II.—THE GOLGI APPARATUS OF FREE-LIVING PROTOZOA.

By H. S. Madhava Rao.

The occurrence of Golgi apparatus has been noted in several classes of the animal kingdom by Ludford and Gatenby. Their presence even in nucleated red blood cells such as the erythrocytes of the *Sauropsida* has been recorded by Bhattacharya and Brambell (*Q.J.M.S.*, 1925, **69**, 167). Among the protozoa, the first record of the presence of Golgi apparatus is that of Hirschler (*Anat. Anzeiger*, 1914, **47**, 289) who succeeded in demonstrating these bodies by the methods used for the metazoan Golgi apparatus; but their presence in protozoa received further confirmation only so late as 1923 by King and Gatenby (*Q.J.M.S.*, 1923, **67**, 381) who described them in *Aldelea orata*, and also by Joyet-Lavergne (*Compt. rend.*, 1923, **177**, 957) who observed them in *Adelina dimidiata* and later in some coccidia (*Ibid.*, 1924, **178**, 136; **79**, 1212) and gregarines (*Compt. rend. Soc. Biol.*, 1924, **90**, 681 and 1220). King (*Q.J.M.S.*, 1926, **70**, 147) described them in *Haplosporidium*.

The Golgi apparatus studied by Joyet-Lavergne (*Compt. rend.*, 1924, **178**, 136 and 2200) indicates a connection with the parabasal type of apparatus described by Duboscq and Grasse in flagellates (*Compt. rend. Soc. Biol.*, 1925, **92**, 154; **93**, 345; *Compt. rend.*, 1925, **180**, 477). King and Gatenby (*Q.J.M.S.*, 1926, **70**, 217) have suggested a homology between certain lipoid inclusions in *Opalina ranarum* and the Golgi apparatus. A similar observation has been made by Sokolska (*Compt. rend. Soc. Biol.*, 1927, **96**, 570) although their homology with the Golgi bodies is not yet conclusively proved. In all cases thoroughly studied, the parabasal body is found to divide at cell division. Kudd (*Archiv. Protistenk.*, 1926, **53**, 191) stated that the body arises *de novo* in *Lophomonas blattarum*, but he does not believe it to be homologous to parabasals of other flagellates. Finally the association of these bodies with the blepharoblast and centro-blepharoblast recalls the arrangement of the Golgi apparatus in relation to the centrosome in metazoa. The parabasal is found to be concerned with secretion. In *Trichomonas batrachorum* and *Tetramastix bufonis* they break off from the chromophobe and fall into the cytoplasm where they seem to be dissolved according to Grasse (*Compt. rend. Soc. Biol.*, 1926, **94**, 1012). A similar observation was made in *Trichonympha chattoni* by Duboscq and Grasse (*Compt. rend. Soc. Biol.*, 1927, **96**, 94). Causey (*Univ. Calif. Pub. Zool.*, 1925, **28**, 225) describes in *Entamoeba* what he considers to be Golgi elements, arising from food vacuoles. Therefore according to him it is to be inferred that the Golgi bodies arise *de novo* in the cytoplasm in *Entamoeba gingivalis*.

Nassonov (*Arch. mik. Anat. Entwick-Mech.*, 1924, **103**, 437) compares the ring-shaped secretory apparatus in *Chilodon* and *Dogielella* with the ring-shaped Golgi bodies found in many metazoan cells. This relationship, however, requires further confirmation since it is believed that the Golgi bodies do not appear *de novo* after cell division.

The hypotheses of Nassonov and Grasse are both very interesting and seem to be supported by weighty evidence. Until further work substantiates their results, however, they cannot be regarded as proved. Observations made on the nature of the Golgi bodies in a number of free-living protozoa form the subject of the present paper.

MATERIAL.

In order to obtain as many species of free-living protozoa as possible, the organisms were isolated from (1) Activated Sludge and (2) Soil. A large quantity of sludge was filtered through a fine sieve and then through muslin, the filtrate centrifuged for five minutes at 2,000 revolutions per minute, and the upper clear portion of the liquid decanted. The small quantity of remaining liquid contains numerous organisms.

The protozoa of the soil were isolated by inoculating the soil into the culture medium and allowing sufficient time for growth. The following medium was found to be the best:—Hay infusion, 2 c.c., agar, 1·5 gms. and distilled water, 100 c.c. The isolated organisms have been found to thrive well in malted milk also, 1 gm. being dissolved in 1,000 c.c. of water. As in the previous case the culture was centrifuged and the excess of the liquid decanted.

METHODS.

The Golgi apparatus was observed in the living cell and the observations were confirmed by the several methods used in cytological technique, of which the following were tried.

Mann Kopsch.—A small quantity of the culture solution is transferred to a glass slide, and 3 or 4 drops of Mann's corrosive osmic solution added, the slide being well washed in running water after half an hour. Then 3 or 4 drops of 2 per cent. osmic acid are added and the slide kept in a desiccator containing water; if necessary ice may be added to this. Thus a small quantity of the acid will be sufficient for a number of slides and there will be an economy in the amount of acid used; the method also avoids the use of osmic acid which has been used already.

The slides were exposed to the osmic acid treatment from eight to twelve days, being examined every day. They were then washed for about half an hour in running water, and treated with toluene to remove the blackness from the mitochondria if they were also impregnated. The modifications suggested by Ludford, namely, completing the reduction of osmic acid at higher temperatures were also tried in addition to the Mann Kopsch Altmann combination.

Sjovall.—The organisms on the slides were fixed for half an hour in formalin (20 per cent.), washed for about 15 minutes, 2 per cent. osmic acid added and the slides allowed to remain in the desiccator containing water for about eight to ten days. They were then washed for about half an hour in running water and mounted.

Golgi.—Equal parts of 20 per cent. formalin, a saturated solution of arsenious acid and 96 per cent. alcohol were mixed and a few drops added to the slides containing the culture. Fixation was carried on for about an hour, the slides were then washed quickly and passed into 1 per cent. silver nitrate solution overnight, after which they were treated with the following mixture:—Hydroquinone, 20 gms., sodium sulphate, 5 gms., formalin, 50 c.c., water, 1,000 c.c. They were then washed and bleached in the following solution for about one minute:—Potassium permanganate, 0·5 gm., sulphuric acid, 1 c.c., water, 1,000 c.c. The slides were removed from the permanganate solution just when the colour became slightly yellowish-brown and then washed; this must be done quickly and the permanganate not be allowed to remain on the slide too long. A solution of sulphurous acid (2 to 5 per cent.) could also be used. The method was found quite satisfactory.

Cajal.—This method was modified to work well in the case of the protozoa. Uranium nitrate was omitted and thorium nitrate tried instead. After fixation the slides were immersed in 1·5 per cent. silver nitrate overnight, but the results were not so satisfactory as with the Mann Kopsch method and its modifications.

Da Fano.—Formalin (15 c. c.) and 1 gm. of cobalt nitrate were added to 100 c.c. of water and a few drops of this mixture was added to the slide; after half an hour, the slide was washed quickly and treated with 1·5 per cent. silver nitrate. Hydroquinone mixture was used for reduction. This method was more satisfactory than the previous one.

Hirschler.—The organisms were fixed in modified Champy's medium and impregnated in 2 per cent. osmic acid for about eight or ten days.

Kolatschev.—Fixing was effected in modified Champy as before and impregnation was carried on in 1 per cent. osmic acid at 30° for 40–50 hours.

Lewis.—The smears are treated with Janus green (1/50,000) and exposed to iodine vapour for a second. The Golgi apparatus is stained black instantly. The method is excellent for fresh material and also for rapid work.

DISCUSSION.

The Golgi bodies are believed by most cytologists to be definite cytoplasmic structures, while quite the opposite view, that they are artefacts produced as a result of the fixation and staining of the cell is favoured by some (*cf.* Walker and Allen, *Proc. Roy. Soc.*, 1927, B, **101**, 468).

So far as my own observations on the Golgi bodies of the free-living protozoa are concerned I am inclined to the view that these bodies are true cytoplasmic inclusions, for a positive picture has been obtained by the various osmic and silver nitrate methods generally used in cytological technique for impregnating the Golgi bodies. They may occur as small granules or rodlets, the latter sometimes forming aggregates (*cf.* Fig. 19). These observations with regard to protozoa seem to agree with those of Ludford on the metazoan Golgi apparatus. A secretory function has been attributed to these bodies when present in the metazoan cells, especially the higher vertebrates, by Nassanov, Gatenby, Ludford, Bowen and others, but whether it is so or not in the case of protozoa, I have not yet been able to determine. In a previous paper I have shown that the mitochondria present in some soil protozoa seem to be concerned with the digestive function, and that they do not arise *de novo*, and a similar phenomenon has been observed by Horning in the infusorian *Nyctotherus*. In view of the fact that the mitochondria of the protozoa behave in the same way as they do in the other classes of animals (Horning, *Aust. Journ. Expl. Biol. and Med. Sci.*, 1927, **4**, 75) the Golgi bodies which are allied to mitochondria to a certain extent may also function in the same way among the protozoa as they do in the other animal cells. In the lower forms they occur as granules while in the higher ciliated protozoa they are found with a more organised structure in the cytoplasm. The rodlets and aggregates of these appear to be transitory stages leading finally to the more organised spherical forms.

SUMMARY.

1. A number of species of free-living protozoa have been isolated and the Golgi bodies in each rendered visible by various methods employed in cytological technique.

2. The Golgi bodies are found as granules, aggregates and spheres with a more organised structure.

3. They always occur as definite bodies in the higher ciliated protozoa.

4. They are considered from the evidence adduced in this paper to be definite cytoplasmic structures.

5. The rodlets and aggregates appear to be transitory stages between the simple granules and the spherical bodies.

In conclusion, my thanks are due to Dr. A. Subba Rao for his suggestive criticisms.

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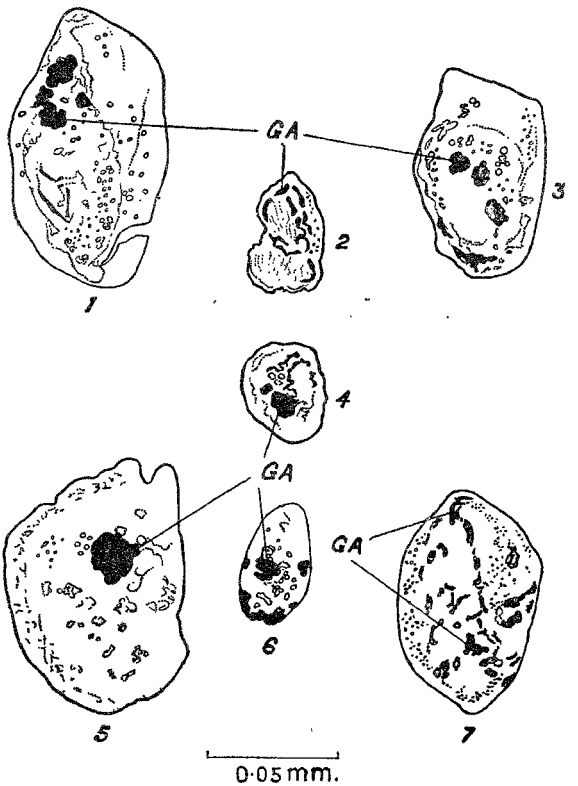
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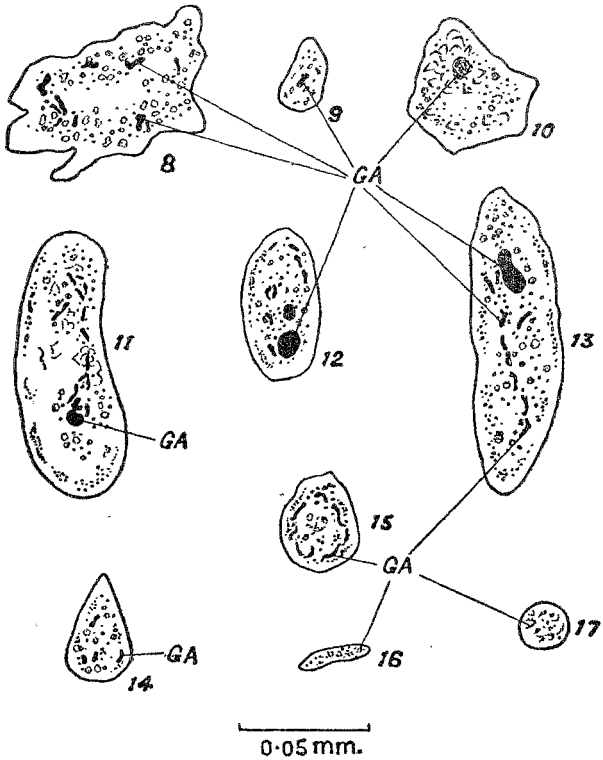
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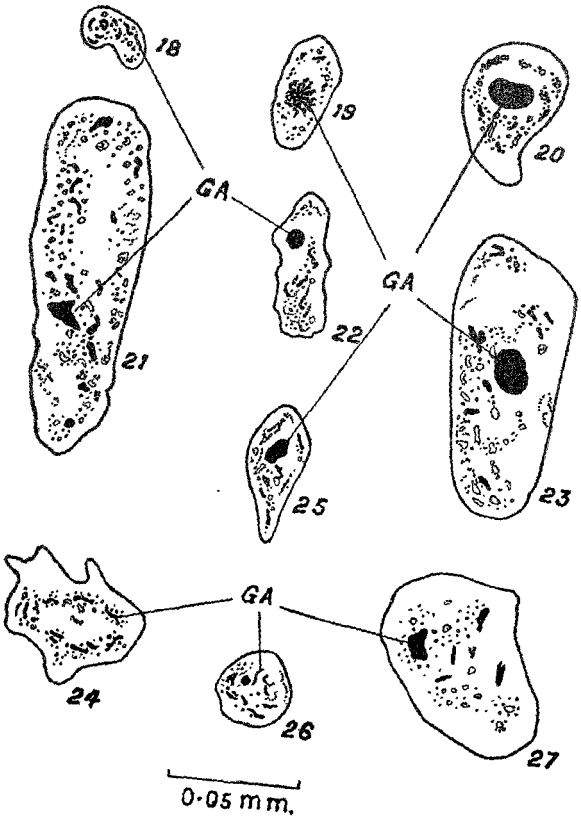
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