Microchemical Studies on the Golgi Apparatus Using Protease-Nile Blue Sulphate Technique I. Golgi Apparatus of Hepatic Cells in the Mouse and the Newt[1)]

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(*With one Plate*)

Introduction

Since Golgi's discovery (1898) of the 'apparato reticolare interno' in the Purkinje cells of *Strix flamma*, the silver-impregnation method has been chiefly employed in the study of that apparatus and many kinds of improvement have occasionally been proposed by many workers. Owing to its capriciousness in impregnation, however, this method has proved to be much inferior to the osmic acid impregnation method invented by Kopsch (1902) in demonstrating the delicate structure of the apparatus. For this reason, the osmic acid technique, gradually improved by the appearance of many excellent modifications, has become lately much in vogue. Though a considerable number of works based on this technique have appeared since then, Parat and his school evidently marked one epoch in consequence of offering their vacuome theory (1924 and thereafter). Since that time skepticism on the morphology of the impregnated apparatus has gradually arisen among cytologists concerning whether such impregnation actually shows the true structure in the live cells or merely an artifact resulting from the mechanical precipitation of metals. It is the morphological discrepancy

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between the vacuomes and the Golgi apparatus that gives rise to the conflicting discussion, though there exists, on the other hand, an absolute coincidence in their location. As pointed out by the vacuome theorists (Parat and Bergeot, 1925; Bensley and Owens, 1929 and others) and by some non-vacuome theorists themselves (Starke, 1895; Beams, 1930; Gatenby, 1931), divergent results are possibly brought about by many unfavourable conditions during impregnation. The chief cause of such results has been believed to be the different degree in impregnation. Surely it is found, in routine work, that a considerable difference is actually brought about by different durations of impregnation. But we confess without hesitation that the familliar Golgi apparatus impregnated with care shows too beautiful a figure to be considered as an artifact. As a matter of fact, despites the theoretical defects of the metalic impregnating methods, many workers still maintain the faithfulness of the adequate metalic impregnation.

Though the specific blackening reaction of the osmic acid to the unsaturated fatty substances has generally been admitted, some workers still claim that this specificity does not hold true in the case of much prolonged osmic acid impregnation. The precipitation produced by this technique, as they believe, is the product of a physical reaction and not that of a chemical one. This claim may well be supported by the fact that the most of the histochemical lipoid tests to the Golgi substance are negative. But on the other hand, contrary to these facts, the solubility of the Golgi apparatus in fat solvents (cf. Eastlick, 1936) and in the dilute acetic acid makes one strongly to suppose the lipoidal nature of the apparatus. Moreover, positive lipoid reactions were obtained by several workers on the dictyosomes of the spermatocytes of certain invertebrate animals, especially by Karpova (1925) on the spermatocytes of *Helix pomatia*. Why the Golgi apparatus fails to reveal the positive histochemical lipoid reactions in almost all kinds of somatic cells still remains obscure. But the most favourable elucidation towards this problem may be the 'histolipoid theory' advanced by Ciaccio (1926 a and b, 1927). Following this theory, some amount of the lipoid substances contained in the animal cells is supposed to be in loose combination with protein, and consequently most of the lipoid reactions are hindered. He termed the lipoid substance in such kind of combination as 'histolipoid' and such a condition as 'masked

condition'. According to him, the lipoid component of the histo-lipoid can be brought to react to the lipoid test after his 'unmasking' procedures. The unmasking procedures consist of 'enzyme method', 'phenol method' and some others. By the application of these methods he succeeded in detecting idiosomic compex in the male germ cells of *Discoglossus*. As the dictyosomes of germ cells are very easily observable, as stated above, whenever the general lipoid tests have been applied, it is no wonder that the idiosomic complex in the male germ cells of *Discoglossus* reveals its apparent lipoidal nature after the unmasking procedures. The general application of these unmasking procedures to the somatic cells to discover the chemical constituents of the Golgi apparatus has not yet been conceived. Indeed it is a matter of very strange fact that this field of study has thus been neglected. By dint of this unmasking procedure, especially by adopting the enzyme method, the present author has succeeded in detecting Golgi apparatus in several kinds of somatic cells of the mouse and the newt. Using this new method and the extraction by fat solvents the present author could also analyse the Golgi apparatus into two essential constituents, *viz.*, a lipoid and a certain protein.

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Material and Method

Livers of the mouse, *Mus musculus* and the newt, *Triturus pyrrhogaster* were employed for the material. It is a general opinion that the morphology of cytoplasmic inclusions in hepatic cells, especially that of the Golgi apparatus, are very susceptible to changes in physiological conditions. The relation of the Golgi apparatus to the bile secretion in various experimentally evoked conditions was clearly illustrated in the schema of Ludford (1926). Such being the case, it is necessary for the present work to keep the animals in absolutely the same condition with respect to the digestion of food. The animals from which the present material was taken were kept hungry for 16 hours after the last feeding.

The pieces of livers were at first submitted to the author's proteolytic enzyme-Nile blue sulphate technique in order to disclose

the fatty substances. The procedure of this method is as follows: The freshly dissected small pieces of livers were fixed in 20% formalin (commercial formalin neutralized with calcium carbonate, 1 part: distilled water 5 parts) for at least 24 hours. Sections were made 30 micra thick by means of the freezing microtom. These sections were in turn digested in one of the following mixtures of proteolytic enzymes, keeping the oven at 37°C:

(A)	Pepsin (Grübler's)	0.3 gm.
	HCl (sp. g. = 1.19)	0.2 c.c.
	Distilled water	100 c.c.
(B)	Trypsin (Grübler's)	0.3 gm.
	Na_2CO_3 (anhydrous)	0.3 gm.
	Distilled water	100 c.c.

The adequate stay in these enzyme solutions is only known from experience. For the present materials (both for mouse and for newt) 8 hours' digestion in the pepsin solution is quite enough to remove the masked protein roughly from the substance comprising the Golgi apparatus. To save time, a denser percentage of the pepsin solution, 0.5% solution for 6 hours for instance, can be used without even slight damage; in this case, however, care must be taken not to carry the digestion to excess. In the trypsin solution almost the same duration of digestion will do, saving that the unmasked Golgi apparatus assumes a very slender contour as will be explained in the next paragraph. The combination of both kinds of enzymes, usually the pepsin first and the trypsin next as the natural mode of digestion in the vertebrates, increases the speed of the digestion. For the present material 2 hours in pepsin followed by 3 hours in trypsin are sufficient to obtain good preparations. Five hours in the second solution is much too long, as the nuclear menbrane is entirely dissolved away.

When the digestion had advanced to an adequate degree, the sections were washed in distilled water for half an hour and stained over night with 0.01% aqueous solution of Nile blue sulphate (Grübler) which is specific for the lipinous substances. Here, special care must be taken to remove completely, a trace of Na_2CO_3 from the sections, because Nile blue sulphate is easily deteriorated by even a trace of it. Then these section were rinsed in 1% aqueous solution of acetic acid for about 2 minutes. The acetic acid thus

applied remove the excess of dye from the sections. It is quite necessary that the blue stain is differentiated until pale blue appearance is reached. After washing thoroughly in the distilled water, the sections were mounted with saturated aqueous solution of dextrose and the margin of the cover glass was sealed with vaselin-paraffin. By this method the Golgi substance stains pinkish blue, while a certain lipoidal substance, perhaps unsaturated, and fat are stained red. The greater part of the blue granules may be supposed to contain saturated fatty acid. The ground substance consisting cytoplasm, though much corroded by enzyme, remains unstained. Aside from its colour tone, the Golgi apparatus is easily detectable by its morphology when it is compared with the one prepared according to the classical osmic acid impregnating method. It may be worth mentioning that the unfavourable artificial change in morphology and location of the Golgi apparatus were never observed in the preparations after the new technique, even when the digestion advanced so excessively that the nuclear membrane was entirely dissolved (Fig. 4). It is needless to say that the sections without such digestion get a diffuse blue staining in which the Golgi apparatus does not stand out.

As a control to the preparations made by the new technique, small pieces of the liver were fixed in Champy's fluid modified after Nassonov for 24 hours, and then washed thoroughly in running water to remove the chrome salts. The pieces thus treated were impregnated in 1% osmic acid solution in the oven kept at 37°C for varying lengths of time between 2 to 9 days. It is the author's experience that 4 days were enough to get a moderate degree of impregnation. After the completion of the impregnation, having been controlled under the microscope following Nassonov's glycerin technique, the peices were put in vials containing distilled water an were left in the oven at 33°C for one day to complete the impregnation. After the ordinary paraffin technique the sections were cut 5 micra in thickness. Other than those which were prepared only by osmic acid impregnaton, post-staining with Altmann's acid fuchsin was also tried in order to demonstrate both mitochondria and the Golgi apparatus in the same preparations.

The estimation of the new technique

The mouse (*Mus musculus*)

Reviewing the literature one finds that investigators are by no means of the same opinion about the morphology and disposition of the Golgi apparatus so far as the hepatic cells of vertebrates are concerned. As to the mammalian hepatic cells, it has been generally accepted that the Golgi apparatus occupies a perinu-

clear position constructing a network, extending its free ends toward the bile capillaries. Sometimes these free ends attain to the bile capillary surrounding the cell, keeping a close relationship between the two. Such a case was drawn typically in the figure of Dornesco (1930) on the hepatic cells of the white mouse. A little later, Makarow (1931) investigated accurately the relation between the Golgi apparatus and bile capillaries in the hepatic cells of the rat, the bat, the frog, and other vertebrates, and found that it followed almost the same plan as observed by Dornesco. Other workers who claim the same view may be enumerated: Ludford (1928) on the mouse, Makarow (1926) on 13 speces of vertebrates, Chlopn an Chlopin (1929) on the hepatic cells cultured in vitro, and Granel (1929) on the guinea pig. Contrary to this view, there are some authors who denied such a relation entirely, namely Kolmer (1916) on a newly born cat, Pappenheimer (1916) on some mammals, Pascual (1924) on a young mouse and on a cat embryo. But the present writer cannot favour the latter view, because such classical works used mainly the simple silver nitrate methods. By a careful examination of the preparations which were made by Kolatchev's method slightly modified, the present author came to the conclusion that the morphology and the disposition of the Golgi apparatus are subjected to variation of the physiological conditions of the animal. Moreover, even the hepatic cells in one and the same individual are not in the same functional stage, but show variations in their fine structures. Holding the view, therefore, that intimate association really exists between the Golgi apparatus and the bile capillaries, there remains room to suppose other phases in which the Golgi apparatus is displaced from the bile capillary occupying a perinuclear position. Such a variation following physiological functional changes experimentally evoked was schematically indicated in the figures of Cramer and Ludford (1926, figs. 10-15). Bowen (1926) also noticed that very slight physiological change caused destruction upon the Golgi net. Of these variant cases in their morphology and disposition, the most common and the ground case for hepatic cells may be the perinuclear Golgi nets. For this reason the present observations were principally restricted to the Golgi apparatus around nucleus.

Fig. 1 is one of these cells impregnated by osmic acid, indicating a typical perinuclear Golgi apparatus. This Golgi apparatus is constructed of incomplete nets, deformed rings and bands, which are all in contact with the nuclear membrane. Its cytoplasm is almost free from Golgi elements, saving a little amount on the periphery facing the bile capillary. Parallel to this impregnation, the same material fixed previously with formalin was cut into sections by means of the freezing method, and were next submitted to the pepsin solution for 8 hours. The digested sections were stained by the Nile blue staining method as indicated in the foregoing paragraph. The lipoidal substance comprised in the Golgi apparatus is disclosed clearly in blue colour with a red tone (Fig. 2).

The most part of the cytoplasm (protein) and some amount of chromatin are removed by this digestion. The nuclear membrane remains. If one compares Fig. 1 with Fig. 2, he is convinced that the Golgi apparatus thus unmasked by the pepsin coincides entirely with that of the osmium impregnating preparation. It is a noteworthy fact that the morphology of the apparatus, especially the thickness of its bands, has never been observed being altered by the pepsin digestion. Its red tone with Nile blue sulphate is due surely to the content of the unsaturated lipoidal substance.

Fig. 3 shows liver cells digested by the trypsin solution for 6 hours and stained with Nile blue sulphate. Here, the greater part of the cellular protein is removed as in the case of digestion by pepsin. The nuclear membrane is not affected by the digestion. As for the Golgi apparatus, the attenuation of its contour is apparent, when compared with that submitted to the pepsin solution (compare Fig. 3 with Fig. 2). In the cells where the digestion with trypsin advances to completion, the attenuation, in other words, the dissolution of some part of the Golgi apparatus, is seen to have taken place. Upon close examination its outline is discernible by the fragments of the lipoidal substance intermittently arranged along the original contour. The substance which is dissolved away from the Golgi apparatus by the trypsin soluiton is not yet substantiated. But two probable suppositions may naturally be deduced as follows: The first is that the lost substance may be trypsin-soluble protein. According to this assumption the Golgi apparatus after pepsin digestion consists of trypsin-soluble protein and lipoidal substances. The second probable supposition is that the dissolved substance may be taken for fat. It is a well-known fact that fat is saponified in the alkaline solution. The trypsin solution used in the present study, though not strong, contains 0.3% of sodium carbonate. Moreover, since the digestion was carried on for as long as 16 hours at 37°C in the oven, it may be supposed with some probability that the fat in the Golgi apparatus was attacked (saponified) by the alkaline solution. In order to determine these two divergent assumptions, the author made the following solubility experiments.

At first the sections cut by means of freezing method were directly rinsed in three fat solvents in succession, namely in aceton, in chloroform, and then in ether, being kept for 16 hours in each fluid. After this procedure for the extraction of fatty substances,

the sections were digested in the pepsin solution. This results is shown in Fig. 5, where almost the same quantity of the Golgi substance still remains as in the original region (compare Fig. 5 with Fig. 2). This fact is enough to show that the lost part of the Golgi apparatus after trypsin treatment is composed for the most part of protein digestible by trypsin, and is not fat or fatty substance. Other sections which were taken from the same material as in the above experiments were next rinsed in aceton for 16 hours keeping at room temperature, and then they were digested in trypsin. As shown in Fig. 6, cells thus treated contain no trace of Golgi substance. From this results it can be concluded, therefore, that the residue of the Golgi apparatus after the trypsin digestion, just as in the case in Fig. 3, must surely be fat or fatty substance unsaponifiable by the very dilute alkaline solution.

The conclusion from what is stated above is that the Golgi apparatus in the hepatic cell of the mouse really exists as such which is impregnated by the osmic acid, and that it consists of protein digestible by trypsin and fat or fatty substance.

The newt (*Triturus pyrrhogaster*)

Nassonov (1926) classified the manner of arrangement of the Golgi apparatuses of hepatic cells of vertebrates into two types; "Maus-Gruppe" and "Eidechse-Gruppe." According to him, the latter group is characteristic for its arrangement of the apparatus along the bile capillary, and this rule is also applicable to Amphibia in general with no allowance for modication in any functional stage. Kraft (1924), Makarow (1931) and Weatherford (1932) obtained reults in full agreement with that of Nassonov. One adverse view appears in the paper of Dornesco (1929 c), where he claimed that in the hepatic cells of *Lacerta vivipara* the Golgi bodies-active chondriosomes complexes always occupied the place between the nucleus and bile capillaries, and moreover his drawing of the Golgi apparatus differs greatly from those of the above mentioned workers. The discussion concerning this point shall be given in the next paragraph. Anyhow, the general opinion about the disposition and the morphology of Golgi apparatus is in complete accordance with the present author's as detailed below.

Fig. 7 is a photograph of hepatic cells from a modified Kopsch preparation of the newt kept in hunger for 16 hours. Two round nuclei are seen blackened diffusely occupying almost the central region of the cells or a little basal. Many fat globules which get intense blackening by the osmic acid are laid upon the basal connective-tissue membrane. Mitochondria stain faintly black with

the osmic acid and are spread throughout the cytoplasm. The Golgi apparatus assumes the shape of a roughly continuous knobby band with a considerable breadth and fringes the border facing the bile capillary. As clearly shown in Fig. 7, the Golgi bands of both sides of hepatic cells, interposing a bile capillary between them, are frequently seen as double bands running parallel to each other.

The same material as the preparation of Fig. 7 was next fixed in formalin and was submitted to the pepsin technique (Fig. 8). By this technique the nuclei stain blue, and fat globules become pink in colour. The mitochondria are dissolved completely. The Golgi apparatus is stained pale blue in the form of a thick band along the margin facing the bile capillary (shown by arrows in Fig. 8). It must be noted that the Golgi apparatus here disclosed shows absolute conformity in shape and location with that of the osmic impregnation. This fact is enough to prove that the adequate osmic impregnation does not produce artifact augmentation in size. Upon careful examination, though not clear in this photograph, these bands of Golgi apparatus are seen fenetrated with tiny pores, showing an appearance of a distended cage. But the detailed architecture should be left for the future investigation.

The experiments on solubility of the substances composing the Golgi apparatus gave similar results as in the case of the mouse; the coexistence of the content of the protein digestible in trypsin and the lipoidal substance is apparent.

The Golgi apparatus of the newt faciliated this investigation owing to its thick contour and its definite position, though its lipoid content is comparatively poor as shown by difficulty in getting a deep staining.

Discussion

As briefly stated in the introductory paragraph, it is still an unsettled question whether the Golgi apparatus does really exist possessing generally the familiar network structure as appeared in the classical works, or whether such a network is merely an artifact produced by the unfavourable precipitation on and between the 'vacuomes' as claimed by Parat and his associates. This controversy originates from the lack of general technique of histochemistry other than the metallic impregnation methods. Modifying Ciaccio's

unmasking technique for the 'histolipoid', the persent author devised a proteolytic enzyme-Nile blue sulphate technique for general use and found it possible to apply it to the variant kinds of animal cells with a fair degree of success. The estimation of this technique on the hepatic cells has just been detailed in the forgoing paragraph, comparing these preparations with those prepared by a modified Kopsch technique. By the comparison between these two, it may be understood that the impregnated Golgi apparatus is of quite identical morphology with that of the apparatus detected by the present technique. As for the hepatic cells Makarow (1926) demonstrates their Golgi apparatus by applying the iron reaction. This fact indicates that the iron introduced into the hepatic cells as a result of body metabolism deposits upon the Golgi apparatus. According to him, the Golgi net or his "Eisennetz" revealed by the iron reaction breaks up into fragments when certain dyes, including neutral red, or the bile globules are heavily accumulated in the cells. Anyhow, it is evident that the properly impregnated Golgi apparatus never exaggerates its morphology beyond the contour resulting from the fixation. In fact, the artificial alteration of shape from vacuome into net during the process of impregnation could never be experienced. Holding that the net-work in the fixed preparation does truly exist, still the question would naturally arise whether or not the Golgi apparatus really assumes the network structure also in the living cells as well as in the fixed material. To solve this problem many attempts have been undertaken by many workers to stain it vitally or supravitally, but a granular staining in the same region as the Golgi apparatus has only been obtained. Indeed, the vacuome theorists have based their argument upon this fact, and have taken the Golgi apparatus for an artifact structure brought about by the precipitation of metals around the vacuomes during impregnation procedure. But attention must be turned to a few exceptional cases in which the vital staining on the Golgi network is positive. An adequate example for this positive case has been known in cells of the convoluted tubule of the vertebrate kidney when the animal is perfused with the solution of trypan blue. The application of trypan blue to the study of the Golgi apparatus of the kidney cells was first undertaken by Jasswoin (1925) on some amphibians. In his figures the vital staining of the Golgi apparatus is not marked, but the accumulation of dye particles upon the Golgi

apparatus occurs. Nassonov (1926) proposed the "Apparatentheorie" of the Golgi apparatus in which he employed trypan blue as a vital stain on kidney and hepatic cells in order to confirm his view on the participation of the Golgi apparatus in the cellular secretion. In this work, vital staining of the Golgi net occurs only in the convoluted tubule cells of the white mouse, while in the hepatic cells of that animal and *Lacerta* the dye particles alone occupy the same position as the Golgi net which does not come into vision by the vital staining.

As for the hepatic cells, Glasunow (1928) succeeded in the vital staining of the Golgi net by repeated injections of the trypan blue solution up to as many as 30 times. Though it might be feared that the staining of the Golgi net is a symptom of deterioration of the cells, this work is still worth attention. Considering the fact that the trypan blue stains the Golgi apparatus in the living kidney cells, which are excretory in function, one may likewise expect similar stainability of the living hepatic cells with the same dye, which also play some rôle in the excretion of the body.

Dornesco, who holds the vacuome theory, presented a series of papers on the Golgi apparatus of the hepatic cells in several vertebrates (1929 a, b, and c; 1930). Among these works only the last one on the white mouse seems credible. Concerning the other animals, expecially on *Lacerta,* his drawings are very wide from the truth. The vacuomes he saw, which he took for the Golgi apparatus, may certainly correspond with the 'Crinome' of Chlopin or other kinds of globules of metaplasmatic nature. In the fixed material, he failed to observe the Golgi apparatus of the hepatic cells even in *Lacerta,* which has been well known for its characteristic morphology and its disposition. When one considers the fact that the bile droplets appear arranged upon the Golgi apparatus of the hepatic cells, as accurately investigated by Weatherford (1932), it seems to be rather safe to conclude that the globules stained by vital dyes, including 'vacuome', may all belong to the category of secretory products.

The supposed intimate relation between the 'vacuomes' and 'chondriomes actifs', which Dornescs and other vacuome theorists claim, has never been supported by any positive proof. As regards the author's observations made by the digesting technique, the mitochondria were completely digested by the proteolytic enzymes

leaving no visible trace. At the same time, the author could neither differentiate these two supposed elements nor could be see any close association between the Golgi apparatus and mitochondria on the impregnated preparations counterstained by the acid fuchsin.

We have at present only a little knowledge concerning the essential chemical ingredients contained in the Golgi apparatus. In their review of the Golgi apparatus, Kirkman and Severinghaus (1938) consider the protein substance of the Golgi apparatus as a substance close to mucin, reticulin or collagen, because the idiosome in the germ cells of various animals has been known to react frequently to the connective tissue staining method (for example, light green on *Notonecta* by Poisson, 1927; on guinea pig by Papanicolau and Stockard, 1918). Weigl (1910) found that, after bleaching the osmicated preparations of the somatic cells of some vertebrates, the Golgi apparatus took restaining of Weigert's resorcin-fuchsin, a dye widely employed for the staining of the elastic fibres of the connective tissue. Kirkman (1937) also stained the bleached osmic preparations of the anterior hypophysis and cervical spinal ganglion of the guinea pig with anilin blue and obtained positive results, from which he thought that this protein might be of collagenous nature. But the above mentioned data are not sufficient to give an ultimate proof of these chemical substances, because of these staining dyes being not specific for the definite chemical substances but for various others. The lipinous nature of the Golgi apparatus has generally been accepted on account of its osmiophilic property and its solubility in fat solvents. The solubility experiemnts of the present work roughly suggest that the Golgi apparatus consists of a comparatively great amount of a certain protein belonging to "Hemigruppe", which is easily soluble with trypsin, and some amount of fatty substance soluble in aceton and other fat solvents. The further accurate chemical analysis of these substances is now under progress by the present author.

Summary

1. The Golgi apparatuses of the hepatic cells of the white mouse and the newt were demonstrated by the writer's new proteolytic enzyme-Nile blue sulphate technique.

2. The Golgi apparatus prepared by the new technique is in

complete accordance with that impregnated by Kolatchev's method both in morphology and in disposition. From this fact it can be concluded that the classical metallic impregnation techniques, when properly employed, give an exact results.

3. The Golgi apparatus of the present material, at least in the fixed preparations, has no morphological similarity to the 'vacuome'. Referring to the results by previous investigators it is better to suppose that the so-called 'vacuome' likely belongs to the category of secretory products, such as bile granules.

4. Mitochondria of hepatic cells of both animals are completely disolved in the proteolytic enzymes. Nothing like 'chondriomes actifs' after Parat are observable either by the impregnation technique or by the present new technique.

5. As the results obtained from the test of solubility in the proteolytic enzymes and in the fat solvents, the Golgi apparatus is supposed to consist of two essential substances of different kinds, trypsin-soluble protein and fat or fatty acid. These two substances seem to be combined loosely as the 'masked condition' after Ciaccio.

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Explanation of Plate I

All the photographs were taken with a semi-apochromatic oil immersion objective (HI.×, 100) and a 10 × ocular, the magnification being 1000 times. Figs. 1 to 6 are photographs of hepatic cells of a mouse. The techniques are detailed in the paragraph on material and method.

Fig. 1. Hepatic cell prepared by a modification of Kolatchev's method, duration of osmification being 4 days in 1% osmic acid solution keeping at 37°C. Perinuclear incomplete Golgi net is shown.

Fig. 2. Bi-nucleated cell digested by pepsin and stained with Nile-blue sulphate. Golgi apparatus corresponds entirely to that of Fig. 1 in shape and location.

Fig. 3. Two uni-nucleated cells disgested by trypsin. Notice the diminution of volume of the Golgi material when compared with that in Figs. 1 and 2.

Fig. 4. Cell digested by pepsin for 3 hours and by trypsin for 5 hours successively. Nucleus membrane is dissolved completely, while the Golgi apparatus still possesses its original form.

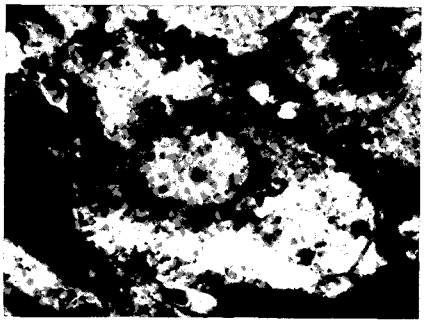
Fig. 5. Cell rinsed previously in aceton, chloroform, and ether in succession, and then digested by pepsin. Note that the Golgi apparatus still remains, though a little diminished and deformed. Cell membrane is lost completely.

Fig. 6. Cells from the section rinsed in aceton and followed by trypsin digestion. Notice that the Golgi apparatus is removed by this procedure leaving no trace.

Figs. 7 and 8 are phtographs of hepatic cells of a newt.

Fig. 7. Cells impregnated by a modification of Kolatchev's method. Golgi apparaus is represented by knobby bands limiting the cell periphery facing bile capillary. Two continuous rows of Golgi bands, running parallel each other are seen, interposing a bile capillary between them. Mitochondria are seen blackened slightly and are spread throughout cytoplasm. Fat globules are heavily blackened, piled on the cell basis underneath the impregnated nuclei.

Fig. 8. Same section as Fig. 7 digested by pepsin. Continuous knobby bands of Golgi apparatus shown by arrows coincide entirely with those in Fig. 7. Nuclei take blue colour of Nile blue sulphate. Fat globules stain red.



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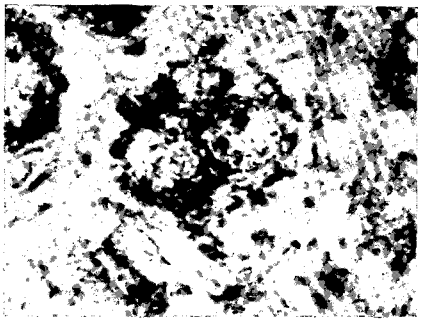

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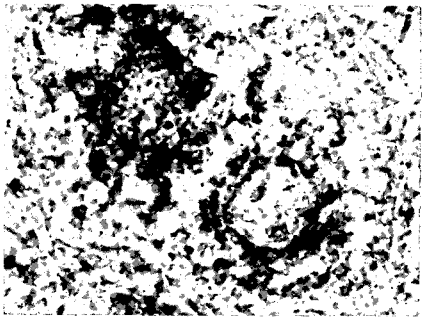

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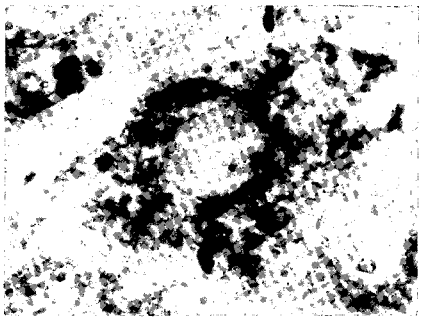

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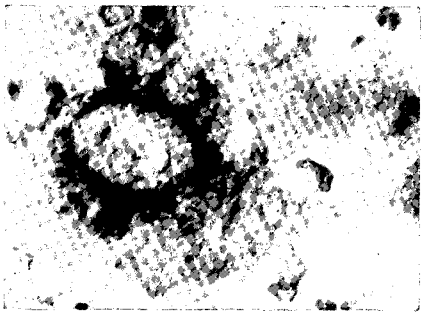

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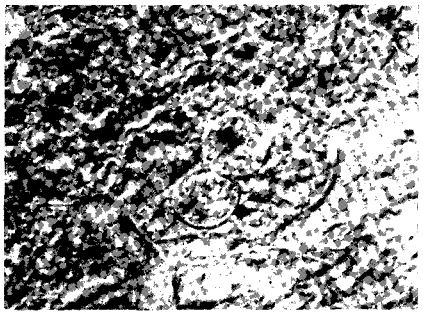

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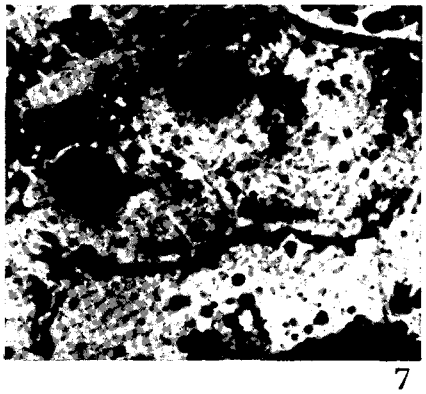

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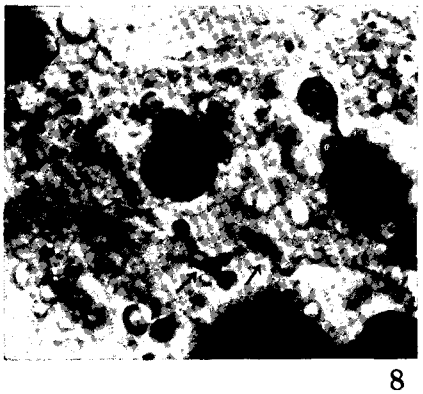

8

S. Tarao photo.

S. Tarao: Golgi Apparatus using Protease-Nile Blue Sulphate Technique