Studies on the Structure of the Golgi Apparatus. I. Cytoplasmic inclusions in the gregarine *Lecudina brasili* (N. Sp.) parasitic in the gut of *Lumbriconereis*

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Introduction

While one of us was going through Hirschler's paper ('27) in connection with the evolution of the net-work-like Golgi apparatus of vertebrate somatic cells, the shapes described and figured resembled those seen in *Lycastis* and other invertebrates (Subramaniam and Gopala Aiyar '36 b & c) studied in this Laboratory. Double rimmed batonettes with the chromophobic part between the two rims and ordinary dictyosomes with the idiosome in relation with the cytoplasm have been figured by Hirschler in various Protozoa. This striking similarity in structure led us to investigate the cytoplasmic inclusions in a gregarine recently described (Ganapati and Gopala Aiyar '37). The studies on the Golgi apparatus in Protozoa have given diverse results and some of the unsettled questions are:— (1) Do the vesicle and batonette forms have an existence; (2) If so, is the batonette derived from the vesicular Golgi bodies; (3) Does the Golgi apparatus stain with neutral red; (4) The relation between the centrosphere, idiosome and the Golgi apparatus.

An analysis of the literature on the cytoplasmic inclusions in Protozoa have already been made (Subramaniam and Gopala Aiyar '37) and it was shown that part of the confusion in Protozoa is due

to the fact that only some criteria for the identification of the Golgi apparatus have been applied in most cases. What we identify as the Golgi apparatus in this paper respond to the tests given for the Golgi apparatus in general (Subramaniam and Gopala Aiyar '37). In addition the Golgi apparatus in the alimentary canal cells can be seen in preparations of *Lumbriconereis*. The morphology and even to some extent the shape and behaviour are similar in *Lecudina*, and we do not believe that lack of agreement in the other groups is due to want of criteria to identify the Golgi apparatus.

Material and Methods

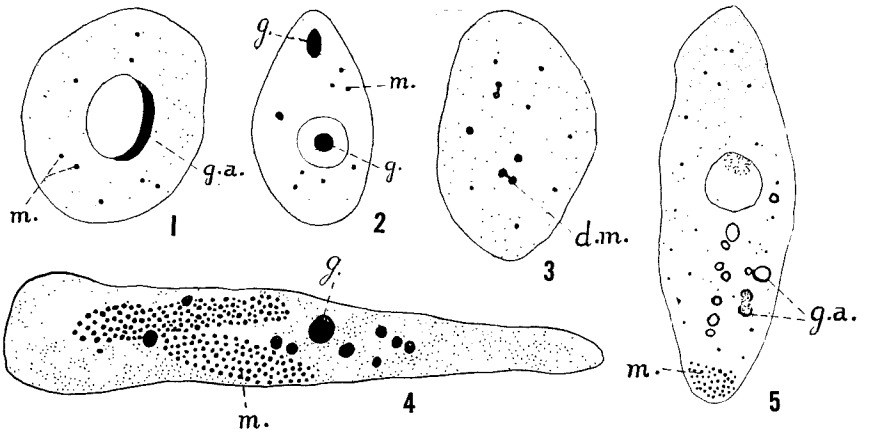
Specimens of *Lumbriconereis* were collected from the mud of the Adyar brackish waters. The worms are small in size not exceeding 3 cms. in length and 1 mm. in girth. The gregarine occurs as an intestinal parasite. The later stages in the life-history of the parasite such as gamete formation and sporulation generally take place outside its host and as such we were not able to obtain sufficient material for a cytological study of these stages. Worms were fixed whole in Champy, Da Fano, Flemming without acetic, Nassonov and Mann's fluid. Nassonov and Mann Kopsch materials were osmicated for varying periods of time in an incubator at 36 deg. C. Da Fano material was toned and stained in alum carmine, and Champy, Nassonov and F. w. a. material with iron haematoxylin. Much of the material had to be discarded owing to the presence of a hyperparasite —*Metchnikovella*—in the gregarine.

The effect of osmication was different in different tissues. Turpentine was employed to remove the extra osmic acid because it permits easy control. The various stages of the gregarine were the first to be bleached, then came the alimentary canal and finally the oogonia and oocytes. Similar staining reactions with iron haematoxylin were also observed.

Mitochondria

Fixation in F. w. a. for 18–24 hours shows the mitochondria alone, while, Champy fixation followed by pyroligneous-chromic mixture and potassium bichromate mordanting shows the Golgi bodies and mitochondria. In Da Fano, often, the mitochondria can be seen as golden brown granules. In the earliest intra-cellular stages (fig. 1) the mitochondria can be seen as a few granules scattered in the cytoplasm. They do not occur collected near the nucleus and all the granules seem to be of equal size. During further growth of the parasite in the cell no appreciable increase in the number of mito-

chondrial granules were observed. When the parasite becomes freed into the alimentary canal lumen some of the mitochondria have been observed to increase in size. This increase in size is soon followed by division (figs. 2*m*. & 3*d.m.*). The divided granules are almost equal in size to the majority of the granules seen in the cytoplasm. As the gregarine grows, the mitochondria also multiply enormously in number. In the free trophozoite they do not seem to have any definite arrangement. In different trophozoites they form concentrations in the anterior (fig. 4 *m*) middle or posterior regions (fig. 5 *m*). Sometimes the majority of the granules form a concentration in some



Figs. 1–5. 1. Intra-cellular stage showing the Golgi apparatus and mitochondria. Nassonov ×1,690. 2. Golgi bodies and mitochondria in a gregarine just becoming free in the alimentary canal lumen. Champy ×1,690. 3. Division of mitochondria. F. w. a. Iron haem. ×1,690. 4. Adult trophozoite. Champy. ×1,690. 5. A growing trophozoite showing mitochondria, nucleolus, dividing Golgi bodies and Golgi vesicles. Mann Kopsch ×2,025. *d. m.* Dividing mitochondria; *g.* Golgi body; *g. a.* Golgi apparatus. *m.* Mitochondria.

region and others may be distributed throughout the cytoplasm. Very few associated individuals were available for study and it appears that during this stage they have a uniform distribution in the cytoplasm.

Intra vital studies were made by teasing out the worms and thus liberating the parasites. The mitochondria are visible as refractive granules occurring scattered uniformly or aggregated in different regions in different trophozoites.

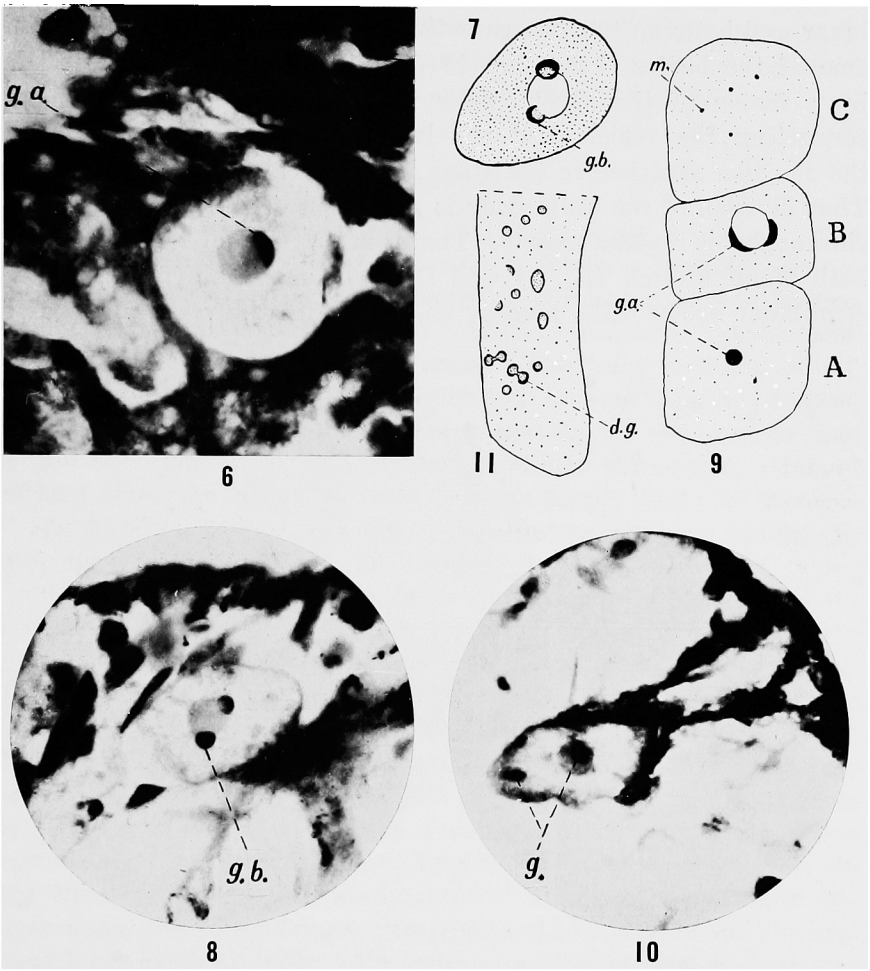
Janus green was used both supra vitally as well as intra vitam. Worms were kept in different dilutions of the dye for varying periods. Keeping the worms in Janus green solution produced only a very slight staining of the mitochondria. In other words they were more clearly visible than in unstained material. The worms when kept for

more than four hours in Janus green showed signs of disintegration, presumably due to the changed artificial medium. Supra vital staining of the gregarines produced a slightly better staining of the mitochondria. But at no time could the mitochondria be said to be stained bright green. Light green colouration was seen in a few of the gregarines and this was the best that we could get. When after staining the parasite with Janus green, the material under the coverslip was irrigated with neutral red, the pale green colouration of the mitochondria could be very clearly made out by contrast.

Golgi Apparatus

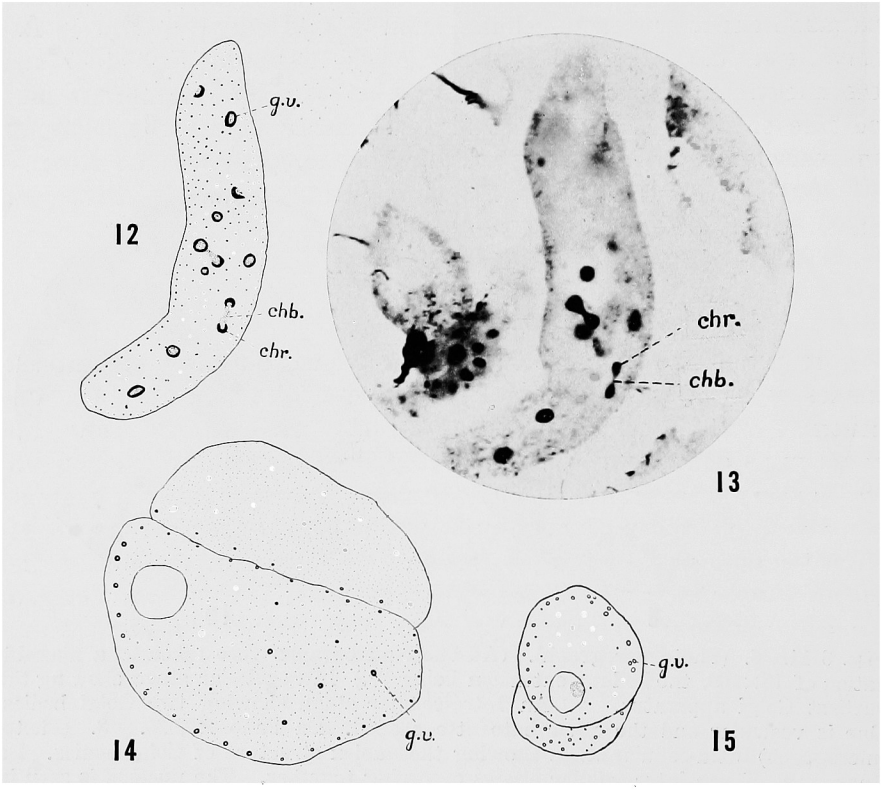
In the intra-cellular stage the Golgi apparatus could be seen as a semi-lunar body appearing as if pasted on to the nuclear membrane (figs. 1.*g. a.* & 6). At this stage no differentiation into chromophilic and chromophobic regions could be made out. There are usually two karyosomes inside the nucleus, but as these are not usually seen at this stage in Nassonov, Da Fano and Mann Kopsch material we are not able to either confirm or refute King and Gatenby's ('23) interesting observation that the excentric nucleolus in each merozoite of *Adelea* is always turned away from the Golgi apparatus and that no exception to this rule has been found to occur. In fig. 7 is shown a slightly older intra-cellular stage, in which the Golgi apparatus has divided into two, both being juxtanuclear. The duplex structure is evident and while in one of the bodies, the chromophilic rim is complete, it is thin in one region. The other is semi-lunar (fig. 8). In fig. 9 is shown three intracellular stages which were found together. The plane of section has apparently passed through different regions of the parasite. In A and C no nucleus is seen and while in A, only a circular bit of the Golgi batonette is present, in C only mitochondria occur. In B the Golgi apparatus is seen as two batonettes plastered on to the nuclear membrane. The duplex structure which was visible during division stages is not seen and apparently is masked. As the parasite is about to get liberated into the gut-lumen, one of the bodies migrates away from the nucleus (figs. 2 & 10). A little later, the other also loses its juxta nuclear position and both begin to divide in the cytoplasm. The duplex structure of the Golgi bodies is seen in well fixed lightly impregnated material where most of the bodies are vesicular. Even in the other cases the duplex structure could be made out even under oil immersion with the aid of powerful illumination. With a 40 objective and 15 eyepiece it is evident. But under oil immersion with ordinary lighting conditions

it is obscured by over impregnation and the resulting opacity. In Champy fixed material mordanted the Golgi bodies appear as spheres which give no evidence of any duplex structure. To get a correct idea about the various changes in the structure of the Golgi bodies



Figs. 6-11. **6.** (Photomicrograph). (All Photomicrographs were taken at a magnification of 15×100, the bellows extension being 9″.) Intra-cellular stage showing the earliest Golgi apparatus. **7.** An Intra-cellular stage showing two Golgi bodies. One is vesicular and the other batonette shaped. Da Fano ×2,025. **8.** (Photomicrograph) Intra-cellular stage showing the duplex structure of Golgi bodies. Da Fano. **9.** Three intra-cellular stages occurring together. The nucleus is seen in B, the Golgi bodies in A and B and Mitochondria in C. The duplex structure of the Golgi appears to be masked. Nassonov ×1,690. **10.** (Photomicrograph). A gregarine becoming free in the alimentary canal. One of the Golgi bodies has migrated away from the nucleus. Nassonov. **11.** A Da Fano preparation showing dumb-bell shaped appearances assumed by Golgi bodies during division. ×1,690. *d.g.* Dividing Golgi bodies; *g. b.* Golgi batonette.

all observations were made from lightly impregnated material, the heavier impregnation being used to check some of the results. Division of Golgi bodies is an interesting process. The vesicle which appears ring-like in optical section becomes dumb-bell shaped by a constriction (fig. 11). The two knobs when they separate are semi-lunar and between the two runs the chromophobic part as a strand. One of the bodies thus formed (figs. 12 & 13) becomes vesicular by a fusion of the chromophilic rim and the chromophobic part connecting the vesicle and the batonette seems to contract into the cup like area before the other batonette also becomes vesicular. Thus division of the Golgi body is a division of its chromophilic and chromophobic regions equally. That this is so could be made out from both Mann Kopsch (fig. 5) and Da Fano material (fig. 12). The semi-lunar bodies often seen by us along with the vesicles appear to



Figs. 12–15. 12. Adult trophozoite showing division of Golgi bodies. Da Fano ×1,500. 13. (Photomicrograph). Adult trophozoite showing division of Golgi bodies. 14. Two gregarines beginning to associate. Nassonov ×1,125. 15. Association stage showing Golgi vesicles. Nassonov ×1,125. *chb.* chromophobic part and *chr.* chromophilic part of the Golgi vesicles and batonettes. *g.v.* Golgi vesicle.

be division stages, the plane of section having passed between the two batonettes in the process of separation. In the trophozoite the Golgi bodies appear scattered in the cytoplasm. Often they are more concentrated in some portion of the cytoplasm (fig. 5).

When two mature individuals begin to associate this irregular distribution is lost and the vesicles become arranged along the periphery of the cytoplasm (fig. 14). The changes in the arrangement during association could be made out from figs. 14 & 15.

Karyosomes

The nature and behaviour of the karyosomes are based on a study of sections of material fixed in Brasil's modified Bouin-Duboscq's fluid and afterwards stained in Heidenhain's iron haematoxylin (Ganapati and Aiyar '37). Usually two karyosomes could be made out in the intra-cellular stage. They may be equal or unequal, stain dark in iron haematoxylin and lie closely applied against the nuclear membrane. In the free gregarine the karyosomes may be free or closely pressed against the nuclear membrane. One of the karyosomes, however, is usually much bigger than the other.

In the association stages only a central karyosome is visible, the other, in all probability, having broken up.

Centrioles

In the nuclear divisions preceding gamete formation a centriole makes its appearance very close to the nuclear membrane. The centriole stains intensely dark with iron haematoxylin, and thin radiating strands of cytoplasm could be seen proceeding from this to the nuclear membrane, forming a cone of attraction. The single centriole apparently divides into two, which ultimately come to occupy the opposite poles of the nucleus. The nuclear membrane disappears and the spindle fibres run from end to end with the chromosomes lying in the middle. No trace of any aster was seen at any stage of division. The opposite poles of the spindle containing the centrioles stain darker than the rest of the spindle, and at this stage, a centrosphere as distinct from a centriole is not clearly visible. As for the origin of the centriole we are tempted to regard it as being extruded from the nucleus prior to division, but we have failed to observe a centriole as such, in an intra-nuclear position, and even if one is present it is difficult to make it out, distinctly from the granules of chromatin inside the nucleus. The spindle, however, appears to have an extra-nuclear origin.

Vital Staining with Neutral Red

A perusal of Hall's papers ('30a, b, '31 & '36) shows that the vacuome (which Hall considers to be the Golgi apparatus) is clearly visible in some cases in unstained material. In *Lecudina* the Golgi bodies could be just made out only in certain cases. Among the mitochondria in some particularly favourable examples light grey bodies could be made out. Due probably to the very slight difference in the refractive index between these bodies and the surrounding cytoplasm, differentiation into chromophobe and chromophile regions could not be made out. The variations in distribution seen in fixed material were also seen in vital material. In the majority of the specimens, however, they have a scattered distribution, more bodies being seen behind the nucleus, than in front of it.

Though experiments with neutral red were carried out on several occasions on gregarines obtained from different localities in Madras the result has always been rather unsatisfactory. Even in very dilute solutions the worms did not live for more than three hours. Segregated neutral red droplets were observed in epidermal cells, intestinal cells, spermatocytes and spermatids of the host showing that failure to stain distinctly, preformed or artificial bodies in the gregarine were not due to any defect in the sample of neutral red employed. The cytoplasm of the parasite was stained in those worms which were dying, but no inclusions were stained. In some specimens the staining of the cytoplasm gave one the impression that the mitochondria were stained by neutral red. That this is not so was evident from the fact that unstained granules were seen when the gregarine was ruptured. In supra vital staining, dilute solutions tinge the cytoplasm light pink and this pink colouration occurred in varying intensities in different regions. Whether these regions were the places where the Golgi bodies occurred, we were not able to make out since these bodies which remain unstained with neutral red could be made out only in rare cases. Long staining with neutral red never produced any visible change and after an hour the gregarines began to show signs of deterioration and death. So we had to try stronger concentrations of the dye to see whether any other bodies could be stained. Even in this case not all specimens behaved in the same way. If the worm was teased out in a strong solution of neutral red the cytoplasm immediately stained followed by the mitochondria, nucleoli and the nucleus itself. Once the nucleus was stained disintegration followed in a few minutes. So worms were teased out in Ringer and the dye in comparatively strong concentration was allowed to diffuse under the cover slip. The first effect of such stain-

ing was to tinge the cytoplasm light pink. The mitochondria could be seen very clearly at this stage. Soon, certain bodies begin to stain lightly, the colouration becoming more and more intense as time passes on. They are bigger than the mitochondria and were taken to be Golgi bodies. Certain appearances suggested division stages. One characteristic of the newly stained bodies were that they presented a duplex structure. An outer rind appeared brick red in certain levels of the objective, while the same region appeared as if only very slightly tinged in certain other levels. At first we thought that both the chromophobic and chromophilic regions stain with the dye. But this view seems to be erroneous as our observations on the staining of the nucleolus would show. The nucleoli in this gregarine occur closely applied against the nuclear membrane, through a considerable period of its life-history. When just before disintegration, the nucleoli are stained, the nuclear membrane in the region of apposition of these bodies had a dark colour. This appearance is an optical illusion because in other regions of the nucleus, the nuclear wall is not even lightly tinged by neutral red. Thus it appears that it is the chromophobic part that stains with neutral red. Golgi bodies remain stained only for a few minutes and is soon followed by the staining of the nucleus and final disintegration. Odette Tuzet ('31) has demonstrated three distinct categories of cytoplasmic inclusions in *Gonospora duboscqui*. 1. Vacuoles stainable with neutral red. 2. Mitochondria stainable with Janus green. 3. Golgi bodies which are not revealed either by neutral red or Janus green. Temporary staining was observed by Joyet-Lavergne ('26) and in our material the chromophobic part is stained only for a very short period. Thus the observations cited above contradict the suggestion of Hall ('30a) that vital staining with neutral red may form an additional criterion for the identification of Golgi in Protozoa.

Discussion

It was shown in a previous paper that the chromophobic part in the double rimmed Golgi batonettes of the eggs of *Lycastis* is between the two chromophilic rims (Subramaniam and Gopala Aiyar '36c). This brought our views into conflict with the views of some workers who use the term 'archoplasm' for the idiosome as though it had something to do with the sphere substance. The theory that in the vertebrate network the idiosome forms a core and is visible in some cases is substantiated by the observations of one of us (Subramaniam '37) on the liver cells of the tree frog *Rhacophorus*. This showed that the circumstantially identical positions occupied by the

idiosome and the sphere in certain stages of the growth of the germ cells are not substantiated in vertebrate somatic cells. This study of the gregarine was made in order to substantiate our conclusion that the chromophilic and chromophobic portions form an entity by themselves having no relation whatever to the sphere.

Before entering into a discussion of the above point we would like to clear some doubts about the duplex structure of the Golgi bodies in Protozoa. Hall saw in *Chromulina* ('30a) and *Trichamoeba* ('30b) crescents and rings but rejects their existence because Parat ('28) has shown that incomplete fixation followed by silvering or osmication resulted in the appearance of such crescents. That such crescents are seen in actual living material is shown by Joyet-Lavergne's experiments on vital staining where he saw small arcs strongly coloured by neutral red, bordering an ovoid mass lightly stained. Thus Joyet-Lavergne whose experiments with neutral red in Protozoa seem to have formed the basis even for Hall's work had seen the duplex structure which Hall and collaborators are not prepared to admit. Some Protozoologists following Hall seem to consider that all crescents are artifacts. Hirschler even in 1914 has shown vesicles and crescents and in eggs it has been shown by one of us (Subramaniam '35; Subramaniam and Gopala Aiyar '36a, b) that a Golgi granule when it enlarges becomes differentiated into a vesicle having chromophilic and chromophobic regions. Rupture of the vesicle gives rise to batonettes in which the chromophobic part is in relation with the cytoplasm. Evidence that the vesicles give rise to batonettes is seen even in *Lecudina*. The Golgi apparatus of the parasite is vesicular and during division the single vesicle gets divided into two batonettes connected by the elongated chromophobic strand. The vesicle is once again formed by the fusion of the open ends of the batonette and the chromophobic part which was in relation with the cytoplasm once again becomes separated from it by the chromophilic portion.

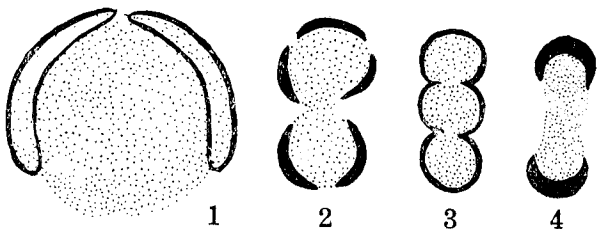


Fig. 16. A diagrammatic representation of the various modes of division of Golgi bodies. 1. *Euschistus* (Bowen '20 reconstituted from description). 2. *Limnaea* (Gatenby '19). 3. *Clibanarius* (Subramaniam '35). 4. *Lecudina*.

In fig. 16 are given the various modes of division of Golgi bodies observed by Bowen ('20), Gatenby ('19) and Subramaniam ('35) and it will be seen that in all cases division of Golgi bodies is

division of both the chromophilic part and the idiosome. This fact goes against any conception of the chromophobic part having any relation to the sphere or archoplasm. This idea that the chromophobic part has something to do with the sphere and centriole is due to Gatenby. In 1919 he defines Archoplasm or Archoplasmic zone as the "dense mass of protoplasmic material gathered around the centrosome in the form of a sphere" and the chondrioplasts thus. "The archoplasm in the germ cells of some animals is often studded over its periphery with a number of banana shaped rodlets or batonettes" (p. 205–206). These definitions were given before the chondrioplasts and acroblasts were identified as Golgi and the duplex structure instead of being treated as a characteristic of these bodies was supposed to be an association between two different cell structures. When the chondrioplasts and acroblasts were recognized as Golgi the definition was carried unaltered. Thus Gatenby ('19 b) speaks of "the Golgi rod and its archoplasm" (p. 451) and also describes the morphology of the apparatus as follows: "It nearly always consists of a sphere or archoplasm, such as is found around a centriole (centrosome) upon which lie several more or less deeply curved batonettes or little rods" (p. 475). In 1920 Gatenby defines archoplasm as a "concentrated region of the cell cytoplasm generally associated with the centrosome and at certain stages with the Golgi elements. The archoplasm seems to have some relation to the amphiaster, but this has not been conclusively established" (p. 132). Gatenby says "With regard to the use of the word Golgi apparatus it is necessary to point out that in young germ cells and in all embryonic cells the apparatus occupies a position surrounding the archoplasm and centrosome, from which the elements of the apparatus are rarely separated" (p. 134). This treatment of the duplex structure as caused by the coming together of two different cytoplasmic structures goes a step further when King ('26) in *Haplosporidium chitonis* describes the Golgi apparatus separating from the sphere. She observed in the sporoblast stage in Champy preparations a chromatic area on one side of the nucleus which sometimes stained violently in iron haematoxylin, but more often appeared as a greyish mass. This according to her is the "spherule" described by Pixell-Goodrich and Debaisieux in the sporoblast and is "the portion of the cytoplasm surrounded by Golgi bodies. This is shown by the similarity in the behaviour of the 'spherule' and Golgi bodies; both lie near the nucleus at first, then, moving away, take up a position near the spore cap. Also in properly impregnated Mann Kopsch preparations, the spore never shows the sphere until the Golgi bodies have broken away as in fig. 20 Pl. 15, while it is shown well in Champy-

Kull and other preparations, where it is not obscured by these bodies" (p. 154). King and Gatenby ('23) mention in *Adelea* "In Pl. 21 fig. 2 is drawn at a very high magnification, a number of these structures, we now identify as the Golgi apparatus; the latter consists as in sponge, coelenterate and many other cells of dictyosomes (or Golgi crescents and bent rods) lying upon the surface of a thickened protoplasmic zone or centrosphere. Whether or not a centrosome exists in *Adelea*, there is certainly a darker (denser) zone associated with the sickle shaped dictyosomes" (p. 383). The centrosome in Protozoa is a much discussed question. It is often assumed that an intra-nuclear centrosome, the centriole, is present in the nuclei of many Protozoa. It is supposed to be embedded in the karyosome and only to become recognizable as centrosomic in nature during nuclear division, when it divides into two parts which separate from one another, though remaining connected for some time by a fibril, a centrodesmose. King and Gatenby ('23) remark that previous workers are mainly against the view that an extra-nuclear centrosome occurs in *Adelea*. Still as the crescents present a duplex structure we believe that the "thickened protoplasmic zone or centrosphere" cannot be a centrosphere, because no case—within our knowledge—is recorded of a 'centrosome' inside the nucleus and the centrosphere in the cytoplasm.

In the somatic cells of vertebrates according to this conception of the relation between the sphere or archoplasm and the Golgi material, a chromophobic component should be present in the space enclosed by the network. Bowen ('26) mentions that such an idiosomic constituent was absolutely lacking in the form here suggested. Bowen's failure to demonstrate a chromophobic component in relation with the Golgi network has also been the experience of most other workers. This relation of the "archoplasm" and the Golgi, if considered temporary, does not explain how a "sphere" can be differentiated inside a vesicle formed by enlargement of a Golgi granule. Nor, can it explain the occurrence of a central chromophobic area in a vesicle which does not arise by fusion of the open ends of a batonette. *Lecudina* itself presents an early Golgi apparatus which has no visible duplex structure and it is from this that the vesicles and batonettes arise. Further, it is not at all clear why during division the sphere substance should also divide regularly if the relation is a casual one, and if one can exist without the other. Bowen ('26) has discussed this question at length and pointed out that the idiosome or the chromophobic part is not even remotely related to a sphere. He showed in insects that the Golgi bodies are not at all concentrated around the central apparatus but are scat-

tered throughout the cell in the form of Golgi bodies, each accompanied by a small mass of idiosomic material. The experience of one of us in eggs where in many cases a Golgi mass occurs without any differentiation, which by rupture gives rise to granules, which when they enlarge into vesicles differentiate a chromophobic area, present evidence that the Golgi apparatus like the mitochondria and chromatin has a duplex structure which justifies the supposition that it is composed of two substances one lipoid and the other protein in character. Such a conception alone can explain the occurrence of hollow tubuar networks observed in vertebrate somatic cells. The observations on *Lycastis* where a double rimmed batonette gives rise to a dictyosome without any chromophobic part shows conclusively that the idiosome and the archoplasm have no relation at all. The search for the duplex structure of the Golgi apparatus in network like Golgi apparatus of vertebrate somatic cells has been circumscribed by this supposed relation between the archoplasm and the Golgi which led investigators to search for it in the area enclosed by the Golgi network and not inside the strands of the network. We entirely agree with Bowen ('26) that the relation between the Golgi and the central apparatus is only topographical, effected perhaps by the same factors as those which organize cellular polarity in general and is not of the nature of an anatomical relation. Viewed in the light of the above interpretation we cannot at all agree with Gatenby's suggestion that the Golgi bodies probably arose in connection with the terminal bead of the flagellum of some primitive flagellate. The very fact that a Golgi apparatus with duplex structure is present in the cytoplasm while the centriole is supposed to be intra-nuclear in *Adelea,* in relation with the entirely different natures of the idiosome and the archoplasm as also the mere topographical relation between the Golgi and the central apparatus render it difficult for us to accept Gatenby's suggestion ('23) that the Golgi apparatus and the centrosome were evolved side by side "or the apparatus from the centrosphere in some way." We believe that investigations in Protozoa have sufficiently shown that the Golgi apparatus did not originate in Protozoa as it was already formed when Protozoa themselves were evolved and hence further work in the line of metazoa applying criteria which define Golgi apparatus in invertebrates in general will clear much of the confusion in this field.

Summary

1. In the intra-cellular stage the mitochondria occur as a few granules. When the parasite becomes freed into the alimentary

canal lumen the mitochondria actively divide in the cytoplasm and occur scattered or in concentrations in different regions of the cytoplasm.

2. The Golgi apparatus occurs as a semi-lunar body appearing pasted on to the nuclear membrane in the intra-cellular stage. When this divides into two the duplex structure becomes evident. When the parasite becomes freed into the alimentary canal lumen the Golgi bodies which are vesicular show the duplex structure. During division the Golgi body becomes dumb-bell shaped. The two knobs when they separate are semi-lunar and between the two runs the chromophobic part as a strand. One of the bodies thus formed becomes vesicular by a fusion of the chromophilic rim and the chromophobic part connecting the vesicle with the batonette seems to contract into the cup-like area before the batonette also becomes vesicular. Thus division of the Golgi body is a division of its chromophilic and chromophobic regions equally.
3. The duplex structure of the Golgi bodies is discussed and it is shown that the chromophobic part has nothing to do with the sphere. The chromophobic and chromophilic portions together form an entity and the idiosome and archoplasm are entirely different structures. The relation between the Golgi and central apparatus is merely topographical and hence it is difficult to accept Gatenby's suggestion that either the Golgi and the central apparatus were evolved side by side or the Golgi from the central apparatus in some way.

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