**Studies on Intramitochondrial Inclusions in the Pancreatic
Acinar Cells of the Japanese Newt, *Triturus
pyrrhogaster*. II. The disappearance of
Intramitochondrial Inclusions in
Re-fed Animals following
Starvation**

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Summary: Intramitochondrial inclusions in pancreatic acinar cells of the re-fed Japanese newt, *Triturus pyrrhogaster*, following starvation were examined with the aid of an electron microscope. Clusters of mitochondria and the close apposition between mitochondria and rough endoplasmic reticulum were observed in the first few hours after re-feeding. Unusual mitochondria, which were observed in the acinar cells of the starved animals, decreased in number and size after re-feeding and finally disappeared. We suggest that intramitochondrial inclusions are temporarily induced as a result of depressed mitochondrial function due to prolonged starvation and also that type III inclusions (crystal or crystalloid) originate from the mitochondrial membrane and matrix.

In the past few decades, considerable attention has been paid to intramitochondrial inclusions with respect to mitochondrial structure and function. However, the mode of formation and especially the fate of these inclusions have been unclear except for those found in frog oocytes (Massover 1971a and b), and rat brown adipocytes (Barnard and Lindberg 1969, Lindgren and Barnard 1972, Suter 1969). The disappearance of inclusions has been reported in rat brown adipocytes in the early hours following birth (Barnard and Lindberg 1969, Lind-

gren and Barnard, 1972, Suter 1969) and in rat hepatocytes during rehydration after prolonged water deprivation (Bartok et al 1975). However, there have not been any precise experimental studies known to us on the process of restoration of the unusual mitochondria to more common forms.

In previous work, we reported the occurrence of unusual mitochondria with three types of inclusions in pancreatic acinar cells of the starved Japanese newt (Taira 1979). Type I inclusion is a localized enlargement of the mitochondrial

matrix or an area devoid of cristae. Type II inclusion is a dense homogeneous body. Type III inclusion is a crystal or crystalloid. Other unusual mitochondria have parallel arrays of cristae. The purpose of this study as an approach to understanding the structure and function of mitochondria is to elucidate more detailed morphological features of these unusual mitochondria which are restored to common forms after re-feeding without any degenerative changes.

Materials and Methods

Japanese newts, *Triturus pyrrhogaster*, of both sexes were collected in the suburbs of Maebashi in the spring, and adapted to the laboratory aquarium at 17°C for one month being fed commercially obtained swine liver regularly every 3 days. Thereafter the animals were starved for 4 weeks (control), then groups comprising five newts each were killed at 3, 6 and 12 hours, and at 1, 2, 4 and 8 days after the first re-feeding. Feedings were successively carried out at 3 and 6 days after the first re-feeding. Preparations of small pancreatic tissues for electron microscopic examination were similar to those described in our previous study (Taira 1979).

Results

Three marked changes were observed in pancreatic acinar cells of the starved animals. Namely, many fat droplets were seen in the basal portion of the cytoplasm, there were unusual types of mitochondria (Figs. 1-3) and vesicular or whorled endoplasmic reticulum (ER).

Zymogen granules decreased in number in the early hours following re-feeding and thereafter they increased quickly. Marked formation of zymogen granules was found in animals on the 2nd day

following re-feeding. The profiles of ER changed from vesicular to tubulo-vesicular forms at 3 hours following re-feeding. Whorls of ER slowly decreased in number and size but were occasionally found in the perinuclear zone even on the 8th day following re-feeding (Taira 1981). Fat droplets also decreased in number and size. Intracisternal granules and annulate lamellae were found in several cells.

As for mitochondrial changes, intramitochondrial granules gradually decreased in number. In early stages following re-feeding clusters of mitochondria were frequently observed (Figs. 4-6). They were located close to each other and smooth-surfaced cisternae of ER were interposed between these mitochondria (Fig. 4). Thereafter these contacting mitochondria decreased in number. No other unusual mitochondria, different from those observed in the previous study (Taira 1979), were seen in the acinar cells of re-fed animals. The situation which restores unusual mitochondria to common forms varied from cell to cell; since all the mitochondria did not change synchronously and the mitochondria in all the cells did not show similar structures at the same stage after refeeding. In the process of restoration inclusions were only found in the mitochondrial matrix, and degenerating mitochondria were never found in any observations in the present experiment.

Type I inclusions (Fig. 5) were found from 3 hours to one day following re-feeding, and thereafter decreased in number. They disappeared more quickly than type III inclusions or parallel arrays of cristae. Their enlarged matrix area was filled with a large amount of amorphous or fine granular substance similar to that found in the matrix of common mitochondria. Type II inclusions (Fig. 7) also disappeared by the 4th day following re-feeding, but their precise process of

recovery to common mitochondria was not demonstrated in the present experiment, since they were not numerous and were difficult to evaluate. Type III crystal inclusions became small and their lattice-like parallel pattern appeared indistinct following re-feeding (Fig. 8). Type III crystalloid inclusions also became small and irregular, and parts looked quite similar to their inner membrane or cristae (Figs. 9 and 10). Mitochondria with parallel arrays of cristae became smaller and shorter than those observed in the starved animals and the frequency of mitochondrial cristae aligned obliquely in their long axis increased (Fig. 11). Some mitochondria had wavy and irregular cristae inclined at various angles to their long axis. By the 2nd day following re-feeding none of these parallel arrays of cristae were found in any mitochondria examined.

Discussion

The present study confirmed the findings of our previous work (Taira 1979) that three types of intramitochondrial inclusions were located only in the mitochondrial matrix. They may be induced during prolonged starvation and restored to common forms following re-feeding. No evidence was found to support the hypothesis that mitochondria with various inclusions degenerate. The restoration from unusual mitochondria to common forms has been reported in a few types of inclusions of rat brown adipocytes (Barnard and Lindberg 1969, Lindgren and Barnard 1972, Suter 1969), hepatocyte (Bartok et al 1973) and follicular cell of rabbit thyroid glands (Nathaniel 1978).

In the first few hours following re-feeding, some mitochondria were positioned in close proximity to each other. Moreover, their outer membranes were sometimes closely in contact with the elongated part of the ER. According to

an earlier report (Bew et al. 1969), clusters of mitochondria have been found in hepatocytes of fasted rats after prolonged water deprivation. In these rats interdigitations of the membranes of adjacent mitochondria often formed chains of mitochondria. There are protrusions of one mitochondria into another. However, the function of these clusters of mitochondria remains to be elucidated.

Close apposition between ER and mitochondria has been reported in oocytes (Kessel 1971), hepatocytes (Bartok et al 1973, Riede and Rohr 1971, Tuchweber et al 1972, Norton et al. 1977) and follicular cells of the thyroid (Nunez et al. 1975). It is difficult to imagine that the close apposition between ER and mitochondria is without functional significance. Recent studies suggest that such mitochondria-ER complexes may operate as functional units in the synthesis an assembly of cytochrome P-450 and possibly in other biosynthetic processes involving these organelles (Meier et al. 1978, Pickett et al. 1981). Our previous and present studies also demonstrated the close apposition between mitochondria and ER. These findings suggest the hypothesis that the raw material of the intramitochondrial inclusion is produced in the ER and transported from the ER into the mitochondria.

Type III crystal inclusions became small after re-feeding. Sometimes the periphery of the crystal inclusion is present in close proximity to the enlarged matrix of the mitochondria. From the previous and present results, it seems reasonable to assume that type III crystal inclusions originate from the mitochondrial matrix.

It is an important finding that type I inclusions were not found in animals starved for 4 weeks but became prominent within the first few hours following re-feeding. In the previous work type I inclusions had been found in one or two

weeke of starvation. Therefore, it seems likely that type I inclusions are a transitional form in the formation of or restoration from type III crystal inclusions. The above-mentioned assumption accords with the findings of mitochondria with enlarged matrix in amphibian oocytes which may be in a temporarily form of intramitochondrial yolk formation (Massover 1971a). Type I inclusions look very similar to mitochondria with enlarged matrix seen in amphibian oocytes, tempting us to conclude that they perform an identical function.

On the other hand, type III crystalloid inclusions may originate from mitochondrial cristae or inner membranes, since the continuity between type III crystalloid inclusions and the mitochondrial inner membranes or cristae was sometimes found. Following re-feeding mitochondria with highly ordered structures of type III crystalloid inclusions seemed to diminish representing a continuity of the inclusions with the mitochondrial membrane. It is clear that mitochondria with parallel arrays of cristae are a variation of mitochondria with common transversed cristae.

From the previous and present studies, it seems reasonable to assume that intramitochondrial inclusions in the newt pancreas originate from mitochondrial membranes and matrix. However, the chemical nature of the inclusions remains to be elucidated.

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Explanation of Figures**Abbreviation**

W, Whorled ER.

Plate I

Fig. 1. The enlarged matrix of a type I inclusion (smaller arrow) in a fasted newt consists of a large amount of fine granular substance. The larger arrow indicates a type III inclusion. The cytoplasm of acinar cells contains tubulo-vesicular and whorled ER(W). The bar represents 0.5μ in all figures. $\times 33,000$.

Plate II

- Fig. 2. A type III crystal inclusion in a fasted newt. It is directly embedded in the matrix without any surrounding membrane or hollow space. The distance between adjacent dense bands is found to be about 130 Å and the dense bands meet at the long axis of the mitochondrion at an angle of about 60°. $\times 46,000$.
- Fig. 3. A type III crystalloid inclusion in a fasted newt. Highly organized structures are discernible within the matrix. $\times 93,000$.

Plate III

- Fig. 4. The close apposition of mitochondria and ER in a re-fed newt (6 hours following re-feeding). Part of the ER degranulates (arrow) and seems to be sandwiched by closely locating mitochondria. $\times 36,000$.
- Fig. 5. Four mitochondria in close contact with each other in a re-fed newt (6 hours following re-feeding). One contains an enlarged matrix (type I inclusion). $\times 36,000$.
- Fig. 6. Parallel arrays of cristae membranes (arrow) are found in one clustered mitochondria (6 hours after re-feeding). $\times 42,000$.
- Fig. 7. Mitochondrion containing dense osmiophilic bodies (type II inclusions) in a re-fed newt (2 days following re-feeding). The degradation process in this type of inclusion is obscure because of their scarcity. $\times 31,000$.

Plate IV

- Fig. 8. The coexistence of type I (smaller arrow) and type III crystal (larger arrows) inclusions in a mitochondrion of a re-fed newt (6 hours following re-feeding). An indistinct parallel pattern seems to represent the residual of type III crystal inclusions. Note the transversely and longitudinally arranged cristae in the mitochondrion. $\times 50,000$.
- Fig. 9. The membranous structures of type III crystalloid inclusions closely resemble mitochondrial cristae (2 days after re-feeding). $\times 45,000$.
- Fig. 10. Residual structures (arrow) may represent a transition from type III crystalloid inclusions to common cristae (3 hours following re-feeding). $\times 44,000$.
- Fig. 11. A mitochondrion contains cristae which meet at various angles along their long axis (2 days after re-feeding). The smaller arrows indicate the faint parallel structures of type III crystal residues. The larger arrow indicates the continuity between the ordinary cristae and dense parallel arrays. $\times 40,000$.