MITOCHONDRIAL CHANGES INDUCED BY IRON ABSORPTION IN THE DUODENAL ABSORPTIVE CELLS OF RATS

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The ultrastructural changes of the duodenal absorptive cells of rats after gastric infusion of ferrous chloride were investigated with the aid of the electron microscope. This paper concerns the occurrence of characteristic dense granules in the space between the double membranes of the mitochondria of the cells.

MATERIALS AND METHODS

Ten young albino rats of Wistar strain were used for this experiment. For 24 hours prior to the experiment all of the animals were put into narrow cages to prevent coprophagia and received nothing but distilled water. Ferrous chloride was obtained by reduction from ferric chloride with ascorbic acid just before administration. About 0.5 ml of the solution containing 3 mg of iron was infused into the stomach of eight rats through a polyethylene tube. Two rats were sacrificed after 30 minutes, 1, 2, and 3 hours, respectively. Another two rats were killed as control without receiving iron solution. Specimens of the duodenum approximately 2 cm distal to the pyloric ring were fixed in buffered 1 per cent osmium tetroxide (pH 7.4) with sucrose added, and then dehydrated in alcohol, embedded in Epon, and sectioned with a Porter-Blum microtome. Most thin sections were stained with uranyl acetate, and were examined by a Hitachi HU-11A type electron microscope.

OBSERVATIONS

The mitochondria in the duodenal absorptive cells of the control rats are similar to those found elsewhere (1, 2). In some specimens mitochondrial particles (1) 200 to 300 A in diameter are found embedded in the mitochondrial matrix.

The main changes observed in the duodenal absorptive cells after infusion of iron concern the mitochondrial outlines which become irregular, and the matrix which is less dense than in controls. The most remarkable change is the appearance of characteristic dense granules in the mitochondria (Figs. 1 to 3). These granules are different from the mitochondrial particles of the control rats in the following respects. Although the mitochondrial particles in the control rats are embedded in the mitochondrial matrix, these dense granules are located in the outer mitochondrial chamber and within the cristae (Figs. 2 and 3). The granules are more dense than mitochondrial particles in the control rats. All mitochondrial particles in the control rats are homogeneous, but the dense granules have an appearance of aggregates of numerous subunits (Fig. 3). The dense granules are 200 to 500 A in diameter and are larger than

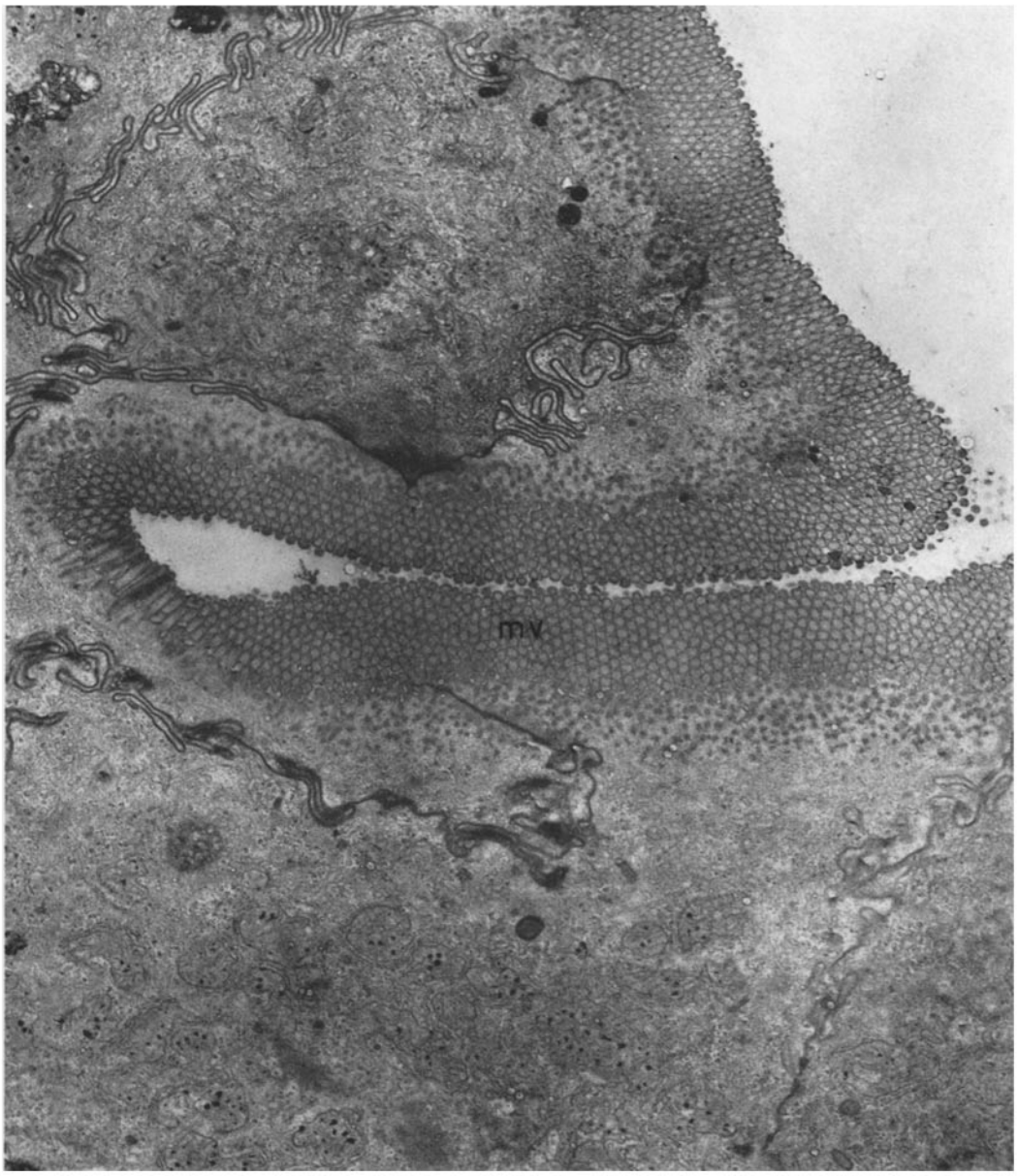

FIGURE 1 A part of the apical portion of several duodenal absorptive cells of a rat that had received gastric infusion of ferrous chloride 1 hour before being killed. Many dense granules are seen in the mitochondria. *mv*, microvilli. × 9,600.

the mitochondrial particles in the control rats. Besides these characteristic dense granules, numerous fine particles are also observed dispersed in the mitochondrial matrix as shown in Fig. 3. These fine particles may have approximately the same size as the subunits composing the dense granules. Both fine particles dispersed in the mitochondrial matrix and subunits composing the dense granules differ morphologically from ferritin.

In specimens obtained 1 and 2 hours after the

iron administration almost all mitochondria have abundant dense granules in many absorptive cells, and in some sections over thirty granules are seen in a mitochondrial profile. Thirty minutes and 3 hours respectively following infusion the dense granules are few in number. These granules are also observed in the jejunal absorptive cells, but not in the gastric epithelial cells.

DISCUSSION

Particulate matter within the mitochondria of many cells has been reported by many investigators (1–4). Weiss (5) observed the increase of the mitochondrial particles in the duodenal absorptive cells of mouse fed large amounts of sodium or potassium. Recently Bessis *et al.* (6) and Policard *et al.* (7) observed ferritin and silica granules in the mitochondria. Their particles and granules were described as existing in the matrix of the mitochondria. Nilsson (8), and Frei and Sheldon (9) observed dense bodies in the mitochondrial cristae in the cuboidal cells of the uterus or in the hyperplastic epidermal cells of the mouse. Moreover, Helander (10) described some dense granules in the mitochondrial cristae of the gastric mucoid cells of the mouse, and Ward (11) observed platelike, hexagonally shaped bodies in the mitochondrial cristae of the amphibian eggs. However, dense bodies and granules observed by these authors are different from the characteristic dense granules we have observed: the former are larger in size, less dense, fewer in number, and more homogeneous than the latter.

Next, a question arises concerning the relation between iron absorption and the changes described. Bessis *et al.* (12) and Richter (13) have described how the mitochondria may participate in normal iron metabolism. It is an indisputable fact that many characteristic dense granules appear in the mitochondria of the duodenal absorptive ceils of the rats after gastric infusion of iron in absorbable state. This evidence suggests that there is a close relation between the appearance of dense granules in the mitochondria and iron absorption. However, because of our failure to demonstrate an iron component in the granules, we hesitate to conclude that the dense granules may represent iron segregated within the mitochondria.

SUMMARY

Many characteristic dense granules are demonstrated in the space between the double membranes of the mitochondria in the duodenal absorptive cells of rats after gastric infusion of iron. These granules are different from the mitochondrial particles in the normal rats in their location, density, substructure, and size. Although the authors can not prove whether the dense granule contains iron or not, they suppose that there is a close relation between the appearance of the granule and iron absorption.

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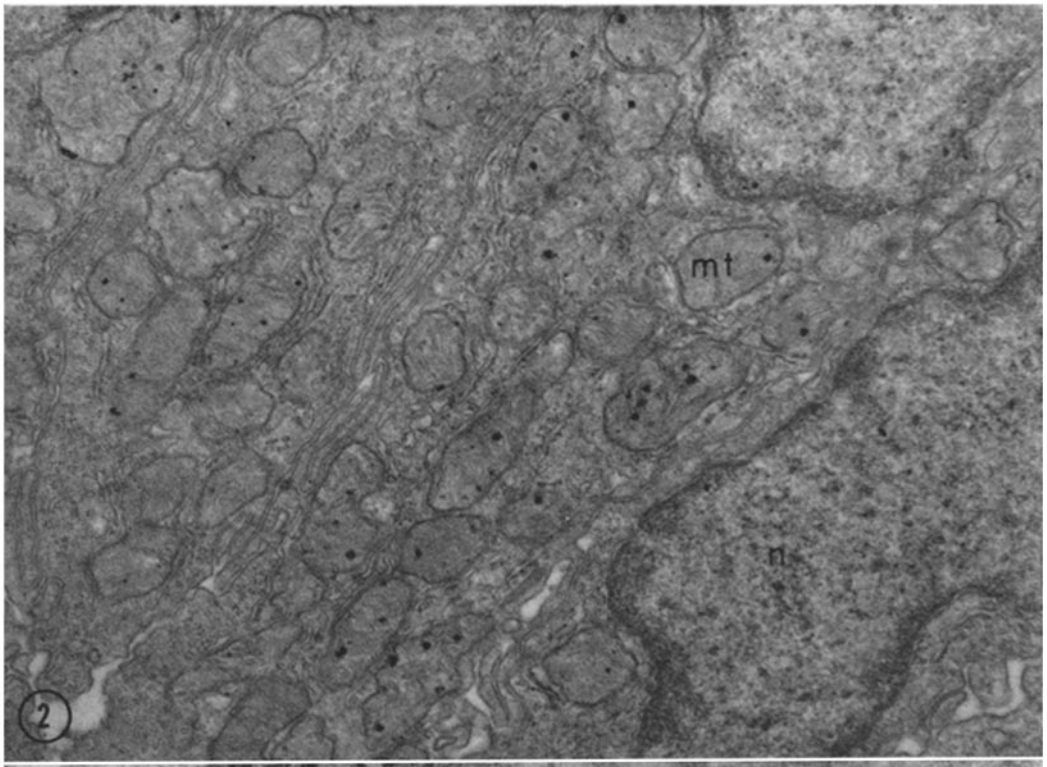
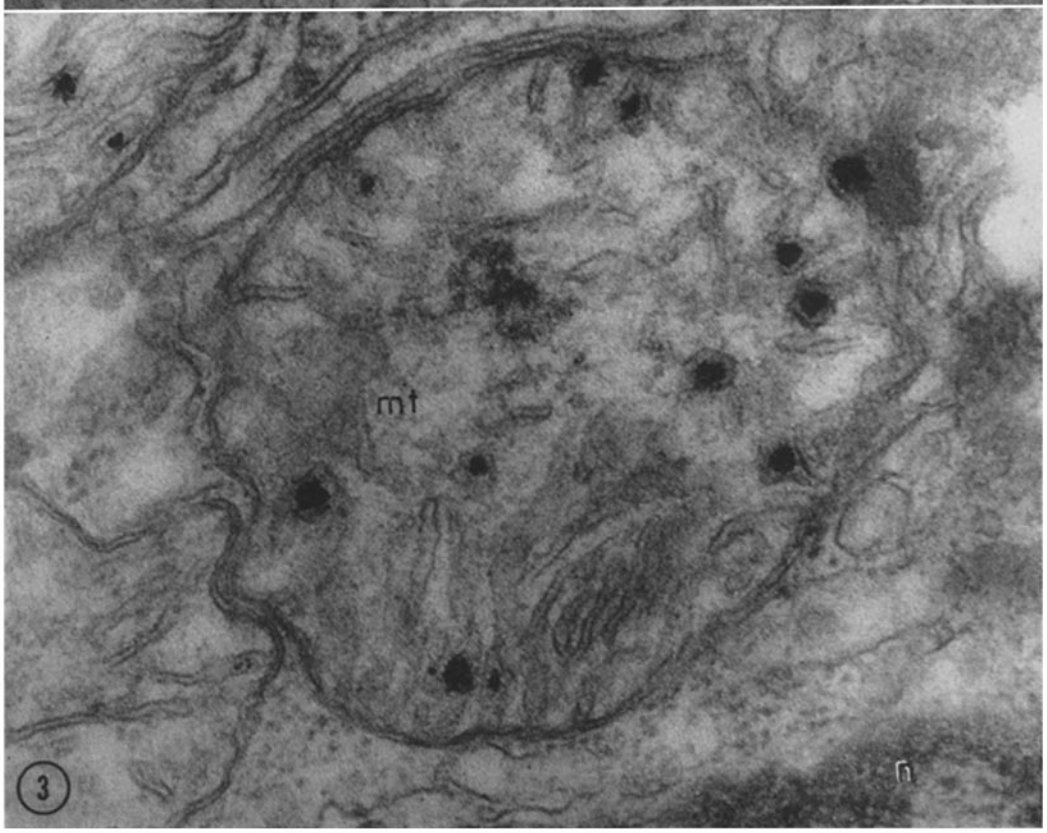
REFERENCES

1. PALADE, G. E., *Anat. Rec.*, 1952, **114**, 427.
2. SJÖSTRAND, F. S., and RHODIN, J., *Exp. Cell Research*, 1953, **4**, 426.
3. ROUILLER, C., *Internat. Rev. Cytol.*, 1960.
4. NOVIKOFF, A. B., and ESSNER, E., *Am. J. Med.*, 1960, **29**, 102.
5. WEISS, J. M., *J. Exp. Med.*, 1955, **102**, 783.
6. BESSIS, M., and BRETON-GORIUS, J., *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 231.
7. POLICARD, A., COLLET, A., DANIELL-MOSSARD, H., and PREGERMAN, S., *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 236.
8. NILSSON, O., *J. Ultrastruct. Research*, 1958, **1**, 375.

FIGURE 2 A part of the basal portion of three duodenal absorptive cells of a rat that had received gastric infusion of ferrous chloride 2 hours before being killed. Note dense granules located in the mitochondrial cristae and between the double limiting membranes of mitochondria (*mt*). *n*, nucleus. × 24,000.

FIGURE 3 The specimen obtained from the same rat as in Fig. 2. A mitochondrion near the nucleus is shown at higher magnification. Twelve granules are seen in this mitochondrial profile. Most of granules are surrounded with a membrane that forms mitochondrial cristae, and have an appearance of aggregates of small subunits. Besides these granules many fine particles are observed in the matrix of the mitochondrion. × 96,000.





9. FREI, J. V., and SHELDON, H., *J. Biophysic. and Biochem. Cytol.*, 1961, **11**, 724.
10. HELANDER, H. F., *J. Ultrastruct. Research*, 1962, suppl. 4, 62.
11. WARD, R. T., *J. Cell Biol.*, 1962, **14**, 309.
12. BESSIS, M., and BRETON-GORIUS, J., *Blood*, 1959, **14**, 423.
13. RICHTER, G. W., *J. Exp. Med.*, 1959, **109**, 197.