Observations on the Life-history of Adelea ovata, *Aimé Schneider, with a Note on a New Gregarine from the Gut of* Lithobius forficatus.

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[Plates 2 and 3.]

At the suggestion of Mr. J. J. Lister, F.R.S., I recently undertook a reinvestigation of the sporozoan parasites occurring in the gut of the centipede, *Lithobius forficatus*. The present paper is the result of these investigations. It is with pleasure that I take this opportunity of expressing my thanks for the advice and assistance I have received not only from Mr. Lister, but also from Mr. Adam Sedgwick, F.R.S., and from Mr. W. S. Perrin.

No less than six protozoan parasites have been recorded from the gut of *Lithobius forficatus*—four Coccidia and two Gregarines. The Cambridge centipede appears to harbour but a single one of the former—namely, *Adelea ovata*, A. Schn.—whilst both the latter* have been observed in rather less than 1 per cent. of the animals examined. It is with the sexual phases of the life cycle of *A. ovata* that the present paper is chiefly concerned.

Previous Work.—*Adelea ovata* was first described† by Aimé Schneider in 1875 [12], and although a part of the sexual process was observed, it was not recognised as such. Schneider published some further particulars in 1892 [14]. In 1897 Schaudinn and Siedlecki‡ published a preliminary account of the Coccidia of the centipede, and later—in 1899—Siedlecki [16] gave a detailed description of the whole life-history of *Adelea ovata*. Subsequent writers have described other species of the genus *Adelea*, and in all cases the life cycle appears to correspond closely with that recorded by Siedlecki. I may say that I am able to confirm most of the results obtained by this writer, but I have come to different conclusions regarding one or two points. The most important of these deals with the formation of the microgametes.

Methods.—The best results have been obtained by adopting Schaudinn's methods [11]. The entire gut was removed, and the epithelial cells and gut-contents spread out upon a coverslip. The films thus obtained were

* Viz., *Actinocephalus dujardini*, A. Schn., and *Echinomera hispida*, A. Schn.

† As a gregarine.

‡ Schaudinn and Siedlecki, 'Sitz. ber. d. d. zool. Gesell.,' 1897.

instantly fixed by immersion in hot sublimate-alcohol containing a trace of acetic acid (Schaudinn). After fixation the films were treated with alcohol containing iodine, and stained in Bütschli's modification of Delafield's hæmatoxylin.* This I prepare by adding 1-per-cent. acetic acid solution to a 0·5-per-cent. solution of the ordinary concentrated Delafield's hæmatoxylin in water until a pink colour is produced. Staining, for all stages except spores, is complete in from 15 to 30 hours.

Giemsa's modification of the Romanowsky-Nocht stain was tried, among others, but it proved to be of little use for delicate nuclear structures, owing to the drying which necessarily takes place.

Transverse sections of the gut were made, but they were found to be unsatisfactory. Moist films are far more useful for examining the Coccidia.

I will now proceed to describe the stages which I have observed and which differ from those hitherto recorded.

(1) Association.—The entire genus *Adelea* is characterised by a precocious association of the gametocytes. This was observed in *A. ovata* as long ago as 1875, although, strangely enough, it was not even suspected of being connected with any sexual process. Association, it may be noted, takes place in the lumen of the gut, and not in the epithelium. Siedlecki, who ultimately interpreted this phase correctly, states that but a single microgametocyte ever attaches itself to a macrogametocyte. Only once did he observe two microgametocytes attached to the same female individual. My observations, on the contrary, lead me to believe that this condition is not uncommon. I have seen upwards of a dozen instances in which two microgametocytes were attached to the same macrogametocyte. Apparently, these microgametocytes do not always develop or become attached simultaneously, for they may be found in different stages of maturity (*cf.* Plate 3, fig. 3). Association in which more than one microgametocyte takes part has been described by Laveran† in the closely allied form *Klossia helicina*.

An association of two microgametocytes with one another as described in *A. mesnili* (Pérez) and *A. zonula* (Moroff), has never come under my observation.

(2) Formation of Microgametes.—The only detailed account of the genesis of the male gametes of *A. ovata* is that given by Siedlecki. He states that the nucleus of the microgametocyte divides into two daughter-nuclei, each of which then divides in a plane at right angles to that of the first division, so that four nuclei result. These nuclei then develop into the

* Bütschli, 'Zeitschr. f. wiss. Mik.,' ix, 2, 1892, p. 197.

† Laveran, 'C. R. Soc. Biol. Paris,' 1898 (Novembre).

four microgametes, one of which is destined to fertilise the macrogamete; the remaining three perish. In the first nuclear division it was believed that the quantity of chromatin was reduced, whereas the number of chromosomes was reduced in the second division. I am convinced that this is not a correct interpretation of this particular gametogenesis; and the inference that the maturation of these gametes approaches the metazoan type is, to my mind, quite unjustifiable.

To follow the formation of the male gametes from the beginning, it is necessary to describe in detail a merozoite of the male type. Such a merozoite is shown in the accompanying figure (text-fig. 1).

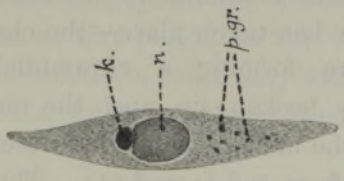

Fig. 1.

It is seen to consist of a somewhat fusiform (or sometimes falciform) body, containing a nucleus (*n.*) and a large peripheral karyosome (*k.*). Sometimes two or even three karyosomes are present, but the usual number is one. Occasionally it appears to be extranuclear. At one end of the merozoite—usually that remote from the karyosome—a number of granules is usually, though not always, present. These are the so-called "pigment granules" (*p. gr.*). I am inclined to believe that they are really chromatin granules. They stain deeply with hæmatoxylin, and are coloured red by Giemsa's stain. They are never present in macromerozoites.

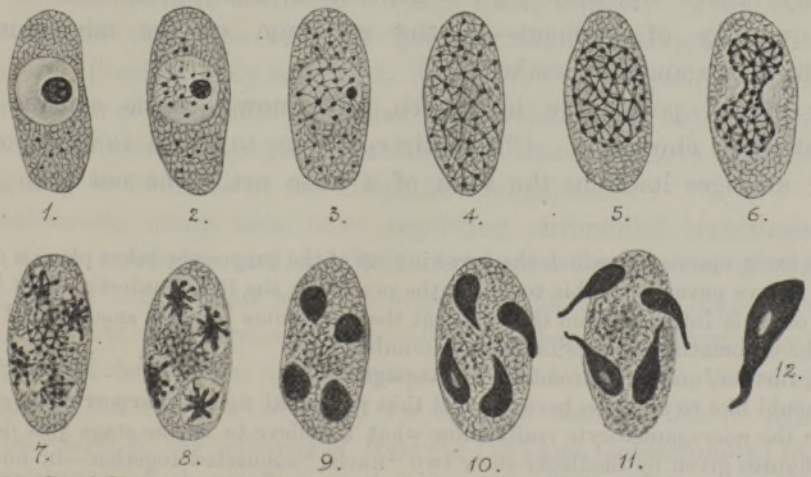

Fig. 2.—Semi-diagrammatic Representation of Formation of Microgametes. The macrogametocyte is not shown.

A micromerozoite, such as that just described, may increase in size and become a microgametocyte (text-fig. 2 (1)). It then becomes attached to a macrogametocyte in the usual manner. During its transformation into a microgametocyte, the karyosome travels to the centre of the nucleus, and is apparently reinforced by the nuclear chromatin. A part of this latter, however, is usually to be found lying free in the clear nucleus in the form of granules.

The next step towards the formation of the microgametes consists in a dispersal of the karyosome and the formation of a chromatic network in the nucleus. This is shown in (2) text-fig. 2, and may take place before association occurs. Further breaking up* of the karyosome now ensues—provided that association has taken place—the chromatin particles invading the cytoplasm and there forming a chromidial† net (3). When the karyosome is completely broken up, and the outline of the nucleus has completely disappeared, the microgametocyte is seen to contain a chromidial net completely filling it from end to end (4). The foregoing stages (1 to 4) may be conveniently termed the *analytic* phase. Those which follow constitute what I may call the *synthetic* phase, in which four new nuclei are built up from the chromatic elements of the old nucleus. Synthesis begins with the gathering up of the chromidial net into a more or less rounded mass (5) which soon becomes constricted in the middle, giving rise to a dumb-bell shaped reticulum (6). If deeply stained, it has the appearance of a dividing nucleus.‡ The ends of the dumb-bell now extend transversely, and the chromatin becomes localised at the four poles so formed (7). At the same time, the intermediate portion becomes diffuse; it finally breaks up, undergoes degeneration, and is left behind—with a small quantity of pigment—in the residuum of the microgametocyte when the microgametes forsake it.

Four special points, we have seen, have now become centres for the aggregation of chromatin. Chromatin continues to travel to each point, and at first arranges itself in the form of a loose net. The net then resolves

* The exact manner in which the breaking up of the karyosome takes place is unknown to me. I have never been able to watch the process in the living animal. That breaking up does occur is inferred from the fact that the karyosome becomes smaller and smaller, whilst the chromatin granules increase in number.

† See, further, under "Chromidia," next page.

‡ I should like to mention here the fact that published figures purporting to show two nuclei in the microgametocyte really show what I believe to be the stage just described. All the figures given by Siedlecki show two "nuclei" connected together—in no case are they completely separate. Siedlecki's "corpuscule intermédiaire" is, I believe, a more deeply stained portion of the intervening network.

itself into a stellate mass of chromatin. Hence the highly-characteristic figure of a microgametocyte containing four star-shaped nuclei is presented (8). The stars finally become rounded off (9)—the spherical nuclei so formed afterwards elongating to form the microgametes, which apparently consist entirely of chromatin ("chromatozoites") (10—11). Many of the microgametes enclose a clear space resembling the vacuole described by Schaudinn in *Coccidium schubergi*. A mature microgamete is shown on a larger scale in text-fig. 2 (12).

It will be seen from the foregoing that my account of the formation of the male gametes differs considerably from that of previous writers.

It now becomes an interesting point to determine whether a similar chromidial condition occurs at any period in the life-history of the female forms. So far, I have been unable to reach any satisfactory conclusion with regard to this. However, the following observations seem worth recording in this connection :—

(*a*) A single instance has occurred in which the individuals of a group of macromerozoites were found to have their nuclei in a chromidial condition (see Plate 3, fig. 6).

(*b*) Since the stellate nuclei of the microgametocyte are formed by synthesis from chromidia, it is possible that the stellate nuclei of the macroschizont may have a similar origin. Although, up to the present, I have not been able to demonstrate that this is the case, nevertheless, I think that the macroschizont, of which fig. 5, Plate 3, is an exact copy, probably shows a stage in which neuclei are being synthesised by the concentration of chromidia at various points. It should be noticed that a considerable quantity of chromatin is scattered through the cytoplasm. I am, of course, ready to admit that this may be quite a wrong interpretation of the appearances. Possibly they are due to degenerative changes. Nevertheless, the phenomenon of chromidia-formation has assumed such importance, owing to recent work in other groups of the Protozoa, that I believe the conditions described may be found to be not altogether insignificant.

Chromidia.—So many new facts regarding chromidial structures have recently come to light, and so little notice has been taken of them in England, that I think a few words on chromidia in general may not be out of place here. As it is obviously impossible to treat the subject fully in the present paper, I have given a short list of papers bearing on the subject in the literature list (B) at the end; for a more detailed list of references I refer the reader to the writings of Hertwig [21] and Goldschmidt [18, 19].

The conception of chromidia was first formulated by Richard Hertwig in 1902 [20], and resulted from his observations on *Actinosphærium*, *Arcella*, etc.

Since then, many other "chromidial" nuclear phenomena have been described. Indeed, it is now a matter of some difficulty to give a definition of chromidia which will embrace all conditions so described. In its widest sense, the term is applied to all chromatin substance (*i.e.*, all matter which takes up nuclear stains) which lies within the cell and which is not gathered together in the form of a definite nucleus. Chromatin granules of diverse shapes and sizes, strands, networks—all these come in this category; and, moreover, the physiology of these structures seems to be no less heterogeneous than their morphology. For chromidia seem to be formed sometimes as the result of degeneration, sometimes as a form of nuclear division, sometimes as katabolic products of nuclear activity—sometimes (as in bacteria), being the normal vegetative stage of the neucleus—and so on.

Many new terms have been introduced for these various forms of chromidia. Since many of these have not received general acceptance, and since many are, I think, quite unjustifiable, owing to our present ignorance of the causes underlying the different phenomena, I have used but two terms in my description of the nuclear changes in *A. ovata*,—"*chromidia*" for separate chromatin granules lying in the cytoplasm, and "*chromidial net*" for a collection of chromatin granules united by a reticulum. Amongst the terms which are more frequently met with in papers on this subject the following may be mentioned:—The name "*Sporetium*" (Goldschmidt) has been given to chromidial structures formed previous to gamete formation. "*Protogonoplasm*" (Calkins) and "*Idiochromidium*" (Goldschmidt) are other terms applied to the same structures. Then, again, we have such terms as "*Trophochromidium*" and "*Chromidium*" (*sensu stricto*) applied by Goldschmidt to chromidial structures of a vegetative (as opposed to reproductive) nature. Other terms, such as "distributed nucleus" (Calkins), etc., require no special explanation.

A chromidial condition of the nucleus may occur, apparently, not only in all the chief subdivisions of the Protozoa, but also in some Metazoa and in Bacteria.

The great interest which attaches to chromidia arises from the fact that investigation of these structures has revealed a "Doppelkernigkeit," or nuclear dimorphism, in many Protozoa. That is to say, two distinct elements can be recognised in many protozoan nuclei—one, a "*propagative*" element, the other, a "*somatic*." The propagative seems specially liable to separate from the somatic as chromidia (*cf.* Schaudinn, Neresheimer, etc.). The heterokaryote condition of the Ciliata thus finds a parallel in many other Protozoa. To the single nucleus, combining the chromatic elements proper both to the soma and reproduction, is given the name "*amphinucleus*."

From the foregoing remarks, I think it will be evident that the subject of chromidia is one of great importance, but not one on which we are at present, owing to our lack of knowledge, able to dogmatise. I will conclude by quoting Mesnil. "Il faut," says this writer, "bien se garder de trop généraliser, et chaque cas où le protoplasme se charge de granulations chromatiques doit être étudié avec soin à ce nouveau point de vue."

Note on a New Gregarine from the Gut of Lithobius forficatus.

Whilst examining centipedes for stages in the life-history of *A. ovata*, I came across the trophozoites of a gregarine which, I think, has not been recorded hitherto. The trophozoites were very numerous, but no spores or other stages could be found. Hence it is at present impossible to refer the animal to its proper systematic position.

Only one centipede which I examined contained these gregarines, but in this particular case they were very numerous. The Lithobius infected was in a dying condition just before I examined it, possibly owing to the harmful effects of the parasite.

The gregarine has an exceedingly simple structure (see fig. 3). There is

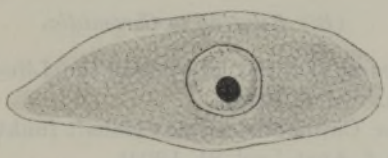

Fig. 3.

no epimerite, and the body is non-septate and usually slightly more pointed at one end than at the other. A large nucleus containing a big karyosome is situated more or less in the middle of the animal: its position varies. The average length is 127 μ, breadth 48 μ. The measurements were made from permanent preparations.

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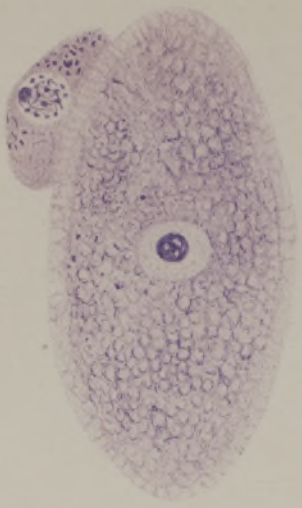

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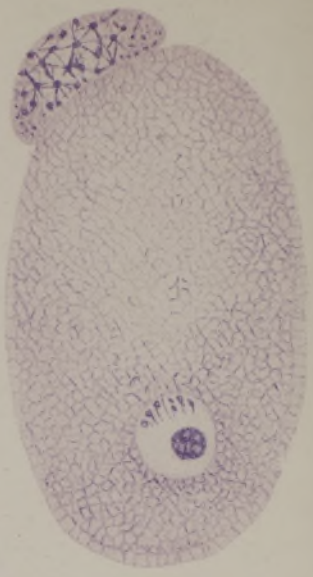

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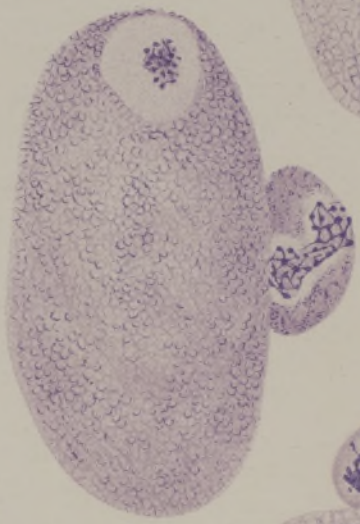

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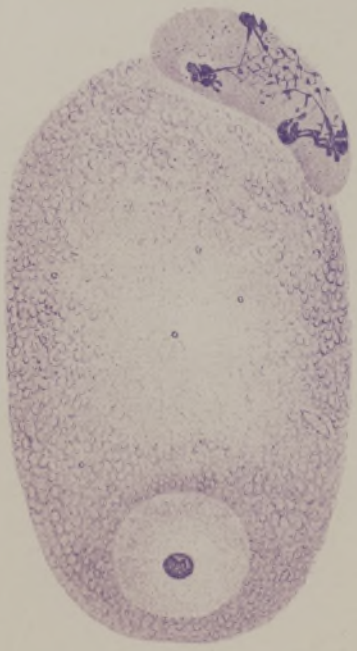

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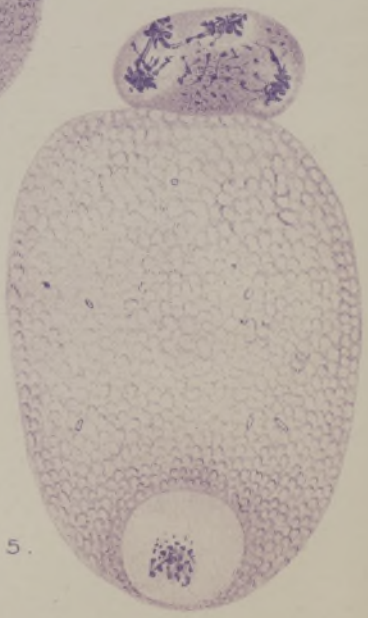

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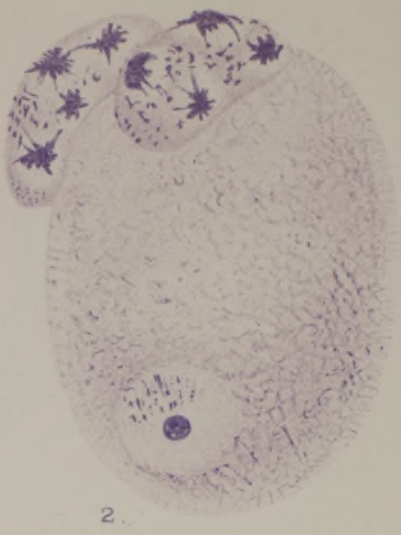

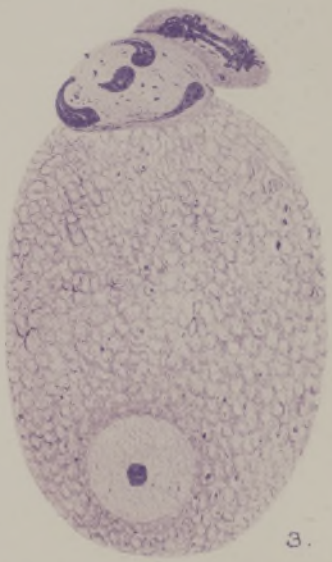

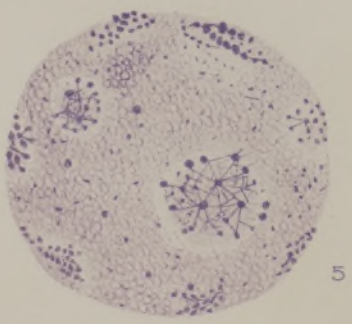

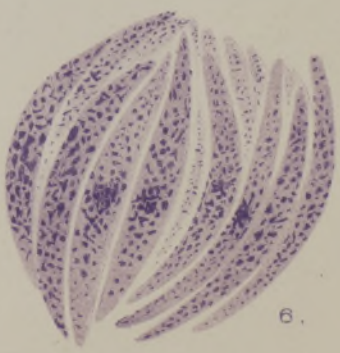

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DESCRIPTION OF PLATES.

[All figures are drawn from permanent preparations stained with hæmatoxylin. Drawings made under a 3-mm. apochromatic objective (Zeiss) with compensating-ocular 12.]

Plate 2.

Fig. 1.—Microgametocyte associating with macrogametocyte (lying free in gut). Karyosome of microgametocyte has partially broken up to form a chromidial net.

Fig. 2.—In this figure the chromidial net is seen distributed throughout the whole of the microgametocyte.

Fig. 3.—The chromidial net of the microgametocyte has become gathered together into a dumbbell-shaped network.

Fig. 4.—Stage in formation of four nuclei. The developing nuclei are still connected with the chromidial net.

Fig. 5.—The four nuclei are showing signs of becoming separated as star-shaped masses of chromatin. Part of the network is still visible.

Plate 3.

Fig. 1.—Association of two microgametocytes with a single macrogametocyte. A chromidial net is seen in both the former.

Fig. 2.—Association of two microgametocytes (each containing four characteristic star-shaped nuclei) with one macrogametocyte.

Fig. 3.—Two microgametocytes associating with one macrogametocyte. In one, four nearly ripe microgametes are seen; in the other, a dumbbell-shaped network is present.

Fig. 4.—Four mature male gametes formed. A chromatic spindle is seen in the macrogamete.

Fig. 5.—Macroschizont, the nuclei in the form of networks. Numerous chromatin particles are scattered through the cytoplasm.

Fig. 6.—Group of macromerozoites with nuclei in a chromidial condition.