Retroperfusion of the heart with a solution of glucose, insulin and potassium during anoxic arrest. J Thorac Cardiovasc Surg **67**: 364–370, 1974.
67) Lolley DM, Ray JF III, et al: Importance of preoperative myocardial glycogen levels in human cardiac preservation. Preliminary report. J Thorac Cardiovasc Surg **78**: 678–687, 1979.
68) Lowe JE, Jennings RB, et al: Cardiac rigor mortis in dogs. J Moll Cell Cardiol **11**: 1017–1031, 1979.
69) Lundell DC, Laks H, et al: The importance of myocardial protection in combined aortic valve replacement and myocardial revascularization. Ann Thorac Surg **28**: 501–508, 1979.
70) MacGregor DC, Wilson GJ, et al: Ischemic contracture of the left ventricle. Production and prevention. J Thorac Cardiovasc Surg **70**: 945–954, 1975.
71) MacGregor DC, Wilson GJ, et al: Intramyocaridal carbon dioxide tension: a guide to the safe period of anoxic arrest of the heart. J Thorac Cariovasc Surg **68**: 101–107, 1974.
72) Magovern GJ, Jr, Flaherty JT, et al: Failure of blood cardioplegia to protect myocardium at lower temperatures. Circulation **66** (Suppl I): I-60-I-67, 1982.
73) Mansour TE: Studies on heart phosphofructokinase purification, inhibition and activation. J Biol Chem **238**: 2285–2292, 1963.
74) McFarland JA, Thomas LB, et al: Myocardial necrosis following elective cardiac arrest induced with potassium citrate. J Thorac Cardiovasc Surg **40**: 200–208, 1960.
75) McIntosh HD, Garcia JA: The first decade of aortocoronary bypass surgery, 1967–1977: a review. Circulation **57**: 405–431, 1978.
76) Melrose DG, Dreyer B, et al: Elective cardiac arrest. Lancet **2**: 21–22, 1955.
77) Ogilby JD, Apstein CS: Preservation of myocardial compliance and reversal of contracture ("stone heart") during ischemic arrest by applied intermittent ventricular stretch. Am J Cardiol **46**: 397–404, 1980.
78) Randall HM Jr, Cohen JJ: Anaerobic CO_2 production by dog kidney in vitro. Am J Physiol **211**(2): 493–505, 1966.
79) Roberts AJ, Spies SM, et al: Serial assessment of left ventricular performance following coronary artery bypass grafting. Eealy postoperative results with myocardial protection afforded by multidose hypothermic potassium crystalloid cardioplegia. J Thorac Cardiovasc Surg **81**: 69–84, 1981.
80) Roberts AJ, Abel RM, et al: Advantages of hypothermic potassium cardioplegia and superiority of continuous versus intermittent aortic cross-clamping. J Thorac Cardiovasc Surg **79**: 44–58, 1980.
81) Roe BB, Hutchinson JC, et al: Myocardial protection with cold ischemic potassium-induced cardioplegia.

J Thorac Cardiovasc Surg **73**: 366–374, 1977.
82) Rousou JH, Dobbs WA, et al: Fluosol cardioplegia—a method of optimizing aerobic metabolism during arrest. Circulation **64** (Suppl) II: II-55-II-59, 1982.
83) Salerno TA, Wasan SM, et al: Glucose substrate in myocardial protection. J Thorac Cardiovasc Surg **79**: 59–62, 1980.
84) Scheuer J, Stezoski SM: Protective role of increased myocardial glycogen stores in cardiac anoxia in the rat. Circ Res **27**: 835–849, 1970.
85) Scheuer J: Myocardial metabolism in cardiac hypoxia. Am J Cardiol **19**: 385–392, 1967.
86) Shapira N, Kirsh M, et al: Comparison of the effect of blood cardioplegia to crystalloid cardioplegia on myocardial contractility in man. J Thorac Cardiovasc Surg **80**: 647–655, 1980.
87) Shea TM, Watson RM, et al: Anaerobic myocardial metabolism. Am J Physiol **203**: 463–469, 1962.
88) Sunamori M, Harrison CE Jr: Myocardial respiration and edema following hypothermic cardioplegia and anoxic arrest. J Thorac Cardiovasc Surg **78**: 208–216, 1979.
89) Taber RE, Morales AR, et al: Myocardial necrosis and the postoperative low cardiac output syndrome. Ann Thorac Surg **4**: 12–28, 1967.
90) Trigt PV, Jones RN, et al: Sonomicrometric determination of ischemic contracture of the left ventricle. J Thorac Cardiovasc Surg **83**: 298–305, 1982.
91) Tyers GFO, Todd GJ, et al: The mechanism of myocardial damage following potassium citrate (Melrose) cardioplegia. Surgery **78**: 45–53, 1975.
92) Tyras DH, Barner HB, et al: Long-term results of myocardial revascularization. Am J Cardiol **44**: 1290–1296, 1979.
93) Ui M: A role of phosphofructokinase in PH-dependent regulation of glycolysis. Biochim Biophys Acta **124**: 310–322, 1966.
94) Walters FJM, Chir B, et al: Intramyocardial pH as an index of myocardial metabolism during cardiac surgery. J Thorac Cardiovasc Surg **78**: 319–330, 1979.
95) Williamson JR: Glycolytic control mechanism. II. Kinetics of intermediates changes during the aerobic-anoxic transition in perfused rat heart. J Biol Chem **24**: 5026–5036, 1966.
96) Willman VL, Cooper T, et al: Depression of ventricular function following elective cardiac arrest with potassium citrate. Surgery **46**: 792–796, 1959.
97) Wilson GJ, Robertson JM, et al: Intramyocaridal pH during elective arrest of the heart: relative effects of hypothermia versus potassium cardioplegia on anaerobic metabolism. Ann Thorac Surg **30**: 472–481, 1980.
98) Wollenberger A, Krause EG: Metabolic control characteristics of the acutely ischemic myocardium. Am J Cardiol **22**: 349–359, 1968.
99) Wright RN, Levitsky S, et al: Beneficial effects of potassium cardioplegia during intermittent aortic cross-clamping and reperfusion. J Surg Res **24**: 201–209, 1978.
100) Wukasch DC, Reul GJ, et al: The "stone heart" syndrome. Surgery **72**: 1071–1080, 1972.
101) Zumbro GL Jr, Tillman L, et al: A comparison between propranolol and hypothermia in preventing ischemic contracture of the left ventricle (stone heart). Ann Thorac Surg **25**: 541–550, 1978.

和文抄録

心筋保護に関する実験研究
特に Cold Blood Potassium Cardioplegia について
I　形態的及び生化学的考察

京都大学医学部外科学教室第2講座（指導：日笠頼則教授）
白　石　義　定

最近の心手術成績の向上は，診断技術，患者管理技術，手術手技，体外循環技術などの進歩・発展に負うところが大きいが，それのみならず，術中の心筋保護法の改良・進歩が果す役割を看過することはできない．

現在，hypothermic potassium cardioplegia with topical cooling が冠血行遮断時における心筋保護法として一般的に施行され，良好な成績が得られている．しかしながら，細胞の基礎代謝率を減じ，高エネルギー燐酸化合物の消費を抑制するといった消極的な方法では ATP の減少を防止することはできない．

glucose-insulin-potassium cardioplegia (GIKC) は，エネルギー基質としてブドウ糖を投与し嫌気性代謝を介して ATP 産生を図る方法であるが，嫌気性解糖によって産生される ATP は極めて少なく，細胞内 ATP 含量の減少を軽減することは困難である．著者は，虚血時にも間歇的に酸素とエネルギー基質を細胞に供給し，好気性代謝を介してより多量の ATP 産生を得る方法として，いわゆる cold blood potassium cardioplegia (CBKC) を心筋保護法として導入し，その心保護効果を，血液成分を含まない GIKC と比較検討したところ以下の結果が得られた．

1. GIKC の1例を除き stone heart の発生が認められなかった．
2. 虚血導入後早期からミトコンドリアの破壊及びグリコーゲン顆粒の減少が観察された．細胞構造の破壊は心内膜側心筋に最も著しく，心外膜側心筋，中間部心筋層の破壊は比較的軽度だった．この結果から，細胞内各微少器官及び心筋各層間に虚血に対する抵抗力の差異が存在することが示唆された．
3. 虚血時間の延長に伴い，CBKC においても，ATP 及び adenylate energy charge の減少を余儀なくされたが，GIKC よりも常に高値を維持することができた．
4. しかしながら，CBKC において，細胞内に二酸化炭素，ラクテートの蓄積，pH の低下が認められた事実は，虚血細胞に対する酸素供給が充分でなく嫌気性代謝を余儀なくされていることを示唆していた．

結　論

sanguineous cardioplegia (CBKC) は心筋保護手段として asanguineous cardioplegia (GIKC) よりも優れている．しかしながら，cold blood potassium cardioplegia によっても虚血心筋は嫌気性代謝を免れ得ず，ATP の減少を防止することができない．従って，より優れた心筋保護法の開発が必要である．