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CONTENTS.

I.—A STUDY OF THE SYMBIOTIC FUNGUS FROM THE MYSORE LAC INSECT.

By M. Sreenivasaya and S. Mahdihassan.

Lac insects are normally associated with intracellular organisms, ellipsoidal and yeast-like in appearance. The organism is a fungus and its presence is a sign of vigorous health in the insect. Similar symbiotic associates have been recorded from time to time but the physiological function of the symbionts has not been the subject of much study. Keeble has suggested that they utilise the excretory nitrogen of the host while Portier ascribes to them a more important role, since he found that they produce reactive ketones and aldehydes on fermentation.

The organism was first isolated from the Mysore lac-insect on prune-agar medium, the reaction of which was distinctly acid to litmus. It was then grown in three series of artificial media; the first series consisting of carbohydrates, the second of nitrogenous compounds, while in the third a study was made of the necessary inorganic constituents.

In the carbohydrate series, various sugars were employed in equivalent proportions, as also starch, inulin and gum arabic. Various nitrogen compounds, amino-acids, amides, purines, nitrates etc. were used in the second series as the source of nitrogen, in such quantities that the nitrogen-content of the medium was kept constant. With regard to the inorganic constituents, the table explains itself.

After eight weeks' growth the solutions were analysed in the following manner. The solution was made up to the original volume and then filtered through a bed of previously washed and ignited kieselguhr. The residue was washed repeatedly with hot water to free it from the gummy medium and the precipitate together with the kieselguhr bed was dried in the hot air oven at 90° to constant weight, ignited and again weighed. The difference was taken as the weight of the fungus crop.

The filtrate, of which the first portions were rejected, was used for determining rotation and viscosity, the latter being measured with an Ostwald's viscosimeter at 25°, making the usual precautions. The viscosity has been taken as a measure of the gum-content of the medium.

TABLE I.

Carbohydrate Series.

No.	Source of carbon	Yeast crop in grams	Rotation in 10 cm. tube	Viscosity at 25°
1	No Sugar ...	...	...	1·30
2	Glucose ...	74·6	...	1·38
3	Fructose ...	56·4	...	1·43
4	Sucrose ...	75·4	...	1·47
5	Maltose ...	118·0	...	1·46
6	Lactose ...	40·0	+0·25	1·33
7	Arabinose ...	24·8	...	1·38
8	Xylose	51·6	...	1·38
9	Starch	25·0	?	2·37
10	Inulin	7·4	−0·81	1·96
11	Gum arabic ...	127·6	−0·25 (Initial, +1·05)	1·86

From the above it follows that among the sugars, maltose yields the highest crop: gum arabic, however, serves as well if not better. Maltose and sucrose are hardly distinguishable in yielding the highest gum-content as shown by the greatest viscosity. Lactose is not quantitatively utilised by the organism, the galactose portion of the molecule perhaps being left untouched; unfortunately the galactose medium became contaminated and this point has not yet been made clear. Inulin is not utilised by the organism, while only a part of the gum arabic appears to be attacked, the laevo-component being left untouched or appearing as a result of excretion; further work alone can decide this interesting point.

TABLE II.

Nitrogen Series.

No.	Source of nitrogen	Yeast crop in grams	Viscosity at 25°	Rotation in 10 cm tube
1	No nitrogen (not inoculated) ...	...	1·32	+0·95°
2	No nitrogen ...	25·5	1·36	+0·50
3	Glycine	130·0	2·51	+0·19
4	Alanine	63·4	2·10	+0·34
5	Leucine	56·3	2·13	+0·43
6	Tyrosine ...	112·3	2·15	+0·21
7	Asparagine ...	204·0	2·18	...
8	Acetamide ...	313·4	3·25	...
9	Urea	169·2	3·10	...
10	Uric acid ...	323·6	3·33	...
11	Hippuric acid ...	73·5	2·20	+0·29
12	Nitrate	153·0	2·51	+0·10
13	Ammonium sulphate	267·0	3·10	...

Uric acid and acetamide yield the heaviest crops of yeast, ammonium sulphate coming next. When such a diversity of compounds serve as sources of nitrogen it is difficult to draw any definite conclusion. Sugar is quantitatively utilised in the presence of asparagine, acetamide, urea, uric acid and ammonium sulphate. The greatest quantity of gum is produced with uric acid and the nitrogen series generally yield a higher gum value. The utilisation of uric acid is interesting in view of Keeble's suggestion that a function of the organism is to remove the excretory nitrogen of the host.

TABLE III.

Inorganic Constituents Series.

No.	Medium	Yeast crop in grams	Rotation in 10 cm. tube	Viscosity at 25°
1	Normal	156·8	...	1·41
2	Nitrogen free ...	25·5	+0·50	1·32
3	Phosphorus free ...	5·8	+0·93	1·31
4	Calcium free ...	168·6	...	1·35
5	Magnesium free ...	266·8	...	1·42
6	Potassium free ...	149·0	+0·12	1·37
7	Sodium free ...	166·8	...	1·39
8	Calcium excess ...	136·0	+0·16	1·43
9	Magnesium excess ...	110·6	...	1·33
10	Normal (not inoculated) ...	...	+0·95	1·30

The magnesium-free medium yields the highest crop of the fungus and, with the medium containing excess of calcium, has the highest gum-content. The absence of nitrogen, phosphorus or potassium affects the carbohydrate metabolism and the sugar is not quantitatively utilised.

A large scale fermentation was carried out to study the products, the medium being composed of:—Potassium nitrate, 20 gms., hydrogen potassium phosphate, 10 gms., sodium chloride, 5 gms., magnesium sulphate, 5 gms., glucose (commercial), 100 gms., water, 2 litres.

The time allowed for fermentation was two months. The fermented mash was filtered through a kieselguhr bed and the clear filtrate distilled on a sand-bath. The first 200 c.c. were collected and found to have the following properties:—It reduces ammoniacal silver nitrate but not Fehling's solution; it answers to the iodoform reaction and with phenylhydrazine gives a yellow precipitate. All these tests point to the presence of an aldehyde or ketone, but further work is necessary to identify the constituents.

The kieselguhr fungus crop was ground up with quartz sand and extracted with ether. A light yellow oil has been obtained, which has the property of drying, or absorbing oxygen from the air. A micro-analysis of the product will throw some light on the nature of the oil. Lastly it may be stated that the comparative bio-combustion experiments carried out with the Mysore symbionts as well as *S. Cerevisiæ* showed that the carbon dioxide produced by the former was only one-third that given out by the latter.

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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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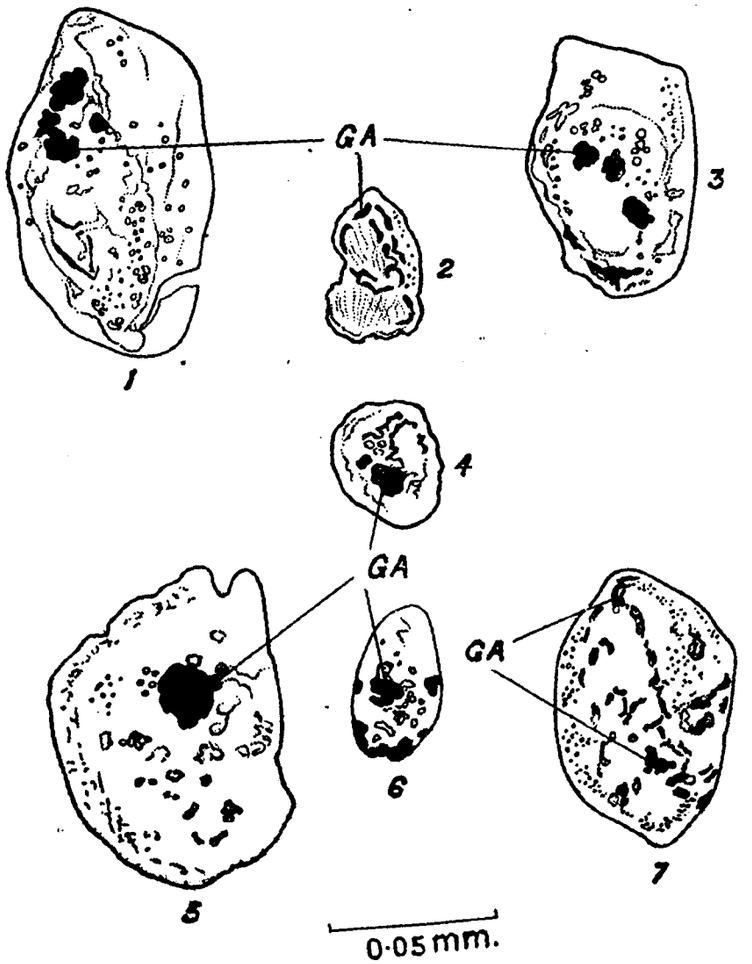

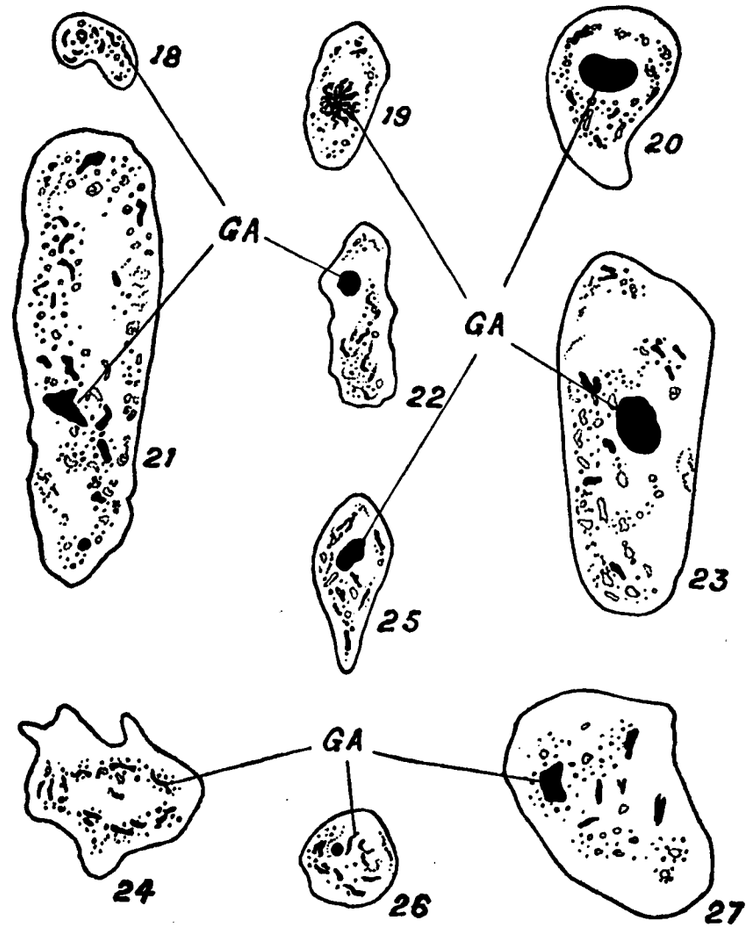

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