Fine Structures of Cardiac Muscle in the Thiamine- and Potassium-Deficient Rats

ARATA MIWA

The present study was designed to elucidate the fine structures of the myocardium of rats kept on combined-deficient diet of thiamine and potassium for four weeks.

When thiamine alone was deficient, the myocardial cells were characterized by the swelling of mitochondria with fragmented cristae.

While potassium was depleted, the findings were characterized by the dilatation of sarcoplasmic reticulum.

In the combined deficiency group, the mitochondria showed less change than that of the thiamine depleted and the sarcoplasmic reticulum was less affected than the potassium depleted rats.

These findings in the combined deficiency group suggest that thiamine deficiency decreases or prevents electrolyte imbalance and potassium depletion may cover thiamine deficiency.

In 1937, Schrader, Prickett, and Salmon called attention to the myocardial necrosis seen in rats on potassium-deficient diet. Thomas, Mylon, and Winternitz reported a similar myocardial lesion in rats but attributed it to a combined deficiency of potassium and vitamin B. Darrow and Miller later reported that myocardial lesions produced by injections of desoxy-corticosterone acetate in rats could not be distinguished from those resulted from diets low in potassium, and were not aggravated by suboptimal amount of thiamine in the diet.

Since 1949 progress has been made in the understanding of the fine structure of the normal myocardium.

In addition, there have been studies on the fine structure of the cardiac muscle of thiamine- and potassium-deficient rats however little work on the combined deficiency of thiamine and potassium has been done.

The main purpose of the present study is to elucidate electron microscopically the fine structure of the cardiac muscle of rats fed by combined-deficient diet of thiamine and potassium.

Material and Methods

Male albino rats of Wistar strain, weighing 50 to 60g, were used. Before the experiments, all the rats were fed with the control diet for three days and divided into five groups of five rats each; I) the control rats were fed with the basal diet (Table I) with added vitamin (Table II), II) the thiamine-deficient rats were fed with the diet in that thiamine was omitted from the control diet, III) the potassium-deficient rats were fed with the similar diet to the control except that the salt mixture from HEPPEL (Table III) was substituted for McCollem's salt mixture, IV) the combined-deficient rats of thiamine and potassium were fed with the diet in which thiamine was omitted from the potassium-deficient diet, V) the recovery experiments on the rats, kept for four weeks on the thiamine-deficient diet, were carried out using the diet which thiamine content was adjusted to the control diet by adding optimal amount of thiamine to the thiamine-free diet.

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Third Department of Internal Medicine, Nagoya University School of Medicine, Nagoya, Japan.
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diet.

Distilled water was used in the groups III and IV.

On the twenty-eighth day of the programmed feeding, the rats of each group were anesthetized with inhalation of ethyl-ether. Under the above anesthesia, immediately after midline thoracotomy, the myocardium of both ventricles was cut into small pieces and fixed in osmium tetroxide solution with sodium phosphate (Millonig)18 After fixation of one hour and a half the pieces were dehydrated in a graded ethyl alcohol and embedded in Epon 812 (Lufi).19

Ultrathin sections were made with LKB ultratome using glass knives and stained with saturated uranil acetate (Watson)20 followed by lead citrate (reynolds)21. Stained sections were examined with an electron microscope HU-11 D type and JEM-7 type.

Furthermore, specimens from the myocardium of each group were fixed in 10% formalin and stained with hematoxylin and eosin and with periodic acid Schiff.

RESULTS

I) Weights (Fig.1)

The control rats weighed an average of 64.2g at the beginning of the experiments and weighed 91.2g, 132.2g, and 149g after 14, 21, and 28 days, respectively.

The thiamine-deficient rats' weight averaged 67g at the beginning, then after making a slightly skewed corn shaped upward curve, the peak being the 14th day, came down to almost the same weight as that of the beginning on the 28th day.

The potassium-deficient rats gradually increased their weight, the final average weight being 21.5% more than the initial.

The combined-deficient rats lost an average of about 0.58% of the starting weight.

![Fig. 1. Average weight curves for four groups of rats.](image)

II) Observations under the light microscope

The myocardium in the thiamine-deficient rats revealed the swelling of the nucleus and the atrophy of the fibers.

The myocardium in the potassium-deficient rats revealed swelling of the nucleus as well as loss of the cross striation, and in addition, occasional focal necrosis of the myocardium.

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On the combined deficiency group occasional findings were swelling of the nucleus and poorly discernible cross striation.

The myocardial PAS positive-substance decrease was about the same in the three groups mentioned above.

III) Observations under the electron microscope
1) Control rats' myocardium (Fig.2 to Fig.5)

The individual fibers were delimited by the sarcolemma, which was a complex structure consisting of a dense cell surface unit membrane and was invested on its external aspect by a less dense basement membrane layer of fairly uniform width (Figs. 2, 4, 5).

The nucleus (Fig.4) was oval and located near the sarcolemma. The nuclear membrane consisted of two parallel membranes. Shallow invaginations of the nuclear membrane were frequently observed. No direct communication between the outer perinuclear membrane and the sarcoplasmic reticulum was demonstrated.

The golgi apparatus was occasionally observed near the nucleus.

Mitochondria (Figs. 2, 3, 4, 5) were quite numerous in the rat cardiac muscle, and measured 0.47 to 4.8μ (average 1.41μ) in length and 0.2 to 2.27μ (average 0.88μ) in width. They were abundant between the myofibrils and often aggregated near the nucleus or beneath the sarcolemma. They were generally oval to rod-shaped though variable in their shape and size; being demarcated by double membranes and having well preserved numerous cristae inside.

The intramitochondrial granules were seen in some mitochondria within the matrix of the organellae (Figs. 2, 3, 5).

The sarcoplasmic reticulum consisted of a network of membrane-limited intracellular channels (Figs. 2, 3, 4, 5). These channels which were present throughout the cardiac muscle cells, formed two component systems; one being the transverse system and the other the longitudinal system. The triads were occasionally seen.

The intercalated discs represented the modified cell membranes of adjacent cardiac muscle cells and were located for the most part at right angle to the long axis of the fibers (Fig.2).

The discs exhibited three different structural forms (Fig.2). The first type consisted of two cell membranes separated by an intercellular space of variable width. The second type consisted of two dense parallel cell membranes separated by a narrow and uniform intercellular space, and the parallel section being short. The third type consisted of desmosome.

The myofibrils were longitudinally divided into sarcomeres, showing a periodic band pattern when fixed at relaxed state (Fig.5). The sarcomere was delineated by two adjacent dense Z lines, which were arranged face to face (Figs.2, 3, 5), and was composed of two types of myofilaments; thick and thin myofilaments (Figs.4, 5). An I band extended toward the center of the sarcomere from each Z line (Fig.5). The central portion of sarcomere was subdivided into two lateral A band with a central H disc, which was bisected by a central M line (Fig.5). I band and H disc were absent when fixed at contracted state (Figs. 2, 3).

Glycogen granules were numerous found in the cytoplasm and were approximately 125Å to 200Å in diameter (Figs. 2, 3, 4, 5).

Lipid droplets were occasionally seen adjacent to mitochondria.

Blood capillaries (Fig.5) were rich in the extracellular space, and collagen fibrils (Fig.2) and fibroblasts were seen.

These observations on the normal rat myocardium are in agreement with earlier workers
2) Thiamine deficient rats' myocardium (Fig.6 to Fig.8)

In the thiamine deficiency group, the myocardial cells were characterized by swelling and rupture of the mitochondria and by having twisted and fragmented cristae (Figs. 6, 7, 8).

Mitochondria were 0.4 to 9.0μ (average 2.21μ) in length and 0.33 to 4.0μ (average 1.27μ) in width, and the glycogen granules decreased in number (Figs. 6, 7, 8).

Vesicles and tubules of the sarcoplasmic reticulum were not dilated.

No dilatation of the extracellular space was observed (Figs. 6, 8) and the lipid droplets were scanty (Fig. 8).

The lysosomes were not found.

3) Potassium deficient rats' myocardium (Fig. 9 to Fig. 12)

The most conspicuous change was a marked dilatation of the sarcoplasmic reticulum (Figs. 9, 10).

The partial dilatation of the extracellular spaces and the intermyofibrillar cytoplasmics with a decrease in their density were occasionally observed (Figs. 9, 10).

The intermyofibrillar space became wider (Fig. 10).

The obvious disruption and disorganization of the myofilament made a poorly discernible band
pattern (Figs. 9, 10).

The disruption of the intercalated disc was occasionally seen (Fig. 10).

Lipid droplets were found immediately adjacent to mitochondria (Figs. 11, 12).

Lysosomes were rarely seen in the cytoplasm (Fig. 12).

The changes of the mitochondria were observed as the slight swelling and the diminution of electron density in their matrix, and mitochondria measured 0.4 to 4.67 \( \mu \) (average 1.73 \( \mu \)) in length and 0.33 to 2.4 \( \mu \) (average 1.05 \( \mu \)) in width, but above changes were less compared with the thiamine deficiency group (Figs. 9, 11).

Decrease in glycogen granule number was similar to that of the thiamine deficiency group (Figs. 9, 10, 11, 12).

4) Combined deficient rats’ myocardium (Fig. 13 to Fig. 15)

The dilatation of the sarcoplasmic reticulum was less in degree compared with the potassium deficiency group (Figs. 13, 14).

Mitochondria measured 0.67 to 6.13\( \mu \) (average 1.74 \( \mu \)) in length and 0.33 to 2.73\( \mu \) (average 1.09 \( \mu \)) in width.

As to mitochondria (Figs. 13, 14, 15), lipid droplet number, and lysosome (Fig. 15), their changes were almost the same seen in the potassium deficiency group.

The decrease in the glycogen granule number was about the same as was observed in the two previously mentioned groups (Figs. 13, 14, 15).

No changes on the myofibrils and on the extracellular spaces were observed (Figs. 13, 14, 15).

5) Rats’ myocardium in the recovery experiments from the thiamine deficiency (Fig. 16).

No swelling and rupture of the mitochondria nor twisting and fragmentation of cristae, observed in the myocardium of the thiamine-deficient rats, were noted.

Mitochondria measured 0.53 to 4.27\( \mu \) (average 1.35 \( \mu \)) in length and 0.27 to 2.13\( \mu \) (average 0.84 \( \mu \)) in width.

No decrease of glycogen granules was observed.

6) As to nucleus, sarcolemma, collagen fibril, and intramitochondrial granule, no changes were observed in any of three groups kept on a deficient diet.

Discussion and Conclusions:

1) Thiamine deficiency group

YAMADA\(^{12}\) suggested that the alternations of the myocardial mitochondria in thiamine-deficient rats are most likely the result of abnormal myocardial metabolism.

On the other hand, YOSHITOSHI et al\(^{13}\) detected no change, comparing with the control, of the myocardial mitochondria in thiamine-deficient rats.

Meanwhile, SUZUKI\(^{14}\) suggested that the mitochondrial changes in the myocardium of thiamine-deficient rat result from the disturbance of carbohydrate metabolism.

The results in this experiment are in the same line with YAMADA\(^{12}\)’s and SUZUKI\(^{14}\)’s findings, but not with YOSHITOSHI et al\(^{13}\)’s.

It is known that thiamine pyrophosphate catalyzes the reaction pyruvate to acetyl CoA, and \( \alpha \)-ketoglutarate to succinate as well as the transketolase reaction.

OLSON\(^{22}\) reported that metabolic process in cardiac muscle may be divided into three phases; (1) energy liberation, (2) energy conservation, (3) energy utilization.

As are well known, the glycolytic reactions which produce pyruvate and lactate from glucose occur in the cytoplasm. The oxidation of fatty acid, pyruvate, and certain amino acid occur in the mitochondrion.

The enzymes of the tricarboxylic acid cycle, the hydrogen transport enzymes, and the associated enzymes which catalyze oxidative phosphorylation are known to be located in the mitochondrion.

The main reactions of energy production (including energy liberation and energy conservation) occur in the mitochondrion.

Therefore, it is evident that thiamine deficiency results in the disturbance of energy production.

ARCOS and ARGUS\(^{23}\) reported that ATP inhibits and reverses the swelling of the heart mitochondria.

It is generally recognized that disturbance in oxidative phosphorylation accelerates glycolysis.

The ultrastructural changes in the myocardium of thiamine-deficient rats were not observed when thiamine was administered for twenty-eight days following twenty-eight days of thiamine-free diet.

The above mentioned observation strongly suggests that the changes of mitochondria and the decrease of glycogen granules in the thiamine deficiency group are reversible.

It may be concluded from the results in this experiment that the marked changes in the myocardium of thiamine-deficient rats, namely, the

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swelling of mitochondria with twisted and fragmented cristae and the decrease of glycogen granules, result from the disturbance of oxidative phosphorylation.

2) Potassium deficiency group

In 1937, Schrader et al. first recognized cardiac necrosis in connection with potassium deficiency. Pochel suggested that an increase in the number of lipid droplet and the dilatation of the sarcoplasmic reticulum, both recognized in the myocardium of rats in potassium deficiency, result from the disorder of carbohydrate metabolism and the electrolyte imbalance, respectively.

Nickerson et al. reported that the electrolyte-steroid myocardial lesions in rats were due to simple intracellular potassium deficiency, and their incidence and severity were well correlated with the degree of myocardial potassium depletion below the threshold value of approximately 72 mEq/kg wet weight.

Prioreshi reported data on rats that he felt refuted the role of potassium deficiency in the production of electrolyte-steroid myocardial lesion. Molnar et al. suggested that the dilatation of the sarcoplasmic reticulum and the disruption of myofilaments in the myocardium of the potassium-depleted rats may result from the electrolyte imbalance.

D'Acostino suggested that both of the abnormalities in rats' myocardium, namely, the intramitochondrial inclusions and the moderate dilatation of the sarcoplasmic reticulum, both produced by simultaneous administration of 9α-fluorocortisol and sodium phosphate, may be due to the electrolyte disturbances.

It is known that potassium catalyzes the reaction phosphoenolpyruvate to pyruvate and affects conductive system in the cardiac muscle.

There have been many reports on the role of potassium in protein synthesis. Rinehart et al. has reported that dietary potassium deficiency on chicks decreases incorporation of the labeled leucine into skeletal muscle protein.

Summarizing the sarcoplasmic reticulum functions, it is the pathways for metabolites and ions, and it also contains ATPase and ADPase.

According to Toura, an associate, who used the same rats used in this work, only a negligible difference in the myocardial potassium to serum potassium ratio was noted between the control group (77.20 mEq/kg wet weight: 5.39 mEq/L \( \equiv 14.3 : 1 \)) and the thiamine deficiency group (72.20 : 4.87 \( \equiv 14.8 : 1 \)), while the above ratio in the potassium deficiency group was 70.27 : 2.35 \( \equiv 29.9 : 1 \) and was higher than that of the combined deficiency group (71.86 : 3.88 \( \equiv 18.5 : 1 \)).

It is the understanding of the author that the dilatation of sarcoplasmic reticulum and the changes of myofibril are the results of electrolyte imbalance.

The so-called myocardial necrosis was not observed in this study and it may be because of the relatively high concentration of potassium in myocardium (70.27 ± 1.15 mEq/kg wet weight).

In the potassium deficiency group, the changes of the mitochondria were less in degree compared with the thiamine deficiency group; and this observation is realized as showing less disturbed energy production in the mitochondria than in thiamine deficiency group.

It has been reported in the field of biochemistry that increase of glycogenolysis and impairment of cellular glucose uptake take place in potassium deficiency.

Applying this to the result of observation so far described, it seems reasonable to understand the decrease of glycogen granules and the increase of lipid droplets from two aspects, namely (1) energy utilization has increased markedly for the purpose of maintaining the intramyocardial potassium level and (2) carbohydrate metabolism has been disturbed due to less potassium in the myocardium.

The changes in this potassium deficiency group with their striking dissimilarities to the changes in the thiamine deficiency group suggest that there are significant differences in the pathogenic mechanisms.

3) Thiamine- and potassium-combined deficiency group

Thomas et al. reported that a deficiency of crystalline vitamin B6 hydrochloride, when coupled with a potassium deficiency, led to myocardial lesions in rats, while coupled deficiency of thiamine and potassium together produced no significant changes.

Results on this 'combined deficiency group' indicate much the same as their findings and disagree Darrow et al.'s mentioned previously, and may elucidate in detail relation between thiamine and potassium.

In this combined deficiency group, the changes of the mitochondria, the dilatation of the sarco-

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plasmic reticulum, the decrease of the glycogen granules, the increase of the lipid droplets and the appearance of the lysosomes were observed.

But, it is worthy of note that changes in the mitochondria are less compared with the thiamine deficiency group; while dilatation of the sarcoplasmic reticulum being less and changes of the myofibrils none compared with the potassium deficiency group.

TUCKER et al.\textsuperscript{35} reported that myocardial lesions in rats were associated with the decrease of potassium in serum and, when the serum potassium reached a level of 4.0 mEq/L, approximately 13\% of the rats developed myocardial lesions.

It is felt that the normal finding of the myofibrils may be due to higher potassium concentration in both serum and myocardium of this group than in those of potassium deficiency group.

Results obtained suggest that thiamine deficiency decreases or prevents the electrolyte imbalance; and thiamine deficiency may be prevented or compensated by the low potassium level as suggested in this study.

**Summary**

The fine structures in myocardium of rats under the thiamine- and/or potassium-deficient diet of four weeks duration were presented.

When thiamine was deficient, swelling and rupture of mitochondria with twisted and fragmented cristae were the characteristics of myocardial cells. The matrix of mitochondria diminished in electron density.

In the potassium deficiency group, the findings were characterized by the dilatation of sarcoplasmic reticulum and the poorly discernible band pattern of myofilaments. The diminution of electron density in mitochondrial matrix and slight swelling of mitochondria were recognized.

In the group of thiamine- and potassium-combined deficiency, the changes of mitochondria were less compared with the thiamine deficiency group and the dilatation of sarcoplasmic reticulum was also less compared with the potassium deficiency group.

The appearances of lipid droplet and lysosome in both potassium and thiamine-potassium combined deficiency group were almost identical.

Glycogen granules decreased in number in all the three groups.

In the recovery experiment on the thiamine deficiency group, the changes of mitochondria and the decrease of glycogen granules were reversible.

In each group, no change of nucleus, of collagen fibrils, sarcolemma and of intramitochondrial granules was observed.

These findings in the thiamine and potassium deficiency group suggest that the changes of mitochondria and sarcoplasmic reticulum result from disturbance of oxidative phosphorylation and electrolyte imbalance, respectively.

However, the findings in the thiamine-potassium combined deficiency group suggest that thiamine deficiency decreases or prevents the electrolyte imbalance; and thiamine deficiency may be prevented or compensated by the low potassium level as suggested in this study.

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EXPLANATION OF PLATES (Fig. 2 to Fig. 16)

Abbreviations
A : A band
BM : Basement membrane
Co : Collagen fibril
Cp : Capillary
Ed : Endothel
Gl : Glycogen granule
H : H disc
I : I band
ID : Intercalated disc
L : Longitudinal system of sarcoplasmic reticulum
Li : Lipid droplet
Ly : Lysosome
M : M line
MF : Myofibril
Mf : Myofilament
Mt : Mitochondrion
N : Nucleus
RBC : Red blood corpuscle
SI : Sarcolemma
SR : Sarcoplasmic reticulum
T : Transverse system of sarcoplasmic reticulum
Z : Z line

Fig. 2. Myocardium, longitudinal section, of a control rat.
Electron micrograph delineating the general cytologic features of cardiac muscle.
On both sides, contiguous myocardial cells are limited by the sarcolemma, which is invested on its external aspect by a basement membrane.
Many mitochondria with numerous cristae lie between the myofibrils and close to the sarcolemma. No specific localization of mitochondria in relation to the band pattern is observed.
The myofibrils demonstrate A band, Z and M lines, when fixed at contracted state.
An intercalated disc is seen coursing in an irregular fashion from the middle left side of the figure to the upper right.
Three types of disc structure are present.
The first consists of two adjacent cell membranes separated by an intercellular space of variable width (1). The second, of two dense cell membranes with a narrow intercellular space of constant width (2). This type is oriented parallel to the longitudinal axis of the myofibrils.
The third, of desmosomes (3).
The junction between disc and sarcolemma is noted in the upper right side of the figure (an arrow).
Many small vesicles in the cytoplasm are the sarcoplasmic reticulum.
Fine, dense glycogen granules are distributed throughout the cytoplasm.
The collagen fibrils are occasionally seen in the extracellular spaces. \( \times 20,000 \)

Fig. 3. Myocardium, longitudinal section, of a control rat.
Group of mitochondria having numerous cristae lie between the myofibrils. The myofibrils demonstrate A band, Z and M lines; fixed at contracted state.
Small vesicles and tubules in the cytoplasm are the sarcoplasmic reticulum.
A large number of glycogen granules are distributed throughout the cytoplasm. \( \times 20,000 \)

Fig. 4. Myocardium, of a control rat.
Electron micrograph showing a transverse section through an A band.
Both thick and thin myofilaments are observed in an 'Honey Comb' arrangement.
The nucleus contains finely dispersed electron dense granules.
The nuclear envelope consists of two parallel membranes.
Sarcolemma, mitochondria, glycogen granules, and longitudinal system of sarcoplasmic reticulum are observed as on Figs. 2 and 3. \( \times 40,000 \)

Fig. 5. Myocardium, longitudinal section, of a control rat.
The myofibrils are divided into sarcomeres (Z to Z line).
The sarcomeres show Z line, I band, A band, H disc, and M line, when fixed at relaxed state. The M line is indicated by an short arrow.

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Both thin and thick myofilaments are evident only in the A band proper.
The I band consists of thin myofilaments.
A capillary is seen in the extracellular space.
The endothel of the capillary contains numerous pinocytotic vesicles. $\times 20,000$

**Fig. 6.** Myocardium, longitudinal section, of a rat. Four weeks of thiamine-deficient diet.
Electron micrograph showing the most predominant change of mitochondria.
The mitochondria with twisted and fragmented cristae are remarkably swollen.
The matrix of mitochondria has lost electron density and the glycogen granule number becomes less.
Note structureless areas of cyst-like appearance in the mitochondria (arrows).
No change on sarcoplasmic reticulum, extracellular space, myofibril, intercalated disc, and sarcolemma is observed.
No lipid droplets are noted. $\times 20,000$

**Fig. 7.** Myocardium, oblique section, of a rat. Four weeks of thiamine-deficient diet.
Electron micrograph showing changes of mitochondria and decreased number of glycogen granules.
Note the swelling of mitochondria with twisted and fragmented cristae, and structureless areas of cyst-like appearance (indicated by arrows) in the matrix.
Glycogen granules are sparsely distributed in the cytoplasm. $\times 20,000$

**Fig. 8.** Myocardium, longitudinal section, of a rat. Four weeks of thiamine-deficient diet.
Similar to Figs. 6 and 7; changes in appearance of mitochondria and decreased number of glycogen granules are observed.
One giant mitochondrion is occupying the right side of the figure, measuring about 4.0 $\mu$ in length and about 1.8 $\mu$ in width.
The structureless area of the mitochondrial matrix is indicated by an arrow.
At the lower right corner of the figure, adjacent to mitochondria, a lipid droplet is present. $\times 20,000$

**Fig. 9.** Myocardium, longitudinal section, of a rat. Four weeks of potassium-deficient diet.
Markedly dilated sarcoplasmic reticulum and the cytoplasm, reduced in its electron density, are visualized; most likely due to intracellular edema.
The extracellular space is partially dilated (indicated by an arrow).
The myofibrils have poorly discernible band pattern, but do not show disruption.
The mitochondria with twisted cristae are slightly swollen and the mitochondrial matrix diminishes in electron density.
The mitochondria are less affected than those of the thiamine deficiency group.
No rupture of sarcolemma, nor glycogen granules are observed. $\times 20,000$

**Fig. 10.** Myocardium, longitudinal section, of a rat. Four weeks of potassium-deficient diet.
Myofibrils are widely separated (single arrows) with disruption of myofilaments (double arrows) and show poorly discernible band pattern.
The intercalated disc also is disrupted.
The sarcoplasmic reticulum is dilated.
No glycogen granules are seen. $\times 20,000$

**Fig. 11.** Myocardium, oblique section, of a rat. Four weeks of potassium-deficient diet.
The degenerative change of mitochondria in this group is most apparent in this particular figure, though the change is less compared with that of thiamine deficiency group.
Similar to Fig. 9, the mitochondria with twisted cristae are slightly swollen and the mitochondrial matrix diminishes in electron density.
Some of the mitochondria show fragmented cristae and structureless areas of cyst-like appearance (indicated by arrows).
Lipid droplets are seen adjacent to mitochondria.
Glycogen granules are sparsely distributed in the cytoplasm.
No changes of the myofibril, the sarcoplasmic reticulum, and of the intercalated disc are found. $\times 20,000$

**Fig. 12.** Myocardium, longitudinal section, of a rat. Four weeks of potassium-deficient diet.
In this figure, as are observed frequently in this group, a lysosome and two lipid droplets are present.
Lysosome is seen between the sarcolemma and the mitochondria.
Lipid droplets also are found adjacent to mitochondria similar to Fig. 11.
Glycogen granules are sparsely distributed in the cytoplasm. $\times 20,000$

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Fig. 13. Myocardium, transverse section, of a rat. Four weeks of combined-deficient diet of thiamine and potassium.

The sarcoplasmic reticulum is dilated, however, less in degree compared with the potassium deficiency group.
Slightly swollen mitochondria and decrease in electron density of mitochondrial matrix, similar in extent as in the potassium deficiency group, are observed.
Glycogen granules are sparsely distributed in the cytoplasm similar to Figs. 6, 11, and 12.
No changes of the extracellular space (an arrow), the myofibrils, and of the sarcolemma are observed. × 20,000

Fig. 14. Myocardium, oblique section, of a rat. Four weeks of combined-deficient diet of thiamine and potassium.

The sarcoplasmic reticulum is slightly dilated.
Glycogen granules are sparsely distributed in the cytoplasm.
No change in other organellae. × 20,000

Fig. 15. Myocardium, longitudinal section, of a rat. Four weeks of combined-deficient diet of thiamine and potassium.

In the upper right quadrant of the figure, two lysosomes are seen in the cytoplasm, surrounded by the mitochondria.
Glycogen granules are sparsely distributed in the cytoplasm similar to Figs. 13 and 14.
No changes of the other organellae are observed. × 20,000

Fig. 16. Myocardium, longitudinal section, of a rat. After four weeks of 'recovery diet', (see text).

No structural changes are detectable compared with the control rat myocardium. × 20,000
Fig. 7.

Fig. 8.

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Fig. 13.

Fig. 14.

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Fig. 15.

Fig. 16.

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