Microchemical Studies on the Golgi Apparatus, V. Proteins contained in the Golgi Substance of Nerve Cells and of Hepatic Cells in the Guinea Pig[1)]

By

Sirô Tarao

Zoological Institute, Faculty of Science, Hokkaido Imperial University, Sapporo

(*With 3 Plates*)

Through the combining procedure of the test of solubility in various organic solvents and the subsequent treatment with protease-Nile blue sulphate technique, it has been ascertained by the present author that the Golgi substance is generaly composed of two essential elements, protein and lipoid (Tarao 1939 a, b, 1940, and 1942 a, b). Particularly speaking, the protein is easily digestible in trypsin solution and the lipoid is soluble in cold acetone. The lipoid element varies somewhat according to the kinds of tissue cells. For example, the lipoid in the Golgi apparatus in the pancreatic acinar cells remanins insoluble in cold acetone and is entirely disolved in alcohol.

Though established roughly the above facts, the further accurate knowledges concerning the chemical nature of the apparatus remain to be studied in future. The present author has attempted some histochemical reactions, especially on the protein elements of the Golgi substance. The data obtained by these reactions will be explained on the accompanying photomicrographs in the plates. Though not perfect, it would be enough to suppose the nature of protein in the Golgi apparatus.

Concerning the lipoids and other fatty substances, a paper is now under preparation to present accurate informations. For this purpose, the extraction of the Golgi substance in the test tube is

1) Contribution No. 176 from the Zoological Institute, Faculty of Science, Hokkaido Imperial University, Sapporo.

highly needed. The crude substance has been recently obtained by successfully applying the present author's protease-Nile blue sulphate technique (Tarao 1942 b).

The description of the history, as regards the chemical components consisting the Golgi apparatus, is entirely omitted here. About this concern the reader should refer to the second paper of the present series of investigations.

These series of works have proceeded under the direction of Prof. Kan Oguma to whom the present author wishes to express here his grateful acknowledgement. Thanks must be directed towards Dr. S. Makino for his hearty encouragement throughout the work. The funds expended in this study were furnished in part by the Scientific Research Expenditure of the Department of Education and partly by the grant of the Foundation for the Promotion of Scientific Research of Japan.

Material and Methods

The spinal ganglions and the liver from adult guinea pigs were used as the material in the present study. They were dissected out from the animals directly after killing. Sections of both fresh and fixed material were made 30 micron thick by means of the freezing microtome.

As fixing reagents prior to the execution of reactions, formalin diluted five times with distilled water, 3% aqueous solution of potassium bichromate, saturated aqueous solution of corrossive sublimate, and saturated aqueous solution of ammonium sulphate were employed. Commercial formalin was neutralized with calcium carbonate. Sections from the sublimate material were thoroughly washed with sodium chloride solution in order to remove the residual sublimate. After these fixing procedures the sections were all washed in distilled water, renewing it several times. Especially after fixing with potassium bichromate the traces of the reagent must be completely removed by washing in water.

The subsequent colour reactions of proteins were attempted following formulae appearing in the ordinary text book of biochemistry. These formulae are given as follows.

(1) *Biuret reaction.* The section was plunged in 3 c.c. of 2.5% sodium hydroxide solution adding one drop of 1% aqueous solu-

tion of copper sulphate, and then mounted in this mixture under the cover slip. By the presence of the peptide linkages in the protein, pink or violet colour reaction appears. When the peptide linkage is long, the colour is blue violet, and when the linkage is short, the colour is pink. For this reason, proteoses and peptones appear in a rose tone and other complex proteins mainly in violet colour. Exceptionally the egg-albumin gives a distinct rose tint.

(2) *Xanthoproteic reaction.* Upon the section on the slide glass one drop of 30% nitric acid was added, and then it was boiled for half a minute over the flame. When benzen rings are present in the protein, yellow colour develops. Especially this reaction is prominent by the presence of tyrosine, tryptophane, and phenylalanine. Oleic acid reacts in a similar manner. The stronger percent of nitric acid and the excessive duration of boiling must be avoided, because they both produce the distortion of the preparation.

(3) *Ninhydrin reaction.* Two drops of 0.2% aqueous solution of nynhydrin were added upon the section on the slide glass, and it was boiled for a moment over the flame. Then the solution was drained off by absorbing with filter paper. For the observation the section was mounted in glycerin. The solution of ninhydrin must be always fresh; it contaminates in a week after the preparation. By this reaction all the protein derivatives possessing one free carboxyl and one free amino group in its structure reveal a blue colour.

(4) *Millon's reaction.* The original Millon's reagent was employed. The sections were gently heated over the flame with one or two drops of the reagent. The only known substance derived from protein that gives red colour is tyrosine. The protein is wholly precipitated by the mercury, which then combines with the tyrosin. This compound becomes red on treatment with nitrous acid. The fixing result by this reagent is rather bad for the observation on details. The observation was made on the preparation mounted in glycerin.

(5) *Liebermann's reaction.* As the reagent for this reaction concentrated hydrochloric acid is employed. To avoid the destructive effect upon the microscopical structure, a little weaker percent is favourable. In this study the usual concentrated hydrochloric acid (M. W.=36.468) was diluted as 4:1 with water. By gentle boiling

over the flame the tryptophane and other indole derivatives give a deep violet or a blue black colour.

(6) *Acree-Rosenheim's reaction.* This reaction is a modification of Liebermann's reaction. In the present work the reagent consists of a drop of commercial formalin and four drops of concentrated hydrochloric acid. Upon heating the tryptophane and other substances which contain an indole group, deep violet or blue black colour appears.

(7) *Glutathion reaction.* The cystin, or oxidised glutathion, which possesses -S-S- group was reduced by 10% aqueous solution of potassium cyanide prior to the reaction with sodium nitroprusside. The stay in the potassium cyanide is about 3 minutes, during this time all the glutathion converts into reduced form. Then the sections were transfered into 20% aqueous solution of sodium nitroprusside to which one drop of 10% ammonia solution was added. The observation is better made in this mixture as soon as possible, because the rose tint which is the positive proof for the existence of the glutathion fades very soon. The formalin used in this technique must be neutralized with a certain alkali.

(8) *Sulphur reaction.* The sulphur which is contained in the form of cystin or cystein is detectable in the mixture of lead acetate and caustic soda. In the present study this mixture was made up of equal volume of 5% lead acetate and 10% of caustic soda. On heating for a moment, the sulphur in the tissue, usually contained as cystin or cystein, gives a black colour. It is better make the observation in glycerin.

(9) *Arginin reaction.* When arginin is contained in the protein, its guanidin atom group reacts as a red colour in the mixture of alcoholic alpha naphthol solution alkalified with sodium hydroxide and sodium hypochlorite. The naphthol solution used was 0.1% in 70% alcohol.

Observations

Control preparations through cytological techniques

The examination of the results obtained by the chemical reactions highly necessitates the control preparations through cytological techniques in order to make sure if the reactions have taken place upon the cytolasmic inclusions. Although the cytoplasmic in-

clusions of these materials have been often illustrated by photomicrographs in the foregoing papers (viz. 1938, 1939, 1940, and 1932 a, b), it would be convenient for the reader's examination of this paper to give the figures photomicrographed in the plate (Pl. I, Figs. 1–6). The brief explanation about them is made as follows.

Fig. 1 shows the Golgi apparatus in the nerve cells which are impregnated with osmium after Kolatschev's method. The Golgi apparatus is distinctly demonstrated assuming the diffused type in large cells and a reticular feature around the nuclei in small cells. No other cellular inclusions are apparent. The nerve cell in Fig. 2 is submited to the trypsin-Nile blue sulphate technique. By means of this technique the Golgi apparatus and the Nissl bodies are demonstrated. The former are, concerning the morphology and location, quite identical to those in Fig. 1. Fig. 3 indicates a nerve cell which is fixed with Champy's fluid, chromed after Benda's method and then stained with Heidenhain's hematoxylin. Here, besides very minute granular mitochondria spread uniformly throughout the cytoplasm, the Nissl bodies are demonstrated as blocky masses. The latter assume almost the same morphology as those in Fig. 2.

As for the hepatic cells the techniques applied are the same as in the case of nerve cells except the protease-Nile blue sulphate technique. For this material the enzyme employed is pepsin-hydrochloric acid. The Golgi apparatus impregnated with osmic acid has an appearance of irregular masses or dots gathering mostly around the nuclei (Fig. 4). As for those disclosed by the pepsin-Nile blue sulphate technique, their natural appearance is very finely preserved. They appear as irregular masses arranged almost radially from the nuclei (Fig. 5). The mitochondria are of typical granular shape occupying for the most part the perinuclear region. Some of them are arranged radially from the nucleus to the cell periphery (Fig. 6).

In both kinds of cells, nerve cells and hepatic cells, the mitochondria are so minutes that it is almost impossible to make an exact identification of them in the preparations which are subject to the severe distortion wrought by the reagents employed in the chemical reactions.

Results on the fresh material

Through the course of sectioning upon the freezing microtome the material and the sections were always rinsed in the normal

saline of 0.85%. The subsequent procedures of reactions must be done in due times which are only known from experience. The observation should be made as soon as possible, because the colour tints revealed by the reagents fade very soon as time elapses owing to the diffusion of the detected substances. If the observations are a little delayed the possitive reactions often fail from sight.

(1) *Biuret reaction.* The application of this reaction upon the fresh material, both nerve cells and hepatic cells, is quite impossible, because by the action of the sodium hydroxide contained in the reagent the myelin form grows from the lipoidal substances in the tissue making the preparations obscure. Interesting to say, this formation of myelin form does not occur in the case of the fixed material as seen in the next section.

(2) *Xanthoproteic reaction.* By the violent action of the concentrated nitric acid the cytoplasm reveals a coarse texture which stains in a weak yellow colour. The cytoplasm of the nerve cell is rather non-reactive. When the preparation is carefully examined, the irregularly shaped bodies are observed to be tinged in a somewhat brown colour. These bodies are identifiable to be the Golgi apparatus from their morphology and from their topography. Especially in the nerve cells this reaction on the Golgi apparatus is comparatively evident. It is impossible practically to take photographs, as the staining intensity is below the differentiation from the surrounding cytoplasm to be photographed.

(3) *Ninhydrin reaction.* In this case the fixation is performed by heating, so that the cytoplasm is not fixed nicely. But it is quite sure that in both materials the Golgi apparatus is positive to this reaction staining faint blue. Especially in small nerve cells and in most of hepatic cells the blue reaction appears in the juxtanuclear region where the Golgi apparatus also exists. Other cytoplasmic elements do not react enough for the observation.

(4) *Millon's reaction.* When the diaphragm of the condensor is adjusted to allow a weak light to enter, a weak reaction is seen everywhere in the cytoplasm of both kinds of cells. Owing to the distorting effect upon the cytoplasm by reagent and the weakness in reaction, it is not sure that these reacting portions correspond to the Golgi apparatus and other inclusions.

(5) *Liebermann's reaction and Acree-Rosenheim's reaction.* These reactions were found to be quite useless for the fresh materials,

because the cellular structure was completely damaged owing to the severe action by the concentrated hydrochloric acid.

(6) *Glutathion reaction.* By the application of the method which detects the total amount of substances with sulfohydril group, cystin and cystein, the Golgi apparatuses of nerve cells and hepatic cells are tinged in a weak red colour. The intensity of the colour is far weaker than in the case of the fixed material. The mitochondria seem to be reactive too. The sulphur reaction using lead acetate and caustic soda is not applicable on the fresh material, as the macerating action of the concentrated caustic soda of the reagent makes the structure obscure.

(7) *Arginin reaction.* This reaction was attempted upon the fresh material with the result that no particular cellular elements were discerned owing to the very weak staining reaction.

Results on the fixed material

(1) *Biuret reaction.* The myelin form which disturbs the observation in fresh material is not produced in the fixed one in general, saving ammonium sulphate material in which the myelin form grows very slowly. The most excellent result is obtained in the formol material. In the nerve cells the Golgi apparatus is distinguished in a pinkish violet colour, distributed throughout the cytoplasm in the large cells and clumping around the nuclei in the small cells (cf. Fig. 7 with Figs. 1 and 2). Nissl bodies, though not markedly differentiated from the cytoplasm, seem to be weakly reactive in a purple colour. Mitochondria are supposed to be negative or less reactive if any. In hepatic cells, though a little fainter, the Golgi apparatuses are also quite distinctly reactive to this reaction (cf. Fig. 15 with Figs. 4 and 5). Mitochondria in hepatic cells are quite negative in reaction. In both kinds of cells the pink-staining of the Golgi apparatuses by the biuret reaction indicates that the apparatuses contain a protein of a short peptide linkage, in other words a comparatively simple protein or the like. In the material fixed with potassium bichromate or corrosive sublimate the mode of reaction is almost the same as in the case of formalin, though much weaker.

(2) *Xanthoprotein reaction.* Of all the fixing fluids, the corrosive sublimate is only available for this reaction. The Golgi

apparatus in the nerve cells fixed with corrosive sublimate reacts positively in brown colour. Although the cytoplasm staining in weakly yellow is very badly fixed, the Golgi apparatus stands out in a degree to bear the observation. The identification of other cellular elements is quite impossible. The examination of the preparation of hepatic cells treated with this reagent is difficult because of the bad fixation. The xanthoprotein reaction is not applicable upon the preparations fixed with other fixatives for the contamination by the reagent. But it is certain by the close examination that vage images which are supposed to be the Golgi apparatus are observable in the formol and ammonium sulphate preparations.

(3) *Ninhydrin reaction.* The fixatives recommendable for this reaction are formol and potassium bichromate. As regards the nerve cells, the formol fixation gives the most excellent result (Fig. 9). In the cytoplasm the blue staining is observed restricted upon the Golgi apparatus. In the potassium bichromate preparations the blue staining is a little less intense (Fig. 10). In this case the cytoplasm is stained diffusely blue. It is not apparent whether the Nissl bodies are reactive or not to this reaction, but one seems to be able to say that the reaction is not appreciable enough. In the hepatic cells good results are also yielded by the fixation with formol and potassium bichromate. In both kinds of prpeparations the blue staining of the Golgi elements which are arranged radially from the nuclei is very distinct (Figs. 16, 17, and 18). Upon close examination it is realised that the mitochondria are also reactive to the ninhydrin, though not strongly enough to be photographed.

(4) *Millon's reaction.* This reagent exerts an effect of severe shrinkage upon the cell body, which makes the precise observation almost impossible. The only known facts are that, in the preparations fixed with formol and corrosive sublimate, the Golgi apparatus and the mitochondria exibit weakly red staining. The former elements suffer vacuolation, by the reagent small spaces arising in each of them around which a red staining substance, presumably the tyrosin, attaches. This vacuolation is most easily observable around the nuclei of the nerve cells. In the nerve cells the Nissl bodies are also tinged in pink colour. The preparations of hepatic cells are almost useless for the observation.

(5) *Liebermann's reaction.* Only in the nerve cells the fine structure is observable. The fixation with formol gives the best

result for this reaction. Two photomicrographs are given in Plate II (Figs. 11 and 12), where the Golgi apparatuses appear assuming irregular bodies stained blue. Though not clear in the photographs, the Golgi bodies are each vacuolated by the reagent and the reacting substance stained blue adheres to the periphery of vacuoles. In the preparations fixed with corrosive sublimate or potassium bichromate the Golgi apparatuses, especially in the former, are also found stained blue in a less degree than in the formol preparation. There is some suspicion that the Nissl bodies are also positive in reaction. In Fig. 11 the stained Nissl bodies are seen here and there. When examined carefully, the mitochondria seem to be reactive, though not certainly.

(6) *Acree-Rosenheim's reaction.* This reaction has almost the same meaning as Liebermann's reaction, which indicates the existence of tryptophane and other indole derivatives. This reaction affords good results in hepatic cells; not good ones for the nerve cells. When compared Fig. 19 which shows the result of this reaction in hepatic cells with Fig. 5 a, the controling preparation with pepsin-Nile blue sulphate technique, it may be realized without explanation that the Golgi apparatus is apparently positive in reaction. The mitochondria are not reactive at all.

By Liebermann's and Acree-Rosenheim's reaction the existence of tryptophane or the derivative having indole group in the Golgi apparatuses of the nerve cells and of the hepatic cells, has been established. Besides these reactions the present author attempted the reaction with bromine water upon the preparations. As expected naturally, the Golgi apparatuses of both kinds of cells, also the Nissl bodies in the nerve cells, stain in weakly reddish violet colour which indicates the presence of free tryptophane.

(7) *Glutathion reaction.* In the fixed sections, especially in those fixed with formol, the alkalified sodium nitroprusside displays a distinct positive result. The Golgi apparatuses in both kinds of cells are coloured in pink corresponding exactly to those in the cytological preparations (Figs. 13, 14, 20, 21; cf. these with Figs. in Pl. I). The mitochondria stain more reddish, distributing themselves either on the area of the Golgi apparatus or in no association with them. The Nissl bodies seem to contain no glutathion of appreciable amount. Judging from the colour intensity, it comes to a conclusion that the mitochondria contain most abundant sulfo-

hydril groups, then the Golgi apparatus in a less amount. The figures of these two kinds of cytoplasmic inclusions are so clear cut, staining in pink colour, that there is no room for doubt of the existence of the glutathion in them. The localisation of the glutathion upon the mitochondria has been claimed by Joyet-Lavergne (1927, 1929, and 1935). Aside from the question of the cellular oxydo-reduction system, the localisation of the glutathion on the mitochondria, again on the Golgi apparatus, seems to have been established here.

(8) *Sulphur reaction.* To make sure the data obtained by the glutathion reaction and to detect the protein containing cystin, the present author attempted the sulphur reaction using lead acetate and caustic soda. By macerating action of the caustic soda the preparations are unsuitable for detailed observation. In the hepatic cell fixed with formol, however, the Golgi apparatus is observable in black colour by this sulphur reaction (Fig. 22). Though this reaction does not give a clear cut contour of the apparatus, the distribution of them corresponds entirely to those disclosed by the glutathion reaction. The difference between the glutathion reaction and the sulphur reaction is that by the latter the mitochondria are not markedly coloured as by the former.

(9) *Arginine reaction.* Both in nerve cells and in hepatic cells the Golgi apparatuses react in pink colour. The mitochondria rather stain more intensely. These data mean the fact that the Golgi apparatus and mitochondria are both laden with some amount of arginine.

Comments and Conclusion

It may be convenient for the readers' understanding to insert a section in which the meanings of the data hitherto obtained by the colour reactions are explained.

It can be naturally surmised that Golgi substance contains, as the protein components, several kinds of proteins and not only one kind. Another probable assumption is that the protein components of the Golgi substance are variable according to the kinds of tissues or animals. But it is at the same time supposed that it consists of a certain predominant protein which characterises the nature of the Golgi apparatus physico-chemically.

As stated in the foregoing section, the preponderating protein in the Golgi apparatus in the nerve cells and in the hepatic cells

reacts positively to almost all the colour reactions of proteins. It would be too bold to lead a conclusion from the above data which lack in the analysis f other amino acids. Nevertheless, since the protein in the Golgi apparatus reveals colours microscopically by the reagents, it must be admitted that the amino acids indicated by the reactions are considerably abundant in the Golgi apparatus.

The apparent positive staining by the biuret reaction means the existence of protein. The rose colour of the Golgi apparatus by this reaction indicates that the peptide linkage is not long. The blue staining by the ninhydrin reaction is supporting the existence of proteins and their derivatives.

The red colour of the Golgi apparatus and the mitochondria by the Millon's reagent is showing the existence of tyrosin in them. Liebermann's reaction and Acree-Rosenheim's reaction are both positive on the Golgi apparatus, indicating the existence of the tryptophane and the indole derivatives. The aromatic substances, that are represented by tyrosine, tryptophane, and phenylalanine, in the protein constituting the Golgi apparatus, are on the other hand disclosed by the xanthoprotein reaction. Thus it has been established that tyrosine and tryptophane really exist in the protein of the Golgi apparatus. One thing that must not be overlooked is that the Golgi apparatus, the Nissl bodies as well, react positively to the bromine water in a weakly reddish violet colour. This positive reaction is caused by the presence of free tryptophane in the apparatus. Judging from this fact the protein in the Golgi apparatus seems to be partly decomposed to its amino acids or other derivatives.

The distinct red staining on the Golgi apparatus and on the mitochondria by the glutathion reaction, together with the positive staining with the sulphur reaction using lead acetate alkalified with ammonia, is due to the presence of cystin and cystein in them. The intensity of the staining is considerably high, so that it makes us suppose that these cellular inclusions contain plenty of these sulphur-containing amino acids.

By the arginine reaction the apparatus, mitochondria too, are coloured in pink. The significance of this positive reaction on the apparatus is the presence of the arginine to some amount. Summarizing briefly the above data, the protein of the Golgi apparatus is made up of a predominating amount of cystin, cystein, tyrosin, tryptophane, and arginine in a complex combination. On the other

hand the protein of the Golgi apparatus is partially decomposed in the free amino acids or their derivatives.

Discussion

Reviewing the literature, several investigators are mentionable who hold the view that the Golgi apparatus is of the protein nature. But their claim are based unexceptionally upon the staining properties with the dye solutions, and not a claim has been known to be made from the chemical view-point. The present author is intending to criticise their claims and to state his own opinion on the ground of the data obtained in the present study.

Papanicolaou and Stockard (1918) could stain the idiosome-complex of the spermatid of *Cavia cobaya* with acid fuchsin, which usually stains collagenous fibres. Kirkman (1937) stained the Golgi apparatus in the basophilic cells of anterior hypophysis in deep blue with anilin blue after Champy fixation. This dye is also known to stain deeply the collagenous fibers. From these data they supposed that the Golgi apparatus has in its consisting protein a collagenous substance. Referring to the present author's digesting experiments, their assumption must be entirely denied, because the collagenous substance is easily digestible in the pepsin-hydrochloric acid solution and it resists against the action of trypsin; the properties of solubility of the protein constituting the Golgi apparatus in these two kinds of enzymes are in general quite contradictory (see Tarao 1939, 1940, and 1942 a, b). Particularly speaking, the protein in the Golgi apparatus is not digestible by pepsin and is easily soluble in trypsin.

As for the keratin, it must be noted that it resembles very much in the quantity of each kind of amino acid to the protein in the Golgi apparatus, especially in the point that they both contain much cystin and the like, some amount of tyrosine, tryptophane, and arginine. For all of them the keratin is very resistent to the digestive enzymes. Another screloprotein, the elastin, remains to be discussed. Concerning this protein Weigl's 'fuchselin method' must be mentioned in which he used Weigert's resorcin fuchsin after bleaching the sections fixed with Champy's fluid in staining the Golgi apparatus-like structure in cells of a number of tissues. This dye is familiar to us for its specific staining upon elastin fibres.

But this protein cannot be predominant in the protein of the Golgi apparatus, because the former differs from the latter in the point that it lacks in cystin and tryptophane. Moreover, the elastin is easily digestible with pepsin-hydrochloric solution.

Poisson (1927) could stain with light green the idiosome in the spermatide of *Notonecta* fixed with Bouin's fluid, concluding that the Golgi apparatus contains a mucin-like substance. The glucoproteins, the mucin being a member of them, are provided with one common significant nature which is not the case with the protein in the Golgi apparatus. This is the liable solubility in pepsin-hydrochloric acid solution. This difference is enough to reject the identification of the protein in the apparatus to be the mucin or other glucoproteins.

Every protein classified in the phosphoproteins cannot be regarded as the chief protein in the Golgi apparatus, because these proteins are easily precipitated with diluted acetic acid, and moreover they are easily digestible in the pepsin-hydrochloric acid solution. The characters concerning these points of the protein in the Golgi apparatus are quite adverse. In spite of the exceeding speediness of dissolution in the trypsin solution, the identification with any protein of this group must be abandoned.

The Golgi apparatus does not contain the nucleoproteids, because it has never been known to be reactive to the Feulgen's reaction for the thymonucleic acid, and moreover it is liable to be attacked by the diluted acetic acid. Whereas the nucleoproteids are precipitated in the diluted acetic acid and are stained by the general basic dyes which usually do not stain the Golgi apparatus.

As regards the simple proteins, among which the screloproteins have been already brought up for discussion, the proteins probable in the Golgi apparatus are histons, albumins, and globulins.

Among these simple proteins, histons are out of question, because they lack in cystin, tyrosine, and tryptophane, which are found microchemically abundant in the Golgi apparatus. Moreover, the histons are digestible in pepsin forming histopeptone.

The albumins resemble very much to the protein in the Golgi apparatus in the proportion of the quantity of each amino acid; they both contain a lot of cystin, tyrosin, tryptophane, and arginine. But the albumins are generally characterised by the difficulty in

digestion in the trypsin solution, which digests the protein in the Golgi apparatus very soon.

Finally the globulins remain for discussion. It is very astonishing to see that the protein component in the Golgi apparatus coincides fully with the globulin in chemical and in physical properties. At first, in the point of the quantity of each amino acid, they resemble each other, both containing considerable amount of cystin, tryptophane, tyrosin, and arginine. Secondly the globulins are not digestible in pepsin-hydrochloric acid solution. They are digested in pepsin only in the swelling condition. Contrary to the digestion with pepsin, trypsin digests them very easily. These properties for the enzyme solutions are common with the protein in the Golgi apparatus. Thirdly, in the dilute acetic acid the globlulins swell and are dissolved gradually (see Cole 1933, p. 89). This property of the globulins reminds us of the well known fact that the Golgi apparatus is dissolved away in the fixatives containing acetic acid. These similarities in the chemical and in the physical characters are presenting the basis for the argument that the predominating protein in the Golgi apparatus is a kind of globulin.

It would be interesting to mention here the well known fact that the fibrinogen or the fibrin, kinds of globulins, adsorb the proteolytic enzymes. While living, they get rid of digestion by this enzymes thanks to a certain condition. They are very liable to be digested by the autolysis when they are precipitated from blood serum. If the supposed globulin in the Golgi substance can adsorb proteolytic enzymes as the fibrinogen does, the phenomenon of perforation of the sperm into ovum with the perforatorium, which is originated from the acrosome, is easily understood. Moreover, it was reported by Joyet-Lavergne (1925) that the sporozoites of the Sporozoa, which penetrate the intestinal epithelium cells of the hosts, are provided with the Golgi substance at the tip of the perforatory terminals of cells. At the same time he could demonstrate the Golgi substance at the tips of the microgamates of the same group of Protozoa. By means of these terminal organs they can perform the conjugation with macrogamates. Thus it has been supposed by Joyet-Lavergne that at the penetration of one cell into another the Golgi substance which can secrete or adsorb the enzymes plays a rôle of dissolving the region of entrance.

The idea that the elaboration of the secreting granules is per-

formed by the Golgi apparatus, especially at the secretion of the prozymogen granules of proteolytic enzymes from the stomach, intestine, pancreas and from others, has been generally held morphologically. Since the Golgi apparatus contains a kind of globulin, the possibility of the production of the proteolytic enzymes from the apparatus is to be supposed, though not restricted to this cell organ. In relation to this problem, attention must be paid to the fact that several enzymes have been supposed to be belonging to the globulin series; viz. trypsin (Northrop and Kunitz 1932), chymotrypsin (Kunitz and Northrop 1933), pancreatic lipase (Glick and King 1933), pancreatic amylase (Caldwell, Booker, and Sherman 1931), and urease (Summer 1932).

Though not sufficient enough in chemical analysis, the supposition of the presence of globulin in the Glogi apparatus would cast some elucidation towards the explanation of many biological phenomena having association with the Golgi apparatus.

Summary

1. The nerve cells and the hepatic cells of guinea pig were employed for the microchemical study on the protein in the Golgi apparatus. The study was made applying the chief colour reactions for the proteins on both fresh and fixed material.

2. Almost all the protein reactions are positive in more or less degree upon the Golgi apparatus. From this fact it has been established that the Golgi apparatus consists of certain proteins.

3. The general protein reaction, such as biuret reaction and ninhydrin reaction, are markedly positive. By the biuret reaction the Golgi apparatus is coloured in pinkish violet. Of the special protein reactions, Millon's, Liebermann's, Acree-Rosenheim's, glutathion, sulphur, and arginine reaction are outstanding in staining the Golgi apparatus. It is supposed from these data that the protein preponderating in the Golgi apparatus consists mainly of tyrosin, tryptophane, cystin, and arginine. Presuming from the colour tone of these reactions, these amino acids are contained in the Golgi apparatus to a considerable amount.

4. Judging from these colour reactions for proteins and the hitherto established natures, such as insolubility in pepsin, solubility in trypsin, and solubility in the diluted acetic acid, the predominating

protein in the Golgi apparatus can be supposed to be a kind of globulin. Discussion concerning the globulin nature of the Golgi apparatus is made in the text.

5. The Golgi apparatus is stained in weakly reddish violet by the bromine water. This is indicating the presence of free tryptophane in the Golgi apparatus. It can be supposed from this fact that the protein in the Golgi apparatus is partly decomposed into its simple compounds.

6. The mitochondria and the Nissl bodies display colours by these reactions generally in less degree. The former are reactive particularly to the glutathion reaction and Millon's reaction.

Literature cited

CALDWELL, M. L., L. E. BOOKER and H. C. SHERMAN 1931. Crystalline amylase. Science, vol. 74, p. 37.

COLE, S. W. 1933. Practical physiological chemistry. Cambridge, 9. ed.

GLICK, D. and C. G. KING 1933. The protein nature of enzymes. An investigation of pancreatic lipase. Jour. Amer. Chem. Soc., vol. 55, p. 2445.

JOYET-LAVERGNE, PH. 1925a. L'avéoline et la constitution du cytoplasme dans les Sporozoaires. C. r. Soc. Biol., t. 92, p. 1427.

———— 1925b. L'appareil de Golgi des sporozoites de Grégarines. C. r. Soc. Biol., t. 92, p. 1185.

———— 1925c. Recherches sur le cytoplasme des Sporozoaires. Arch. d'Anat. micr., t. 22, p. 105.

———— 1927. Sur les rapports entre le glutathion et le chondriome. C. r. Acad. Sci., t. 184, p. 1587.

———— 1929. Glutathion et chondriome. Protoplasma, Bd. 6, S. 84.

———— 1935. Recherches sur la catalyse des oxydo-reductions dans la cellule vivante. Protoplasma, Bd. 23, S. 51.

KIRKMAN, H. 1937. The anterior hypophysis of the guinea pig; a statistical analysis of its cell types. Amer. Jour. Anat., vol. 61, p. 233.

KUNITZ, M. and J. H. NORTHROP 1933. Isolation of a crystalline protein from pancreas and its conversion into new crystalline proteolytic enzyme by trypsin. Science, vol. 78, p. 558.

NORTHROP, J. H. and M. KUNITZ 1932. Crystalline trypsin. I. Isolation and tests of purity. Jour. Gen. Physiol., vol. 16, p. 267, 295.

PAPANICOLAOU, G. N. and C. R. STOCKARD 1918. The development of the idiosome in the germ-cells of the guinea-pig. Amer. Jour. Anat., vol. 24, p. 37.

POISSON, R. 1927. Recherches sur quelques processus observés dans les éléments sexuels jeunes de *Notonecta maculata* FAB. Arch. Zool. exp., t. 66, p. 23.

TARAO, S. 1939a. Note on distribution of the fatty substance in the spinal ganglion cell of the guinea pig. Zool. Mag., vol. 50, p. 289.

———— 1939b. Microchemical studies on the Golgi apparatus using protease-Nile blue sulphate technique, I. Golgi apparatus of hepatic cells in the mouse and the newt. Jour. Fac. Sci. Hokkaido Imp. Univ., Ser. VI, Zool., vol. 7, p. 1.

———— 1940. Microchemical studies on the Golgi apparatus using protease-Nile blue sulphate technique, II. Golgi apparatus of pancreatic acinar cells in the mouse in fixed and living condition. Cytologia, vol. 11, p. 261.

———— 1942a. Microchemical studies on the Golgi apparatus using protease-Nile blue sulphate technique, III. Golgi apparatus of nerve cells in the guinea pig following various modes of fixation. (to be pressed in Cytologia in near future).

———— 1942b. Microchemical studies on the Golgi apparatus using protease-Nile blue sulphate technique, IV. The extraction of the Golgi substance from nerve cells in the guinea pig. (to be pressed in Cytologia in near future).

Explanation of Plates

All the photomicrographs were taken under the same magnifications of $\times 1000$. The techniques are detailed in the section of material and methods. In the superimposed figures only Golgi elements are sketched.

Plate I

Fig. 1. Nerve cells prepared by Kolatschev's method. Golgi apparatus alone are demonstrated; in the large cell they are distributed evenly in the cytoplasm, in the small cells they gather around the nuclei.

Fig. 2. Large nerve cell from the formol material. Preparation is made by employing trypsin-Nile blue sulphate technique. Golgi apparatus and Nissl bodies are apparent.

Fig. 3. Large nerve cells prepared by means of Champy's fluid-Benda's chroming-Heidenhain's hematoxylin method. Nissl bodies and mitochondria are demonstrated.

Fig. 4. Hepatic cells prepared by Kolatschev's method. Golgi apparatuses are blackened attaching the nuclei.

Fig. 5. Hepatic cells from the formol material, subsequently digested by pepsin and stained with Nile blue sulphate. Golgi apparatuses are arranged almost in the same manner as in Fig. 4.

Fig. 6. Hepatic cells prepared by means of Champy-Benda's chroming-Heidenhain's hematoxylin method. Granular mitochondria and Nissl bodies are shown.

Plate II

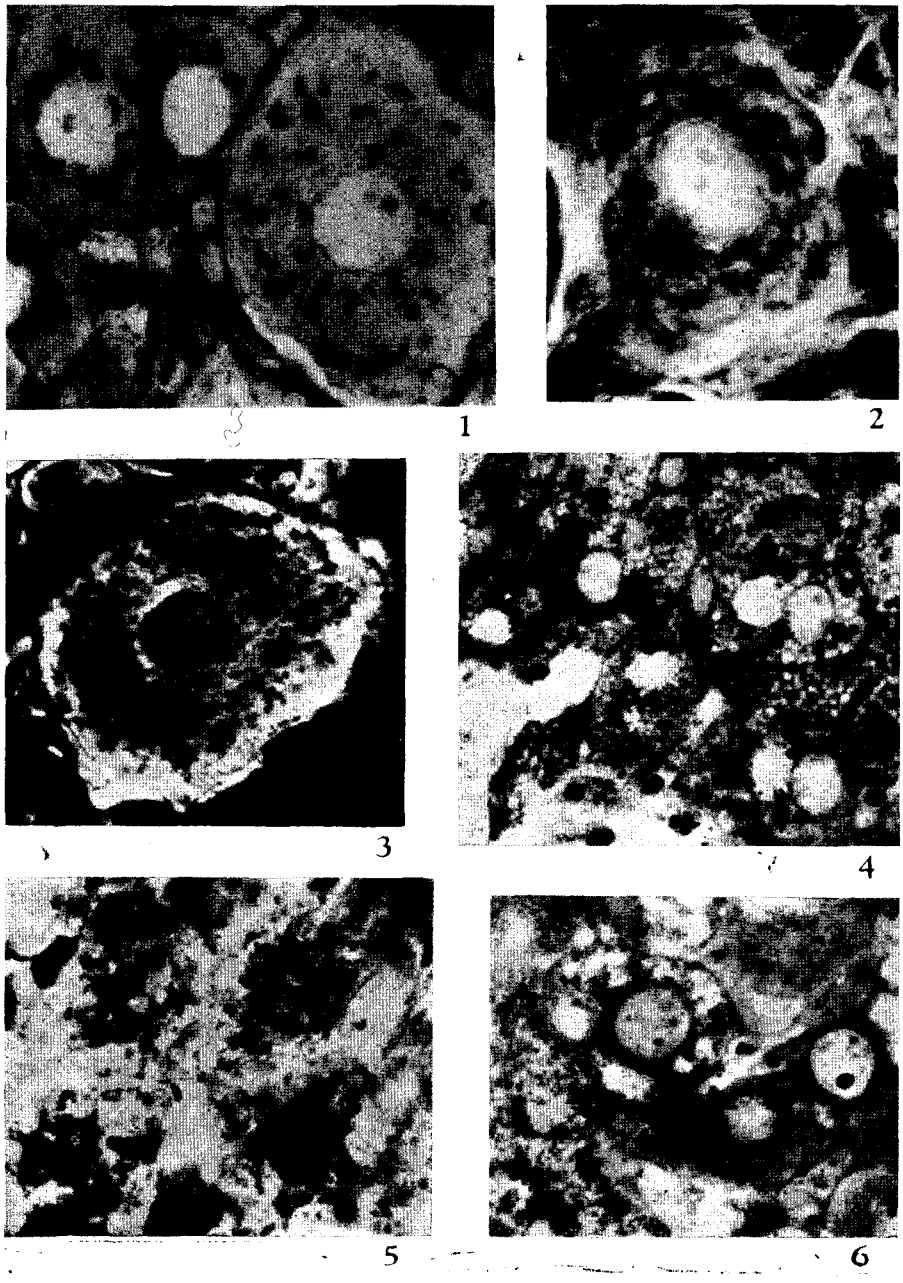
Photomicrographs of nerve cells fixed in various fixatives and treated by reagents of protein reactions. Particular explanations appear in the paragraph of results on the fixed material.

Fig. 7. Preparation fixed with formol and submitted to biuret reaction. Golgi apparatus reacts intensely, and Nissl bodies weakly.
Fig. 8. Prepared by the combinating procedure of corrosive sublimate-xanthoprotein reaction. Golgi apparatus is reactive.
Fig. 9. Prepared by the combinating procedure of formol-ninhydrin reaction. Golgi apparatus is stained blue.
Fig. 10. Prepared by the combinating procedure of potassium bichromate-ninhydrin reaction. Though not intense, the reaction is positive similarly as above.
Figs. 11 and 12. Prepared by the combinating procedure of formol-Liebermann's reaction. Golgi apparatus and Nissl bodies are reactive.
Fig. 13. Prepared by the combinating procedure of formal-glutathion reaction. Mitochondria and Golgi apparatus respond to the reaction.
Fig. 14. Prepared by the combinating procedure of potassium bichromate-glutathion reaction. Result is same as above.

Plate III

Photomicrographs of hepatic cells fixed in various fixatives and treated by reagents of the protein reactions. Particular explanations appear in the paragraph of results on the fixed material.

Fig. 15. Preparation fixed with formol and submitted to biuret reaction. Golgi apparatus reacts distinctly.
Fig. 16. Prepared by the combinating procedure of formol-ninhydrin recation. Golgi apparatus is stained blue.
Figs. 17 and 18. Prepared by the combinating procedure of potassium bichromate-ninhydrin reaction. Golgi apparatus reacts in blue.
Fig. 19. Prepared by the combinating procedure of formol-Acree-Rosenheim's reaction. Golgi apparatus responds to the reaction.
Fig. 20. Prepared by the combinating procedure of formol-sulphur reaction using lead acetate and caustic soda. Golgi apparatus appears, and mitochondria are not apparent.



S. Tarao photo.

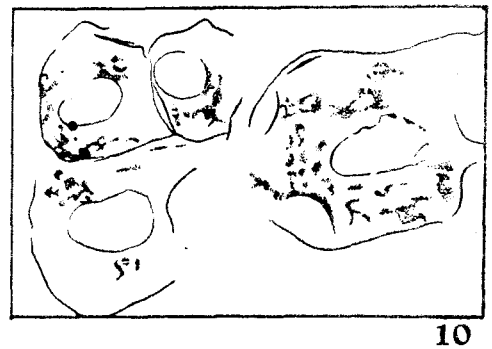
S. Tarao: Microchemical studies on the Golgi apparatus V

Superimposed drawings on figures of Pl. II.

7

8

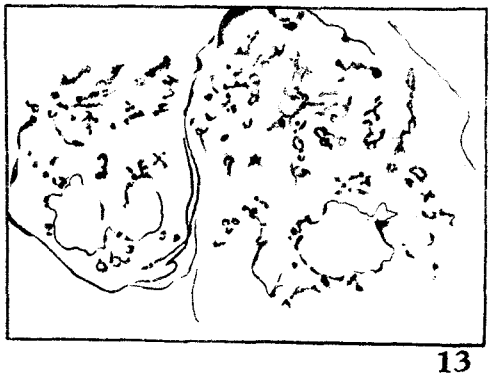
9



10

11

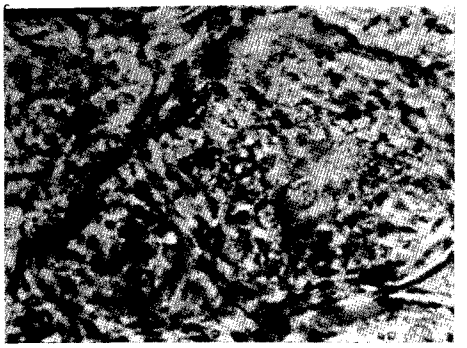
12



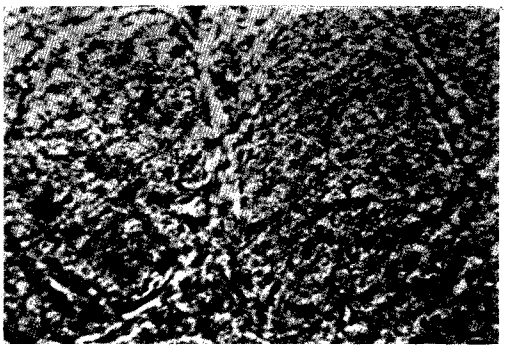
13

14

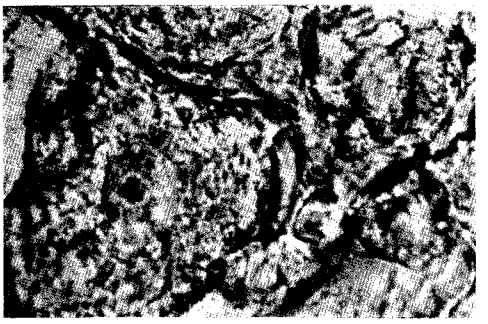
S. Tarao del.



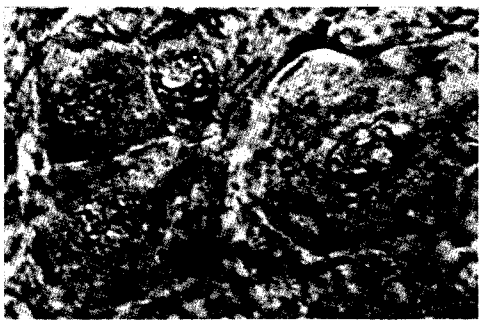
7



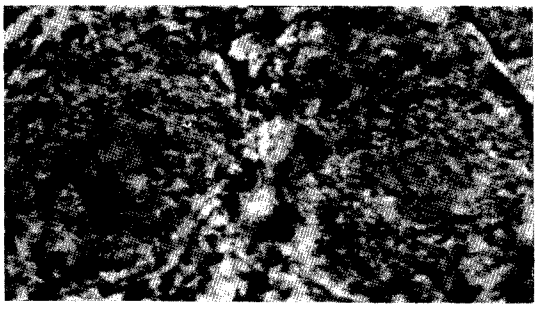
8



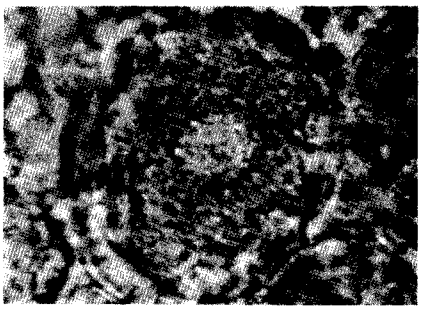
9



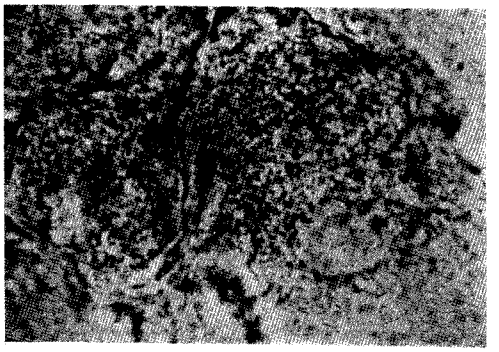
10



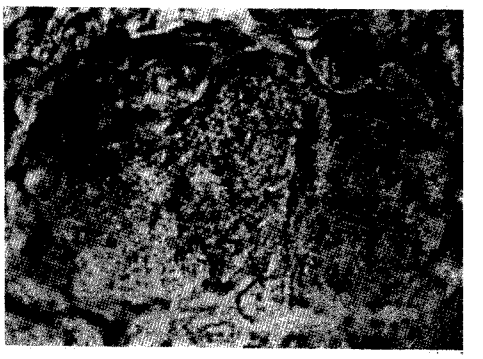
11



12



13

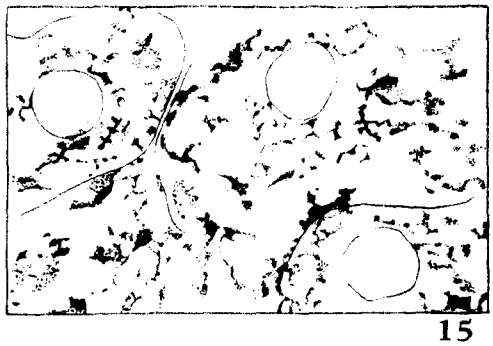


14

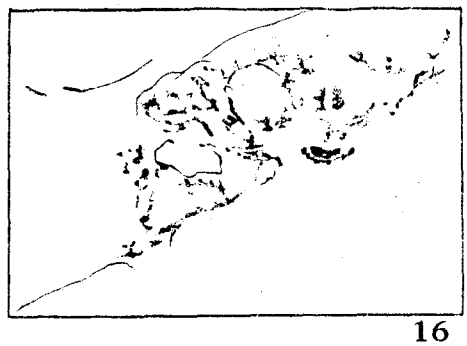
S. Tarao photo.

S. Tarao: Microchemical studies on the Golgi apparatus V

Superimposed drawings on figures of Pl. III.



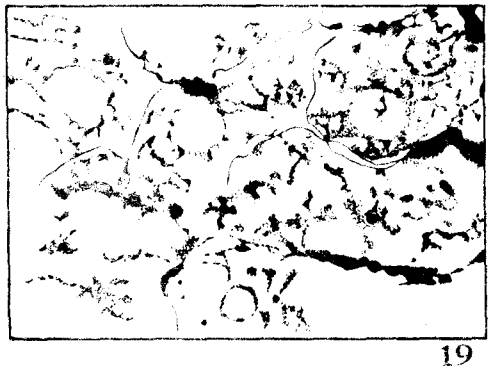
15



16

17

18



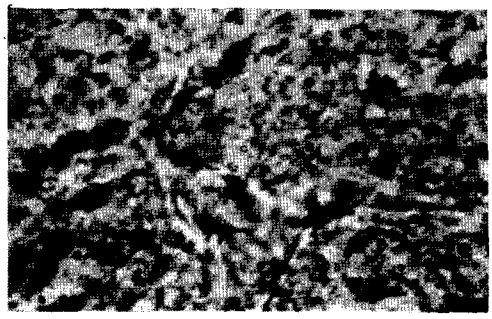
19

20

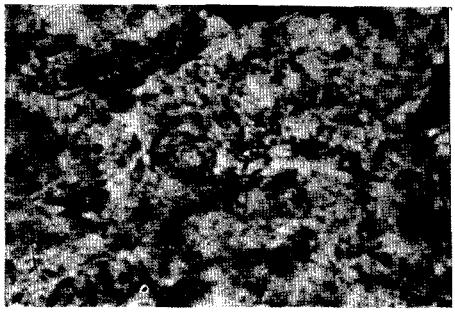
21

22

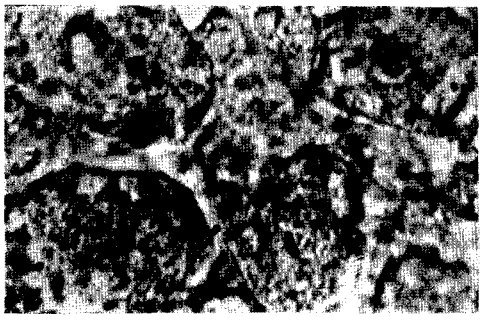
S. Tarao del.



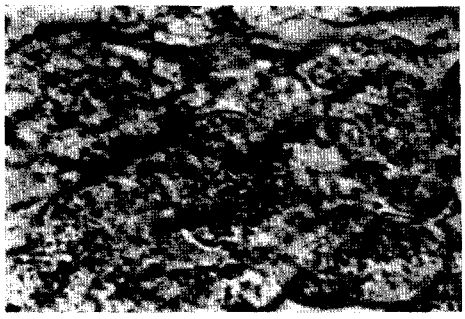
15



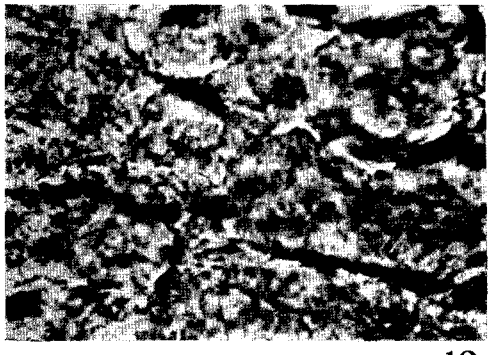
16



17

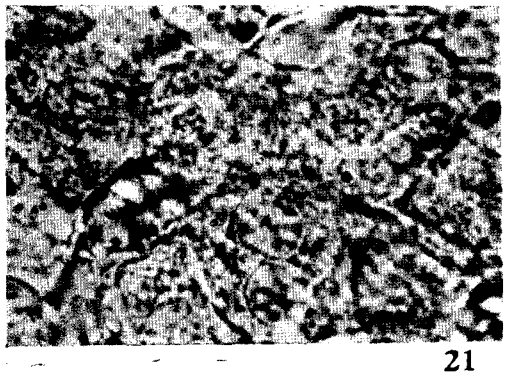


18

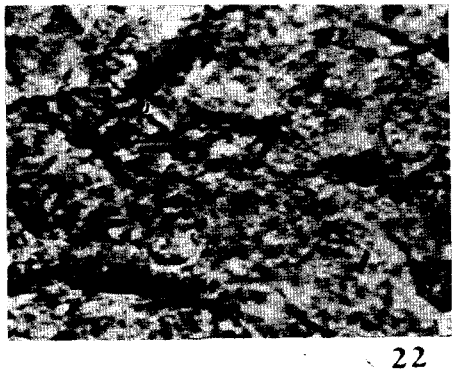


19

20



21



22

S. Tarao photo.

S. Tarao: Microchemical studies on the Golgi apparatus V