Studies on Vital Reaction to the Systemic Hypothernia.

—Histopathological Changes of the Heart. (Report 1)

Yoshihiro SHUDA

Department of Pathology (Director: Prof. Dr. S. Hosokawa) and Department of Surgery (Director: Prof. Dr. A. Matsumoto), Yamaguchi Medical School, Ube, Japan

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INTRODUCTION

Many studies have been done with regard to the effects of cooling to mammals that are able to carry on the homeostatic function of the organism only within the extent of the decided temperature. Bell (1812) cooled locally in an attempt to check the pain.[1] Smith and Fay attempted to apply their knowledges of hypothermia to the treatments of cancer patients at first, expecting to inhibit the growth and spread of tumor cells.[1] Their investigations stimulated many research-workers to examine the effects of cooling on laboratory animals.

Pioneers in this field were Dill and Forbes (1941), Fuhrman and Crismon (1947)[2], and Bigelow et al (1950).[3,4]

Mc Quiston (1949)[5] indicated the possibility of application of hypothermia for the heart surgery to the hypoxaemic child. For years, cardiologists have been searching for the safer ways of shutting off the circulation, during operation of the heart. At the normal body temperature, circulatory occlusion for 1 to $2\frac{1}{2}$ minutes or longer, is impossible, and results in irreversible changes due to anoxia to the vital organs, especially to the cerebelum and heart. Bigelow et al (1950)[3] succeeded in keeping cooled dogs alive, despite of exclusion of the heart from the circulation for fifteen minutes or more. Lewis and Taufic (1953)[6] applied clinically hypothermia to direct vision cardiac surgery at first. Delorme (1952) made a way to the present deep hypothermia, by means of cooling the blood directly.[7] Abundant reports subsequent to the forerunning as above, accelerated to apply hypothermia to clinical surgery. On the other hand the workers encountered accidental occurrence of ventricular fibrillation during the cooling and rewarming processes. In deeper hypothermia, ventricular fibrillation occurred frequently. In this respect, an attention has been paid to the lower limit of body temperature in the hypothermia.

And, since Watanabe et al[8] recently indicated the possibility of circulatory occulsion for one hour or more at 20°C. below, workers have afresh paid their respects to hypothermia. Hitherto, the foundamental studies on the vital reac-

tions to the induced hypothermia have numerously been done from views of the function, but on the contrary, the morphological studies[2, 9] have been rather scarce in numbers and contents. When hypothermia is applied to surgery, prolonged hypothermic condition may practically be required.

However, the reports[11, 12] about the prolonged hypothermia were a few and poor. Even if functional reaction of the organism to induced hypothermia is reversible, morphological changes may irreversibly be remained in the vital organ. The mechanism of hypothermic, post-hypothermic, and so-called rewarming death has satisfactorily not been explained yet. For these reasons, the author intended to elucidate the pathological changes of the heart of the rat induced the prolonged hypothermia, comparing the relationship between the lower limit of body temperature and the length of survival following hypothermia.

MATERIALS AND METHODS

The materials used were apparently healthy adult albino rats weighing 170–200 g. So, rats at the rectal temperature of above 38°C. were excluded from the experiment. The experimental rats were kept with the standard diets for 4 days before experiment. They were anesthetized with ether, for the purpose of premedication and prevention of shivering. Thereafter, they were cooled in an ice bath (−3 to 0°C.), and were gradually rewarmed by the heat of the electric lamp. This experiment was divided into 4 groups, according to the rectal temperature as follows:

1) 29°C, 2) 25°C, 3) 20°C, 4) 15°C.

Cooling temperature in 4 groups was maintained for 3 hours. Moreover, the difference of each temperature during the maintenance of cooling was kept within ±0.5°C.

Artificial respiration was not used through the processes of cooling and rewarming at any rectal temperatures. Rats were sacrificed, and then, were observed at various intervals as described below.

a) Immediately after cooling, b) Immediately after rewarming, c) Twenty four hours after rewarming, d) Forty eight hours after rewarming, e) Three days after rewarming, d) Five days after rewarming, g) Seven days after rewarming, h) Nine days after rewarming, i) Eleven days after rewarming.

Rats were sacrificed after anesthesia with ether, and the hearts were extirpated to examine histologically in detail. The specimens of the hearts were fixed in 10% formalin, sectioned, and submitted to a battery of stains, i.e., H.E., Van Gieson, Azan, and Phosphotungstic acid. On the other hand, histochemical examination, i.e., Sudan IV, and P.A.S. staining, was partially performed.

OBSERVATION

In the heart, degenerative changes of the myofibers were commonly observed in each group. The author intends to explain at first the general outline of the above mentioned degenerative changes, and subsequently to describe about the findings in each group.

a) So-called eosinophilic degeneration;

The cytoplasma of heart muscle fiber was well stained with eosin. and the stained figure was homogenously seen throughout the muscle fiber.

b) Thinning of sarcoplasm.

Thickness of individual muscle fiber was within normal range, but interfibril space of muscle fiber appeared to be somewhat extended, so, at the first appearance, the structure of sarcoplasm was seen to be thin.

c) Edematous swelling of the muscle fiber.

The figure of this degeneration was considered to have severer change than the thinning of the sarcoplasma mentioned above. The individual myofiber was somewhat swollen edematously, and in addition, interfibril space of myofiber appeared to be slightly extended, partially in company with perinuclear vacuolization.

d) Vacuolization in the muscle fiber.

Various sized vacuolization was noted in the cytoplasma of myofiber.

e) Fatty degeneration was not evident, even after the temperature was fallen down to the maximal lower limit in the present experiment. And fatty droplets were only scattered in the myocardial fibers around the foci of myocaridal necrosis.

f) Glycogen generally showed no significant decrease in the various cooling condition. And it was moderately preserved in the subepicardial muscle layer. Glycogen, however, was relatively recognized in rich amount near the necrotic area.

In general, although the so-called eosinophilic degeneration of myofibers appeared scatteringly in a few fibers of the myocardium, this degeneration also appeared massively.

Thinning of sarcoplasma and edematous swelling of the myofibers were often found in groups of fibers.

Control group (6 rats)

The rats were anesthetized with ether until they stopped automatic movements. At this time their rectal temperature was 35.8—36.2°C. And then they were sacrificed. Their hearts were extirpated to examine.

The histological findings:

In each portion of the ventricle, fragmentatio myocardii was scatteringly noted in slight degree. But, myocardial degeneration, i. e., so-called eosinophilic

degeneration and edematous swelling of the myofibers were not found in any eases. Moreover, myocardial necrosis and cellular reaction were not revealed anywhere. Even in the atrial myocardium, no histological changes were evident anywhere.

The first group

In rats sacrificed immediately after cooling, myocardial degenerations, i. e., so-called eosinophilic degeneration, thinning of sarcoplasma, edematous swelling, and pyknosis of nuclei in the muscle fibers were slightly revealed in the subendo-epicardial muscle layers. But these findings were hardly recognized in the middle layer of the myocardium. Vacuolization was rarely seen. Moreover, fragmentatio myocardii and disappearance of cross-striation were scatteringly observed in each myocardial muscle layer. However, no myocardial necrosis was found anywhere.

Moderate dilatation and congestion of blood vessels in the heart were noticed. In rats sacrificed immediately after rewarming, myocardial changes were almost similar to those seen in the rats sacrificed immediately after cooling. In 24 hours after rewarming, these myocardial degenerations, as described above, were observed somewhat increasingly in general, especially so-called eosinophilic degeneration in the subendocardial muscle layer. However, circulatory disturbances, such as congestion and dilatation of blood vessels, showed thenceforth slight declining tendency. In 48 hours after rewarming, these myocardial degenerations increased moderately, but the myocardial necrosis was not observed anywhere yet. In rats sacrificed at regular time intervals, varying from 3 days through 7 days after rewarming, myocardial degenerations appeared in strong decrease, but were remained only subendocardially. 9 days or 11 days later, myocardial changes were not revealed.

The 2nd group

Myocardial changes observed immediately after cooling in this group were almost similar to those recognized immediately after cooling in the first group. But, so-called eosinophilic degeneration in the fibers was slightly scattered even in the middle layer of myocardium.

Circulatory disturbances developed further, in comparison with those seen in the first group. By means of rewarming, thinning of sarcoplasma, edematous swelling and pyknosis of nuclei in muscle fibers showed slight increasing tendency. In results, edematous swelling appeared massively and extensively. Circulatory disturbances became severer. In 24 hours after rewarming, these degenerations, especially so-called eosinophilic degeneration and vacuolization in the myofibers increased slightly. But, dilatation and congestion of blood vessels showed gradual decline ever since. In 48 hours after rewarming, myocardial degeneration developed further generally. However, since 3 days after rewarming, these myocardial changes showed strong declining tendency.

Nine days later, myocardial degeneration were slightly scattered. Therefore, 11 days later, no myocardial changes were recognized anywhere.

The 3rd group

In rats sacrificed immediately after cooling, myocardial degenerations, including so-called eosinophilic degeneration, edematous swelling, thinning of sarcoplasma, vacuolization and pyknosis of nuclei were moderately revealed in each myocardial muscle layer, especially in the subendo-epicardial muscle layer. The degree of these degenerations in this group was somewhat intenser than that recognized in the 2nd group.

Particularly so-called eosinophilic degeneration was found with greater frequency and massively in the subendocardial muscle layer. However, myocardial necrosis was not noted yet. Dilatation and congestion of blood vessels became severer, comparing with those observed in the 2nd groups. In addition to these degenerations, fragmentatio myocardii and disappearance of cross-striation were distributed in each layer. By rewarming, these myocardial changes in general became distinct increasingly in slight degree. And, even subendocardial hemorrhage was seen. In 24 hours after rewarming, these degenerations increased progressively, and 48 hours later became prominent ultimately. Circulatory disturbances decreased gradually ever since 24 to 48 hours after rewarming.

During 3 to 5 days after rewarming, myocardial degenerations in the subendo-epicardial muscle layers decreased remarkably, especially so-called eosinophilic degeneration.

Thereafter, during 7 to 9 days after rewarming, these changes became slight decreasingly. Even 9 days later, so-called eosinophilic degeneration, thinning of sarcoplasma, and edematous swelling were slightly and scatteringly seen in the myocardium. However, 11 days later, these myocardial changes were rarely noted, and circulatory disturbances were not observed anywhere.

The 4th group

In rats, sacrificed immediately after cooling, the myocardial changes consisted of myocardial degenerations similar to those seen in the 3rd group. It was characteristic that the degree of myocardial degenerations increased suddenly, comparing with those seen in the 3rd group. Of course, these degenerations occurred not only strongly in the subendo-epicardial muscle layers, but also moderately in the middle layer of myocardium. Especially, so-called eosinophilic degeneration was seen scatteringly in each layer and observed in groups of 15 or more muscle fibers. At the first time, 2 to 3 necrotic muscle fibers made their appearances distinct at this temperature of cooling. Dilatation and congestion of blood vessels were strongly noted. Moreover, subendocardial hemorrhage was found with greater frequency. By means of rewarming, these myocardial changes increased slightly, or were similar to those observed before rewarming. Therefore, in occasional cases several necrotic muscle fibers appeared focally.

In 24 hours after rewarming, these myocardial degenerations increased remarkably, and were intensively revealed.

Myocardial necrosis also increased suddenly and remarkably, which occurred in the subendocardial layer with greater frequency, although it appeared in each muscle layer. Necrosis occurred in many myofibers (about 15 or more), and appeared as focal necrosis.

The areas of necrotic foci were replaced with the cellular reaction which characteristically increased in numbers and sizes. Therefore, such cellular reaction became focal in nature. Foci of cellular reaction consisted of lymphocytes, monocytes, neutrophilic leucocytes, endothelial cells, Anitschkow's cells, fibroblasts, and myogenic cells.

Anitschkow's cells could easily be observed in the necrotic foci adjacent to the blood vessels, or in the subendo-epicardial muscle layers. Morphologic characteristics of this cell were observed in their nuclei. Chromatin of the nucleus occupies almost the entire long axis of the nucleus centrally, and was seen just as a brush of test tube. Myogenic cell, seemingly representing attempt at muscle regeneration, was large, and had basophilic cytoplasma. And the nucleus was relatively large and spindle in shape. In 48 hours after rewarming, myocardial degenerations and necrosis became somewhat increasingly distinct in each myocardial layer. And proliferation of fibroblasts was recognized, though slightly, in the foci of cellular reaction. However, during 3 to 5 days after rewarming, myocardial changes in general were remarkably decreased, but so-called eosinophilic degeneration was still remained moderately. Occurrence of necrosis was rarely seen, so that necrosis was not observed focally, although a few necrotic muscle fibers could be detected. Therefore, poor cellular reaction was noted. A few cells were observed only surrounding the necrotic fibers. During 7 to 9 days after rewarming, myocardial degenerations decreased gradually. These changes still remained slightly in the subendo-epicardial muscle layers. But, no necrosis was noted at all.

Proliferation of granulation tissue was moderately noted. In 11 days after rewarming, these myocardial changes generally became decreasingly slight. Therefore, so-called eosinophilic degeneration, thinning of sarcoplasma, edematous swelling, and pyknosis of nuclei were slightly and scatteringly seen only in the subendoepicardial muscle layers. And production of granulation tissue was noted, but no fibrous scar formation was observed yet. The necrosis and circulatory disturbances were not observed anywhere.

DISCUSSION AND SUMMARY

At present, hypothermia at above 25°C has positively been recommended at least, and it has been accepted by most investigators that systemic hypothermia

is not only noninjurious, but rather safer procedure in the fields of application to intracardiac surgery and general surgery. It has been said that in the hypothermia of rectal temperature at 25°C, various changes in the vital organs which were resulted in hypothermia could almost be recovered.[3, 13, 14, 15] However, these opinions are often depended on the observations in the cases immediately rewarmed, without consideration of the hypothermic durations. Moreover, these assumptions originated almost from physiologic or biochemistric results. From the views of hypothermic duration, the influences of hypothermia upon the organism have nearly been studied. Particularly, pathohistological studies on prolonged hypothermia were a few,[10, 16, 17] hitherto.

The author should naturally, pay respects to hypothermic duration. Therefore, the findings observed only in the cases immediately rewarmed without consideration of hypothermic duration, are insufficent to explain the significance of hypothermia. For these reasons, the present experiment is intended to observe prolonged hypothermia (3 hours), at various survival times, varying from immediately after cooling through 11 days after rewarming, in accordance with the problems of the lower limit of temperature. And the author histologically studied the heart which might suffer very easily from hypoxia or anoxia among the vital organs. The present observations were summarized as follows: in rats sacrificed, immediately after cooling for 3 hours duration at 20°C, or above, and at various intervals after rewarming, such myocardial degenerations as so-called eosinophilic degeneration, thinning of sarcoplasma, edematous swelling, vacuolization, and pyknosis of nuclei were found in the muscle fibers of the heart. But, neither myocardial necrosis nor subsquent cellular reaction were revealed anywhere. Sarajas[17] reported that in dogs sacrificed immediately, or at the termination of moderate (26 to 27.5°C) hypothermia of 1 to 4 hours duration, there were signs of necrosis, mainly hyalinization of cytoplasm with concomitant pyknosis of nuclei. And cellular phenomena were seen in such affected areas. On the other hand, the present observations manifested that even in rats sacrificed immediately after cooling for 3 hours duration at 15°C, such apparent and large necrosis and cellular reaction as Sarajas's data[17] were not noted yet, but a few necrotic fibers were only revealed scatteringly in the myocardium in occasional cases. And the cellular reaction was seen only surrounding the necrotic fibers, and were a few in numbers, or not. In contrast, myocardial degenerations could moderately be found. The occurrences of myocardial changes in general became frequent, in accordance with the length of survival time after rewarming. The findings observed immediately after rewarming were almost similar or somewhat intenser, than those observed before rewarming. In 24 hours later, for the first time, apparent necrotic foci with concomitant of more intenser cellular reaction were scatteringly found. And in 48 hours later, these changes became ultimate. These findings were the most

interesting changes. Thereafter, myocardial lesions showed reduction and disappearance, and finally were replaced with scar tissues, in accordance with the length of survival time. But, Sarajas[17] observed myocardial necrosis and cellular phenomena in the heart until 7 to 14 days after rewarming. And the earlier reports have not attached to the relation between the stages of development or healing in the myocardial necrosis and the length of survival, varying from immediately after cooling, to the day sacrificed. The present study showed that general myocardial lesions developed until 48 hours after rewarming, and became remarkable ultimately. Since 3 days, these changes had remarkably tended to declining.

Hayashi[19] also has not attached in detail to the relation between the degree of the myocardial necrosis and the length of survival. However, he demonstrated that in dogs sacrificed in 14 days following hypothermia of 2 hours duration at 25°C, vacuolization, cloudy swelling, and poor staining nuclei were seen in the myofibers of interventricular septum, and that in dogs sacrificed at 40 days following hypothermia of 4 hours duration at 20°C, granular degeneration, vacuolization, and irregular arrangement of heart muscles were remarkabl seen in the epicardium, without proliferation of fibrous connective tissues and collagenous fibers. Omori[9] stated that in a few days sacrificed immediately after cooling, subendocardial hemorrhage, edema of the interstitium, and hyperemia and congestion of blood vessels were noted, and that these changes were moderately increased by rewarming. And he showed pyknosis of nuclei, vacuolization of cytoplasm, and occasionally small necrotic focus in the myocardium, especially in the subendocardium, and showed edema and vacuolization in muscle fibers of the impulse conducting system. But, circulatory disturbances which were temporarily increased, almost disappeared in about 24 hours later.

He pointed out that myocardial changes, as mentioned above, were hardly found in a week or more after hypothermia and circulatory occulsion.

Such circulatory disturbances in the present experiment increased slightly after rewarming. But these became decreasingly slight since 24 hours. Sarajas [16, 18] also demonstrated vacuolization and pyknosis of nuclei in the heart muscle fibers, adding hyaline or granular myocardial necrosis and hemorrhage. These changes were easily observed in the subendocardium. According to Kawano's Reports,[10] in dogs sacrificed immediately after cooling for 3 hours at 19°C, there were edema and slight congestion in the right ventricle. Edema and spotted myocardial degenerations in the left ventricle were distributed around the blood vessels. After rewarming, edema, relatively larger hemorrhage, and necrotic muscle fibers were occasionally detected in the myocardium of the left ventricle. In dogs sacrificed immediately after cooling for 3 hours at 26°C, edema and small spotted degeneration were seen in the myocardium of both the ventricles. But in 1 to 2 days after rewarming, hemorrhage and necrosis were

not detected in the myocardium, except retatively earlier myocardial lesion and slight congestion.

In the present findings, myocardial lesion was not only seen with the greatest frequency in the subendocardial muscle layer, but also was moderately observed in the subepicardial muscle layer and middle layer of myocardium.

In any portions of subendocardium, including the papillary muscles of both the ventricles, interventricular septum, and impulse conducting system, circulatory disturbances, myocardial degenerations, and apparent necrotic foci with concomitant intense cellular reaction were seen scatteringly. It was easily supposed that these myocardial changes might have relation to the disturbances of cardiac rhythm during the process of cooling or rewarming and the death after rewarming. But, no literatures on these remarkable cellular reaction could be obtained, except only Sarajas's one. According to Sarajas's report,[17] early reactive cellular phenomena consisted of mainly lymphocytes, or lymphocyte-like cells, and occasionally of a few polymorphonuclears, mesenchymal cells, and Anitschkow's myocytes. He also described that in dogs sacrificed and autopsied from 3 to 7 days after the cooling process, the infiltrating cells consisted predominantly of mesenchymal cells, which frequently showed mitotic figures. Polymorphonuclear leucocytes, lymphocytes, and Anitschkow's myocytes were rare or absent. Collagen formation, if present, was not prominent. In 14 days after the survival of hypothermia, there was prominent collagen formation, and moreover definite plasma cells, giant cell, and pigmented macrophages were noted among the infiltrating cells. Finally, the myocardium of the dogs which had survived for 3 years after a deep hypothermia, showed well-defined, contracted fibrotic scar with heteroplastic bone formation.

The focus of the cellular reaction in the present experiment consisted of the cells, i. e. lymphocytes, monocytes, Anitschkow's cells, neutrophilic leucocytes, endothelial cells, fibrobrasts, and myogenic cells. Characteristically myogenic cells appeared, seemingly representing attempts at heart muscle regeneration. These myogenic cells were relatively large and splender, and were composed of large and spindle shaped nucleus and basophilic cytoplasm.

The "myogenic cells" in origin were observed at first in 24 hours after rewarming in the granulation in the heart.

Long since, much have been discussed about the presence or absence of regenerative potency of the heart muscle. The true regeneration of the cardiac muscle fibers has been suggested by Heller, Warthin[20] and Mac-Mahon,[21] and more recently by Linzbach[22] and Henschel.[23] Mönckeberg described giant cells of myogenic origin adjacent to myocardial infarcts, and interpreted these as regeneration attempt.[20] Rubenstone and Saphir[20] demonstrated that the myogenic cells, seemingly representing attempts at muscle regeneration, appeared singly or in the form of the syncytial giant cells. And these cells made their appearances at

4 to 6 days after the heart injury, and remained for 1 month in the reactive granulation to injury. These myogenic cells were either large mononuclear or multinuclear cells. When observed in cross section, these mononuclear cells at the first sight resembled the Anitschkow's cells. However, none showed a perinuclear halo, or the spider leg-like chromatin, and all contained a large amount of basophilic cytoplasma. The multinuclear cells usually had two or three overlapping nuclei.

He also described that these termed "myogenic" cells closely resembled the sarcolemmal cells. The Anitschkow's cells was given attention by Anitschknow in 1913, but prominence of the cell in granulation tissues in the heart had been noted before 1901 by Von Oppel. Thereafter, many investigators pursued the origin and function of Anitschkow's cell. Wenezianowa-Grusdkowa and Saphir et al were able to confirm that the cells were of histiocytic origin, and had phagocytic function. Jaffé also believed that they came from the reticuloendothelial system of the heart. Ehrlich and Lapan reported that Anitschkow's cells originated from subendothelial or peri-vascular fixed cells. The Anitschkow's cell was called "Anitschkow's myocyte or Aschoff cell. However, the Anitschkow's cell is neither "myocyte" nor "myogenic".[20, 24] Rubenstone and Saphir[20] demonstrated that Anitschkow's cell was observed in the heart only. The classic criteria practically applied to permit the diagnosis of Anitschkow's cells were 1) a central chromatin bar which was one-third the thickness of the nucleus in its widest part, the bar at its ends extending almost to the nuclear border edges, 2) the presence of regular, threadlike segmentations, and 3) the absence of any nucleolus. According to the recent reports by Rubenstone and Saphir, in the myocardial lesions Anitschkow's cells made their appearances at about 1 to $1\frac{1}{2}$ weeks within the granulation area and also in the walls of vessels in the heart, and were recognized until 2 months following injury. The Antischkow's cells were conspicuous in the walls of small blood vessels, and in occasional cases appeared in the form of syncytial giant cell.

From the author's experiment, it may be said as follows: It is evident that as regard to their pathogenesis, the myocardial changes are chiefly related to the cooling itself. And the author can point out that these myocardial lesions are accelerated or progressed by means of rewarming procedure and the length of survival time after hypothermia. Consequently, even if the pathohistological findings observed immediately after hypothermia are not significant, myocardial injuries such as degeneration and necrosis, perhaps, become apparent and conspicuous after passage of a certain time following hypothermia.

The so-called eosinophilic degeneration was mainly and characteristically seen among the myocardial degenerations in the present observation. This myocardial degeneration was revealed mainly in groups of fibers, and especially in the subendocardial muscle layer. But, such a degeneration was occasionally observed

in single fiber. Kakefuda (25) described that the incidences of the so-called eosinophilic degeneration are pathogenetically related to the somewhat gradual anoxia in the heart muscles. The more gradual anoxia results in the edematous vacuolization of the muscle fiber. These degenerations as described above were found in the peripheral ischemic zone of infarction, and were mostly recovered reversibly, due to the development of the intercoronary anastomoses. The muscle fibers injured irreversibly were disposed by fibrinolysis or phagocytosis, and finally resulted in "Sarkolemmschläuchen" of muscle fibers. Acellular scar originated in the collapse of "Sarkolemmschläuchen" was poor in the cells (nuclei). On the other hand, in the myocardial lesion due to rapid anoxia, necrotic muscle fibers with basophilic cytoplasma were observed.

And, in this affected regions, fibroblasts and fibrocytes could early be observed. Proliferation of these cells and subsequent collagen were observed, and then the focus was gradually transformed into fibrous scar. Kakefuda also described that the width of the scar was seemingly or really reduced due to 3 factors, i. e. (1) remarkable cicatrical contraction, (2) myocardial regeneration, and (3) compensatory hypertrophy of myofibers around the affected area. In the present study these remarkable myocardial lesions, i. e. necrotic foci with concomitant intense cellular reaction appeared in 24–48 hours after rewarming. Then, necrotic materials were removed by autolysis and by phagocytic cells. Thereafter, in the injured area, 48 hours or so after rewarming, at any time onwards, proliferation of fibroblasts and fibrocytes were recognized.

At 11 days after rewarming, production of granulation tissue was noted, but no fibrous scar formation was observed yet. Also, acellular scar pointed out by Kakefuda was not evidenced.

Von Werz hypothesized that the pronounced leftward shift of the hemoglobin dissociation curve in the cold, with its consequent lowering of oxygen partial pressures, was sufficient to explain death in hypothermia.[26] This hypoxia concepts were supported by the observations presented by Lange, Weiner, and Gold.[27] They stated that myocardial lesions were due to cardiac hypoxia from the standpoints of electrocardiographic changes. Therefore, they attempted to compensate for the lowered oxygen dissociation by increasing the amount of physically dissolved oxygen in plasma, independent of hemoglobin. In result, the electrocardiographic changes were remarkably improved. On the other hand, Bigelow et al[28] in their study on dogs, concluded that tissue hypoxia did not exist during hypothermia, because a) there were no residual effects in rewarmed dogs, b) there is no exaggerated oxygen consumption upon rewarming, and c) no change is observed in oxygen consumption in dogs maintained at 19°C for 0.5 to 4.3 hours. Originally, cardiac muscle, especially of the left ventricle, has a stricking ability to extract oxygen from the blood, comparing with any other tissues.[29]

Against the hypoxia theory of myocardial impairment, Penrod[26] felt that oxygen utilization of the heart was normal at 20°C, since he found no changes in coronary arteriovenous oxygen differences from those in normothermic animals. And he supported the contention that in the cases of the heart at least, no serious tissue hypoxia resulted from the leftward shift of hemoglobin dissociation curve in the cold. Edwards[30] also showed that coronary arteriovenous oxygen differences remained unchanged from the normothermic state, confirming Penrod's works. And he described that, although coronary blood flow and myocardial oxygen consumption decreased, the ratio oxygen consumption of the heart to the total oxygen consumption increased during hypothermia. This fact represents a possible safety mechanism of the heart against the serious consequences of myocardial anoxia.

Takeda[31] also confirmed the mechanism of the heart as described above, and demonstrated that there were no evidences of anoxia electrocardiographically or histologically during hypothermia at 20°C at least.

Sarajas's data[17, 18] furnished the evidence for the views that the lesions are possibly due to focal disturbances of the myocardial blood supply rather than to a systemic anoxemia or hypoxemia. Therefore, Sarajas's observations suggested that the lesions were true infarction, from the views of the situations and the attitudes of necrosis. And these are further supported by the facts that the lesions were mainly focal in nature, and were rather sharply delineated. In addition, he found frequently some narrowing processes of an apparently inflammatory nature in the myocardial arterioles. The occurrence of micro-embolisms in hypothermic dogs has been demonstrated by Gelin and Löfström.[18]

Although the occurrence of micro-embolism was not evident in the present hypothermic rats, the focal disturbances of the myocardial blood supply were noted. Therefore, the author assumed that focal disturbance may probably be caused by partially the functional constriction of arterioles, as stated by Kawano[10] and Suwa.[32] About the changes of the blood vessel in the myocardium, edematous changes in the wall of the arterioles were observed by Nakayama,[14] and perivascular edema was pointed out by Sarajas.[16] The present observation demonstrated perivascular edema, too.

Myocardial changes which were moderately seen in the present experiment, appeared to be due not only to the focal disturbances in the myocardial blood supply, but also to hypoxemia or anoxaemia. Therefore hypoxaemia or anoxaemia seemed to exist, as a matter of course, because artificial respiration was not used during the processes of cooling and rewarming at any temperatures, especially even at 15°C. The rectal temperature of 15°C in rat was almost lower limit to keep respiration. From these reasons, the conspicuous myocardial changes in the present experiment can be explained.

CONCLUSION

For the purpose of research on the lower limit of body temperature in prolonged hypothermia, myocardial changes in rats induced hypothermia for 3 hours at various rectal temperatures up to 15°C, were studied pathohistologically in accordance with the length of survival after rewarming. And the present study obtained the following conclusions.

1) In cases of hypothermia at 20°C or above, although myocardial degenerations were moderately observed, and were frequently seen in the subendo-epicardial muscle layers, and especially in the subendocardial muscle layer, myocardial necrosis was not observed yet at any time after cooling.

2) Myocardial degenerations which were seen in the present experiment, included so-called eosinophilic degeneration, thinning of sarcoplasma, edematous swelling, vacuolization, and pyknosis of the nucleus in the myofiber. In these myocardial degenerations, so-called eosinophilic degeneration in the myofibers was characteristic, and this degeneration often appeared massively or scatteringly.

3) When rectal temperature fell to 15°C, myocardial changes became severe. In addition to myocardial degenerations, necrotic muscle fibers were already revealed even in the cases sacrificed immediately after cooling.

4) In cases of hypothermia at 15°C or above, histologic changes of the heart muscle generally increased in accordance with the length of survival after cooling. It was notable that in 24–48 hours after rewarming, myocardial changes became remarkably apparent, thenceforth the changes showed a gradual declining tendency.

5) In rats sacrificed immediately after cooling of 15°C, necrosis were only observed in a few numbers of the myofibers. However, in 24–48 hours after rewarming, these necrosis appeared massively and focally in the myocardium. And the areas of necrotic foci were replaced with intense cellular reaction. The necrotic foci with concomitant cellular reaction were distributed in each myocardial layer, especially in the subendocardial muscle layer.

6) The focus of cellular reaction consisted of the cells, i. e. lymphocytes, monocytes, neutrophilic leucocytes, Anitschkow's cells, endothelial cells, and myogenic cells, seemingly representing attempts at heart muscle regeneration.

7) Circulatory disturbances, such as congestion and dilatation of the blood vessels, were strongly observed in each hypothermia of 15°C or above. And even subendocardial hemorrhage was frequently found. These circulatory disturbances showed slight increasing tendency by means of rewarming, but were reduced gradually since 24 hours after rewarming.

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References of this report are presented together with the Report 2.

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Table 1.

	No.	Portion \ Change	So-called eosinophilic degeneration	Thinning of sarcoplasma	Edematous swelling	Vacuolization	Necrosis	Fragmentatio myocardii	Loss of cross striation	Cellular reaction	Dilatation of blood vessel	Bleeding	Proliferation of granulation tissue	Edema of the interstitium	Degeneration of nucleus
Control	1	Ventricle	—	—	—	—	—	±	—	—	—	—	—	—	—
	2		—	—	—	—	—	—	—	—	—	—	—	—	—
	3		—	—	—	—	—	±	—	—	—	—	—	—	—
	4	Apex	—	—	—	—	—	—	—	—	—	—	—	—	—
	5	middle portion of Ventricle	—	—	—	—	—	±	—	—	—	—	—	—	—
	6	Atrium	—	—	—	—	—	±	—	—	—	—	—	—	—

Table 2.

Temperature of cooling	Time of Sacrifice	No. / Change	So-called eosinophilic degeneration	Thinning of sarcoplasma	Edematous swelling	Vacuolization	Necrosis	Fragmentatio myocardii	Loss of cross striation	Cellular reaction	Dilatation of blood vessel	Bleeding	Proliferation of granulation tissue	Edema of the interstitium	Degeneration of nucleus
29°C	immediately after cooling	68	±	±	±	−	−	−	±	−	+	−	−	−	±
		113	−	+	−	−	−	±	−	−	+	−	−	−	−
	immediately after rewarming	111	−	−	−	−	−	−	±	−	+	−	−	−	±
		112	±	±	±	−	−	±	±	−	+	−	−	±	±
	24 hours after rewarming	117	+	+	+	±	−	−	±	−	±	−	−	±	±
		118	±	±	−	−	−	−	±	−	+	−	−	−	−
	48 hours after rewarming	67	±	−	−	−	−	−	−	−	−	−	−	−	−
		114	+	±	+	−	−	±	±	−	±	−	−	±	+
	3 days after rewarming	137	−	±	±	±	−	−	−	−	−	−	−	−	±
		138	±	−	±	−	−	−	−	−	−	−	−	−	−
	5 days after rewarming	139	±	±	−	−	−	+	±	−	−	−	−	−	±
		140	−	±	±	−	−	±	−	−	−	−	−	−	−
	7 days after rewarming	119	−	±	−	−	−	−	−	−	−	−	−	−	−
		120	−	−	−	−	−	−	−	−	−	−	−	−	−
	9 days after rewarming	135	−	±	−	−	−	±	−	−	−	−	−	−	−
		136	−	−	−	−	−	−	−	−	−	−	−	−	−
	11 days after rewarming	115	−	−	−	−	−	±	−	−	−	−	−	−	−
		116	−	−	−	−	−	±	−	−	−	−	−	−	−
25°C	immediately after cooling	123	±	±	±	−	−	−	±	−	+	−	−	−	±
		124	±	±	±	−	−	−	±	−	+	−	−	±	±
	immediately after rewarming	125	±	±	+	±	−	−	±	−	+	−	−	±	±
		126	+	±	±	−	−	−	±	−	+	−	−	±	±
	24 hours after rewarming	121	±	±	±	±	−	±	±	−	±	−	−	−	±
		122	+	±	+	−	−	±	−	−	+	−	−	±	+
	48 hours after rewarming	69	±	±	+	−	−	−	±	−	±	−	−	±	±
		141	+	+	++	−	−	−	±	−	±	−	−	±	+
	3 days after rewarming	131	±	±	±	−	−	±	−	−	−	−	−	−	±
		132	−	−	±	−	−	−	−	−	±	−	−	−	−

Table 3.

Temperature of cooling	Time of Sacrifice	No. \ change	So-called eosinophilic degeneration	Thinning of sarcoplasma	Edematous swelling	Vacuolization	Necrosis	Fragmentatio myocardii	Loss of cross striation	Cellular reaction	Dilatation of blood vessel	Bleeding	Proliferation of granulation tissue	Edema of the interstitium	Degeneration of nucleus
25°C	5 days after rewarming	13	±	±	±	−	−	−	±	−	−	−	−	−	±
		142	−	±	−	±	−	−	−	−	−	−	−	−	−
	7 days after rewarming	129	−	−	−	±	−	−	−	−	−	−	−	−	−
		130	−	±	±	−	−	±	−	−	−	−	−	−	−
	9 days after rewarming	133	−	±	±	−	−	−	−	−	−	−	−	−	−
		134	−	−	−	−	−	±	−	−	−	−	−	−	−
	11 days after rewarming	127	−	−	−	−	−	±	−	−	−	−	−	−	−
		128	−	−	−	−	−	−	−	−	−	−	−	−	−
20°C	immediately after cooling	6	++	±	++	±	−	+	+	−	++	±	−	+	++
		101	±	±	±	−	−	±	±	−	+++	±	−	−	±
		102	+	±	±	−	−	±	±	−	++	−	−	±	+
	immediately after rewarming	7	++	±	++	±	−	±	+	−	+++	±	−	+	++
		103	++	±	+	−	−	−	±	−	++	−	−	±	+
	24 hours after rewarming	19	++	++	++	−	−	±	+	−	+	±	−	+	++
		70	++	+	++	±	−	−	+	−	+	−	−	+	++
		16	+	+	±	−	−	−	±	−	+	−	−	−	+
	48 hours after rewarming	66	++	+	++	±	−	−	+	−	+	−	−	+	++
		49	++	±	+	−	−	±	+	−	+	−	−	±	++
		54	++	±	+	±	−	−	+	−	+	−	−	−	+
	3 days after rewarming	107	+	±	±	−	−	−	±	−	−	−	−	−	+
		108	±	±	−	−	−	−	±	−	±	−	−	−	−
	5 days after rewarming	109	+	±	±	±	−	−	±	−	−	−	−	±	+
		110	±	−	−	−	−	+	−	−	−	−	−	−	−
	7 days after rewarming	104	−	±	−	−	−	±	−	−	−	−	−	−	−
		105	±	±	±	±	−	±	±	−	−	−	−	−	±
	9 days after rewarming	143	−	±	−	−	−	−	−	−	−	−	−	−	−
		144	±	±	−	±	−	±	−	−	−	−	−	−	±
	11 days after rewarming	78	−	−	−	−	−	±	−	−	−	−	−	−	−
		106	−	±	−	−	−	±	−	−	−	−	−	−	−

Table 4.

Temperature of cooling	Time of Sacrifice	No. \ Change	So-called eosinophilic degeneration	Thinning of sarcoplasma	Edematous swelling	Vacuolization	Necrosis	Fragmentatio myocardii	Loss of cross striation	Cellular reaction	Dilatation of blood vessel	Bleeding	Proliferation of granulation tissue	Edema of the interstitium	Degeneration of nucleus
15°C	immediately after cooling	8	++	+	++	−	−	−	+	−	++	−	−	+	++
		11	++	+	++	±	±	−	+	−	+++	±	−	+	++
		29	++	±	++	−	±	−	+	±	+++	±	−	+	++
	immediately after rewarming	38	++	+	++	−	±	±	±	±	+++	±	−	+	++
		39	++	+	++	−	−	±	±	−	++	−	−	±	+
		53	++	+	++	±	±	±	+	±	+++	±	−	+	++
	24 hours after rewarming	20	+++	++	++	±	++	±	+	++	++	−	−	+	++
		34	+++	+	++	−	+++	−	+	+++	++	−	−	+	++
		15	++	+	++	−	++	−	±	++	++	±	−	+	++
	48 hours after rewarming	22	++	++	+++	±	+++	±	+	+++	++	±	±	+	++
		24	+++	+	+++	±	+++	±	+	+++	+	−	±	+	++
		27	++	+	++	−	+	−	+	+	+	−	−	±	++
	3 days after rewarming	86	++	+	++	±	±	−	±	±	+	−	−	−	++
		87	+	+	±	±	+	±	±	+	±	±	±	−	+
		88	+	±	+	−	±	−	±	±	+	−	−	−	+
	5 days after rewarming	43	++	+	+	±	±	−	±	±	±	−	±	−	++
		90	+	±	±	−	±	−	±	−	±	−	±	−	+
		92	+	±	±	−	−	±	−	−	−	−	−	−	±
	7 days after rewarming	42	+	+	±	−	±	+	±	±	−	−	±	−	+
		79	±	++	−	−	−	+	±	±	−	−	+	−	±
		80	±	+	±	−	−	+	−	−	−	−	−	−	±
	9 days after rewarming	55	±	+	−	−	−	+	±	−	−	−	−	−	±
		81	±	±	±	±	±	±	−	±	−	−	+	−	±
		82	−	±	−	−	+	+	±	±	−	−	++	−	−
	11 days after rewarming	56	−	±	−	−	−	−	−	−	−	−	−	−	−
		77	±	±	±	−	−	±	±	±	−	−	+	−	±
		89	−	−	±	±	−	−	−	−	−	−	−	−	±

EXPLANATION OF PLATES

PLATE I

Fig. 1. So-called eosinophilic degeneration (↑) of myofibers is seen scatteringly in the middle layer of myocardium. 48 hours after hypothermia for 3 hours duration at 15°C.

Fig. 2. So-called eosionphilic degeneration (↑) is seen in groups of myofibers in the subendocardium. 48 hours after hypothermia for 3 hours duration at 15°C.

Fig. 3. So-called eosinophilic degeneration of myofibers. (↑) 24 hours after hypothermia for 3 hours duration at 15°C.

Fig. 4. Cellular reaction in the focal myocardial necrosis in the subendocardium. 48 hours after hypothermia for 3 hours duration at 15°C.

Fig. 5. Focal necrosis with concomitant the reactive cells: lymphocytes, monocytes, and Anitschkow's cells (arrow). 48 hours after hypothermia for 3 hours duration at 15°C.

Fig. 6. Cellular reaction in the necrotic area. Myogenic cells (large nuclei) are seen among the reactive cells. 48 hours after hypothermia for 3 hours duration at 15°C.

PLATE II

Fig. 7. So-called eosinophilic degeneration (↑) is seen around the focal necrosis in the subendocardium. 48 hours after hypothermia for 3 hours duration at 15°C.

Fig. 8. Remarkable cellular reaction. 48 hours after hypothermia for 3 hours duration at 15°C. (P.A.S.)

Fig. 9. Glycogen granules are observed in the myofibers near the necrotic focus. (↑) 48 hours after hypothermia for 3 hours duration at 15°C. (P.A.S.)

Fig. 10. Vacuolization and edematous swelling (↑) of myofibers. Immediately after cooling for 3 hours duration of 15°C.

Fig. 11. Dilatation of blood vessels and so-called eosinophilic degeneration. 24 hours after hypothermia for 3 hours duration at 15°C.

Fig. 12. Proliferation of endothelial cells in the capillaries. 48 hours after hypothermia for 3 hours duration at 15°C.

PLATE I

Fig. 1.

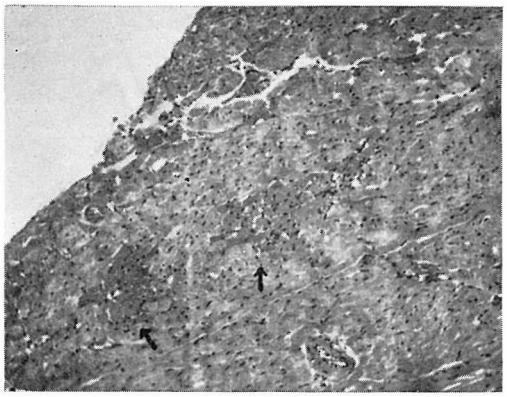

Fig. 2.

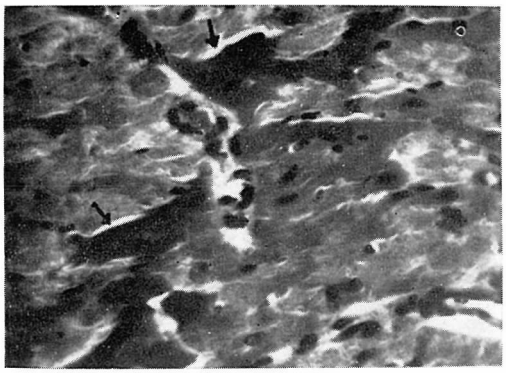

Fig. 3.

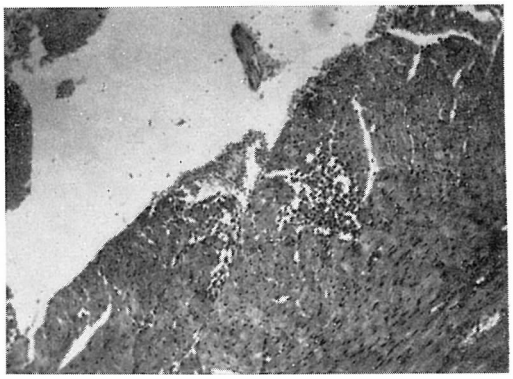

Fig. 4.

Fig. 5.

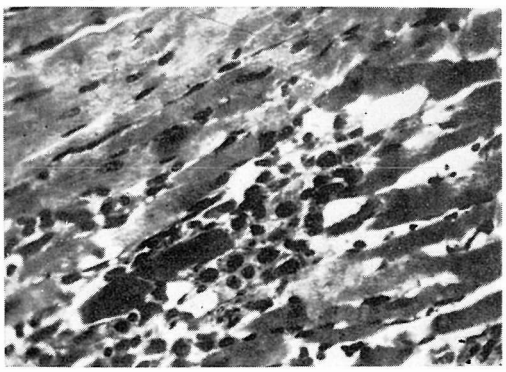

Fig. 6.

PLATE II

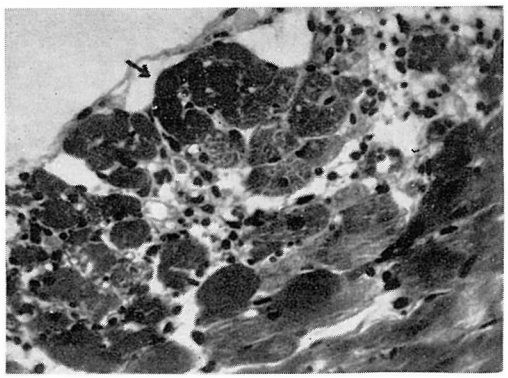

Fig. 7.

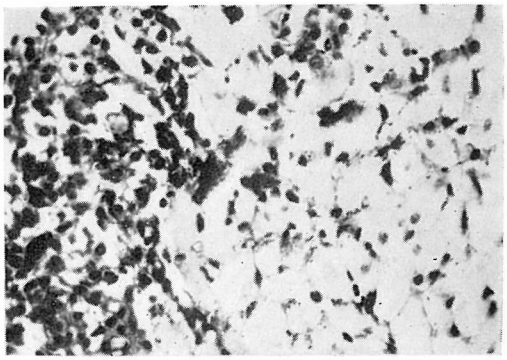

Fig. 8.

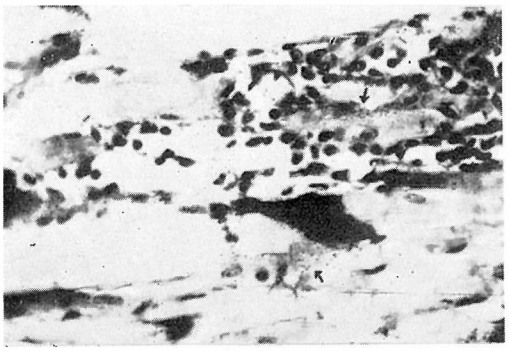

Fig. 9.

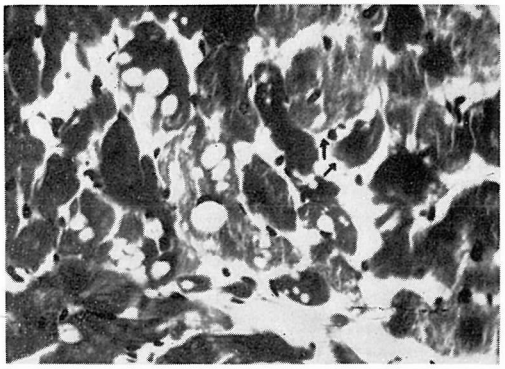

Fig. 10.

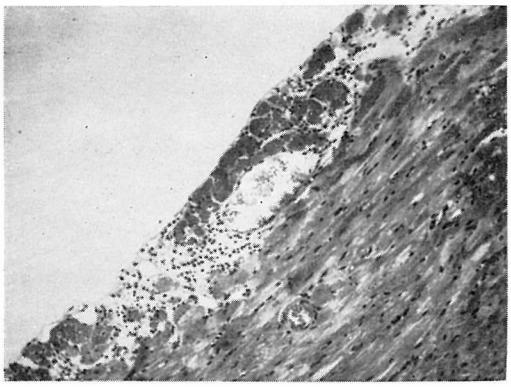

Fig. 11.

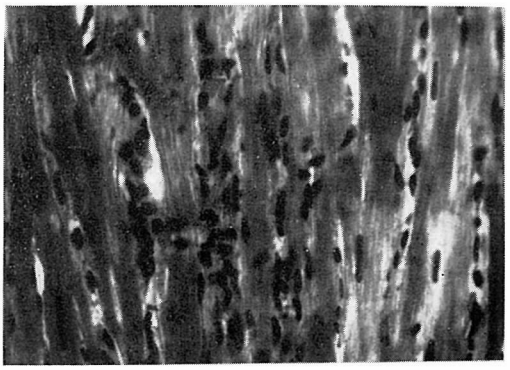

Fig. 12.