(Biological Laboratory, University College, New York University.)

Osmiphilic inclusions similar to Golgi apparatus in the flagellates, *Chromulina*, *Chilomonas* and *Astasia*.

By

Richard P. Hall.

(With plate 2—3.)

Introduction and technic.

It has been shown that, in certain species of Sporozoa (JOYET-LAVERGNE, 1926; COWDRY and SCOTT, 1928) and in several types of metazoan cells (COVELL and SCOTT, 1928; DAWSON, 1928; PARAT, 1928), cytoplasmic inclusions resembling the Golgi apparatus may be stained vitally with neutral red. In the writer's attempts to apply these methods to several free-living flagellates, it has been found that certain neutral-red-stainable inclusions in *Peranema trichophorum* (HALL, 1928, 1929), *Chromulina* sp. (Chrysomonadida) and *Astasia* sp. (Euglenida) are demonstrable also with some of the common methods of osmic impregnation for demonstration of the GOLGI apparatus.

In vital staining, clean glass slides are filmed with a solution of the dye in absolute alcohol, even spreading being obtained by tilting the slide. After the film has dried a drop of the culture is added, and a coverslip placed over the drop. The edges of the coverslip are sealed with melted vaseline. In such preparations the flagellates usually live several days, provided the concentration of the dye is sub-lethal.

The following dyes were prepared in 1 % solutions in absolute alcohol: alizarine blue S, brilliant cresyl blue, Congo red, indulin, Meldola blue, methylene blue, neutral red, neutral violet, Nile blue BB, Nile blue sulfate, pyronin and rhodamine. The 1 % solutions of brilliant cresyl blue and neutral red were diluted 1:15 with absolute alcohol for usable stock solutions, while Janus green B was prepared as a saturated solution in absolute alcohol. These stock solutions of the various dyes were diluted to different degrees in attempts to find effective non-toxic concentrations.

Permanent preparations were made by the MANN-KOPSCH (WEIGL) method of osmic impregnation (for details, see BOWEN, 1928 b), and by fixation in MANN-KOPSCH fluid followed by staining in iron-hematoxylin. In both cases the centrifuge method was used in concentrating and handling material.

Inclusions stainable with vital dyes.

In *Chromulina* sp., stained with neutral red, a number of small globules take the dye. These globules were sometimes more abundant in the anterior half of the organism, and sometimes more or less uniformly distributed. Inclusions similar in size and distribution were also stained in the same manner with brilliant cresyl blue and neutral violet. On account of their similarity, it was assumed that the globules stained with the latter two dyes are probably the same as the inclusions stained with neutral red. Because of its higher visibility, neutral red was used more extensively for demonstration of these inclusions.

In vital staining with a mixture of Janus green B and neutral red, two types of inclusions were observed in *Chromulina*. Granules stained in Janus green were consistently smaller than those stained with neutral red and, furthermore, they showed Brownian movement while the neutral-red-stainable inclusions were motionless.

In *Astasia* sp. similar small spherical inclusions were stained with neutral red, brilliant cresyl blue and neutral violet. These globules were relatively few in number and most of them were usually seen in the anterior half or two-thirds of the organism. Smaller and more numerous granules, stainable in Janus green and presumably mitochondria, were also observed scattered through the cytoplasm. In this flagellate, however, Brownian movement of the mitochondria was not observed.

Osmication of inclusions stained with neutral red.

In order to determine the direct effect of osmic vapor upon the neutral-red-stainable inclusions, coverslips were filmed with the dye, and a drop of the *Chromulina*-culture placed on the film. This was then examined, organisms up, under the microscope to determine penetration of the neutral red. The smear was then inverted over a welled slide containing a few drops of 2 % osmic solution. Almost immediately the cytoplasmic background became light pink in color, due, evidently, to diffuse penetration of the neutral red after death of the organisms.

The neutral-red globules, however, remained bright red in color for 5 minutes, or longer in many cases. After 10—20 minutes these inclusions appeared 'muddy' red. Two hours later the cytoplasm still retained some of the neutral red, while the neutral-red globules were becoming light brown in color. Twenty hours later the globules were definitely gray in appearance. After 48 hours the cytoplasm had developed a pale brownish tint, while the globules were distinctly blackened.

The same procedure was repeated with *Astasia* sp. The behavior of the neutral-red globules, both in regard to appearance and time of reaction, was found to be quite similar to that described for the same inclusions of *Chromulina*.

Results of osmic impregnation.

In *Chromulina* (Pl. 2 Fig. 10), after osmic impregnation and bleaching in hydrogen peroxide, small blackened globules are to be seen. These globules resemble in distribution, shape and relative size the inclusions stained vitally with neutral red and also blackened with osmic vapor after staining with neutral red. It seems quite probable, therefore, that the same inclusions are involved in all three cases. The number of these globules is somewhat variable from flagellate to flagellate. In many cases, the globules are found mostly in the anterior half of the organism; in other specimens, the globules are irregularly distributed throughout the cytoplasm. Occasionally, vacuoles may also remain blackened, just as in *Pernanema trichophorum* (HALL, 1928, 1929). As a rule, the globules are uniformly blackened. In some instances (Pl. 2 Fig 10), however, slightly different inclusions were observed. Instead of uniformly blackened spheres, blackened crescents or almost complete rims of small 'vacuoles' were seen bordering less densely impregnated sub-

stance. The inclusions resemble those described in various other flagellates, Sporozoa and amebas (Table 1, dct). In this connection, PARAT'S (1928) observations on the relation of degree of fixation to the appearance of such inclusions are of interest. This author found that incomplete fixation followed by osmic impregnation resulted in the appearance of such 'crescents', while, with longer periods of fixation, such inclusions appeared as uniformly impregnated globules. It is possible that these observations may explain the occurence of such 'crescents' in *Chromulina*.

In *Astasia* sp. Pl. 1 Figs. 1—3) similar small osmiphilic globules are demonstrated by osmic impregnation. In this case, just as in *Chromulina*, the osmiphilic globules resemble closely the inclusions stained vitally with neutral red and subsequently blackened by exposure to osmic vapor. After 5-day impregnation only the globules are blackened. After 10—14-day impregnation many of the vacuoles, or 'alveoli', also show peripheral blackening. After prolonged treatment with hydrogen peroxide most of the vacuoles (Fig. 2), and sometimes all of them (Fig. 3), are bleached, leaving the small globules still impregnated. If the time of bleaching is relatively short, most of the vacuoles remain blackened (Fig. 1). Similar reaction of the vacuoles to osmic impregnation has been reported for *Peranema trichophorum* (HALL, 1928, 1929). In both *Astasia* and *Peranema*, therefore, the osmiphilic globules are consistently blackened, whereas the vacuoles blacken at a later stage of impregnation and may be bleached more or less completely by treatment with hydrogen peroxide.

In addition to the densely blackened osmiphilic globules in *Astasia*, smaller spherical inclusions are sometimes seen as grayish bodies scattered through the cytoplasm (Pl. 2 Fig. 8). These are possibly to be regarded as mitochondria, incompletely bleached or else only partially impregnated, since they resemble the inclusions, demonstrated by other methods, which are described below as mitochondria. Similar behavior of the mitochondria was noted in a few instances in *Peranema trichophorum* (HALL, 1928, 1929).

In *Chilomonas paramecium* (Pl. 1 Figs. 4—6; Pl. 2 Fig. 11), osmic impregnation followed by the usual bleaching in hydrogen-peroxide demonstrates similar osmiphilic inclusions. In rare instances (Figs. 4, 5) only a few — sometimes only two or three — of the globules remain impregnated after prolonged bleaching (45 minutes in Figs. 4 and 5). The contractile vacuole (Figs. 4, 5, 11) often remains blackened, as described by NASSONOW (1924). Occasionally, two (Fig. 5) or three

blackened vacuoles, instead of a single large one, are to be seen near the gullet. In many other specimens (Fig. 6), the contractile vacuole is not blackened at all.

The organisms shown in Figs. 4 and 5 are unusual in that only a few globules have remained blackened; in the majority of specimens in which the contractile vacuole is impregnated, the number of globules is more like that represented in Fig. 6. In a random sample of 100 flagellates, the results of osmic impregnation were as follows: 39 in which both globules and contractile vacuole (or vacuoles in the region of the gullet) were blackened; 15 in which the globules, contractile vacuole and other cytoplasmic vacuoles were blackened; 9 in which the globules and cytoplasmic vacuoles, but not the contractile vacuole, remained blackened; 25 in which only the globules remained blackened; 3 in which the globules and cytoplasmic vacuoles were bleached completely, while the contractile vacuole was only partly bleached; and 9 in which the globules and all vacuoles were completely bleached. In so far as such a small random sample may be significant, it appears that the globules are more consistently impregnated than is the contractile vacuole. The contractile vacuoles which were blackened included various stages of systole and diastole, and also groups of several smaller vacuoles which were possibly stages in the formation of the new contractile vacuole after systole.

Hematoxylin staining after osmic impregnation.

After Mann-Kopsch fixation followed by iron hematoxylin, small granular inclusions are to be seen in *Astasia* (Pl. 2 Fig. 7), *Chromulina* (Fig. 12) and *Chilomonas* (Fig. 9). These are probably to be considered mitochondria, since they resemble in size, distribution and relative number the inclusions stained vitally with Janus green, rather than the neutral-red globules. In *Peranema trichophorum* (Hall, 1928, 1929) this method of fixation and staining also demonstrated the mitochondria to the exclusion of the neutral-red-stainable globules. In *Peranema* the mitochondria appeared as short rods, peripherally located and arranged in spiral rows, so that there could be no question as to their identity and location. In the three flagellates described in the present paper, however, the mitochondria resemble the osmiphilic globules in shape and distribution, and the only obvious differences in appearance in permanent preparations are the relative ones of size and number. The mitochondria in each species seem to be more numerous, and they are nearly always appreciably smaller than the osmiphilic globules.

Discussion.

There is no general agreement as to what constitutes the GOLGI apparatus in the various groups of Protozoa and, in attempting to identify such inclusions in any particular group, one is confronted with the difficulty that there is as yet no established basis of comparison which might be applied to Protozoa in general. In the Phytomastigophora, for example, GRASSÉ (1926 a, b) concluded that the stigma of *Euglena* is the homologue of the GOLGI apparatus, while NASSONOV (1924) suggested the same interpretation for the contractile vacuole of *Chilomonas paramecium* (and also several ciliates). According to KING (1927), however, "until more work has been done substantiating their results, we cannot regard either of them as proved". GRASSÉ's contention that the stigma of *Euglena* represents the GOLGI apparatus offers no advantage for identification of GOLGI material in the case of flagellates without such an organelle. And MANGENOT (1926), furthermore, maintains that GRASSÉ's interpretation of the stigma is incorrect and is quite incompatible with the known characteristics and origin of this organelle.

The view of GRASSÉ (1926 b) that the parabasal body of the Zoomastigophora is homologous with the GOLGI apparatus cannot be extended to those flagellates which have no parabasal body. Furthermore, it is not entirely certain that GRASSÉ is justified in considering the parabasal body a part of the GOLGI apparatus. This organelle is stained with Janus green B in high dilutions, and in this respect resembles mitochondria (*Tetramastix bufonis*, *Herpetomonas jaculum*, *H. pyrrhocoris*, GRASSÉ, 1926; *Crithidia gerridis*, BECKER, 1923; *Trypanosoma lewisi*, SHIPLEY, 1916). GRASSÉ (1926 b) found that "les fixateurs dits mitochondriaux le fixent en meme temps que le chondriome", and that impregnation with osmic takes places very slowly, even at 30° C (60 days or more in *Trichomonas batrachorum*, *Trypanosoma brucei* and *Pyrsonympha*). The parabasal body seems to be demonstrated readily (GRASSÉ, 1926 b) by fixation in osmic vapor and staining in hematoxylin; and, while this method often stains mitochondria, it is not generally accepted as a method of demonstrating GOLGI material. In addition, PARAT (1928) questions GRASSÉ's conclusion that the parabasal body should be identified with the GOLGI apparatus: "... nous ne pouvons souscrire à cette opinion que l'appareil parabasal est un appareil de GOLGI." It seems therefore, that there is still a reasonable doubt as to the correctness of GRASSÉ's

view that the parabasal body is to be homologized with the GOLGI apparatus of Metazoa.

The question as to whether the contractile vacuole is to be regarded as the GOLGI apparatus is likewise still unsettled. In the case of *Chilomonas paramecium*, the writer has verified NASSONOV'S (1924) description of osmic impregnation of this organelle. It has been found, however, that impregnation of this vacuole is much less constant than impregnation of the small osmiphilic globules demonstrated in *Chilomonas*, and that cytoplasmic vacuoles other than the contractile vacuoles are also commonly blackened. In a series of 100 flagellates examined at random, the globules (after osmic impregnation followed by bleaching in hydrogen peroxide) were blackened in 88 %; the contractile vacuole, in 54 %; other cytoplasmic vacuoles, in 24 %; the globules alone, in 25 %; the contractile vacuole alone, in none. In three cases, however, the contractile vacuole was almost completely bleached, appearing as a light grayish-brown ring, while none of the globules or other vacuoles remained blackened. These observations seem to indicate that in prolonged bleaching after osmic impregnation, the ordinary cytoplasmic vacuoles are the first to be bleached and the contractile vacuole is next, while the small globules are the last to lose the osmic impregnation. In *Peranema trichophorum* (HALL, 1928, 1929) and *Astasia* the contractile vacuole is not blackened, and GRASSÉ (1926 b) found the same thing true for *Euglena proxima*. Among the ciliates, PARK (1929) has shown that the contractile vacuole is not blackened in *Stentor coeruleus* and *Leucophrys patula*. Furthermore, work now in progress in the author's laboratory shows that the same thing is true for several other species of ciliates, which do, however, show neutral-red-stainable osmiphilic globules similar to those of *Peranema*. Other ciliates, however, undoubtedly possess contractile vacuoles which blacken in osmic impregnation (NASSONOV, 1924, 1925; GELEI, 1928; KRASCHENINNIKOW, 1929).

The identification of such specialized structures as examples of the GOLGI apparatus evidently has certain disadvantages, and the more logical course seems to involve the selection of a generalized organization of GOLGI material as a standard of comparison. So far, such a standard is offered only by the dispersed GOLGI apparatus of the Sporozoa, since „in this group alone is there anything like agreement as to the correctness of the identification of the material in question with the metazoan GOLGI material" (BOWEN, 1928 c). It is significant, however, that such discrete inclusions have been

demonstrated in a number of other Protozoa. In table 1 are listed a number of the species, other than those described in the present paper, in which such discrete inclusions, demonstrable by osmic or silver impregnation, have been reported; in some cases, as indicated, these inclusions have not been interpreted as GOLGI apparatus by the respective authors.

Table I.

Species	Form	Vital dyes	Author
Trypanoplasma helicis [2]	dct, gl	NR, DV, GV	HIRSCHLER, 1927
T. dendrocoeli [2]	" "	" " "	" "
Leptomonas ctenocephali [1]	gl	NR	LWOFF & LWOFF, 1929
Lophomonas blattarum [2]	dct, gl	"	HIRSCHLER, 1927
Euglena proxima [3]	gl	no data	GRASSÉ, 1926
Peranema trichophorum [1]	"	NR, NV, BCB	HALL, 1928, 1929
Endamoeba blattae [1]	dct, gl	no data	HIRSCHLER, 1927
Plasmodium praecox [1]	gl, f, n	NR	COWDRY & SCOTT, 1928
Monocystis ascidiae [1]	vesicles	no data	HIRSCHLER, 1914
Monocystis agilis [1]	dct, gl	" "	HIRSCHLER, 1927
Gregarina polymorpha [1]	" "	" "	" "
Aggregata eberthi [1]	gl, scat, or. ag.	" "	JOYET-LAVERGNE, 1925
Adelina dimidiata [1]	gl, gr	" "	" " "
Steinina ovalis [1]	" "	NR	" " 1926
Gregarina polymorpha [1]	" "	NR data	" " "
Gregarina cuneata [1]	" "	NR	" " "
Opalina ranarum [1]	dct	no data	SOKOLSKA, 1927
Opalina ranarum [2]	"	" "	HORNING, 1926
Stentor coeruleus [1]	gl, on macrn	" "	PARK, 1929
Leucophrys patula [1]	" " "	" "	" "
Paramecium [3]	gl	" "	GELEI, 1928

Interpretations: [1] indicates species in which the inclusions were interpreted as similar to Golgi apparatus; [2] species in which the inclusions were described as mitochondria; [3] species in which no interpretation was offered for the inclusions described or figured. Form of inclusions: ag aggregates; dct crescent-shaped inclusions (‚dictyosomes'); f filaments; gl globules; gr granules; macrn meronucleus n networks; scat scattered. Vital dyes: BCB, brilliant cresyl blue; DV dahlia violet; GV gentian violet; NR neutral red; NV neutral violet.

As the table shows, discrete vesicular or globular inclusions demonstrable by osmic or silver impregnation have been described in all four groups of the Protozoa, and the acceptance of such inclusions as typical protozoan GOLGI apparatus would permit some degree of correlation in the study of the GOLGI material in Mastigophora, Sarcodina, Sporozoa and Infusoria.

It might be questioned whether these osmiphilic globules and dictyosome-like inclusions in Protozoa, other than Sporozoa, should

be identified as discrete GOLGI bodies. Assuming that the Sporozoan types may be used as a standard for Protozoa, the inclusions in the other species listed are similar in morphology, intracellular distribution and physico-chemical reactions, to one variety or another of the Sporozoan GOLGI apparatus. In *Peranema trichophorum* (HALL, 1928, 1929), for example, the osmiphilic globules are readily distinguished from the mitochondria with vital dyes and methods of osmic impregnation, or by fixation with osmic and staining with hematoxylin. Furthermore, they are stained vitally with neutral red and are not readily demonstrated with Janus green. This reaction to neutral red is also characteristic of the 'GOLGI apparatus' of some of the Sporozoa (*Greyarina cuneata, G. polymorpha, Steinina ovalis*, JOYET-LAVERGNE, 1926; *Plasmodium praecox, Haemoproteus columbae*, COWDRY and SCOTT, 1928). CHATTON and GRASSÉ (1928), in the dinoflagellate *Polykrikos schwartzi*, have described clusters of elongated osmiphilic vesicles around each centrosome. It is assumed by the authors that these structures represent the GOLGI elements and are similar to the osmiphilic bodies described by HIRSCHLER (1927), but their reaction to neutral red is not mentioned. HIRSCHLER, however, reported that these inclusions in *Trypanoplasma* and *Lophomonas* were stained vitally with neutral red. LWOFF and LWOFF (1929) have also described in *Leptomonas ctenocephali* small globules which are stained vitally with neutral red and are blackened in silver impregnation. They conclude that "les caracteres de ces elements permettent de les homologeur au vacuome au sens de GUILLIERMOND et PARAT."

There may be some objection, however, to an application of the results of vital staining to identification of GOLGI material. BOWEN (1928 a), for example, disparages the use of neutral red for this purpose in metazoan cells, as the following quotation shows: "... the classical GOLGI material is certainly not stainable with neutral red." On the other hand, it must be admitted that there are able cytologists who are not in accord with Bowen on this point, and who even go so far as to believe that the 'classical' GOLGI apparatus may be a fusion product of discrete inclusions, either neutral-red-stainable globules (COVELL and SCOTT, 1928; COWDRY and SCOTT, 1928), or in part of modified mitochondria (the "lepidosomes" of PARAT, 1928). DAWSON, in addition, has shown that the neutral-red-stainable 'segregation apparatus' of the amphibian erythrocyte may be demonstrated by osmic impregnation. Furthermore, BOWEN (1928 c), in a paper published five months later than the one just cited, has

changed his opinion from "certainly not" to "probably not" in regard to the action of neutral red on metazoan GOLGI material.

Whatever may be the status of vital or subravital staining in demonstration of the metazoan GOLGI apparatus, there appears to be no such problem in the Protozoa. Since little is known about this phase of protozoan cytology, the GOLGI material lacks the venerable antiquity necessary to the foundation of any concept of a 'classical GOLGI apparatus' in the Protozoa. Hence, even BOWEN (1928 c) is careful not to question the use of vital dyes in these organisms: "In gregarines ... LOYET-LAVERGNE (1926) [1]) finds that after the slow action of a sufficiently dilute neutral red solution, small red arcs (and also granules) [1], strongly colored, appear. ... These bodies correspond exactly with the morphology, size and location of the GOLGI bodies demonstrated by methods of fixation and staining, and are similarly interpreted. This is one, perhaps the only, fairly clear cut case of vital staining of the GOLGI apparatus in the Protozoa."

There seems, therefore, to be no objection to the use of neutral red as a means of identifying GOLGI material within the group of Protozoa. And, on the basis of similarities in morphology, osmic impregnation, intracellular distribution and vital staining with neutral red, these discrete inclusions of other protozoan classes may permissibly be identified with the GOLGI apparatus of the Sporozoa.

An additional question might be raised as to the adequacy of the basis for identifying the 'GOLGI apparatus' of Sporozoa with that of the Metazoa, and this question involves the identity of any and all protozoan GOLGI material with that of Metazoa. While neutral red may be used as a means of identification among the Protozoa themselves, BOWEN will not admit that this vital dye stains the GOLGI apparatus of Metazoa. Hence, this criterion cannot be applied without objection, and it is necessary to look elsewhere for indications of homology.

According to BOWEN (1926), "the important thing is that the GOLGI apparatus is a substance, the exact modeling of which in the cell is purely a matter of secondary interest". This statement, together with BOWEN's (1928 b) conclusion that "of all known methods for demonstrating GOLGI material, that of osmic impregnation is by far the best", might readily permit identification of sporozoan GOLGI material with that of Metazoa on the basis of reaction to

[1]) Author's insert, based on JOYET-LAVERGNE's statements.

osmic impregnation. In the same paper, however, BOWEN (1928 b) sweeps away this usable criterion with still another ruling — — — "the criteria for identification of the GOLGI apparatus in any given case must be based upon its morphology and behavior, not upon its staining capacity". This final decree leaves only "morphology and behavior" as the fundamental criteria for identification of GOLGI material. Applying these to Sporozoa, it is seen that in certain cases the GOLGI apparatus in this group of Protozoa resembles morphologically the discrete GOLGI elements ('dictyosomes,' etc.) described in many metazoan cells. The criterion of "behavior" fails to offer any adequate parallel between Sporozoa and Metazoa. If one accepts BOWEN's (1928 a) contention that "staining capacity" must not be relied upon, there is thus left only the similarity in shape between the Sporozoan GOLGI elements and the discrete metazoan GOLGI bodies (which vary in both size and form). The dispersed Sporozoan type is not even remotely similar in morphology to the GOLGI apparatus originally described by GOLGI.

If one accepts (as BOWEN does) the 'GOLGI apparatus' of Sporozoa as homologous with that of the Metazoa in spite of the scanty evidence offered by BOWEN's single available criterion, "morphology," then the logical deduction seems to be that any set of granular, globular, elongated or crescent-shaped inclusions in the Protozoa may be considered GOLGI apparatus, provided, first, that they are blackened in osmic impregnation (and withstand the usual bleaching methods) and, second, that they may be distinguished from chondriosomes. An additional criterion, that of vital staining with neutral red, may perhaps be used to identify the GOLGI material of other Protozoa with that found in Sporozoa.

With the present limited knowledge of the protozoan GOLGI apparatus any generalization must be purely tentative, but it seems that there is some basis for considering these scattered globules, described in Mastigophora, Sarcodina, Sporozoa and Infusoria, as typical GOLGI material of Protozoa. They exhibit certain common characteristics in their shape, intracellular distribution and reaction to osmic, and in certain species belonging to three of the groups it has been shown that these inclusions are stained vitally with neutral red. These globules may be seen in loose aggregates (as in *Aggregata eberthi*, JOYET-LAVERGNE, 1925) in addition to their usual distribution in the cell, they may fuse into filaments or nets (e. g., *Plasmodium praecox*, COWDRY and SCOTT, 1928), and they may differ somewhat in appearance; but the general characteristics seem

to remain unaltered. It is possible that, in different species, these inclusions may vary in functional significance within the cell, and that the only features in common are their physico-chemical characteristics. But this would seem to be no serious objection to considering all of them GOLGI material. Even in the Metazoa it seems too much to expect that the behavior and functions of the GOLGI apparatus should always be identical in the cells of all tissues from all types of animals. In fact, the cytological evidence, so far as it goes, indicates that the opposite may be true. For example, the GOLGI apparatus exhibits a certain type of "behavior" in spermatogenesis, another type in secretory cells, and perhaps so on through the various tissues. And one important fact that must not be overlooked is, that even in metazoan cells there may sometimes be no visible morphological changes which can be interpreted as "behavior" of GOLGI apparatus. Accordingly, differences in "behavior" may or may not be observed in protozoan GOLGI apparatus, and it would seem unnecessary that such „behavior", if any, should parallel that characteristic of any particular type of metazoan cell.

It is still possible, of course, that these discrete inclusions of Protozoa are not GOLGI bodies at all in the metazoan sense, and that they may be merely metabolic products which show the characteristics conventionally associated with GOLGI material. Until more is known about this aspect of protozoan cytology, such a question cannot be settled one way or another. In the meantime, whether we accept the rather convincing arguments of Parat or follow the somewhat bewildering dictates of BOWEN, there is no reason why these discrete inclusions of Protozoa may not be accepted as true GOLGI material; since, so far as the present criteria for identification of the metazoan GOLGI apparatus extend, these inclusions of Protozoa satisfy all essential requirements.

Summary.

It has been shown that, in *Chromulina* sp., *Astasia* sp. and *Chilomonas paramecium*, there are certain globular inclusions which are blackened in osmic impregnation and resist prolonged bleaching in hydrogen peroxide. In both *Chromulina* and *Astasia* these inclusions are stained vitally with neutral red, brilliant cresyl blue and neutral violet. In each species it was found that, after staining them vitally with neutral red, the globules could be blackened under direct observation by exposure to osmic vapor in hanging-

drop preparations. In vital staining with a mixture of Janus green and neutral red, the neutral-red globules were readily distinguished from smaller and more numerous granules, presumably mitochondria, which were stained with Janus green. In osmic-fixation followed by iron-hematoxylin, the smaller and more numerous granules were demonstrated, and not the larger neutral-red-stainable globules.

The examination at random of a series of *Chilomonas paramecium* (impregnated in osmic and then bleached) showed the osmiphilic globules blackened in 88 %; the contractile vacuole, in 54 %; the globules alone, in 25 %. Insofar as such a sample may be significant, it seems that the osmiphilic globules in *Chilomonas* are more consistently impregnated than is the contractile vacuole.

It is pointed out that such osmiphilic globules have been described in all four groups of the Protozoa and that, so far as the present criteria for identification of the metazoan Golgi apparatus extend, these inclusions of Protozoa satisfy all essential requirements.

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Description of Plates.

Plate 2—3.

Plate 2.

Fig. 1. *Astasia* sp. showing a number of osmiphilic globules and eight blackened vacuoles, or alveoli; Mann-Kopsch osmic impregnation, bleached in hydrogen-peroxide. 2700 : 1.

Fig. 2. *Astasia* sp. with similar globules and one blackened and one partly bleached vacuole; technic as in fig. 1. 2700 : 1.

Fig. 3. *Astasia* sp. showing only the blackened globules remaining after 45 minutes in hydrogen-peroxide; Mann-Kopsch impregnation. 2700 : 1.

Fig. 4. *Chilomonas paramecium*, showing a single blackened vacuole near the gullet, and only two small osmiphilic globules; Mann-Kopsch impregnation; bleached in hydrogen-peroxide. 2700 : 1.

Fig. 5. *Chilomonas paramecium* with two blackened vacuoles and an osmiphilic globule near the gullet, and a fused chain of three or four globules near the posterior end of the nucleus; technic as in fig. 4. 2700 : 1.

Fig. 6. *Chilomonas paramecium* with a number of osmiphilic globules, but no blackened vacuoles; technic as in fig. 4. 2700 : 1.

Plate 3.

Fig. 7. *Astasia* sp., optical section showing small granules stained with hematoxylin after fixation in MANN-KOPSCH. 2700 : 1.

Fig. 8. *Astasia* sp. showing osmiphilic globules and smaller partly bleached granules (mitochondria?). MANN-KOPSCH osmic impregnation; sleached in hydrogen-peroxide. 2700 : 1.

Fig. 9. *Chilomonas paramecium* showing small granules, probably mitochondria; technic as in fig. 7. 2700 : 1.

Fig. 10. *Chromulina* sp., osmiphilic globules and 'crescents' shown; technic as in fig. 8. 2700 : 1.

Fig. 11. *Chilomonas paramecium*, osmiphilic globules, cytoplasmic globules and contractile (?) vacuole blackened; technic as in fig. 8. 2700 : 1.

Fig. 12. *Chromulina* sp., small granules stained with hematoxylin after MANN-KOPSCH fixation. 2700 : 1.

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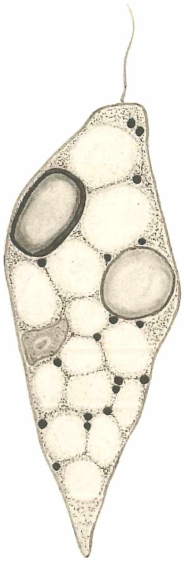

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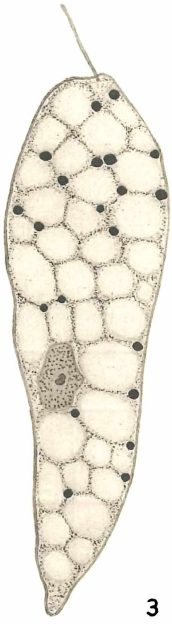

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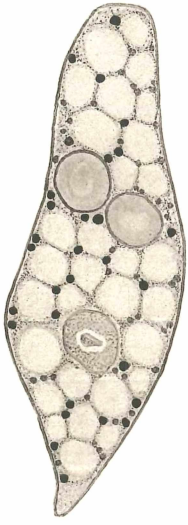

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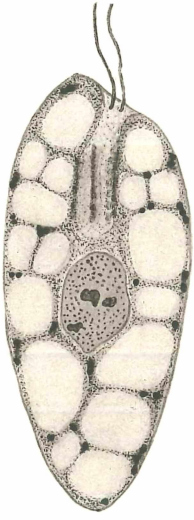

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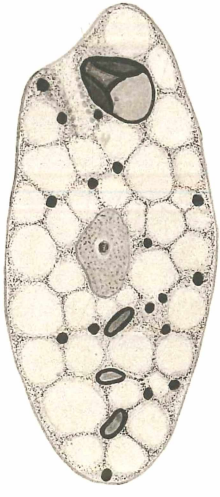

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VERLAG VON GUSTAV FISCHER IN JENA.