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On the Iron Component of the Golgi Apparatus¹⁾

By

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(With 3 Plates)

Reviewing the literature, one can expect the possibility of absorption of metallic substances upon the Golgi apparatus. Among these cases, that of iron may be important from the physiological point of view. Iron has been known to combine with many physiologically important substances in cellular metabolism; *i.e.* hemoglobin, peroxidase, cytochrome, catalase, *etc.*

In respect to the problem of the Golgi apparatus, Makarov (1931) seems to be the first who found the absorption of iron upon the apparatus, when the iron had been taken into the body in the form of the ester of saccharic acid. In this case, the localization of this iron compound, which is made visible by Prussian blue reaction, was strictly corresponding to the networks of the apparatus; hence he termed this figure "Eisennetz." Van Tier (1940) made similar experiments concerning the Golgi bodies in the intestinal epithelial cells of *Ascaris*. In this work, he also found the phenomenon of absorption of iron upon this cell organ. But the above two cases dealt with the absorption of artificially injected iron compounds. The distribution of iron in the intact cells, observed in cytological order, has never been known to the present author. The difficulty in this line of study lies in the obscurity of chemical reaction in the preparations. This obscurity of reaction is probably due to the condition of iron compound. The inorganic iron salts are easily detectable by the routine iron reaction, *i.e.* Prussian blue reaction. But, on the contrary, the organic compounds which are normally linked, or masked, by the proteins or lipinous substances, reveal far weaker reactions. Such ambiguous reaction causes frequently to overlook the presence of iron compounds, especially in protoplasm. But, if special care is taken, a faint blue color of masked iron is observable. For instance, the hemoglobin in the red cells reacts by the routine technique. To make sure about the distribution of masked iron in the protoplasm, the present author attempted the incineration method, by which the iron compounds are all converted into the form of ferric oxide. The iron ash was differentiated from the other kinds of ashes by its reddish color. The rela-

1) Contribution No. 26, from Biological Section, Tokyo Woman's Christian College. This work was preliminarily reported at the annual meeting of the zoological society of Japan, held in November 1953, in Kyoto.

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tionship between the iron ash and the Golgi apparatus is detailed below.

Material and methods

Throughout the study the materials were taken from albino rats. Nerve cells in the spinal ganglion, liver cells, and pancreatic cells were the main materials. As the control, Kolatchev's method of osmium tetroxide impregnation was employed. For the detection of iron salts, the material was fixed by means of the freezing-drying method.¹⁾ The result of this fixation proved to be excellent. The sections were cut 10 micra in thickness. To avoid the artificially induced iron which may come from the blade of the microtome, the writer covered the blade with a film of paraffin or wax.

Prior to the incineration of the sections, the bare slide glasses were heated up to 600°C and kept at this temperature for several minutes. By this procedure the alkali salts in slide glasses can be driven off to some degree. Under the microscope the dusty particles of alkali salts were seen covering the surface of the slides. These particles were removed altogether with a cloth, and then the paraffine sections were affixed on them by pressing them with a cork stopper, without using egg albumen. The paraffin was removed in pure benzol, and then the material was subjected to following procedures.

The heating in the electric furnace was performed by a gradual rising of temperature in the following manner.

135°C	15 min.
200°C	10 min.
300°C	10 min.
530°C	20 min.
550°C	5 min.

Thus, it takes about one hour to get adequate temperature for the incineration of the sections. To obtain a better spodogram, it was found that the maintenance of the temperature between 530°—550°C for 10—15 min. was indispensable. After cooling the furnace, the ash on the slide was covered with a cover slip carefully, and the margin was sealed with wax.

Observations

a) *Nerve cells*: In the spinal ganglion, there are two kinds of nerve cells: large nerve cells in which the Golgi bodies assume a dispersed form, and small nerve cells in which the Golgi apparatus assumes networks around the nuclei (Pl. VI, Fig. 1). Among these two kinds of cells, the small cells are preferable for observation, because the Golgi apparatus can be easily differentiated from the other cytoplasmic inclusions by its topography. In the incinerated preparation, a cluster of coarse granules is found around the nucleus of the small cell (Pl. VI, Figs. 2, 3). These granules are refringent and glisten in a pale red color under

1) Dr. M. Nakajima of the Tokyo Educational University kindly gave me the opportunity of using the apparatus.

dark field illumination. Another kind of coarse granule which has no appreciable color, is found in the cytoplasm, especially in the peripheral region. This kind of granule is also found in the intercellular spaces. The cytoplasm is generally occupied with glimmering minute granules which have pale blue color. The same cells which are subjected to the Prussian blue reaction show the weak blue reacting area around the nuclei (Pl. VI, Fig. 4). This blue area is taken for the locus of the Golgi network.

b) *Liver cells*: In liver cells the distribution of the cellular elements is apt to alter according to the physiological activity. For this reason, material to be compared must be cut from the same area of the same animal. The Golgi apparatus is represented in the liver cells by bodies with irregular shape, which are usually found in the juxta-nuclear region and along the bile capillaries (Pl. IV, Fig 5). Sometimes they connect these two regions forming a bridge between them. In the spodogram of the same material, granules with weakly red color showed the same distribution as the control which was impregnated with osmic acid (Pl. VI, Fig. 6, Pl. V, Fig. 7). In the Prussian blue preparation, the reacting area was also found in the same place (Pl. V, Fig. 8), although the blue color is considerably weaker. As for the ash image of the other areas in the cytoplasm, it is almost the same as the nerve cell detailed above.

c) *Pancreatic cells and cells of the intestinal epithelium*: In the pancreatic cells, the morphology of the Golgi apparatus varies considerably with the stages of secretion. But at any stage, the apparatus is located in the cytoplasm on the border line toward the secretory area which contains secretory granules (Pl. V, Fig. 9). Ashes of brownish color occupy the area almost corresponding to the Golgi zone in the control impregnated with osmic acid (Pl. V, Figs. 10, 11). In contrast to the Golgi zone, the secretory area contains strongly refractive ash granules without any color. The ash image of the cytoplasm reveals a granular structure of pale blue color. By the application of Prussian blue reaction, a slight positive reaction was obtained in the area corresponding to the Golgi apparatus (Pl. V, Fig. 12).

As for the cells of the intestinal epithelium, the result of the fixing by freezing-drying method was not successful. Among many fixatives which do not contain metallic salts, the combination of alcohol and chloroform of equal parts gave the best result. Though the cause remains unknown, the ash image of the intestinal epithelium was quite unsuccessful. Aside from the ash image, the Prussian blue test gave a considerably positive reaction (Pl. VI, Fig. 13). In the intestinal epithelial cell, the Golgi apparatus has been known generally to occupy the position between the basal nucleus and the secretory area. A blue body with an irregular shape which corresponds exactly to the Golgi apparatus of the control was found in this preparation.

d) *Liver cells of the newt*: As was shown by the present author (Tarao, 1939), the Golgi apparatus in the liver cells of the newt has a characteristic feature

of bands which entangles along the bile capillaries (Pl. VI, Fig. 14). In these cells, there are many lipinous droplets which are blackened with osmic acid. These droplets are easily distinguishable from the Golgi elements by dissolving the former in turpentine oil. By the examination of the ash image, it was found that intensely red ashes were mingled with brown or white ashes which formed the matrix of the Golgi band (Pl. VI, Figs. 15, 16, 17). These bands were also reactive to the Prussian blue test for iron salt (Pl. VI, Fig. 18). Along the intercellular spaces or blood sinuses supplied from the portal veins, strongly refractive white ash granules are deposited. Throughout the cytoplasm, pale blue minute granules are distributed evenly and sparsely.

Discussion

In the living cytoplasm, metallic elements are usually in combination with proteins or lipinous substances. In such cases, they are non or weakly reactive to specific tests. Even in the fixed materials the reactions are not intense. The metals in such cases have been thought to be in the masked condition. This masked iron was disclosed by the incineration of the sections. It has been found in this work that the masked iron becomes considerably reactive to the Prussian blue test after the material is fixed by means of the freezing-drying method.

The combination of freezing-drying method and microincineration was first developed by Scott (1933).¹⁾ He proved that the shift of ions might be overcome by dehydrating tissues at temperatures ranging from -38°C to -78°C . In the present work, the temperature was kept at nearly -30°C using a mixture of dry ice and ether. Special care was taken to cut out materials as small as possible, and these very small fragments were plunged into the freezing mixture. For these reasons, a severe artificial shift of the mineral elements in the cellular details could be almost avoided.

The optic differentiation of the mineral elements in the spodiogram has been frequently discussed (Policard, 1929; Scott, 1951). They found that calcium and magnesium compounds give a white amorphous ash, the differentiation between them being impossible from visual appearances alone. Sodium and potassium compounds give minute pale blue ashes. The color of ferric oxide, which can be obtained by heating the iron compounds at about 500°C , ranges from a yellow to a reddish tinge. According to Policard (1929), the deep coloration indicates the presence of free iron. The reddish or brownish color of the incinerated Golgi area may show the presence of iron compounds in the apparatus. This fact is supported by the positive reaction to Prussian blue test in the same area.

Contrary to the claim by Scott (1951), Nissl bodies are observed to be poor in calcium salts (Pl. IV, Figs. 2, 3). In the Golgi band in the liver cells of the newt, there is a fairly large volume of free iron as shown by the presence of the ash

1) Refer to Horning's description (1951).

image with intense red color. This fact can be naturally conjectured from the function of liver concerning the break-down of hemoglobin into bile pigments and free iron.

What kind of iron compound really is in the Golgi apparatus is unknown at present. Yet one may suppose that the physiologically active iron compounds are associated with such cell structures as mitochondria. Future investigations shall be directed along these lines.

Summary

1. The microincineration method was applied to frozen dried materials: ganglion cells, liver cells, pancreatic cells, intestinal epithelial cells of the rat, and liver cells of the newt.

2. The spodogram of these cells indicates that the Golgi zones contain ashes of ferric oxide. This result means that the Golgi apparatus is generally associated with iron compounds which are masked by proteins or lipinous substances. Moreover, these iron compounds on the apparatus may play important rôles in cellular physiology, and they are not mere waste products of cellular metabolism.

3. The presence of iron compounds upon the Golgi apparatus is on the other side supported by the positive results of the Prussian blue test of ferric compounds.

4. In contrast to the Golgi apparatus in most kinds of cells where the masked iron is found, free iron is shown in the liver cells in the newt. In the latter material, the ash of the Golgi apparatus contains intensely red amorphous masses, which are presumed to be the products of the decomposition of hemoglobin.

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Explanations of Plates IV to VI

All the microphotographs are taken at the same magnification of $\times 800$. Golgi apparatuses are indicated by arrows.

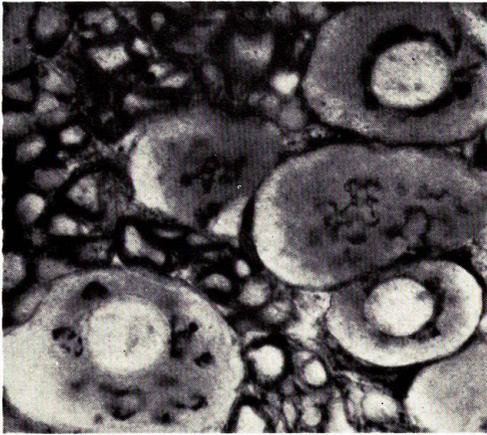
Plate IV, Figs. 1-4. Spinal ganglion cells of rat. Fig. 1. Kolatchev preparation as the control. Perinuclear Golgi network is seen. Fig. 2. Ash image of the Golgi apparatus in the dark field. Fig. 3. Drawing of ash image in natural color, Golgi ashes being tinged reddish. Fig. 4. Frozen-dried material subjected to Prussian blue test.

Plate IV, Fig. 5 to Plate V, Fig. 8. Liver cells of rat. Fig. 5. Kolatchev preparation. Fig. 6. Ash image of cells. Fig. 7. Drawing in natural color, the apparatus is colored reddish. Fig. 8. Prussian blue preparation.

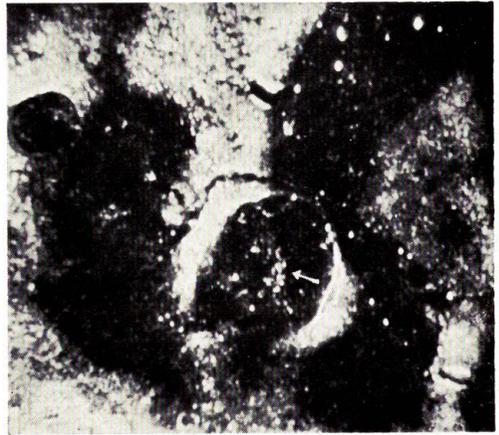
Plate V, Figs. 9-12. Pancreatic cells of rat. Fig. 9. Kolatchev preparation. Fig. 10. Ash preparation. Fig. 11. Ash image in natural color.

Plate VI, Fig. 13. Cells of intestinal epithelium subjected to the Prussian blue test for iron.

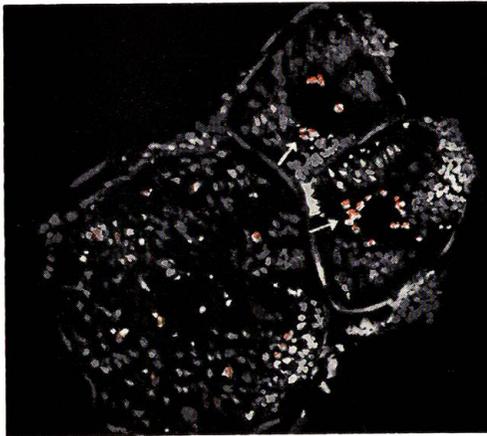
Plate VI, Figs. 14-18. Liver cells of newt. Fig. 14. Kolatchev preparation. Figs. 15, 16. Incineration preparations. Fig. 17. Drawing of the ash. The Golgi apparatus contains intensely red masses of free iron. Fig 18. Prussian blue preparation.



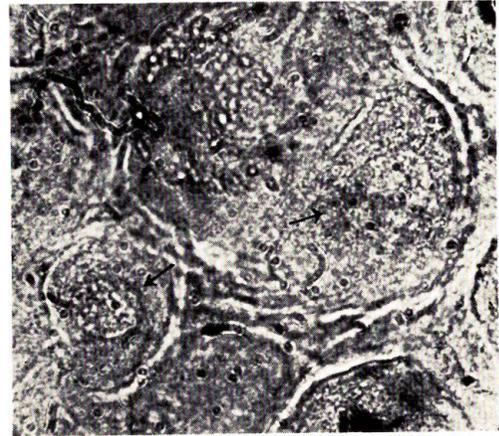
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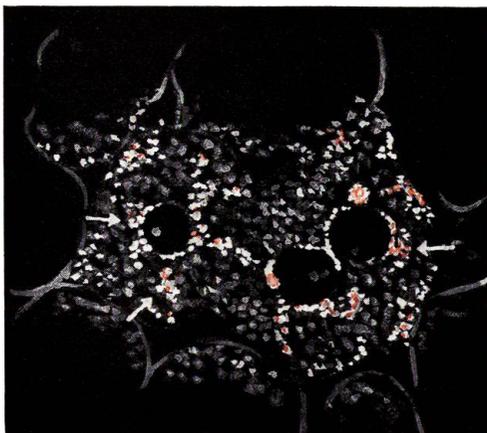
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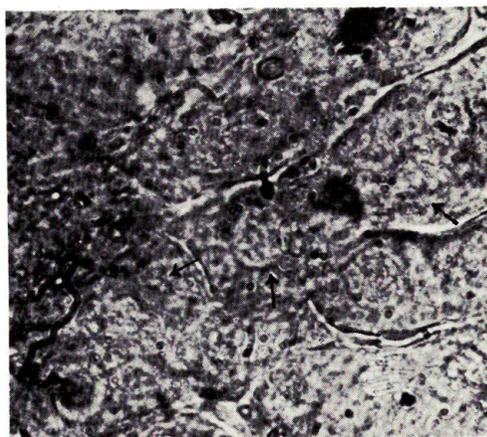
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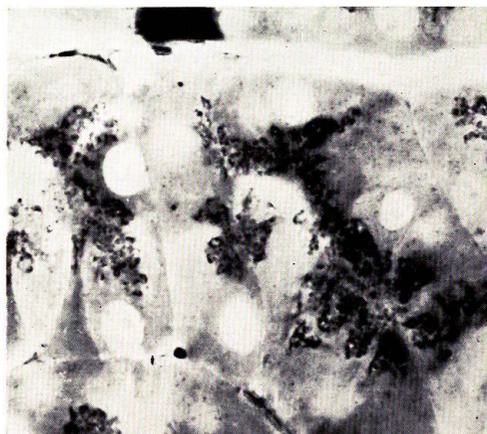
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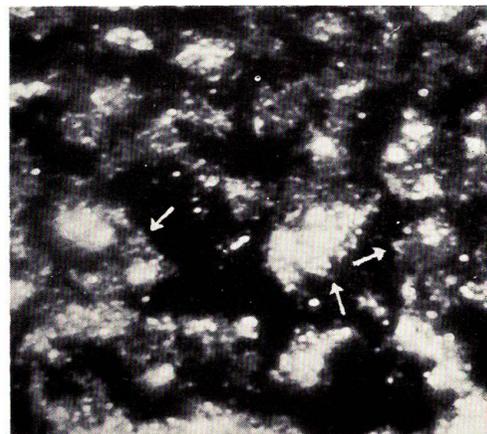
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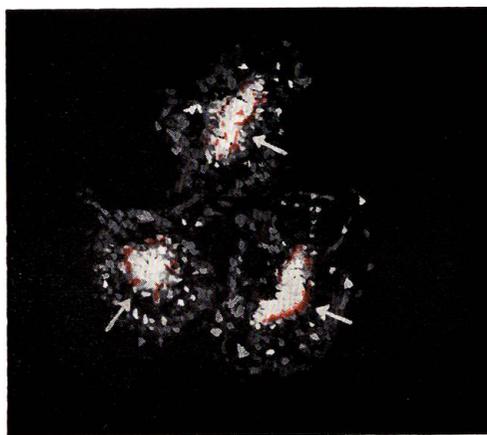
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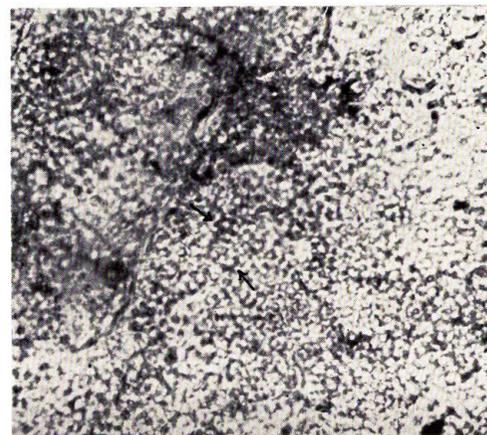
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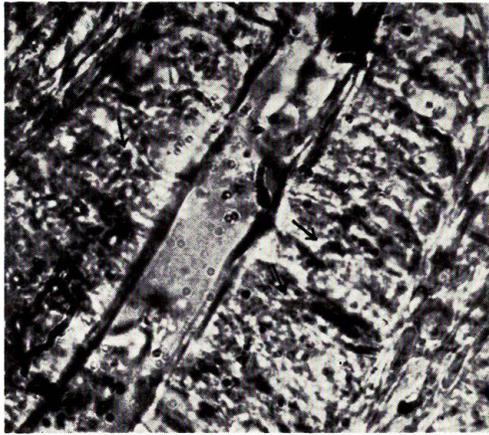
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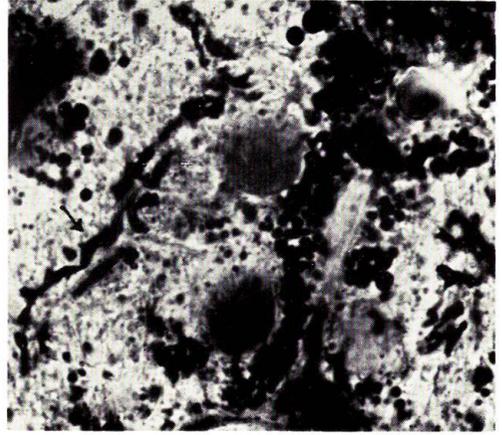
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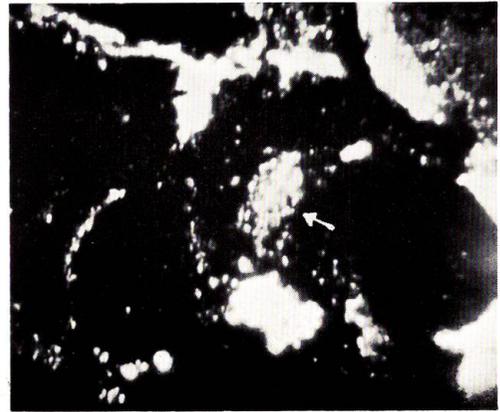
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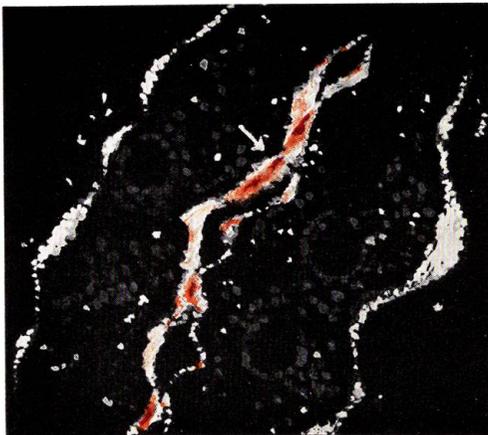
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