Problems on the Golgi Apparatus with Special Reference to its Chemical Components and its Functions

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Concerning the structure of the Golgi apparatus, there have been a considerable number of works. Yet, still now, the opinions of the workers are by no means unanimous, though many modern methods have been utilized. But it seems to me that the conclusion is gradually coming near to an end by the images obtained through the electron microscope; especially those of Sjöstrand who enterprised to make clear the real feature of the Golgi apparatus by fixing the material using freezing-drying technique and embeding them in the metacrylate polymerized by the ultraviolet ray at low temperature, and stained by means of PTA (phosphotungstic acid) staining method. Upon the completion of such a technique we can expect to reveal a perfect natural image of the apparatus. The problem of morphology of the Golgi apparatus has a close relationship to the problem of its function, therefore it would probably favourable to make an explanation about the architecture of the apparatus at the beginning.

## 1. Constructing Chemical Substances

As for the structure of the Golgi apparatus, the photographs of Dalton and Felix (1953, 1954) and those of Sjöstrand (1956) taken by the electron microscope seem to be most plausible. The conclusion deduced from them were: (1) The Golgi apparatus are not an artefact as the result of production of myelin figure. (2) The so-called "lipochondria" are the different cell element from the Golgi apparatus, though Ries and Baker claimed that they are one and the same cellular element. (3) The osmiophilic parts of the apparatus are composed of parallel lamella. (4) The vaculoar system have a close association with the Golgi apparatus. (5) The precursor of

the zymogen granules are produced in the Golgi zone, and the covering membranes of these granules are continuous to the lamellar membrane of the apparatus. According to Sjöstrand, the unit of this lamellar structure is of double nature, each membrane having a breadth of 60Å. This γ-cytomembrane, termed by Sjöstrand, assumes the same feature also in the material fixed by freezing-drying method. It is Sjöstrand's opinion that the osmiophilic part is of protein nature and the chromophobic part is of lipid nature. But as regards the chemical nature, it must be determined by the cytochmical data.

The most conflicting discussions have been focused upon the substances which attach to the wall of tubes (vacuoles). This part is meant by the chromophilic part, or " conduit de Holmgren ", or a group of "γ-cytomembrane " of Sjöstrand. It has unexceptionally 3–5 pairs of γ-cytomembranes, the thickness being 0.1–0.3 μ in all. So that, even from the the order of its breadth, the chromophilic part of the Golgi apparatus observed by the ordinary light microscope may be identified with this lamellar structure. This substance has been generally taken for the combination of protein and lipid (Eestlick, 1936; Tarao, 1941; Schneider-Kuff, 1954; Dalton-Felix, 1954 *et al.*). As for its lipinous element, many varieties of substances have been found in different organs and in different animals. For example, Tarao found that the lipid in the Golgi apparatus was generally soluble in cold aceton, but in pancreatic cells the lipid there was observed to be insoluble in acetone and soluble in alcohol. Recently, Nagatani (1957) found sphingomyelin in the abdominal gland cells in the newt. Here, one thing we must take attention to is that the Golgi substance should not be mistaken for lipochondria.

There have been confusing controversies concerning the difference between the Golgi apparatus and the so-called " vacuomes ", or in other word lipochondria, which are stained in the living cells with basic dyes. Recently, Baker (1949) and Thomas (1948) *et al.* still stick to the vacuome theory in the form of modern style. Having

a relationship to the secretory function of the apparatus, Hirsch (1939 etc.) held the lipochondria (his " Präsubstanz ") to be a transitional substance in the course of morphological change of the Golgi apparatus during secretion (" Systemtheorie "). The lipinous substance in the Golgi apparatus is in the state of " histolipoid " after Ciaccio (1927), and one cannot detect it by means of the ordinary cytochemical techniques. To remove the protein from this lipinous element, Tarao (1938, 1939 etc.) digested the sections of formalin marerial in HCl-pepsin solution or in Na_2CO_3-trypsin solution at 37°C, and then stained them with Nile blue sulphate and differentiated by diluted acetic acid. By means of this demasking procedure, he could stain the genuine Golgi substance, which is only stained faintly in the living condition by this dye. Such a demasking phenomenon of protein from fatty substances occurs naturally, for example, in the maturation process of germ cells of animals in general. These phenomena have been called " lipophanerosis ". Lipophanerosis has been noticed by many workers, especially by Karpova (1929) in *Helix pomatia*, in " Nebenkern " of male germ cells. The dictyo-
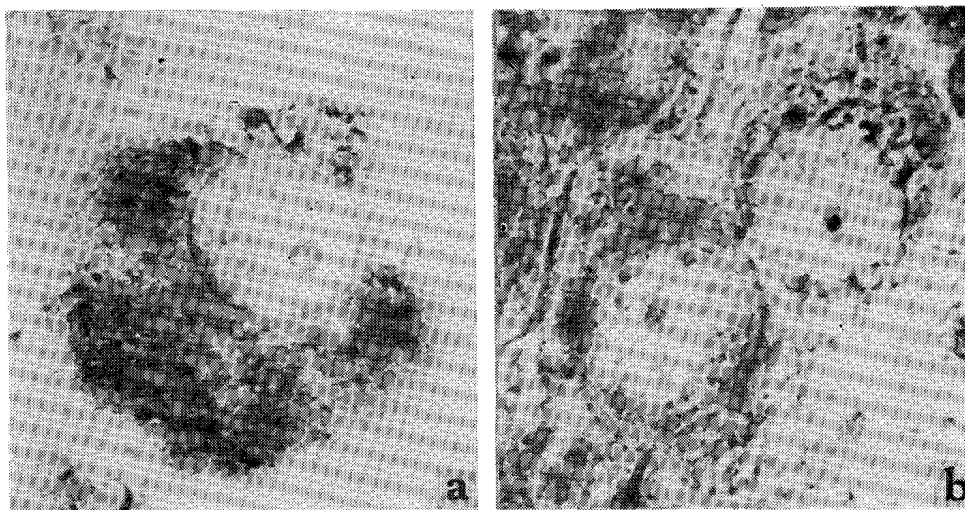
Text-fig. 1. Liver cells of rat subjected to the selenophen poisoning. Fat degeneration occurs in the cells. **a** A cell stained by the lipid staining of Smith-Dietrich. **b** Two cells stained by Nile blue sulphate. In both cases the Golgi apparatus are demasked, thereby their lipid elements are liberated and show lipid reactions with appearance of network (original).

somes there are easily stainable with lipophilic basic dyes in living condition. Moreover, they are also positive to the ordinary lipoid tests. Such an exceptional case in male germ cells can be easily understood by the lipophanerosis. During the sperm formation, the autolysis of the cytoplasm occurs and finally it flows along the tail and is cast off, thus the isolation of lipinous substances from the
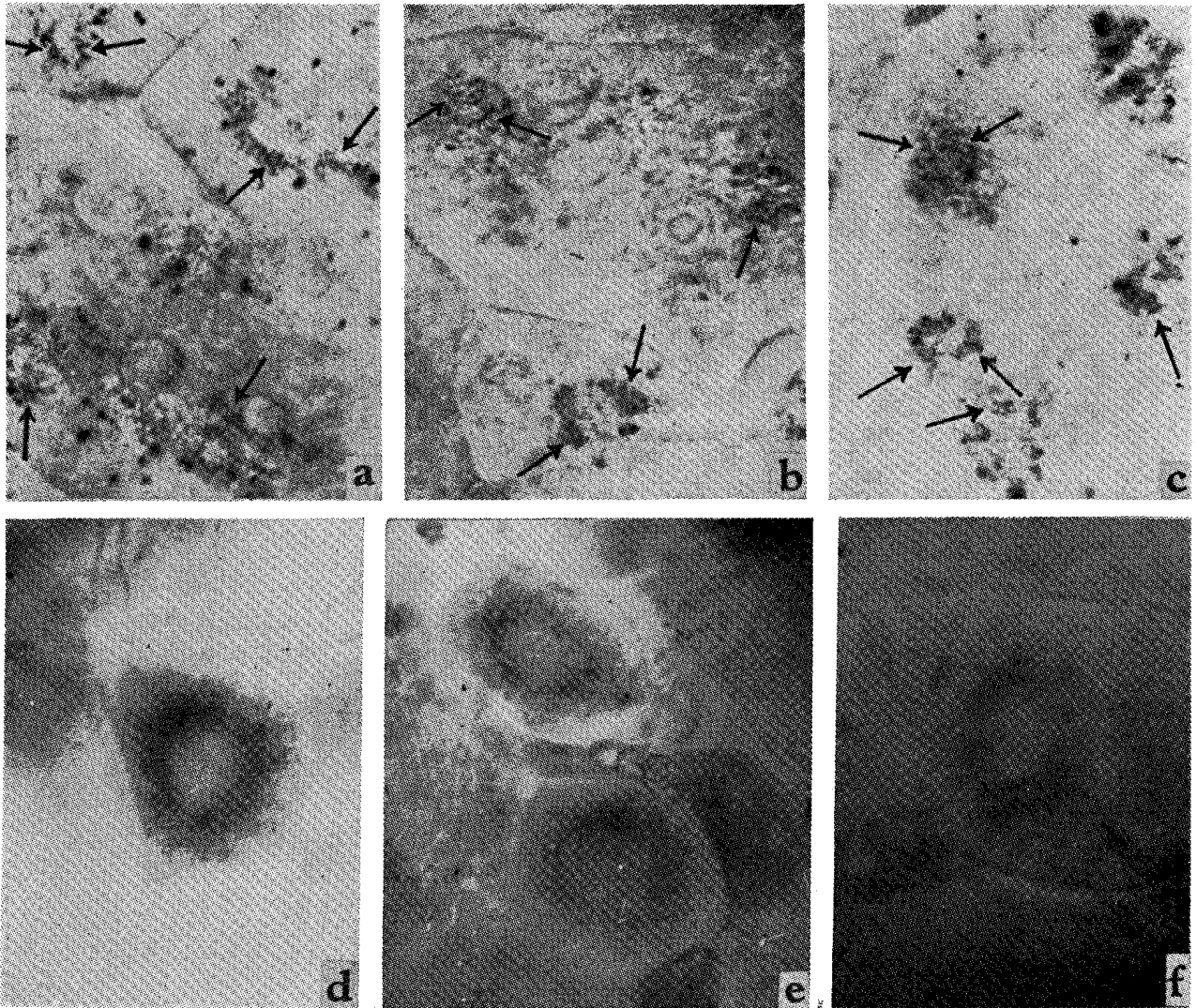
Text-fig. 2. **a-c** Pancreatic cells of mouse which ͺwas injected intraperitoneally with Nile blue sulphate. Note the Golgi apparatus in pale blue, beside which deeply coloured lipochondria are visible. **d-f** Spinal ganglion cells of rat treated with alcali-KCl solution and stained vitally with Nile blue sulphate. The network of the Golgi apparatus around the nuclei are visible stained blue in the classical appearance. The staining of the Golgi apparatus fades after few minutes, and deeply colored lipochondria remain (original).

protein is brought about. Recently, Tarao (1953) has succeeded in the artificial production of lipophanerosis in liver cells of mice by the injection of 0.1–0.35 γ of ⬚ (selenophen) daily. By this injection, fat liver could be obtained after one week. The Golgi apparatus in the liver cells thus treated became positive to Fischler's, Smith-Dietrich's, and Ciaccio's reaction by the autolytic denaturation.

In the normal cells there may be no room for the objections against the conjecture that the free lipinous grobules can be liberated from the Golgi apparatus as the natural phenomenon. Hirsch's " Präsubstanz ", Baker's " simple Golgi ", etc. are the mere metabolic products from the genuine Golgi apparatus. The differentiation of the lipochondria from the Golgi apparatus is difficult owing to the fact that the latter is usually indifferent to the vitally staining dyes. But the writer (Tarao, 1940) successfully stained the apparatus in the pancreatic acinar cells in the mice by the injection of Nile blue sulphate intra-peritoneally. The Golgi apparatus appeared in pale blue by this staining, and a deeply blue staining was obtained on the lipochondria on the other hand. Besides this experiment, the writer (Tarao, 1953) could stain vitally the Golgi apparatus of spinal ganglion cells of rats. The ganglions were homogenized for a moment in alkalic KCl solution (Weber's solution to extract the actomyosin of muscle), and then they were stained with a diluted Nile blue sulphate solution. The Golgi networks surrounding the nuclei appear in blue for several minutes, and then they fade gradually in color; while the lipochondria remain deep blue for quite a long time. The opinion that the lipochondria should be discriminated from the Golgi apparatus has been frequently claimed by the orthodox Golgi theorists (Gatenby and many others), and the positive proofs for this claim have been afforded by these present author's experiments. Dalton and Felix (1955) also stated that the Golgi lamella were ever been stained neither by methylene blue, nor by neutral red in living state. Hanaoka (1955) is of same opinion about the plasma cells. Last year Takagi and Masuda (1956) abandoned their former hypothesis

(lipochondria or myelin theory), and insist in turnth at the fat grobules and lipochondria exist outside of the Golgi zone in fibroblast in tissue culture.

Juding from the above stated data, it is clear that the diversities of termination (lipochondria by Ries, " Präsubstanz " by Hisch, simple Golgi by Baker, binary spheroid system by Thomas, Golgi bodies by Palade and Claude, pudding stone type by Worley, metachondria by Mitamura, and vacuome by Parat and Painlevé) are mere free lipid granules, or to some extent masked by protein or include a water-drop, and they are never the Golgi apparatus itself.

2. Functions

(a) Adsorped substances

To discuss the functions of the Golgi substance, it must be posturated what substanees are realy adsorped to the Golgi substance. The most well known substance is vitamin C. Giroud-Leblond (1934), Bourne (1935), Barnett-Bourne (1941), Tonutti (1937, 1940), Jörvi (1940), and Van Tiel (1940) gave a plenty of data toward its existence. Pfuhl (1941) doubted the specificity of $AgNO_3$-acetic acid to test the vitamin C, and he also doubted the localization of the vitamin C obtained by this test from the reason that such a easily diffusible substance as vitamin C could not tell the true localization after the fixation. But it may be reasonable to say, as Bourne stated, that the vitamin C is sure to be masked or combined with protein and is not in the free condition. Tonutti (1937) injected trypan blue and vitamin C at the same time into animals, and found that the vitamin C granules occupied their position upon the trypan blue granules. Jasswoin (1925), Nassonov (1926) *et al.* observed that the injected trypan blue appeared as granules on the Golgi apparatus, therefore, vitamin C granules were believed by Tonutti to locate on the Golgi apparatus. But reviewing the literature, vitamin C does not always exist in the Golgi apparatus as in the case of abdominal glands of the newt shown by Nagatani (1957) (also Sosa, 1948, 1951, 1952;

Dalton-Felix, 1954). But, in these cases, it is not yet clear if vitamin C does not exist there or exist in the form of oxide. Anyhow, it is doubtless that the vitamin C appears in the Golgi apparatus frequently. This fact has been supported by other angles; *i.e.* in the case of scorbute (Miwa, 1939), the injection of vitamin C (Hirsch, 1931), and the changes by the diuresis (Fischer, 1938).

The other physiologically significant substances which adsorp to the Golgi apparatus are enzymes.

The chemical complex of polysaccharide and protein, which has become gradually believed as a common element in the Golgi apparatus is thought to afford a favourable milieu upon which enzymes are adsorped (Gersch, 1949). Of these enzymes phosphatase has been disclosed cytochemically by many workers (Emmel, 1945; Dean-Dempsy, 1945; Bourne, 1943; Wilocki-Dempsy, 1946 *et al.*). Most of them, detected by Gomori's method, are alcaline phosphatase; yet a few are acid phosphatase. Schneider *et al.* (1953) could isolate the Golgi apparatus of cell of epididymis by means of the ultracentrifuge, and found a great quantity of phosphatase in this layer. This reaction are found to be inhibited by KCN. The phosphatase in the Golgi apparatus may be probably in close relationship to the phosphorylation of sugar and to the infusion of carbohydrates or fat. Aside from the phosphatase, there are possibilities of existence of other enzymes in the Golgi apparatus. This assumption is made rather from the morphological data. Bourne holds an opinion that the Golgi zone is the site of activity of enzymes of hydrolysis. The production of prozymogen granules of glands, especially those in the pancreas, have been thought to be in close association to Golgi apparatus. This relationship is lately made clear by the electron microscope. About the discussions concerning this problem, readers may better refer to the latter part of this paragraph.

The last physiologically important substance in the Golgi apparatus is $Fe^{..}$. In the liver cells of frog, bat, and rat, Makarov

(1931) observed the networks which were tinged blue by Purssian blue reaction; thus he termed this formation " Eisennetz ". These networks were identified as the Golgi apparatus which entangled along the bile canaliculi. The Fe·· substance comes apparently from the hemoglobin in the course of the bile formation. In the intestinal
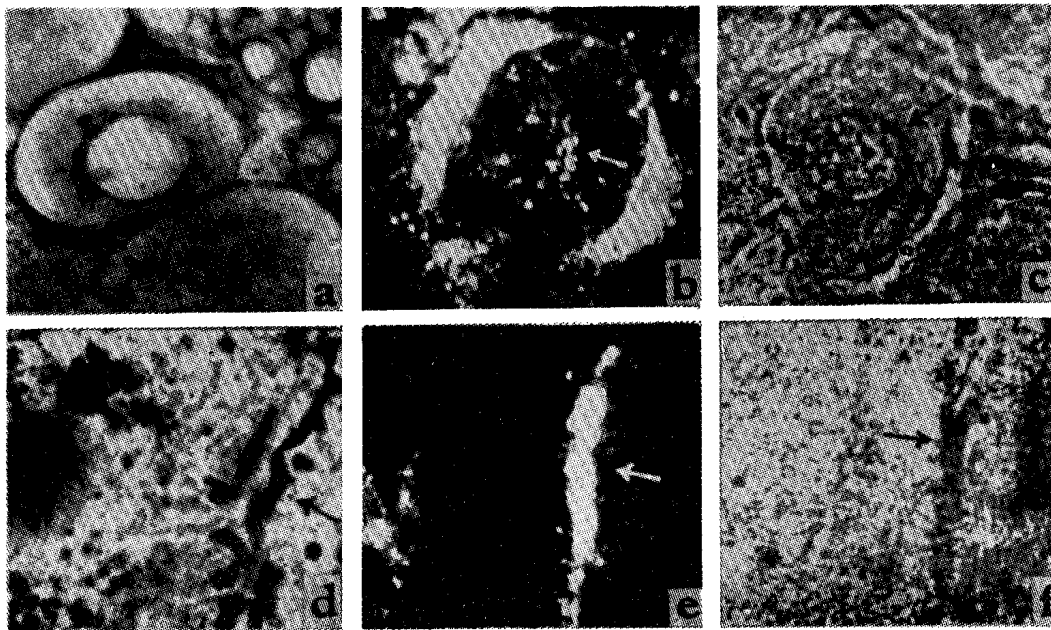
Text-fig. 3. **a-c** Spinal ganglion cells of rat. Golgi apparatus are shown by arrows. **a** Kolatchev preparation as the controle. Note the osmicated Golgi apparatus is seen around the nucleus. **b** Ash image of Golgi apparatus in the same position. The color is weakly brown with the naked eye. **c** Frozen-dried preparation treated with Purssian blue reaction. Perinuclear position which correspond to the Golgi zone is stained blue. **d-f** Liver cells of newt. **d** Kolatchev preparation as the controle. Note, that the Golgi apparatus are seen lining the border of bile canaliculum. **e** Ash image of the Golgi apparatus in the same position. The ash is colored deeply red owing to the free iron salt in the Golgi apparatus. **f** Frozen-dried preparation treated with Purssian blue reaction. A deep blue Golgi band is seen (original).

epithelial cells of *Ascaris*, Hirsch-Bretschneider (1937) observed that Fe·· accumulated in the Golgi apparatus when the animals were fed with Fe-glucuronate. Recently, Tarao (1957) found out the Fe-compounds in the Golgi apparatus in several kinds of cells

(liver, pancreas, intestine, and spinal ganglion) by Purssian blue reaction and at the same time by means of spodography. Especially, in the ash images of these cells, the ash of Fe_2O_3 is observable in brownish or red color. Policard (1938) stated that the free ion (not masked by organic compounds) is revealed in intensely red, while the masked iron colors faintly brown. The free iron ash was observed by the writer in liver cells and intestinal cells. The presence of masked iron which is found in the other cells is supported on the other hand by the weekly positive reaction of Purssian blue. The physiological meaning of thse iron compounds in the Golgi apparatus is not clear at present. There may be many possible suppositions; but it would not unnatural to suppose that this $Fe^{..}$ is comprised in catalase, peroxidase, or in enzymes of cytochrom system. The former two enzymes are highly possible, because it is necessary to counteract H_2O_2 which is generated at the oxidizing destruction of protein. The Golgi apparatus is the zone where the formation of secretory granules is taken place, so that the reconstruction of the protein to form enzymes from the fractions as the products of the break down of protein in the cytoplasm must occur. Other than the above mentioned enzymes, there must be many other enzymes which are not confirmed at present.

(b) Secretory and synthetic potency

The potency of secretion of the Golgi apparatus has been entirely settled today, based upon a considerable number of works made chiefly by Nassonov (1924), Bowen (1929), and Hirsch (1939). The clues which support this theory are almost presented from the morphological data: (1) the changes in volume and in shape of the Golgi apparatus; (2) the development of vacuoles and their transformation into the zymogen granules in the Golgi apparatus. A Series of precise studies were made by Hirsch (1932) in the living pancreatic cells and by Sluiter (1944) on the fixed pancreas. Expecially, Hirsch (1948) found that after the injection of pilocarpin into mice, Golgi vacuoles gradually increase in number till 7 hours

after injection, and the zymogen granules decrease during the first one hour and then increase till 11 hours after injection. He also observed the process of their formation during the starvation. Thus he noticed the close raletionship of the apparatus to the secretion. Möllendorff (1918) formerly insisted upon a hypothesis that in the vital staining the basic dyes usually stain the preforming structures, and the acidic dyes the newly formed granules (secretory granules). Heidenhain made the classical work concerning the secretory phenomenon of the cells lining the convoluted tubules of kidney by the injection of trypan blue. Jasswoin (1925) and Nassonov (1926) also observed the generation of the granules of injected trypan blue from the Golgi apparatus, and they concluded that the Golgi apparatus has the function of secretion.

The secretion may be performed by respective processes according to the natures of the secretory products. Saving the fatty products, it may be supposed that the secretion ought to have to do with three main phenomena: (1) the condensation or adsorption of materials upon tne surface of the Golgi apparatus; (2) the synthesis of the secretory products; (3) the isolation and the imbibision of free water from the bound water of the cytoplasm, namely the swelling up to form the vacuolated granules.

Concerning the first problem it has been supposed that the Golgi apparatus serves as the condensation membrane for various kinds of substances by the morphological observations, and by the fact that the Golgi apparatus has lipinous substances as its composing element. As for the second problem, the secretory products are associated unexceptionally with protein; therefore the synthesis of protein should be discussed. The question if there is RNA in the Golgi apparatus is at present equivocal. Worley (1944) stated that both mitochondria and Golgi apparatus develope from microsomes, accordingly both elements have RNA and phospholipins. On the other hand, Hibbard-Levin (1945) concluded that the epithelial cells of crop in hen do not contain a trace of RNA in the

Golgi area. Finally, Schneider and Kuff (1954) isolated the Golgi substance by means of the ultracentrifuge, and the fraction of the Golgi apparatus contains a pretty content of pentose nucleic acid. This fact is supported from the other side by the photomicrographs of the electron microscope, where many unmature zymogen granules are visible in the Golgi zone. These figures supply a positive proof toward the synthesis of protein. It has been admitted generally that the ergastoplasm has the function of protein synthesis, and it contains greater amount of RNA. The ergastoplasm is constructed of pairs of α-cytomembranes after Sjöstrand (1956), and each pair of membranes are lined with minute opaque granules of 150Å in diameter. In the embryonal cells, where the ergastoplasm is found abundantly, a considerable volume of RNA is contained. Palade-Siekevitz (1955) could separate the fraction of opaque granules by ultracentrifuge, and found that they were rich in RNA. Therefore, they concluded that these granules contain RNA. Strange to say, γ-cytomembranes of Golgi apparatus (after Sjöstrand) are lacking in opaque granules. It is hard for the writer to understand how the the synthesis of protein is performed in the Golgi apparatus. Dr. Sjöstrand who visited Japan last autumn explained to the writer that the opaque granules which contain RNA remove to the Golgi zone where they transform into clear granules of a little larger size. He said that he confirmed this transformation by a considerable number of preparations. But no preparation which is enough to show this transitory form has yet been presented. According to his opinion, these granules coalesce each other to form larger granules, which at once swell up by absorbing water. The covering membranes of the zymogen granules are continuous to the membranes of the Golgi apparatus. He has an idea of imagining that the bleb which contain organic substances and is wrapped by such a double membrane is thought to have been the primitive or standard unit of living organisms, and the nature has adopted this plan everywhere in the construction of organisms. Hirsch (1933, a, b) and

Sluiter (1944) thought that the Golgi apparatus is the place of congregation, and is not the place where the synthesis of protein and the discharge of energy are taken place. But how can we explain about the data of Schneider-Kuff? Most of secreted substances are associated with protein. The physiologically active substances are all constructed with protein and respective prosthetic group. Concerning this question, one must call attention to mitochondria, in which Hogeboom *et al.* found 19% of the whole RNA contained in a cell. Whereas we cannot find opaque granules there. In short, the relation between the protein synthesis and the opaque granules remains still obscure.

Usually, as the secretion becomes active, the volume of Golgi material increases. For examples: Radsma-van Weel (1940) on thyroid gland, Miller-Riddle (1942) on adrenal cortex, Krichesky-Mandel (1943) on uterine gland, De Robertis (1940) on parathroid gland, and Pease-Moon (1938) on adrenal cortex by the influence of ACTH of hypophysis.

Sometimes, digestive enzymes secreted from the Golgi apparatus are thought to be used for purposes other than the digestion. As examples, we can mention the following cases. The so-called acrosomes, which have been taken for the product of the Golgi apparatus, remove to the top of the spenrmatozoon and serve to the penetration into the ovum. Recently, J. Dan (1956) observed by electron microscope the ejaculation of acrosome-substance from the spermatozoa of *Mytilus* into the sea water where the over present. Joyet-Lavergne found the Golgi substance at the tip of sporozoites and microgametes of *Aggregata*. Smyth (1945) also observed " parabasal body ", which corresponds to the Golgi apparatus, at the front region of some parasitic Flagellata.

As for the respiratory enzyme system or metabolic system of nucleic acid, no enzymes have been found in the Golgi apparatus (Schneider-Kuff, 1954). Particularly speaking, cytochrom oxidase, desoxyribonuclease, or enzymes of Krebs' cycle which were found

in mitochondria, do not exist there.

The last problem, about which the writer has taken a special interest, is the isolation of free water by the Golgi apparatus from the surrounding cytoplasm. In plant cells, de Vries discovered tonoplast which covers the vacuome and controle the imbibision of water into vacuome. By the application of writer's proteinase-Nile blue
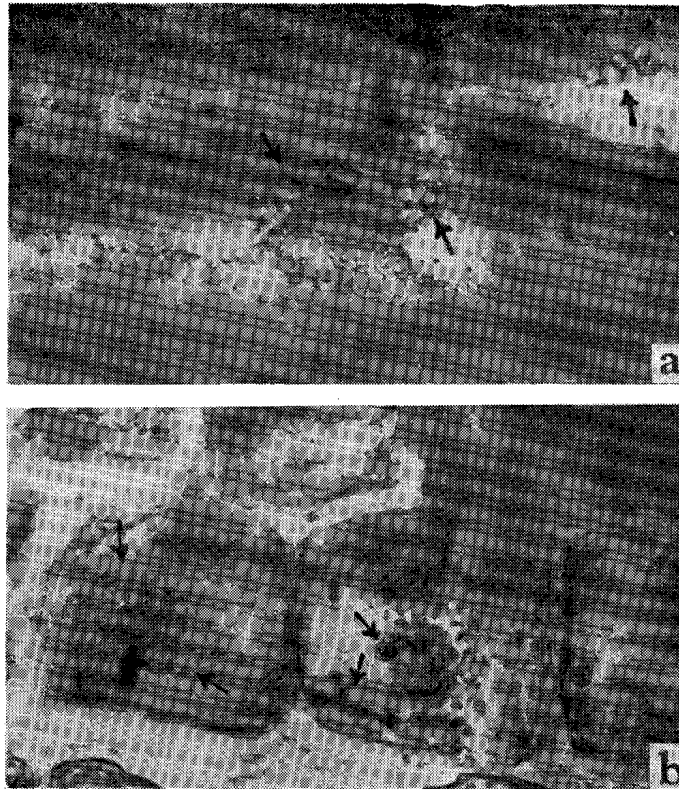

Text-fig. 4. a Epidermal cell of bulb of onion, fixed with formalin, digested with trypsin, and stained with Nile blue sulphate. The tonoplast of vacuoles around the nucleus is stained blue (shown by arrows). b Cells of root tip which are subjected to the same method. Granules and the tonoplast of vacuoles are stained blue (original).

sulphate technique, the tonoplast was observed to be constructed with the lipoprotein. *Amoeba diploidea* were also subjected to the same technique, and the writer could find the lipoprotein membrane covering the contractile vacuole and food vacuole. Such membranes of lipoprotein nature play the rôle of imbibision of free water from the cytoplasm; just in the same manner the Golgi apparatus works this function. The presence of canalicules (or vacuoles) in the

Golgi apparatus was claimed first by Cajal and then by many others. Recently, they were made evident from the data of electron microscopy (Dalton-Felix, Sjöstrand *et al.*). Concerning this problem, one may better refer to the works of Lasfargues-Fine (1950), Lacy (1953), and Adamstone (1952) by the light microscope. In the classical works of Nassonov (1923) and Smith (1945), they observed the Golgi apparatus closely associating to the contractile vacuoles of some ciliates, *Chilodon*, *Dogiella*, and *Paramoecium*. Furthermore, Hirschler observed the dictyosome-like body attaching to the contractile vacuole in the coanocyte of *Spongilla*. It may be natural to suppose that the Golgi apparatus in these cases is the organ of segregation of water. The secretion in glandular cells in general may be thought to belong to the same category of function. As stated above, between the γ-cytomembranes of the Golgi apparatus the formation of the precursors of zymogen granules were observed by Sjöstrand. The swelling of these precursory granules can be only attained by the imbibision of free water from the cytoplasm. The β-cytomembranes after Sjöstrand, which were found sinking deeply from the cell surface in the cells of convoluted tubules of kidney, serve most actively to the imbibision of water from the provisory urine from the glomerulus. The nature and the mechanism of these membrane are unknown. The further investigations about the nature of these membranes are expected in future.

The permeability and the segregation of water have a close relationship to the concentration of mineral salts. Metabolism of water and that of mineral salts go always side by side. If the Golgi apparatus is related to the segregation of water, it must contain mineral salts, especially the presence of sodium ion is highly possible. To make sure about this point, the writer devised a new method. The reagents were applied upon the ashes of microincineration. Interesting to say, at that time, the ashes have never been displaced to other place by the flow of reagents. Of various reagents, zinc-uranium acetate for Na· and sodium-cobalt nitrit for K· were mainly
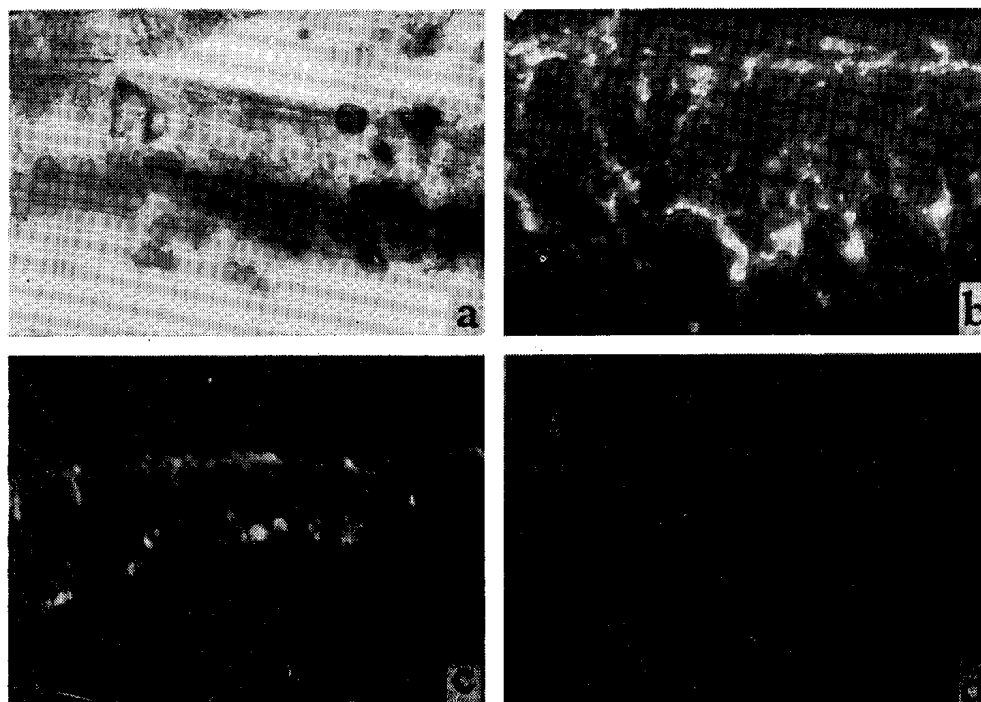
Text-fig. 5. Intestinal epithelial cells of rat. **a** Allen-Bouin preparation stained with hematoxylin and eosin as the controle. **b** Ash image. **c** Ash image submitted to zinc-uranium acetate for the detection of Na ash. **d** Absolute disappearance of Na ash combined with zinc-uranium acetate by 2% HCl (original).

employed. As the results of this method, it is noteworthy that sodium salts are abundantly accumulated on tne Golgi apparatus, while potassium salts are rather poor. The balance between Na· of outer medium and K· of cytoplasm has been found on the all surface, nerve fiber, melanophore etc., where the irritability is taken place. The occurrence of the irritability is usually brought about by the reversal in position of sodium and potassium. There may be some supposition that the Golgi apparatus is related to the cellular irritability by the reversal of K· in the cytoplasm and Na· in the vacuoles of Golgi apparatus. The fact that the Golgi apparatus are very well developed in the nerve cells as compared with other kinds of tissue cells may be selfexplanatory about this mechanism. Another data obtained by the writer which shows the relationship of the Golgi apparatus to the water exchange was seen in liver cells. The Golgi apparatus in the liver cells is usually found around the nuclei

and the bile canaliculi. The bile canaliculi between the liver cells ramify the branches into the cytoplasm of liver cells, attenuating gradually toward the nuclei. Accoudingly, the Golgi apparatus cling along these branches of canaliculi. We can often observe the figure in which the strand of Golgi element along the canaliculum of the cytoplasm of one cell keeps connection with that of other cell. At
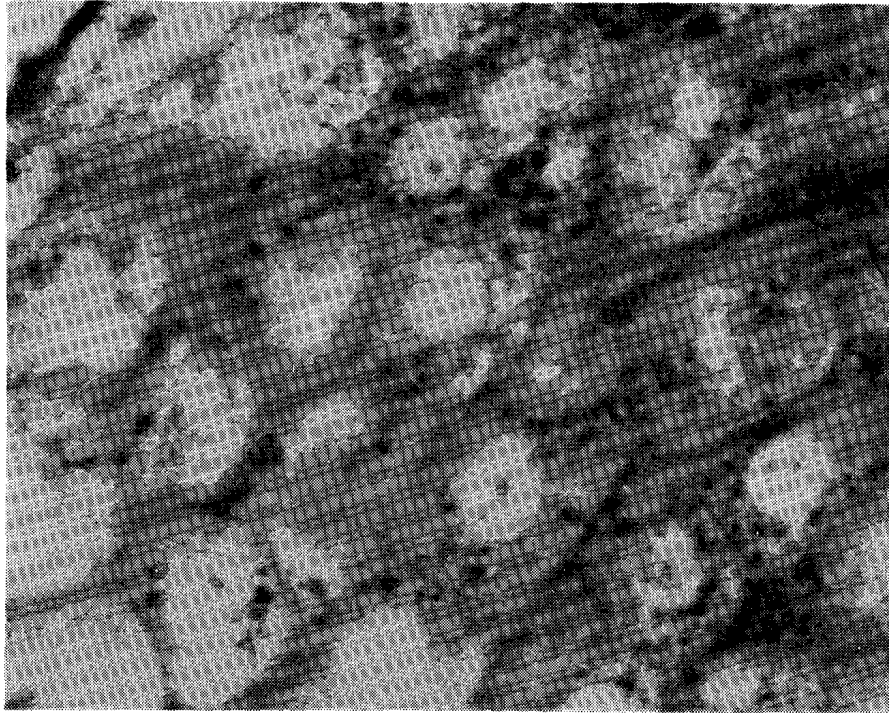
Text-fig. 6. Kolatchev preparation of liver cells of rat. Note that the Golgi apparatus of each cell keep the mutual connection (original).

the secretion of bile, a great quantity of water is indispensable. This imbibision of water is performed by the Golgi apparatus lining the canaliculi and their branches. The present author wishes to stress that the Golgi apparatus is the osmoregulating system of the cell, though other functions are at the same time may be mentionable. The above mentioned facts would sure give a strong impression toward this theory to the readers.

Resumé

In reference to the classical papers concerning the morphological and chemical data, the findings of the modern cytologists and cytochemists are discussed. Owing to the ambiguous term " Lipochondrien ", the confusing controversies between authorox theorists and the modern " vacuome " theorists have been brought about. According to the present author's opinion, which is chiefly based upon the data yielded from the author's " Nile blue sulphate technique " and from the vital staining of pancreatic cells and nerve cells, the so called lipochondria are the liberated lipoid substances from the lipo-protein of the Golgi matrix. This liberation of free lipoid granules from the Golgi apparatus has been frequently observed in several works, which can be mentioned for examples as follows. The dictyosomes of the Nebenkern in male germ cells are the lipoidal bodies demasked by the autolysis during the course of the casting off of the cytoplasm at the sperm formation. Another example can be mentioned in the author's experiment, in which the fat liver of mice was produced by injectioning 0.1–0.35 gamma of selenophen $\underset{Se}{\square}$ daily into the animals. During the course of fat formation the demasked Golgi apparatus was easily demonstrable by means of the ordinary lipoid tests. By examining the photomicrographs taken from the vitally stained pancreatic cells and nerve cells, the differentiation of the lipochondria from the genuine Golgi apparatus may be quite easy. It would be quite natural that the liberation of lipoid granules from the Golgi material usually occurs during the physiological process in the cellular metabolism.

From the data obtained by the modern techniques by a considerable number of investigators, especially from those of electron microscope, one may conclude that the Golgi apparatus are composed of the lipoidferous lamellar structure in which canalicular system (vacuoles) is embedded. On the other hand, it may be almost acceptable that the polysaccharides of various quantities are also comprised. As for the presence of RNA in the Golgi apparatus, there have been diverse opinions. Nevertheless, the presence of RNA seems to be made sure by the analysis of the Golgi substance extracted by means of ultracentrifuge made by Schneider *et al.* It is clearly shown by Sjöstrand in his collective review appeared in International Review of Cytology (1956), the opaque particles which attach to the a-cytomembrane are identified to RNA. On the

contrary, the γ-cytomembranes in the Golgi apparatus lack in this element, yet the Golgi apparatus are generally thought to have the function to produce the secretory granules. The ground matrix of secretory granules are almost protein in nature. This discrepancy is not conclusively solved at present.

In the latter part of this paper, the functions of the Golgi apparatus are dealt with. Concerning the association of vitamin C and phasphatase with the Golgi apparatus, their localization there is almost assured. By several workers, the adsorption of Fe to the Golgi apparatus has been reported. Above all cases, the free iron compounds are always found in the Golgi apparatus of liver cells. Those are disclosed not only by Pursian blue reaction, but also by the red spodogram after the microincineration. As for the general presence of the iron compound in the Golgi apparatus, the present author has succeeded in detecting them in reddish color of their ash image. The reddish color of the ash image has been thought to be the presence of masked iron. The masked iron in the Golgi apparatus, if any, may be considered to indicate the presence of catalase or peroxidase. If the Golgi apparatus has a catalytic action of protein, the presence of catalase or peroxidase would have an important meaning as the antidotal mediators of hydrogen peroxide which is produced by the aminoxidase.

The secretory function of the Golgi apparatus has been approved by many investigators, i.e. Nassonov (1924), Bowen (1927), and Hirsch (1939) et al. The phenomenon of secretion comprises some physiological phenomenon; protein synthesis, condensation, and permeation of water. The protein synthesis has been already discussed. The idea that the Golgi apparatus is the site of condensation has been frequently admitted. The reader may refer to the reviews written by Kirkman-Severinghaus (1928) and by Bourne (1950). The present author has stressed from long ago the idea that the Golgi apparatus has a kind of semipermeable membrane, through which the water and salts permeate easily. Nassonov (1925) and Smyth (1945) demonstrated the Golgi apparatus surrounding the contractile vacuole in Amoeba diploidea. It is quite interesting to mention here that the Golgi apparatus adheres closely to the bile conaliculi of liver cells. These canaliculi are supposed to ramify not only through the interstice of cells, but also into the cytoplasm of cells. In accordance to this fact, the Golgi apparatus distribute themselves so as to keep a connection between neighbouring cells.

The presence of vacuoles in the Golgi apparatus which appear in the electron microphotographs may present a strong supposition toward this idea. The accumulation of salts in the Golgi apparatus may naturally be imagined from this idea. Concerning to this problem, the present author attempted the detection of sodium, potassium, and calcium in them. By means of the newly devised method, in which the author immersed the reagents upon the ash image under the cover slip, the observation of the reactions under the dark field microscope has been succeeded. Above all, the reaction of the sodium oxide to the uranium-zinc acetate has become possible. The resulted sodium uranium-zinc acetate is easily soluble in 2% HCl. This reaction is most conspicuous in the case of pancreatic zymogen granules which have an abundant quantity of sodium salts. The localization of these sodium salts upon the Golgi apparatus is most clearly seen in the intestinal epithelium. The potassiuum salts are comparatively poor in the Golgi apparatus. Calcium salts differ in various tissue cells. The detailed reports should be offered in future.

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