Bull. Fac. Fish. Hokkaido Univ.
23 (3), 151-157. 1972.

Electron Microscopic Studies on the Liver Cells in Pantothenic Acid Deficient Goldfish

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Abstract

Light and electron microscopic studies were done on liver parenchymal cells of goldfish fed on Halver's synthetic diet without calcium pantothenate. The experimental period lasted 52 days from the 20th of October to the 11th of December 1969.

The pantothenic acid deficient fish showed retardation of growth, loss of appetite, localized hemorrhage and slight exophthalmus. Ultrastructurally, the liver parenchymal cells of the vitamin deficient fish exhibited several cytoplasmic changes. The most striking change was found in mitochondria; most of them had become larger in size than those of control and some of them attached closely to one another. Endoplasmic reticulum was less developed as compared with that of the liver cells of the control fish. Furthermore, electron dense, myelinated bodies and amorphous dense bodies appeared also in the cytoplasm of the liver parenchymal cells. No conspicuous distinction in the amount of fat droplets was detectable in the liver cells of both control and experimental ones.

Many investigations concerning vitamin requirement and influence of its deficiency have been done by a number of workers using fishes such as trout, carps and eels.

Pantothenic acid, one of the B-complex vitamins, has been known to be an indispensable vitamin in some fihses. About thirty years ago, Wolf[1] and McLaren et al.[2] demonstrated that pantothenic acid deficiency in the diet causes a disease of gills and retardation of growth in some trout. Recently Ogino[3], who studies on the influence of the lack of that vitamin, revealed that extremely poor growth, a loss of appetite and a hemorrhage of body surface are brought in carp as vitamin deficiency symptoms. In rainbow trout fingerlings, Kitamura et al.[4] also reported pantothenic acid deficiency symptoms such as poor growth, high mortality, and abnormal swimming.

In some warm-blooded animals it is well clarified that pantothenic acid deficient animals show several alterations in their liver cells[5][6]. So far as we are aware of, however, there is no report on the effect of pantothenic acid deficiency upon the ultrastructure of fish liver cells.

The present paper deals with light and electron microscopic studies on the liver cells affected by pantothenic acid deficiency in the goldfish.

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Material and Methods

Goldfish, *Carassius auratus* L., weighing about 4.6 g in average were used as material. They were divided into 2 groups of 15 fish each and kept in aquaria of 58 *l* capacity each, after having been starved for 3 days. Well water, filtered with gauze, was aerated, heated and supplied into each aquarium at the rate of 10 *l* per hour. The water temperature was adjusted to 21±1°C during the experiment. The aquaria were kept clean by washing every three days.

A synthetic diet of Halver and Coates[7] with a slight modication was used as a basal diet, which is shown in Table 1.

One group of the fish were fed on the basal diet as a control and another group with a pantothenic acid deficient diet which was prepared by omitting calcium pantothenate from the basal diet. A 100 g portion of the diet was mixed with 150 m*l* of water at 40–50°C, and stored in a freezer. Only as much diet as

Table 1. Composition of the basal diet

Main mixture		*Mineral mixture		**Vitamin mixture *1/*	
Vitamin-free casein	54 g	USP XII No. 2 plus		Thiamine hydrochloride	6 mg
Gelatin	15	$AlCl_3$	18 mg	Riboflavin	20
Soybean oil	7	$ZnSO_4$	357	Pyridoxine hydrochloride	4
Cod liver oil	2	CuCl	11	Nicotinic acid	80
White dextrin	8	$MnSO_4$	80	Calcium pantothenate	28
α-Cellulose flour	9	KI	17	Inositol	400
Mineral mixture*	4	$CoCl_2$	105	Biotin	0.6
DL-Methionine	1	per 100 g of salt mixture		Folic acid	1.5
L-Tryptophan	0.5			*p*-Aminobenzoic acid	40
Vitamin mixture**				Choline chloride	800
				Ascorbic acid	200
				α-Tocopherol	40
				Menadione	4
				Vitamin B_{12}	0.01

1/ These vitamins were added per 100 g of the main mixture

needed for ten day feeding was prepared. The fish was fed twice a day at the level of approximately 18% of total body weight. The quantities of diet were adjusted every 10 days by weighing both the control and experimental fish. The experimental period lasted 52 days from the 20th of October to the 11th of December 1969. After the experiment the fish were decapitated.

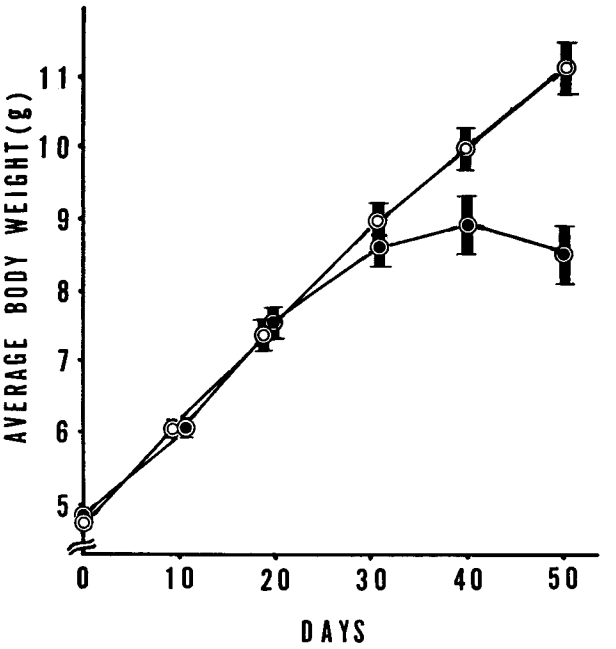
For light microscopic observations, the liver was fixed in Bouin's solution, cut at 5–6 micra in thickness by the routine paraffin method and stained with Delafield's hematoxylin and eosin.

For electron microscopic observations, small pieces of the liver were fixed in Millonig's solution for two hours, dehydrated by graded ethanol and two changes of n-butyl glycidyl ether and embedded in Epon. Sections were cut with glass

knives at 500–800 Å in thickness on a Porter-Blum microtome, stained with uranyl acetate in combination with lead citrate, and examined with a Hitachi HS-7 electron microscope.

Results

Text-figure 1 shows the growth changes of average body weight in control and experimental fish during the period of experiment. Throughout the experimental period, a nearly constant rate of body weight gain was maintained by the fish receiving the basal diet. They remained normal in appearance until the end of the experiment.



Text-figure 1. Growth changes in body weight of goldfish. Each of the marks on the lines shows the mean body weight of the fish fed on basal diet (◎) or on pantothenic acid deficient diet (◉). The range of standard error is indicated by vertical bars.

The fish fed on the pantothenic acid deficient diet showed a retardation of growth due to the loss of appetite after 30 days of feeding and even a slight decrease of average body weight throughout the 50 days. About half of them showed skin lesions after 40 days, having suffered a localized hemorrhage in the dorsal and lateral skin especially around the base of dorsal or pectoral fins. Most of the fish also showed a slight exophthalmus. During the 50 days some of them

began to float on the surface, displaying abnormal swimming behaviour. In this experiment, however, pantothenic acid deficient fish showed no histological abnormality in their gill filament.

Histologically, the cytoplasm of liver parenchymal cells of the fish fed on the basal diet was scarcely stained with hematoxylin except the perinuclear zone where the cytoplasm was stained dark with the dye (PL. I-1). In the dark perinuclear zone of the cells, electron microscopically, there were found many cytoplasmic organelles such as mitochondria, Golgi complexes, endoplasmic reticulum, microbodies and lysosomes. A large mass of glycogen deposit usually occupied the peripheral region of the cytoplasm of parenchymal cells. Mitochondria were various in shape, viz., round, oval, rod-shaped and gourd-shaped, the round ones varying from 0.4 to 0.6 μ in diameter. They had a dense distribution around the nucleus, though some of them were also found buried in the mass of glycogen deposit. An amount of rough surfaced endoplasmic reticulum appeared as stacks of flattened cisternae in the vicinity of the nucleus. Similar granular endoplasmic reticulum was also surrounding the mitochondria. Several Golgi complexes were recognized as a stack of flattened agranular sacs with a number of vesicles of various sizes scattered around it. Lysosomes laying in the cytoplasm were usually of a round shape and were packed with amorphous material of high electron density. Microbodies were round and granular in appearance, being 0.3 to 0.4 μ in size, and were less numerous than the mitochondria. They contained a finely granular substance limited by a single membrane (PL. I-3).

On the other hand, the liver cells of the fish fed on the pantothenic acid deficient diet contained, light microscopically, many small granules stained slightly with eosin in the cytoplasm (PL. I-2). These granules seemed to be large mitochondria as described later. Ultrastructurally, the liver cells of the vitamin deficient fish showed conspicuous changes in cytological features. The most striking change was found in the mitochondria; most of them had become to be round in shape. They were larger in size than those of controls (PL. II-4). These mitochondria, measuring about 1.5 μ in diameter, revealed no apparent change in the matrix density. Mitochondria of rod-shaped type appearing frequently in the liver of the control showed a reduction in number in the liver cells of the experimental fish. It seems likely that most of the mitochondria changed their form into a sphere under the influence of the vitamin deficiency. Several mitochondria were attached closely to one another. The outer membrane of some of the rounded mitochondria showed a toothed-wheel structure (PL. III-6 and 7). There were also some mitochondria with a vacuolar matrix (PL. III-10). The endoplasmic reticulum developed poorly as compared with that of the liver cells of the control in which it constructed the stacks of flattened cisternae in the perinuclear zone. Most of the endoplasmic reticulum appeared as short tubes

and some of them as small vesicles scattered in the cytoplasm (PL. II-4 and 5). Golgi complexes contained a large amount of a very dense material in the associated vesicles and sacs. Electron dense, myelinated bodies were present closely to the Golgi area (PL. III-8), or scattered in the cytoplasm (PL. III-9). Amorphous dense bodies of various sizes, presumably lysosomal in nature, were also encountered in the cytoplasm (PL. II-5).

In the present study, some parenchymal cells of the liver in the fish fed either with the basal diet or with the pantothenic acid deficient diet were observed to contain many fat droplets in their cytoplasm. No conspicuous alterations in the amount of fat droplets were detectable in the liver cells of both the control and the experimental.

Discussion

In the present experiment, pantothenic acid deficient goldfish showed a retardation of growth and a loss of appetite after 30 days of treatment, and further revealed a slight decrease of average body weight during 50 days. Some fish suffered skin lesions mainly appearing as localized hemorrhage, together with the occurrence of exophthalmus and abnormal swimming behaviour after 40–50 days.

From the pantothenic acid deficiency experiment, Wolf[1] came to a conclusion that pantothenic acid deficiency in the diet predisposed the brook trout to an attack of gill disease by making the respiratory epithelium more sensitive to chemical irritations in the water. McLaren et al.[2] reported that the omission of the vitamin caused an early retardation of growth followed by the development of club-shaped gills in rainbow trout. According to Ogino[3], in the carp, extremely poor growth, loss of appetite and hemorrhage of body surface were caused by the vitamin deficiency. In addition, Kitamura[4] observed several pantothenic acid deficiency symptoms such as low growth, high mortality and violent and spiral swimming in rainbow trout fingerlings. The gross symptoms observed in the vitamin deficient goldfish in the present study, therefore, are very similar to those reported by these investigators except that the abnormality of gill filaments was hardly detectable in the goldfish.

In pantothenic acid deficient goldfish, light microscopically, parenchymal cells of the liver contained many small granules stained slightly with eosin, which corresponded to enlarged mitochondria as detected electron microscopically. Ultrastructurally, the liver cells of pantothenic acid deficient fish showed conspicuous alterations in the cytoplasmic organelles. Endoplasmic reticulum developed poorly and appeared as small tubes or small vesicles. Most of the mitochondria became larger in size than those of the control and some of them attached closely to one another. These characteristic features suggest that the mitochondria may fuse one another to increase their size.

So far as the writers are aware of, no report concerning ultrastructural changes of liver cells in pantothenic acid deficient fishes has been published.

In higher animals, however, there are many reports which described symptoms caused by the vitamin deficiency[5)8)9)10)]. Nakamura et al.[6)] observed the liver of pantothenic acid deficient rat by an electron microscope and revealed that a glycogen depletion, the appearance of a large amount of lysosome and an increase in number of microbody are the characteristics of parenchymal cells of the affected liver. They demonstrated further that rough surfaced endoplasmic reticulum had almost disappeared from the cells probably due to the breaking up of their cisternae into small units. Nakamura et al.[11)] reported that an increase in the number and curious deformity of mitochondria, the disappearance of rough surfaced endoplasmic reticulum, and glycogen depletion occurred in rats administered with homopantothenic acid which is known to be one of the antimetabolites of pantothenic acid.

The most striking change in all pantothenic acid deficient animals, therefore, seems to be the change of the mitochondrial form. There are many investigations about the mitochondrial change of the liver induced by various nutritional disorders[12)13)14)15)16)]. The precise mechanisms whereby these enlargement takes place have not been established. So it remains to further more experiments to clarify the mechanism of the mitochondrial deformity in pantothenic acid deficiency.

In higher animals the fatty degeneration is marked change occurring in the liver cells in the case of pantothenic acid deficiency. As demonstrated in the present study, however, no difference in amount of fat droplets of the liver cell in both control and experimental fish is recognized, but it is uncertain whether a long term feeding on the vitamin deficient diet may cause a sign of fatty degeneration in the liver cell of the fish or not.

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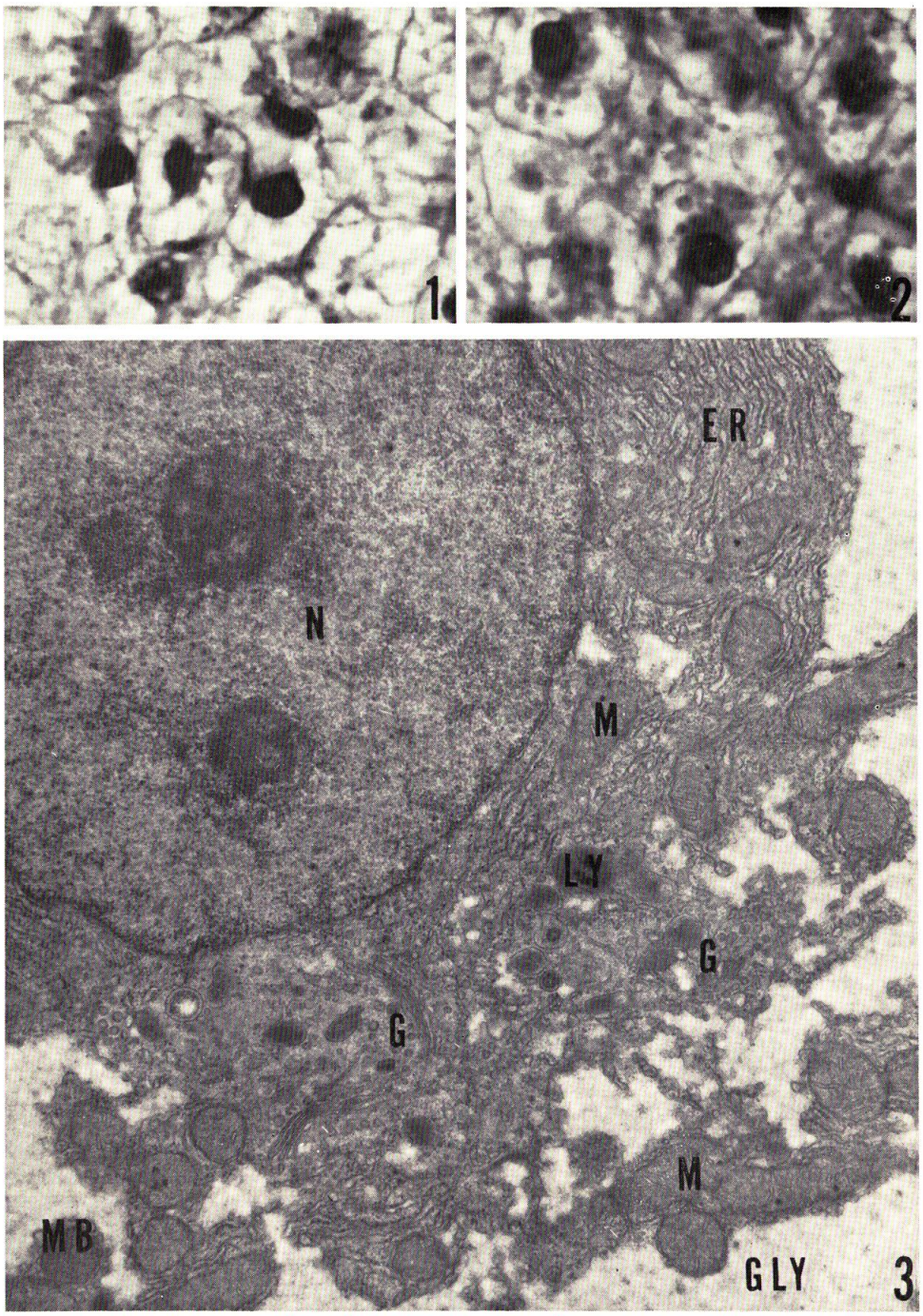
Explanation of Plates

PLATE I

Fig. 1. Photomicrograph of liver parenchymal cells of goldfish fed on basal diet. Hematoxylin and eosin, × 1,200.

Fig. 2. Photomicrograph of liver parenchymal cells of goldfish fed on pantothenic acid deficient diet. Many small granules stained slightly with eosin are seen in the cytoplasm. Hematolxylin and eosin, × 1,200.

Fig. 3. Electronmicrograph of liver parenchymal cells in a normal goldfish. Various cytoplasmic organelles are seen concentrated in the perinuclear region. ER, endoplasmic reticulum; G, Golgi complex; GLY, glycogen deposit; LY, lysosome; M, mitochondrion; MB, microbody; N, nucleus, × 18,000.



ISHII & YAMAMOTO: Liver of pantothenic acid deficient goldfish

PLATE II

Fig. 4. Electronmicrograph of liver parenchymal cells in pantothenic acid deficient goldfish. The mitochondria became larger in size than those of control. Vesicular endoplasmic reticulum of small size was scattered in the cytoplasm. F, fat droplet; G, Golgi complex; M, mitochondrion; N, nucleus. × 14,000.

Fig. 5. Electronmicrograph of liver parenchymal cells in pantothenic acid deficient goldfish. Amorphous dense bodies of various sizes, lysosomal in nature, appear in the cytoplasm. G, Golgi complex; M, mitochondrion, × 12,000.

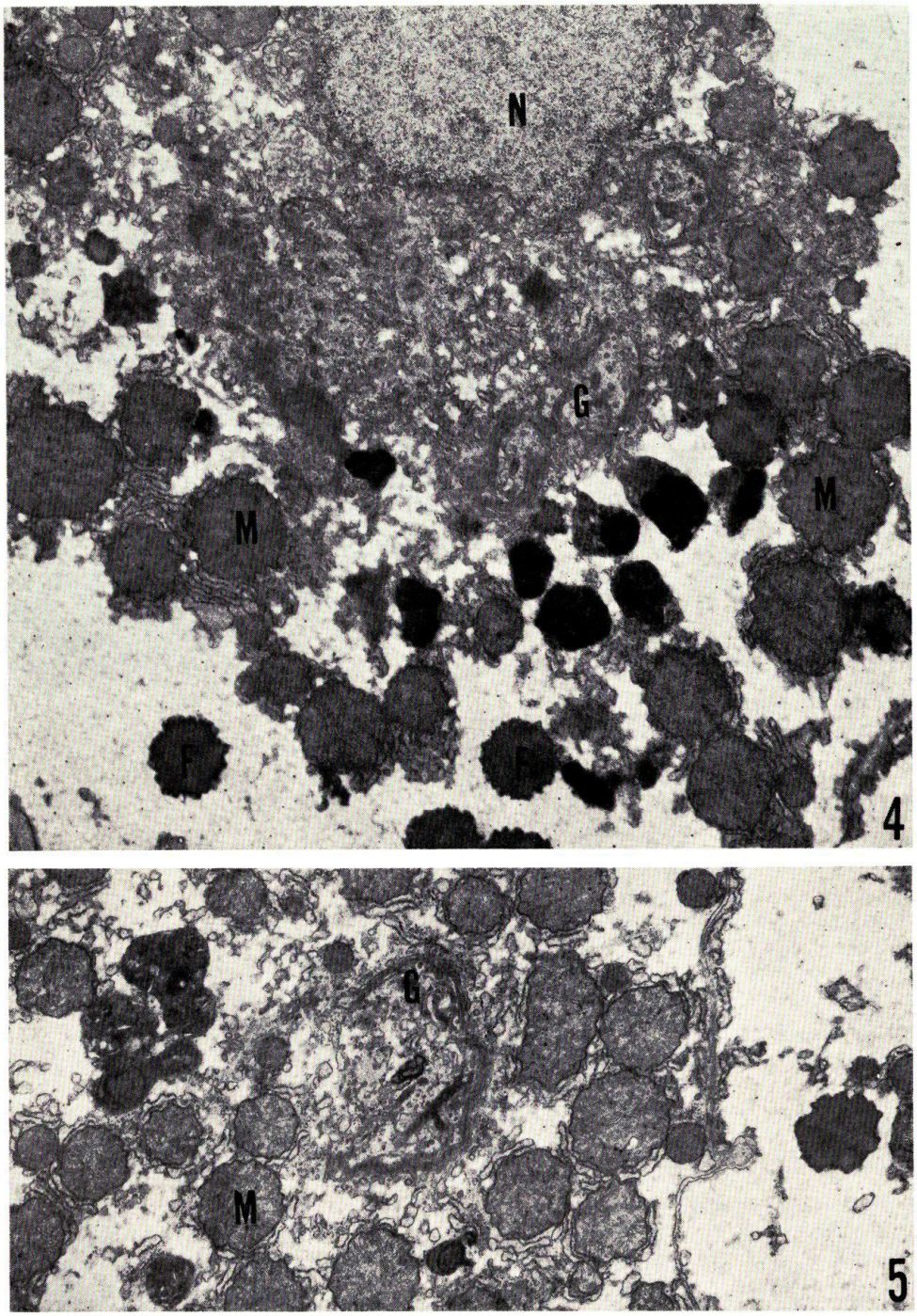


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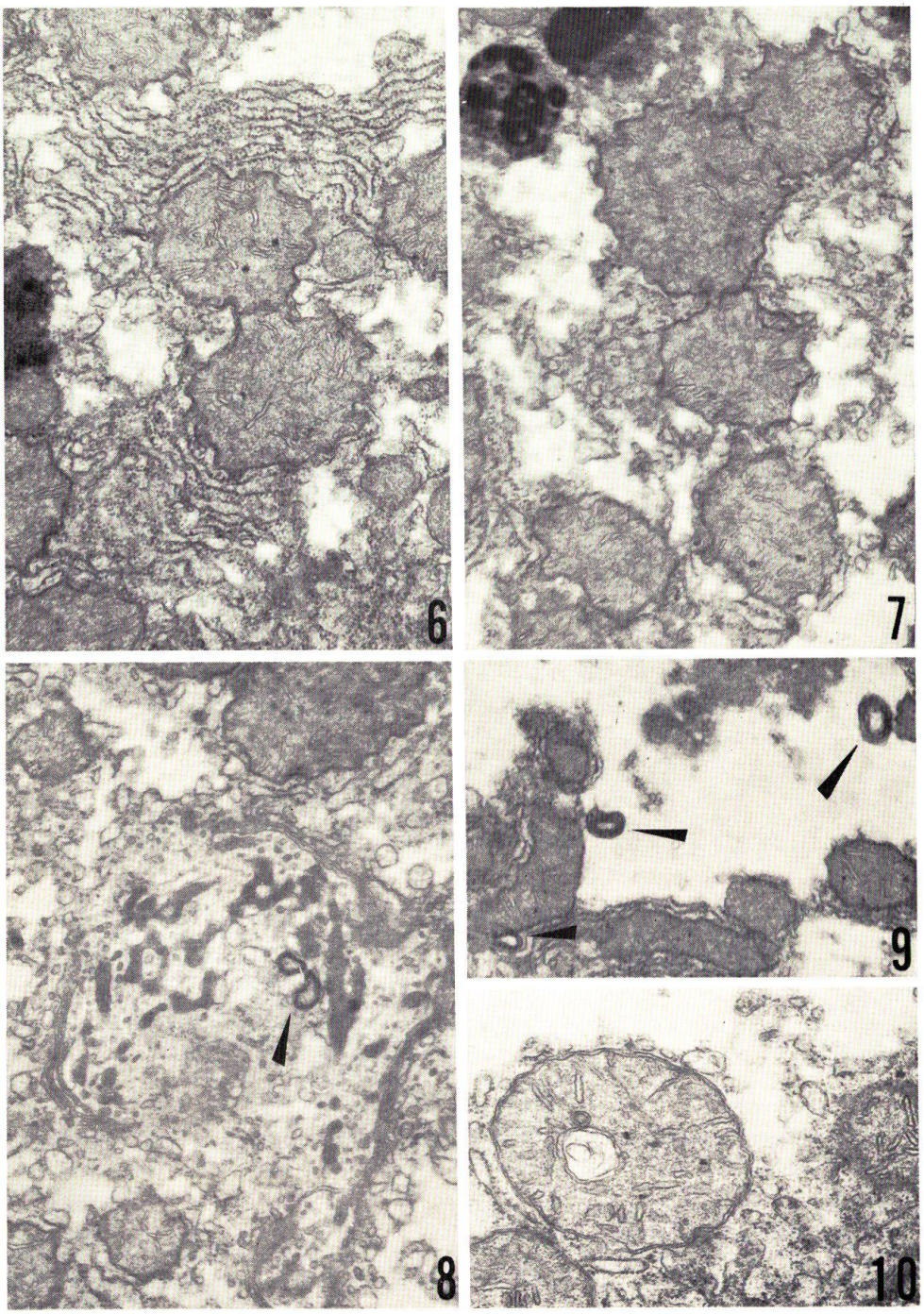
PLATE III

Figs. 6 and 7. High power electronmicrographs of mitochondria in the liver parenchymal cells in pantothenic acid deficient goldfish. Some mitochondria attached closely to one another. Fig. 6, × 31,200; Fig. 7, × 27,600.

Figs. 8 and 9. High power electronmicrographs of liver parenchymal cells in pantothenic acid deficient goldfish. Electron dense, myelinated bodies (arrows) were present closely to the Golgi area (Fig. 8), or scattered in the cytoplasm (Fig. 9). Fig. 8, ×21,700; Fig. 9, × 19,200.

Fig. 10. High power electronmicrograph of liver parenchymal cells of pantothenic acid deficient fish. Some mitochondria contained a vacuole in the matrix. × 29,200.



ISHII & YAMAMOTO: Liver of pantothenic acid deficient goldfish