Notes on the Minute Structure of Pelomyxa palustris (Greeff).

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With Plates 20 and 21.

While working as a student in the laboratory of the Linacre Professor at Oxford, during the summer term of 1893, the opportunity was given me of examining a considerable number of specimens of the interesting fresh-water Rhizopod Pelomyxa palustris, originally described by Professor Greeff in 1867 as Pelobius, and later discussed by him under its present name in the 'Archiv für mikroskopische Anatomie,' Bd. x, 1874. I was enabled, by the kindness of Professor Lankester (to whom my best thanks are due for affording me the material for these observations, as well as for much valuable advice and assistance), to study more than twenty individuals of P. palustris, both in the living state and by means of sections.

Some points with regard to the minute structure which I was able to make out have not, as far as I am aware, been observed before, and others which had been doubtful have received confirmation. It has therefore seemed desirable to bring forward the results of my observations, as far as they go, with the conclusions I have drawn from them, although one or two of these conclusions must be regarded merely as inferences until verified by further investigation. Most of the sections were cut by the skilled hand of my friend Mr. E. A. Minchin, B.A. (to whom I am much indebted for his kind help), and
some were also stained by him. Others cut by Mr. Minchin were prepared by me, and together we obtained a most instructive series, which I studied very carefully. The figures in the accompanying plate are from drawings I made with the camera under high powers, and illustrate the chief points dealt with in these notes. The stains used were Paul Mayer's new "carm-alum" and "paracarmine" ('Mitth. Neapel,' Band x, 1891–3, pp. 489, 491); anilines such as eosin, fuchsin, fuchsin S., orange G., and gentian violet, either alone or in combination; also picro-carmine and hæmatoxylin. The sections varied from 1 to 7 μ in thickness. The only observations of mine which can claim to be new have reference (1) to the appearance of a central mass of doubtful significance in the general protoplasm, (2) to the staining properties of the refringent bodies or "Glanzkörper" of Greeff, and (3) to the perfectly definite jointing staining-reaction of the rod-like bodies (a point suspected but hardly established hitherto), which establish the view that they are bacteria. These points, and others confirmatory of previous observations, are considered separately as follows.

General Structure of the Protoplasm.

The protoplasm of Pelomyxa is well known to be highly vacuolated, and in P. viridis Professor A. G. Bourne ('Quarterly Journal of Microscopical Science,' xxxii, 1891) distinguishes between the large vacuoles, which are comparatively few, and the much more numerous smaller ones which he calls "vesicles," and which, in the species described by him, have chlorophyllogenous contents. The difference in size, though not in colour, between "vacuoles" and "vesicles" obtains in P. palustris, and the numerical proportion of one to the other is much the same. Thus the general character of the protoplasm has been considered as practically identical in the two species. But I was able to make out that, in P. palustris at least, the protoplasm surrounding the vesicles was by no means homogeneous, as stated by Professor Bourne for P. viridis, but showed very distinct structure.
The examination of sections under very high powers lent strong support to the views of Professor Bütschli as to the foam-like structure of protoplasm (‘Mikroskopische Schäume,’ 1892). I was able to confirm the existence of the very fine vacuolisation described by him (loc. cit., pp. 200 and 216) for P. palustris, which probably occurs also in P. viridis, but, as he suggests, may have escaped Professor Bourne’s notice. The vesicles in P. palustris, which corresponded to the smallest seen by Professor Bourne in P. viridis, were easily known by their size as compared with that of the nuclei in both forms; and a foam-like structure, consisting of alveoli of infinitely smaller diameter (\( \frac{1}{3} - 1 \mu \)) than these vesicles, was most distinct in many of the sections. Fig. 11 shows this fine alveolar structure with a nucleus and two vesicles for comparison; in fig. 10 it is seen on a larger scale. The strands of protoplasm bounding these smallest alveoli, and also some of the thin strands between the large vacuoles, appeared to me homogeneous, and comparable with the finest pseudopodia of Rhizopods and the finest strands of vegetable protoplasm described by Bütschli (loc. cit., pp. 67, 79). The peripheral radiate alveolar border of Bütschli, said to be characteristic of froths, was clearly distinguishable in some sections (fig. 2), and the radiate alveolar layer described by him is seen in fig. 9, (a) round a nucleus, and (b) round a refringent body.

Appearance of a Central Mass.

In one individual of Pelomyxa, of which a consecutive series of sections had been cut, a very curious appearance was observable. The animal had been killed with osmic acid, and stained in bulk with carm-alum, a delicate protoplasmic stain. The sections presented a central, more deeply staining, irregularly oval ring of apparently denser protoplasm, which was traceable through many sections. The ring diminished at each end of the series to a small central solid patch of denser protoplasm, and hence apparently represented a more or less spherical or oval mass, reminding one somewhat of a central
capsule. The character of the protoplasm was somewhat different without the ring from that within. External to the ring it was highly vacuolated; from the periphery of the animal inwards areas containing very few nuclei or vacuoles, but consisting almost entirely of small vesicles, extended nearly to the boundary of the ring, in some places running quite up to it. Fig. 1 shows a whole section about the middle of the series, drawn without details, showing merely the general appearance and size of the ring, with the nuclei and largest vacuoles. The dotted regions represent the vesicular areas. The ring itself exhibited the extremely fine alveolar structure above mentioned. Internal to the ring I could make out nothing more than a very finely granular appearance of the protoplasm; alveoli, if present, must have been infinitely small in diameter. Fig. 8 is a high-power view of a portion of the ring; the area marked out by dotted lines is represented on a much larger scale in fig. 10, to show more accurately the character of the alveoli. The capsular appearance was not due to the effect of semi-penetration of the osmic acid used in killing, since an individual treated with the same reagent for the same length of time, but stained with picro-carmine, showed no trace of the structure. Some specimens of P. palustris obtained later, which were killed with alcohol and stained in bulk with carm-alum, showed a slight tendency to the same appearance; there seemed to be a drawing together or central concentration of the protoplasm, though there was no definite ring formed. But these individuals presented, in the living state, quite a different appearance from those examined previously. They were shrunk up into a globular shape, were brownish in colour, and perfectly quiescent, so that on first examination they seemed to be dead. But after they had been under the microscope for a long time, they gradually began to assume a more normal appearance, and very slowly put forth pseudopodia. It was a condition suggesting encystment, but no definite cyst-wall was found. It appeared to me possible that these individuals were in a stage leading on to, or nearly connected with, that seen in the first-mentioned
specimen, and that the quiescent condition may have been preparatory to the production of swarm-spores.

The outward appearance of quiescent individuals certainly seemed somewhat to resemble that described by Greeff (loc. cit.) as preceding reproduction. The protoplasm of all quiescent individuals was filled with sand-particles to such an unusual extent that the cutting of sections was a matter of extreme difficulty.

The "Glanzkörper."

The refringent bodies, or "Glanzkörper" of Greeff, were very numerous in P. palustris. Professor Bourne says he never saw "anything in the protoplasm of P. viridis resembling" them, but it seems to me possible that he might have confounded them with vacuoles of the same size, as in life they were often not easily distinguishable (except by the fact of their extrusion from the body) from these. ¹ Nor were they always recognisable in sections when stains such as carm-alum and picro-carmine were used. Alum-carmine, used by Professor Bourne, probably would not show them up either. But I found that they stained very readily and deeply with fuchsin, eosin, dahlia, solution of iodine in potassium iodide, picric acid dissolved in turpentine, and some other stains, and could thus be picked out with beautiful distinctness. With all stains except picric acid in turpentine they appeared perfectly homogeneous, but with the latter reagent they showed plainly a fine granulation, and sometimes contained a small bright crescentic area which might represent a space or cavity in the interior. From my observations I concluded that they were almost certainly either solid structures or filled with a coagulable fluid. Greeff observed the granulation with acetic acid, and found these bodies to become deep brown with iodine. I found a solution of iodine in potassium iodide to have the same effect. He also thought he could sometimes distinguish in the refringent bodies

¹ I am inclined to regard the chlorophyll-bearing "vesicles" of Bourne's P. viridis as equivalent to the "glanzkörper" of P. palustris.—E. R. L.
some kind of contents and a nucleus, but I found no appearance of internal structure other than that described above.

I am inclined to think that he, too, sometimes confounded refringent bodies with vacuoles, since he describes a falling in of the walls of the former which occurred with some reagents, and I found a crenellation of this kind to be very characteristic of the food-vacuoles under certain conditions. The refringent bodies divide by constriction, and in fig. 6 the process is seen near completion. M. Pénard ('Archives des Sciences physiques et naturelles,' tome xix, 1893) says that colouring-matters have little or no effect upon them, and that they are either structureless or contain vacuoles. Possibly he looked upon the crescentic areas as vacuoles, or, as he evidently used different reagents, he may have failed to distinguish the refringent bodies from the food-vacuoles, which generally have contents of some sort.

The Vacuoles.

The vacuoles proper were of two kinds, viz. (1) large non-contractile vacuoles, which did not stain (figs. 2 and 8), and (2) food vacuoles of varying size, which were found with and without contents. These contents in all cases stained freely with carm-alum and picro-carmine, and the vacuoles were further distinguishable by the greater thickness, and often by the crenellation of their walls (fig. 8). The "vesicles" of Professor Bourne, which greatly outnumber the vacuoles, and must be placed apart on account of their having in P. viridis chlorophyllogenous contents, are perhaps, as suggested by Professor Lankester, not to be regarded as "vacuoles," but as corresponding to the glanzkörper of P. palustris.

The Nuclei.

With regard to these I have nothing new to add. The nuclei were lodged in the nodes of the protoplasmic network, and presented, as described by Greeff and others, a finely granular structure with several nucleoli in the middle, and deeply-staining chromatin granules arranged peripherally in
a more or less ring-like fashion. I was not able to detect any appearance of nuclear division. The radiate alveolar layer round the nuclei and refringent bodies has been noticed above.

The Bacteria.

The rod-like bodies found in profusion in *Pelomyxa* were originally described by Greeff as "crystals," but later observers, firstly Bourne, and after him Pénard (loc. cit.), have been inclined to regard them as symbiotic bacteria. Leidy ("Fresh-water Rhizopods of N. America, 1879") and Greeff both thought they distinguished a "transverse striation" of the rods.

M. Pénard considered the rods as "véritables bactéries." He saw in them "une striation transversale, ou plutôt une apparence de divisions à intervalles réguliers, telles qu'on les trouve dans les algues filamenteuses inférieures." With reagents the rods appeared "nettement divisées en plusieurs parties par des étranglements," or "réduites en quelque sorte à des chapelets, dont les nombres respectifs de grains étaient de 2, 3, ou 4." But he did not make it very clear whether the appearance he saw was one of constriction merely and due to reagents, or jointing, which is rather a different thing. Nor did he state definitely the largest number of divisions seen in a single rod, while his description of some of the longest as "filaments ondulés ou recourbés" rather inclines one to think that some of those he saw might have been really the "algues filamenteuses" to which he compared them, and which are not uncommonly found in the protoplasm of *Pelomyxa*. The rods, as figured in the plate accompanying M. Pénard’s paper, show either transverse striation or a moniliform appearance; the former does not of course amount to jointing, and the latter is very different from anything seen by me.

In a *Pelomyxa* killed with osmic acid, stained in bulk with cærm-alum, and teased up in glycerine, I found that the rods were not constricted, but very distinctly jointed
I obtained the same result with a specimen treated in the same way, but stained with picro-carmine. In the sections, which were mostly double- or treble-stained with anilines such as eosin, fuchsin, fuchsin S., orange G., and dahlia, the rods were deeply stained, and the jointing could be well seen; in some cases the terminal joint could be seen separating off (fig. 7). The rods were always straight and made up of 2, 3, 4, 6, and sometimes even 9 joints. They were highly refringent, and their refractive index seemed to be nearly the same as that of Canada balsam, since in preparations mounted in the latter medium the rods were difficult to see, while in glycerine they were most distinct. The joints had the shape of long prisms, and in a few of the 6-jointed rods a further transverse division of each joint into two was apparent.

In the living Pelomyxa the rods were frequently thrown out into the water, together with refringent bodies and nuclei, a process evidently abnormal and the result of unfavorable conditions. When thus thrown out, the rods exhibited active movement of a kind which has been considered as possibly molecular (Bourne, loc. cit.), but they also travelled round the periphery of the animal. I could not absolutely satisfy myself that the latter movement might not have been due to currents created in the water by the activity of the pseudopodia. Still, taking all the appearances together, it seemed impossible to doubt that the rods were really bacteria.

My friend and fellow-student Mr. M. D. Hill, of New College, Oxford, undertook to prove this by cultivation of the bacteria in suitable media, and some account of his preliminary investigations, which are not yet completed, are appended here.

With regard to the situation of the bacteria, they were scattered more or less throughout the protoplasm, but were, as stated by Greff, especially abundant around and adhered thickly to the walls of the refringent bodies. Fig. 7 shows some of the latter cut through at different levels, and here the rods are plainly seen in situ. The rods alone are represented in fig. 12.
New Species.

If further criticism of M. Pénard's observations on Pelomyxa be permissible, it would seem that he has scarcely sufficient grounds for the establishment of a new species (P. beleostei). He does not mention any definite feature which is not equally characteristic of P. palustris. Size is no criterion, since individuals of P. palustris vary very much in this particular, as also in the presence or absence of sand débris in the protoplasm. The sole real difference appears to be in the structure of the nuclei; but as this also differs in two nuclei from the same animal (according to the figure), and both of these, from their thick walls, size relative to the vesicles, and general appearance, bear far more resemblance to food-vacuoles with contents than to the nuclei of any amœba, it would seem doubtful whether they were really nuclei at all. In a paper published several years ago in the 'Archives Exp. de Zoologie,' Korotneff has given reasons for recognising a second species of Pelomyxa. I have no doubt that the form studied by me is the P. palustris of Greeff.

Addendum.

November 10th, 1893.

In order to furnish, if possible, a conclusive proof of the organic nature of the so-called "rods" of Pelomyxa, Professor Lankester suggested to me that I should attempt to obtain a cultivation of these organisms by means of the usual bacteriological methods. This I did during June, 1893, and the following is a short account of the work.

The investigation was carried on in the laboratory of the Regius Professor of Medicine in the University Museum, with the kind collaboration of Dr. Ritchie, who was then, and is at present, engaged in bacteriological research.

We made a large number of cultivations by teasing up fresh specimens of Pelomyxa in sterilised water, after removing them straight from the pond water in which they were kept,
and "sowing" them in test-tubes containing various media, e.g. blood-serum, beef jelly, bovril, &c. The tubes were kept in the dark at the ordinary temperature of the room, except in some cases where artificial warmth was applied. In all these cases a very mixed culture was obtained, and it was impossible to say whether the growth was derived from the "rods." In one instance, however, we were able to detect a few long bacteria, which probably were those for which we were seeking, but it was found impossible to convince ourselves that this was the case.

Furthermore, at Professor Lankester's suggestion, we tried immersing the animals for a moment, some in dilute corrosive sublimate, others in strong alcohol, in order to kill, if possible, the foreign micro-organisms which would naturally be clinging to the surface of the protoplasm, without injuring those in the interior. Cultures were made in the same way as in the previous experiments, but only one was successful. Here we obtained a pure colony of short rod-like forms, which may have been the "rods" in a more finely divided state than that in which they appear normally in the living Pelomyxa. It is probable that, when supplied with abundant nutriment, the "rods" would break down and multiply so rapidly as not to allow themselves to assume the many-jointed condition which Miss Gould has described.

Other methods were tried, such as the "hanging drop" and "fractional" methods, but where colonies were produced they were of too mixed a character to give conclusive results.

At present, therefore, our results are negative as regards having obtained a demonstrably pure cultivation.

My best thanks are due to Dr. Ritchie for his kindness in putting his apparatus at my disposal, and for his help during the work.

M. D. Hill.
DESCRIPTION OF PLATES 20 & 21,
Illustrating Miss Lilian J. Gould's paper, "Notes on the Minute Structure of Pelomyxa palustris" (Greeff).

Fig. 1.—General view of complete section of Pelomyxa palustris without details. Killed osmic acid, stained in bulk carm-alum. Zeiss, obj. B, comp. oc. 4; camera.  r. Ring of denser protoplasm.  n. Nuclei.  v. Vacuoles.  v. a. Vesicular area.


Fig. 3.—Portion of section. Killed osmic, stained carm-alum, fuchsin, and eosin. Zeiss, obj. E, comp. oc. 4; tube not drawn out; camera.  g. Refringent bodies.  n. Nuclei.  b. Bacteria.  s. Sand and débris.  p. r. b. Peripheral radiate border.  d. Diatom.

Fig. 4.—Teased-up portion of P. palustris in glycerine. Killed osmic, stained carm-alum, fuchsin, and eosin. Zeiss, obj. E, comp. oc. 4; tube not drawn out; camera.  b. Bacteria.  g. Refringent bodies.  n. Nuclei.  pr. Droplets of protoplasm.

Fig. 5.—Teased-up portion in glycerine. Killed osmic, stained picro-carmine. Zeiss, obj. E, comp. oc. 4; tube not drawn out; camera.  b. Bacteria.  g. Refringent bodies.  n. Nuclei.

Fig. 6.—Portion of section. Killed osmic, stained picro-carmine and picric turpentine. Zeiss, obj. E, comp. oc. 4; tube not drawn out; camera.  g. Refringent bodies.  g g. Dividing refringent body.  n. Nuclei.

Fig. 7.—Refringent bodies, to show rods in situ. Killed osmic, stained picro-carmine and dahlia. Zeiss, oil imm. 1/2, comp. oc. 4; tube at 17 mm.; camera.  n. Nuclei.  b. Bacteria.  g. Refringent bodies.  w. Wall of refringent body.

Fig. 8.—Small portion of section, including bit of capsular region.  r r. Width of ring.  i. pr. Protoplasm within ring.  e. pr. Protoplasm external to ring.  f. v. Food-vacuoles.  sq. Alveolar area represented in Fig. 10. Killed osmic, stained carm-alum and dahlia. Zeiss, oil imm. 1/2, comp. oc. 4; tube at 19 mm., finished with comp. oc. 8.; camera.

Fig. 9.—A. Nucleus, with surrounding radiate alveolar layer. Killed osmic, stained carm-alum.  B. Refringent body, with surrounding radiate
alveolar layer. Killed osmic, stained picro-carmine and picric turpentine. Zeiss, oil imm. \( \times 40 \), comp. oc. 8; tube at 19 mm.; camera.

**Fig. 10.**—Protoplasm between vacuoles, to show finest alveolar structure. 
: V. Vacuoles. A. Alveoli.

**Fig. 11.**—Small portion of section. Killed osmic, stained picro-carmine and haematoxylin. Zeiss, oil imm., comp. oc. 8. N. Nucleus. : V. Vacuoles. \( v. v. \) Vesicles corresponding to the chlorophyllogenous vesicles of P. viridis.

**Fig. 12.**—Bacteria. Zeiss, oil imm. \( \times 60 \), comp. oc. 4; tube not drawn out.