OBSERVATIONS ON A NEW GREGARINE, *STYLOCEPHALUS BAHLI*, SP. NOV. FROM THE ALIMENTARY CANAL OF AN INDIAN BEETLE, *GONOCEPHALUM HELOPIOIDES* FRM.

By P. L. Misra, *M.Sc.*

(Zoological Research Laboratory, University of Lucknow.)

(Plate III.)

Contents.

	Page.
Introduction	43
Material and Methods	44
The life-history of *Stylocephalus bahli*, sp. nov.	45
(*a*) Development of the young trophozoite	45
(*b*) The structure of an adult trophozoite	46
(*c*) The sporonts	50
(*d*) Association	51
(i) Normal association	51
(ii) Abnormal association and encystment	53
(*e*) Gamete-formation and anisogamy	53
(*f*) Fertilisation and spore-formation	56
(*g*) Structure of the spores and formation of the sporozoites	57
(*h*) Dehiscence	58
(*i*) Probable mode of infestation	58
Seasonal intensity and site of infestation	59
Polynuclearism	59
Hyperparasitism	59
(*a*) Cytoplasmic Parasitism	59
(*b*) Nuclear Parasitism	60
Effect of the parasite upon the host	60
Systematic Position	61
Movement	61
(*a*) Observations on the movement of *S. bahli*, sp. nov.	61
(*b*) Discussion	66
Summary	68
Acknowledgments	69
References	69

Introduction.

In October 1938, while examining the alimentary canal of the common beetle *Gonocephalum helopioides*, I found that it was heavily infested by a gregarine belonging to the genus *Stylocephalus*[1] which has so far not been recorded from India. A study of the structure and life-history of this gregarine presented several features in which it differs from other species recorded so far, and I have therefore instituted for

[1] As the name *Stylorhynchus* was pre-occupied, *Stylocephalus* has been substituted for it by Ellis (1912).

this gregarine a new species, which I have associated with the name of my Professor Dr. K. N. Bahl of the Lucknow University.

MATERIAL AND METHODS.

From October to March specimens of the beetle *Gonocephalum helopioides* were collected from the University grounds at Lucknow, but during May and June they were found only in moist places, *e.g.*, under shrubs on the banks of the river Gumti. During the rains they become scarce but can be found in hollows of trees and in heaps of cattle dung.

After removing the elytra and clipping off the head and the posterior end of the abdomen, the entire gut was removed from the posterior end. The parasites are sometimes seen even without opening the gut, particularly in starved specimens. The gametocysts are also easily detected within the intestine and the rectum. For making smears the gut was teased in a drop of normal saline solution, the parasites taken out, dried in air for a couple of minutes and fixed in Schaudinn's fluid (half an hour), Carnoy's mixture (five to ten minutes), Sublimate-acetic (seven to twelve minutes) or Bouin's fluid (two to three hours). Ehrlich's haematoxylin, Delafield's haematoxylin and Mann's methyl-blue-eosin were used as stains for the smears. The gametocysts were fixed in the same fixatives and stained in Heidenhain's iron-alum haematoxylin. For sectioning, the gametocysts were fixed in Dobell's modification of Bouin's fluid (one hour on the paraffin bath and twenty-three hours at the room temperature), sectioned at 1-3 μ and stained with iron-alum haematoxylin. Liver tissue was tried as a support but it did not prove satisfactory. I therefore injected the cysts by means of a fine pipette into a piece of mid-gut. It is not essential to tie the cut-ends of the gut. At first, a certain quantity of the fixative was injected into the gut to avoid the action of the gastric juice upon the spores in those cases in which the cyst had automatically ruptured and the spores had come out, and then by a careful manipulation, the cysts or the chains of spores with the cystic wall could be lodged within the mid-gut, which was itself immersed into the fixative. To study the endogenous stages of development, the infected gut was fixed in Brasil's modification of Bouin-Duboscq fluid, Dobell's modification of Bouin, Sublimate-acetic (acetic acid four per cent.) and Gilson's mixture. Washing, dehydration and clearing were carried out in the usual way and paraffin was used as imbedding medium. Sections were cut 2-6 μ thick and were stained with Heidenhain's haemotoxylin or its modification by Dobell and counter-stained with eosin, Orange-G, Van Gieson's picro-säurefuchsin and Chromotrop 2 R. Giemsa's stain and Mallory's triple stain were also tried but they did not yield good results. Of all the preparations I find that those fixed in fixatives containing picric acid and stained with Heidenhain's haematoxylin and Chromotrop 2 R gave by far the best results.

" Culture " of the cysts :—The gametocysts could easily be collected from the faecal matter of the host (their detection being facilitated by using a piece of black paper or black porcelain background) by soaking the faeces in water and pipetting off the cysts. After washing thorough-

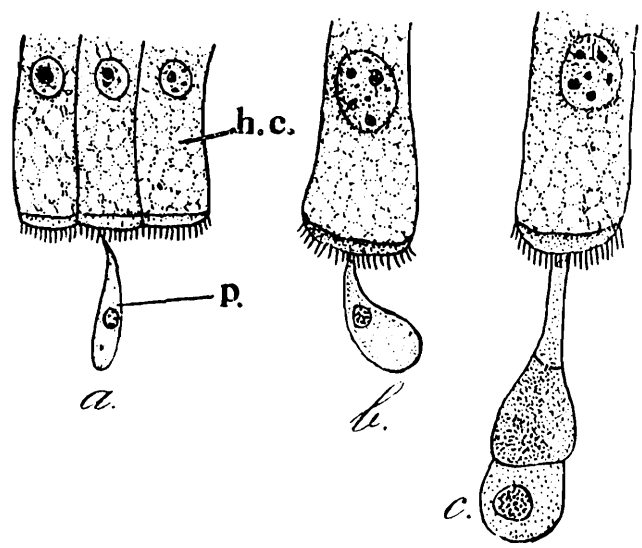
ly in distilled water, the cysts were kept in a drop of normal saline on a coverslip which was inverted upon a cavity slide and placed in a moist chamber. Observations upon cysts in these hanging drops were recorded every fourth hour and cysts at particular stages were fixed for further examination.

Other methods, for example, those adopted for studying the movement of the gregarine, the effect of certain acids and alkalis upon movement, the movement of microgametes, etc., are dealt with in their respective places in the text.

THE LIFE-HISTORY OF *STYLOCEPHALUS BAHLI*, SP. NOV.

(*a*) *Development of the young trophozoite.*

After its liberation inside the lumen of the gut of the host the sporozoite makes its way towards an epithelial cell, secures an attachment by penetrating its rostral end (text-fig. 1*a*) into the cell-wall and commences its development at the expense of the nutrient material of the parasitised cell. The cause of the diffluence of the parasitised cell-wall still remains undetermined although it is generally held that certain toxins produced by the sporozoite are responsible for it. Léger and Duboscq (1903) have described and figured intra-cellular development in *Stylorhynchus longicollis*, in which the sporozoite makes its way into the cell, grows inside it for a certain duration and then evaginates, after which it remains attached to the host-cell as a cephalont. In



TEXT-FIG. 1.—*a*. A sporozoite of *Stylocephalus bahli* attached to an epithelial cell of the host's gut: × 1500. *b*, *c*. Developmental stages of the trophozoites: × 1500.

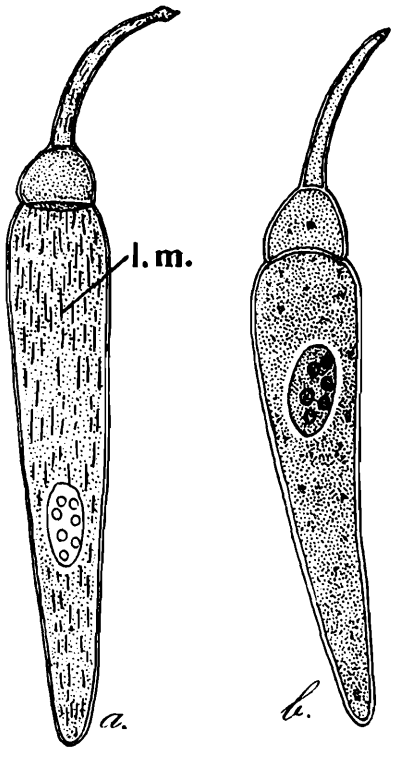
h.c., host-cell; *p.*, parasite.

Stylocephalus bahli, however, no intra-cellular stage could be seen. The parasite grows all the time extra-cellularly. The rostrum of the parasite within the cell-wall forms primarily an attaching organ, the

epimerite (text-fig. 1 *b*, *c*). Later, this function of attachment is, in all probability, superseded by its capacity to absorb the nutrient material out of the host-cell because as growth proceeds the parasitised cell becomes completely disfigured. The youngest trophozoite I could come across measured 15 μ in length and showed faint indications of compartments (Pl. III, fig. 1). As growth proceeds, the compartments become well-defined into epi- proto- and deuto-merites. In younger stages the protomerite is longer than the deutomerite and possesses a comparatively denser cytoplasm. Later, however, the deutomerite becomes enormously dilated and further growth leads to its prolongation, whereby it remains widest immediately behind the septum and tapers gradually towards its posterior extremity. At this stage the host-cell becomes degenerate having its nucleus more or less crumpled and its cytoplasm less dense than that of a normal cell.

(*b*) *The structure of an adult trophozoite.*

The body of a full-grown trophozoite (text-fig. 2 *a*, *b*) is elongated and is divided by septa into epi- proto- and deuto-merites. The epimerite is a hollow, tube-like structure, consisting of two parts: (1) a

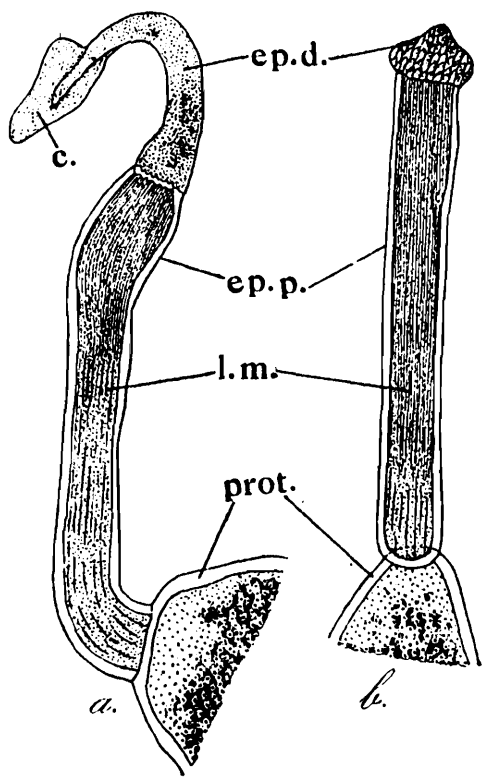


TEXT-FIG. 2.—*a*. An adult cephalont of *S. bahli* examined *in vivo*: ×150. *b*. A cephalont fixed in alcoholic Bouin and stained with Heidenhain's haematoxylin: ×168.

l.m., longitudinal myonemic striations.

proximal and (2) a distal, agreeing in this respect with the epimerite of *S. gladiator* (Blanchard) Watson, but differing from all the other

species of this genus. The distal part is a tongue-like process which remains in intimate contact with the host-cell (text-fig. 3 *a*); when torn apart from its moorings, a crown-like fringe—the torn parts of the host-cell—comes off along with it and obscures the details of its structure. Only rarely could an epimerite be secured, in which its distal end did not carry the remains of the parasitised cell, and in such a case a minute ring could be identified at its extreme distal end. In certain cases well-defined protruberances could be noticed at the end of the epimerite when it was in a contracted condition (text-fig. 3 *b*). The proximal part is retractile and hyaline in appearance. Several longitudinal fibrillae (text-fig. 3 *a*, *b* ; *l.m.*) are seen running along the whole length of the proximal portion of the epimerite to which they impart its power of retractility.



Text-fig. 3.—*a*. Portion of a freshly detached cephalont of *S. bahli* : ×850. *b*. Showing contracted distal portion of the epimerite : ×850.

c., torn off portion of the parasitised cell ; *ep. d.*, distal portion of the epimerite ; *ep. p.*, proximal portion of the epimerite., *l.m.*, longitudinal myonemes ; *prot.*, a portion of the protomerite.

The protomerite of an adult trophozoite is typically broader than long and is conical or sub-conical in shape. Thus it differs from that of the other species of *Stylocephalus*. It is separated from the deutomerite by a fairly thick septum forming a distinct constriction. The cytoplasm has the same characters as those of the deutomerite (*vide infra*) except that it is less dense and does not contain big granules. Sometimes patches of chromatin material are present in its substance. The following tables give the measurements showing the ratio of the length of the protomerite to the total length and also the ratio of its width to that of the deutomerite.

TABLE 1.—*Showing the ratio of the length of the protomerite to the total length of the body.*

Length of the protomerite (L. P.) in microns.	Total length of the body (L. T.) in microns.	Ratio of the length of the protomerite to the total length (L. P. : L. T.).
20	160	1 : 8
20	900	1 : 45
30	162	1 : 5·4
40	1,010	1 : 25·2
48	1,008	1 : 21
50	1,045	1 : 20·9
58	1,054	1 : 18·1
66	896	1 : 13·5
75	1,100	1 : 14·6
75	1,100	1 : 14·6
	Average L. P. : L. T. ::	1 : 18·63

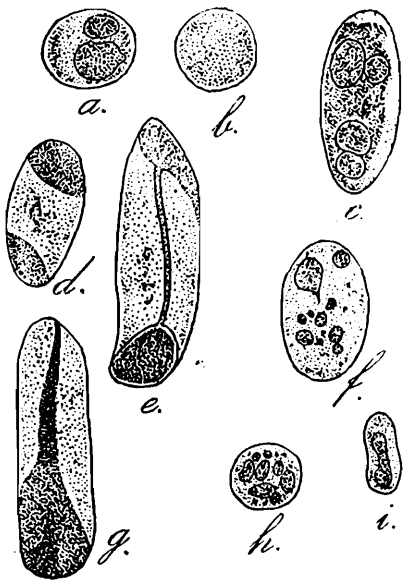
TABLE 2.—*Showing the measurements and ratios of the width of the protomerite to the width of the deutomerite.*

Width of the protomerite (W. P.) in microns.	Width of the deutomerite (W. D.) in microns.	Ratio of the width of the protomerite to the width of the deutomerite. (W. P. : W. D.)
20	30	1 : 1·5
60	76	1 : 1·2
66	91	1 : 1·3
66	91	1 : 1·3
72	120	1 : 1·6
72	120	1 : 1·6
83	94	1 : 1·1
83	132	1 : 1·5
83	132	1 : 1·5
33	40	1 : 1·2
	Average W. P. : W. D. ::	1 : 1·38.

The deutomerite is the largest segment of the body and is circular in cross-section. It is broadest immediately behind the septum and

gradually tapers towards the posterior end but is never sharply pointed. The pellicle is 2·5 μ in thickness. Epicytial longitudinal striations are very clearly discernible in the living condition (text-fig. 2 *a*). The cytoplasm of the deutomerite consists of a semi-fluid matrix, charged with numerous granules of a brownish colour, some of which are fairly large in size. In between these granules there are often certain other granules which are smaller in size and appear pinkish in colour by reflected light. Usually with strong fixatives the general appearance of the cytoplasm appears entirely different from that in the living condition.

In the living condition the nucleus in the deutomerite appears as a translucent area situated anywhere between the septum and the posterior end of the body. Actually it moves from one place to another following the streaming movement of the protoplasm, but its speed is much slower than that of the cytoplasmic current. Further, it may not make a complete circuit but may make a short cut and come back



TEXT-FIG. 4.—*a-i*. Nuclei of *S. bahli* showing various types of accumulations of the chromatin material : × 850.

to the position from which it started. How its movement is controlled is not clear. Ray and Chakravarty (1933) have mentioned a " system of tethering threads " attached to the concave surface of the half-moon-shaped nucleus of *Monoductus lunatus* and maintain that the contractions and relaxations of these threads are responsible for determining its shape and position in the deutomerite. No such arrangement for adjusting its location could be detected in the nucleus of *Stylocephalus bahli*, and the change of its position, presumably, appears to be passive.

The nucleus is very variable in size, shape and structure. In a young cephalont it is spherical, subspherical or ellipsoidal in shape and its size is comparatively bigger in relation to the body as compared with that of the adult forms. A normal full-grown nucleus is always ellipsoidal and measures on an average 57·5 μ × 35 μ. It has a distinct nuclear membrane surrounding the nucleoplasm in which several karyosomes are clearly discernible in the living condition (text-fig. 4 *c*, *f*, *h*). Their number varies from two to ten. The nuclear

membrane takes a deep stain with iron-haematoxylin and appears to be chromatinic in character. In certain cases its boundary was seen to be irregular and shrunken, but this seems to be due to the effect of fixation. The nucleoplasm does not appear uniform in character, certain portions being denser and more granular and exhibiting a stronger affinity for chromatin stains than the others. Sometimes, however, probably prior to nuclear division, the karyosomes were seen to be absorbed and the chromatin masses accumulated at the two ends of the nucleus (text-fig. 4 *d*). These two darkly stained areas might get connected by a thin strand which also stained deeply with chromatin dyes (text-fig. 4 *e*). Further, the accumulation of the chromatin mass might be on one side only, presenting thereby a " geflammte " appearance inside the nucleus (text-fig. 4 *g*), or there might be an intermediate condition between these two extremes, *i.e.*, at one end the accumulation would be very dense and at the other very faint. The significance of these variations in the arrangement of the chromatinic substance in the nucleus is not clear.

(*c*) *The sporonts.*

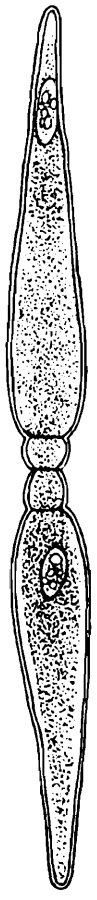
The sporonts can be distinguished from the cephalonts by the absence of the epimerite and by their being charged with greater quantity of reserve granules, which lend the cytoplasm a dark-blue appearance. Further, the sporonts are more inert than the cephalonts as regards locomotion. The sporonts of *S. bahli* are characteristically solitary and measure 200-2000 μ in length. The ratio of the length of the protomerite to the total length in an individual of maximum size is 1 : 37·5. The protomerite is broader than long and the ellipsoidal nucleus lies in the elongated deutomerite, which in its general outline is of the same shape as that of the adult trophozoite. After moving solitarily for some time the sporonts generally unite in pairs and encyst.

As regards the detachment of the epimerite from the protomerite at the time of the formation of the sporont, previous workers have expressed different opinions. For example, Duke (1910) says, " Just at the line of junction between the protomerite and epimerite a bubble-like vacuole appears, which gradually increases in size. .. Having reached a diameter about equal to that of the protomerite the vacuole bursts, and the gregarine is suddenly deprived of its epimerite " Further she says, " This vacuole-formation. in my opinion has a probable bearing on the mooted question regarding the fate of the gregarine epimerite, in the transition from cephalont to sporont." Thus she means that vacuole-formation causes detachment of the epimerite. On the contrary, my observations on *Stylocephalus bahli* prove that the formation of a bubble at the hind end of the detached epimerite and the front end of the protomerite is an after-effect of detachment rather than its cause, and is due to the interaction between two dissimilar media, the saline solution and the cytoplasm. Personally, I believe that it is normally the ageing and its effect on metabolic activities which are responsible for the separation of the epimerite. Léger and Duboscq (1902) have also recorded vacuole-formation in *Pyxinia mo-*

buszi. Frenzel[1] in his observations on several cephalonts, came across some individuals with only a small projection which represents the epimerite on the protomerite ; he concludes that the epimerite slowly degenerates and is absorbed in the same way as a tadpole's tail is absorbed during metamorphosis. A sudden disappearance of the epimerite, according to him, is pathological. Lühe (1904) is of opinion that the falling off of the epimerite is a typical method of cephalonts becoming free. My observations on *S. bahli* coincide with those of Frenzel as I have seen extruded and the so-called absorbed epimerites in the same smear. The variable lengths in the epimerites of *S. bahli* at least are not due to their varying degrees of absorption, but to their degree of retractility. It was noted that when teased the epimerites, either by contact with needles or by the strain imposed upon them by setting apart from their host-cells, retracted partially or wholly, and it is this power of retractility which is responsible for the variations in the lengths of the epimerites.

(*d*) *Association.*

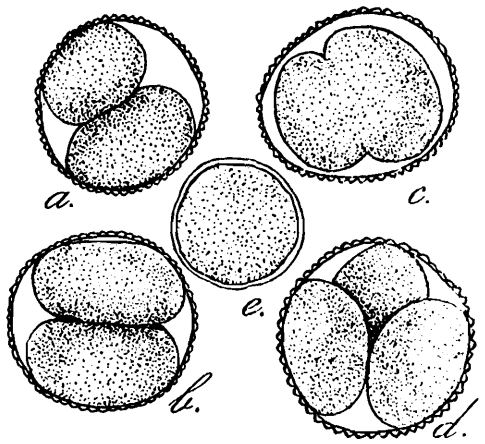
(i) *Normal association.*—Two mature sporonts first associate by their anterior ends (text-fig. 5) forming a pair which keeps moving



Text-fig. 5.—Sporonts of *S. bahli* in association : ×190.

[1] As quoted by Duke, H. L., *Quart. Journ. Micros. Sci.* LV, p. 268 (1910).

for some time and then the two begin to approach closer and closer by their posterior ends and ultimately round off in a common cyst secreted from their outer surfaces (text-fig. 6 *a*, *b*, *c*). While rounding a process of concentration of cytoplasm takes place as is evinced by the diameter of the cyst which becomes much less than the length of the associates. In due course, elevations arise on the cystic wall forming numerous pointed, chitinoid protuberances. An examination of



TEXT-FIG. 6.—Gametocysts of *S. bahli* seen in fresh faecal matter: ×90.

a-c. represent normal association; *d.*, a triple association; *e.*, single individual encysted and devoid of chitinoid protuberances on the cystic wall.

freshly formed cysts revealed that the contained gametocytes are either of the same or of unequal size (text-fig. 6 *a*, *b*). This inequality of size indicates sexual differentiation of the sporonts. Still more cogent and convincing proof of sex-differentiation, however, becomes available on an examination of permanent preparations of these cysts in which nuclear division has taken place (Pl. III, fig. 7). The nucleus of the male gametocyte divides earlier and with greater rapidity than that of the female. Thus, dissimilarity begins at this stage and leads to an undoubted case of anisogamy, in which the male contains only the motile microgametes and the female non-motile macrogametes.

As regards unequal gametocytes encysting together in a common cyst, Woodcock (1906) says, "Probably, in any case, the associates require to be of about equal age and size if the union is to be successful." Berndt (1902) says that no true cyst-wall is formed in those cases of syzygy in which the members are of considerable difference, and ultimately they die off. Siedlecki, Léger and Brasil have quoted examples where the sporoblasts have been produced by one member of the couple in the usual manner, the other remaining stationary and ultimately dying off. Further, Woodcock (1906) says: "One gregarine of a couple can in certain cases, apparently, exert sufficient influence upon its associate to induce it to commence sporulation, although it itself may not be ripe enough to do so and as a result not only does it not benefit by the stimulus or "Reiz" of the other, but, this on the contrary, appears to harm it and it succumbs instead." My observations on *S. bahli* do not absolutely conform with the views and observations of the authors mentioned above. No doubt cysts containing unequal associates can be seen to disintegrate at times, but the dis-

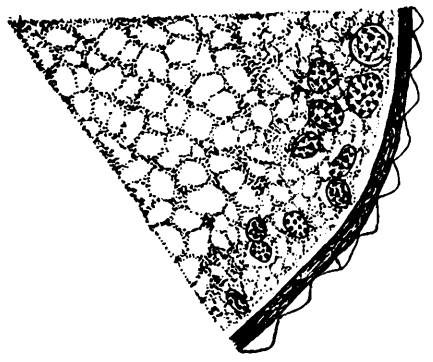
integration is not confined to them only. Couples of equal sizes have also been seen by me to disintegrate, probably on account of certain inhibitory factors. The formation of gametes, their mutual fusion, zygote- and spore-formation was clearly noted even in those cases where one member of the couple was approximately half in diameter as compared with its partner. Nuclear division was, however, set up earlier in the smaller member and microgametes were formed out of its substance indicating that it was really the microgametocyte. The only difference that could be detected in cases of unequal association lay in the size of the residual cytoplasm of the gametocyst which in this case was smaller in comparison to that found in equal but fully matured associates. Usually, the normal gametocysts measure 208 μ—352 μ × 80 μ—320 μ and are spherical, sub-spherical, or egg-shaped in appearance (text-fig. 6 *a*, *b*, *c*).

(ii) *Abnormal association and encystment.*—Not infrequently some gametocysts taken from the faecal matter within the rectum or outside were found to possess three individuals encysted together (text-fig. 6 *d*). Such abnormal cysts have already been recorded by Kunstler (1892) in *Diplocystis schneideri*, Cuénot (1901) in *Diplocystis* sp., Berndt (1902) in *Gregarina cuneata*, Woodcock (1906) in *Cystobia irregularis*, Cunningham (1907) and Robinson (1910) in *Kalpidorhynchus arenicolae*, Bastin (1919) in *Monocystis agilis*, Mary Vincent (1922) in *Pyxinia anobii*, Bhatia and Setna (1926) in *Monocystis matthai*, and Setna and Bhatia (1934) in *Hirmocystis parapeneopsisi*. Cuénot has recorded instances of triple association, which are very rare, in *Diplocystis*, one of which had apparently produced sporoblasts; but Woodcock (1906) remarks, "Judging from his (Cuénot's) fig. 47, however, sporulation would not seem to have been successful, the sporoblasts being extremely minute and scarcely visible, very different from the well-developed layer in the normal cysts figured" Bastin has seen similar abnormal cysts of *Monocystis agilis*, and was able to notice the formation of gametes. Bhatia and Setna have detected fully-developed spores in a case of triple association in *Monocystis matthai* and thus have supported Cuénot's and Bastin's observations. On no occasion could I observe in the triple association of *S. bahli* either complete gamete-formation or any other advanced stage of sporulation. Only twice nuclear divisions were seen to have taken place partially in two out of the three associates, but in all cases they ultimately degenerated.

Encystment of a single individual has also been encountered at times by watching mature sporonts in saline on a slide. After moving for some time the sporont becomes less vigorous, contracts gradually, till it becomes globular and then secretes a wall round itself (text-fig. 6 *e*). The whole process takes from one-and-a-half to four hours when kept in normal saline solution. No protuberances could be seen on the wall of such an encysted individual. Such individuals ultimately perish and in this respect my observations confirm those of Brasil (1905) who recorded a similar fate for solitary encystment in *Gonospora* and *Urospora*. Siedlecki, Cuénot, Berndt, Léger and Cunningham, on the other hand, have asserted that they never encountered cases of solitary encystment.

(*e*) *Gamete-formation and anisogamy.*

After encystment the nucleus of each gametocyte soon prepares to divide. The nuclear membrane disappears and the chromatin mass becomes comparatively dense. The actual chromosome cycle could not be traced, but it was noticed that the nucleus of each gametocyte repeatedly divides into several daughter nuclei, which subsequently migrate to the periphery (text-fig. 7). This division, as already men-



TEXT-FIG. 7.—Portion of the section of the gametocyst of *S. bahli* showing nuclear migration to the periphery: × *ca.* 1000.

tioned, begins and is completed earlier in the male gametocyte than in the female (Pl. III, fig. 7, *m.*, *f.*). The cytoplasm of each gametocyte segments around each nucleus and thus the gametes are formed. The whole process of gamete-formation takes from eight to twenty hours from the time the freshly extruded cysts are kept in the moist chamber. This duration depends chiefly upon temperature, as I find that the period decreases with the rise of temperature, the optimum results having been obtained at 37°C.

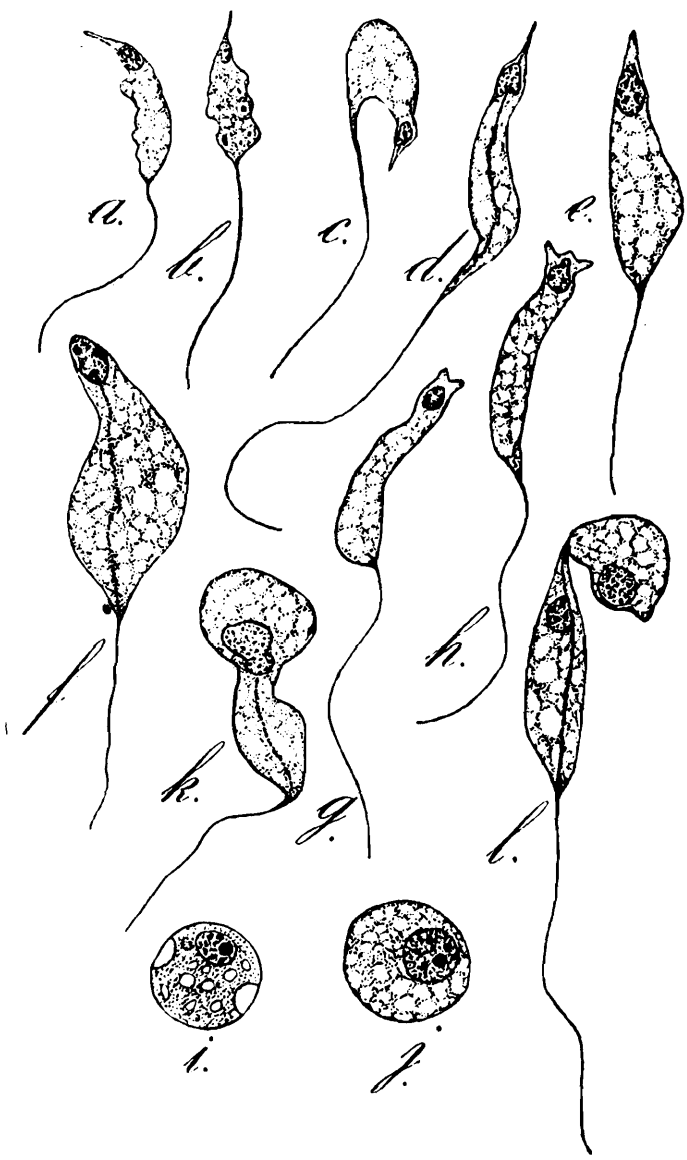
Anisogamy:—An examination of smears obtained by puncturing the mature gametocysts revealed that the gametes could be distinguished into: (i) the microgametes, and (ii) the macrogametes.

(i) The microgametes:—All the microgametes are not of the same kind. On a close examination three types can be distinguished, *viz.*,

(*a*) Normal, fusiform, but sterile microgametes.
(*b*) Normal, pyriform, fertile microgametes.
(*c*) Abnormal microgametes.

A fresh preparation, in a slightly albuminated physiological saline, of a ruptured gametocyst in which gametes have formed, shows that there are two kinds of actively moving microgametes: (i) The first kind of microgametes are fusiform in shape and are fewer in number than (ii) the second type, which are pyriform in shape, 14 to 21 μ in length and more abundant but less active than the first type. The fusiform microgametes have generally two or three prominences (text-fig. 8 *g*, *h*) on the head and are sterile (*vide infra*); while the pyriform microgametes (text-fig. 8 *a-e*) have only one anterior prolongation, the perforatorium on their heads, and are fertile and appear to possess an acute sensitivity for tracing out the receptive spot of the macrogamete (Pl. III, fig. 2, ×).

Fixed and stained preparations revealed the nucleus of the microgametes lying within the head, and containing three to five deeply staining bodies in a homogeneous and faintly staining nucleoplasm. The cytoplasm is vacuolated and granular in character. An axial filament, easily seen to arise from the perforatorium (text-fig. 8 *d*, *l*), traverses the whole length of the body and continues behind as a whip-like tail, which helps the microgamete in its movement.



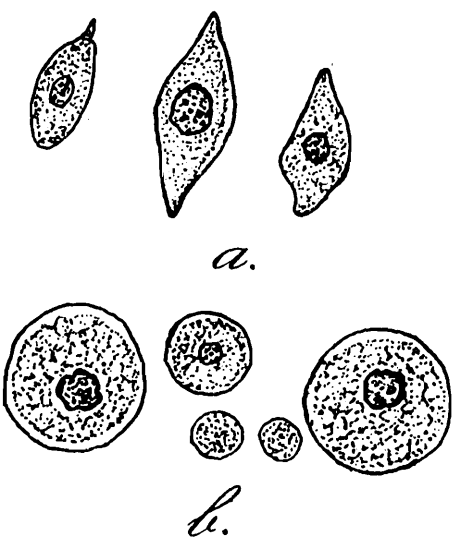
TEXT-FIG. 8.—*a-e*. Normal, pyriform microgametes of *S. bahli* : × 1333. *f*. An abnormal microgamete : × 1333. *g*, *h*. Fusiform, sterile microgametes : × 1333. *i*. A macrogamete : × 1750. *j*. An abnormal macrogamete : × 1750. *k*. Fusion of a microgamete with a macrogamete : × 1750. *l*. A microgamete attached to a zygote : × 1375.

Further, abnormal microgametes (text-fig. 8 *f*) have also been encountered. These are distinguished by their bigger size, blunt anterior end and a fairly stout and stumpy posterior tail. They degenerated ultimately.

(ii) The macrogametes :—They are more or less spherical bodies, measuring 6 μ to 8 μ in diameter. The spherical nucleus is 2 μ in diameter with a well-marked nuclear membrane ; it possesses an eccentrically placed karyosome and four or five chromatoid bodies (text-fig.

8 *i*). The cytoplasm is very much vacuolated and is packed up with reserve granules. There are two thin, hyaline areas at the periphery forming receptive spots, which permit as well as facilitate the entrance of the microgametes. Sometimes, abnormal macrogametes (text-fig. 8 *j*), comparatively bigger than the normal ones, were also met with in some of the preparations.

Degenerating gametes:—Some preparations revealed small bodies which on closer examination proved to be degenerating gametes of varying sizes. Tail-less, spindle-shaped bodies with crumpled nuclei

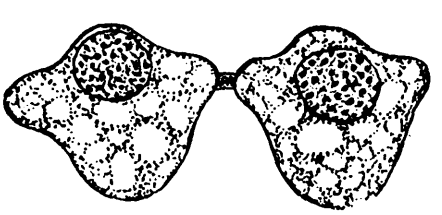


TEXT-FIG. 9.—*a*. Degenerating microgametes: × *ca.* 2800. *b*. Degenerating macrogametes: × *ca.* 2800.

were apparently degenerating microgametes (text-fig. 9 *a*), while other bodies (text-fig. 9 *b*) in which the nucleus had shrunken and the cytoplasm contracted, with a consequent decrease in size were recognised as the degenerating macrogametes.

(*f*) *Fertilisation and spore-formation.*

Coupling of the gametes takes place after the dissolution of the partition between the male and the female chambers when the microgametes rush towards the macrogametes. All the microgametes however, do not go into the female chamber, but there is a displacement of the macrogametes from the female chamber into the male chamber, with the result that coupling can be observed simultaneously in both the compartments. The microgametes agitate violently to and fro, and as soon as they come in contact with suitable partners, mating takes place quickly. The fertile microgametes generally pierce through the receptive spots (Pl. III, fig. 2, ×) of the macrogametes, only one finding entrance into the body of the latter. The nucleus of the microgamete

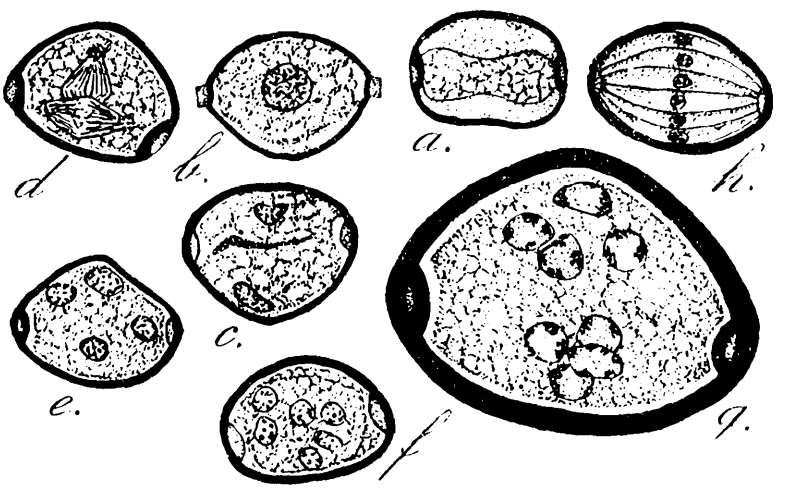


TEXT-FIG. 10.—Two zygotes of *S. bahli* attached with each other: × 4000.

approaches that of the macrogamete and fuses with it (text-fig. 8 *k*). It takes two to eight minutes for a microgamete to fuse completely with the body of the macrogamete. The act of fertilisation being thus accomplished, the fertilised macrogamete or the zygote slightly elongates (text-fig. 10), secretes a wall around it and becomes a spore (text-fig. 11 *a*, *b*). While examining a fresh preparation in which the microgametes were still moving within the cyst, it was found that the fertilised macrogametes became gradually arranged in chains; sometimes enchainment of even unfertilised macrogametes also takes place, in which case the microgametes could be seen making their way into the chains and fertilising the macrogametes there. Some microgametes were observed to remain active from six to fourteen hours after the complete enchainment of spores. These were, no doubt, mostly the sterile microgametes, which later on degenerate. The duration of complete sporulation was noted to be 48-60 hours.

(*g*) *Structure of the spores and formation of the sporozoites.*

The hat- or pouch-shaped spores (text-fig. 11 *a*, *b*) measure 11× 7·5 μ, and are arranged in chains which show a coiling tendency if de-



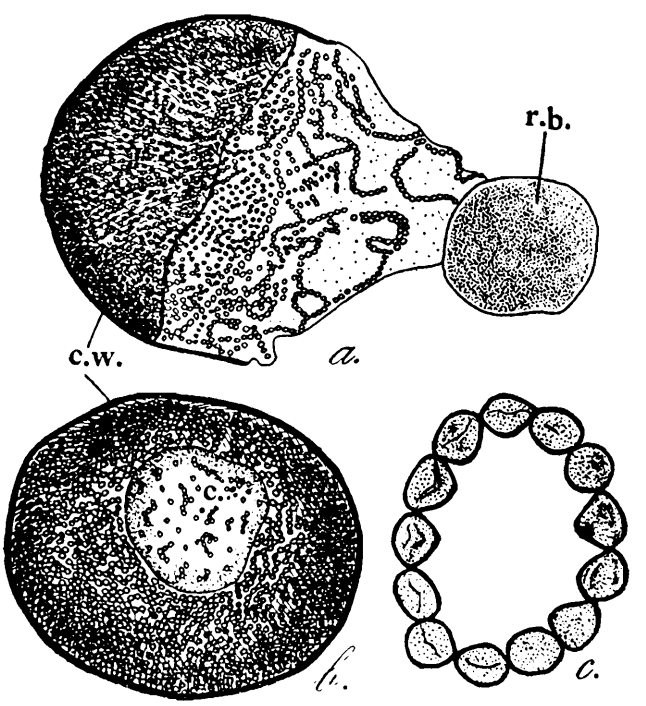
TEXT-FIG. 11.—*a*, *b*.—Spores: ×1727. *c-f*. Spores showing nuclear division: ×1727. *g*. A spore with eight nuclei: ×3454. *h*. A mature spore with sporozoites: ×1727.

tached away from the cyst. The cystic wall is 0·5 μ thick. It is brownish in colour and becomes almost black after the formation of sporozoites. The cytoplasm of the spore is alveolar in appearance and possesses a marked affinity for chromatin stains, indicating the presence of chromatoid granules in it. The nucleus of the spore soon divides mitotically into two (text-fig. 11 *c*), the plane of division being at right angles to the long axis of the spore. The two daughter nuclei again divide mitotically into two each, and thus a quadri-nucleate stage is reached (text-fig. 11 *d*, *e*). At this stage, any two of the four may divide first whereby a six-nucleate stage (text-fig. 11 *f*) can be made out, or all the four may divide simultaneously and form eight nuclei (text-fig. 11 *g*). The latter seems to be the general tendency. At this stage, the chromatin of the daughter nuclei remains concentrated in patches at the periphery and the rest of the nucleoplasm stains faintly. The cytoplasm of each spore segments around the nuclei in such a way that the

segmented bodies, *i.e.*, the sporozoites lie parallel to each other along their long axes and also parallel to the long axis of the spore (text-fig. 11 *h*). Moreover, when viewed laterally, the nuclei of the sporozoites are arranged in a line, at right angles to the length of the sporozoites. Typically the sporozoite is spindle-shaped and measures 10 μ in length and 2 μ in width at its widest central region. Its cytoplasm is homogeneous, while the centrally situated nucleus is more or less spherical and possesses four or five deeply staining granules.

(*h*) *Dehiscence.*

After spore-formation, the residual cytoplasm acquires a wall around it forming a pseudocyst which under favourable conditions of warmth and moisture, swells up and causes the rupture of the cyst. The residual cytoplasm, thus, seems to be hygroscopic in nature. The rupture of the cyst is at times so violent as to liberate not only the spores but



TEXT FIG. 12.—*a*. A ruptured cyst showing the chains of spores: ×46. *b*. A gametocyst in which a lid-like portion has cleft apart leaving an outlet for the spores: ×188. *c*. A chain of spores magnified: ×500.

c., cavity; *c. w.*, cystic wall; *r. b.*, residual body.

also to throw out the cystic residue (text-fig. 12 *a*). On certain occasions, however, it was found that a well-defined lid was thrown off and the spores came out of it (text-fig. 12 *b*). At other times, it was observed that the cystic residue disintegrated and the dehiscence of spores, in such cases, was caused by a simple rupture, presumably caused by the pressure of the fluid formed by the dissolution of the cystic residue.

(*i*) *Probable mode of infestation.*

Infestation is purely accidental and involves only a single host to complete the life-cycle. When the host, *Gonocephalum helopioides* takes in food contaminated with infective spores, the sporozoites are liberated into the gut by the action of the gastric juices upon the spores and make their way towards the epithelial cells of the alimentary canal, where they commence their further development.

SEASONAL INTENSITY AND SITE OF INFESTATION.

The host-gut was found heavily infested during winter. The seat of infestation is usually the mid-gut and the intestine, but in cases of heavy infestation the parasites could be found right from the oesophagus to the rectum and in such cases the lumen of the posterior part of the intestine was entirely occluded by them (Pl. III, fig. 8). From March to June, gametocyst-formation is at its best and this process seems to be correlated with the rise of temperature; its optimum effect being during April, May and June. During July and August sporonts are often met with though not in abundance. An increase in the degree of infestation was noted during the latter part of September and reached its climax in October and November. On an average, 97 per cent. of the beetles were infected.

POLYNUCLEARISM.

There is a great divergence of opinion with regard to the occurrence of the phenomenon of polynuclearism in gregarines. Berndt (1902) has recorded the presence of certain patches, which stain darkly with chromatin dyes, specially in the protomerite. Comes (1907) noted similar patches in *Stenophora* and regarded them as metabolic products, not nuclear in origin. Schellack (1907) has reported the occurrence of darkly-staining areas in the epimerite of *Echinomera hispida*, while Duke (1910) detected such patches throughout the body of *Metamera schubergi*. In *S. bahli* one or two patches, having a strong affinity for chromatin stains, were noted in the protomerite as well as in the deutomerite (Pl. III, figs. 4, 5). However, the occurrence of the so-called several nuclei in the body of the gregarines should be regarded as abnormal. As regards their origin it is difficult to make a suggestion, but it is possible that an increase in the nuclear material causes a disturbance in the kern-plasma relation, whereby nuclear extrusion takes place and the diffused chromatin particles flow along with the cytoplasm and ultimately aggregate into definite patches in a particular part or parts of the organism.

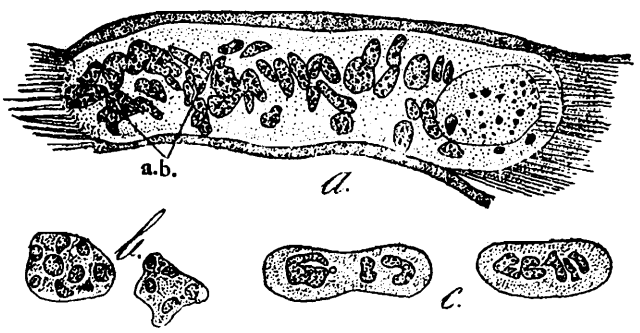
HYPERPARASITISM.

A noteworthy phenomenon of hyperparasitism in *S. bahli* deserves special mention. Under pathological conditions this gregarine appears

to be susceptible to certain infections which may be classed as (*a*) cytoplasmic, and (*b*) nuclear.

(*a*) *Cytoplasmic parasitism.*—A longitudinal or oblique section (Pl. III, fig. 6; text-fig. 13 *a*) of an infected gregarine after staining with Heidenhain's haematoxylin showed the whole of the cytoplasmic area in the deutomerite having been parasitised by numerous multinucleate amoeboid bodies (text-fig. 13 *b*)—probably *Metchnikovella*. These could not be diagnosed as no spores were met with. Their exact systematic position needs further investigation.

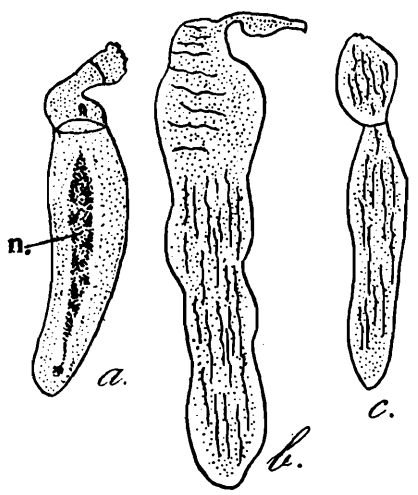
(*b*) *Nuclear parasitism.*—In certain cases nuclei could be observed to have been infected with a fungus, probably belonging to the family



TEXT-FIG. 13.—*a*. An oblique section of the parasite showing multinucleate amoeboid bodies in its cytoplasm: ×*ca.* 950. *b*. Two multinucleate amoeboid bodies magnified: ×*ca.* 2300. *c*. Two parasitised nuclei: ×600.

a. b., amoeboid bodies.

Chytridiaceae (text-fig. 13 *c*). The infection seems to begin at the centre of the nucleus, and to proceed to the periphery, thereby causing a dis-



TEXT-FIG. 14.—*a*. A cephalont of *S. bahli* showing degenerating parasitised nucleus (*n.*): ×90. *b, c*. Degenerating individuals in which the nucleus has vanished: ×65.

solution of the nuclear membrane (Pl. III, fig. 3). The infected nucleus gradually degenerates, and the gregarine loses its metabolic activities, till at last it perishes (text-fig. 14 *a-c*).

Effect of the parasite upon the host.

Inspite of the fact that the host is heavily infested, it does not seem to suffer any serious damage. Only the parasitised epithelial cells of the gut-wall undergo some change and appear abnormal on account of a deficiency in their cytoplasmic contents, and sometimes owing to the atrophy of their nuclei. In some cases, where occlusion occurs, the parasites must be inhibiting the passage of food-material from the anterior to the posterior end of the gut of the beetles. But, nevertheless, the host does not succumb, as it is hardy enough to withstand starvation for a long period during which the parasites encyst and pass down to the rectum leaving the passage clear once more for food.

Systematic Position.

The solitary nature of the sporonts, dehiscence by pseudocyst, spores hat-shaped, brown or black in colour and disposed in chains are characters which assign this gregarine to the family Stylocephalidae Ellis. The epimerite with a dialated papilla at the end of a long slender neck, cysts beset with small papillae and hat-shaped spores determine its assignment to the genus *Stylocephalus*. The species *S. bahli* differs from the hitherto described species of *Stylocephalus* in possessing the following features :—The epimerite is peculiar and consists of two parts : (i) a distal tongue-like portion and (ii) a proximal tubular portion which is hyaline and retractile. In this respect it partially resembles *S. gladiator* (Blanchard) Watson, but differs from the latter in having the apical portion of the epimerite not longer than the rest of the body in the adult condition, and also in possessing a fairly large size ; the maximum size of *S. gladiator* being 720 $\mu \times 70$ μ, while that of *S. bahli* is 2000 $\mu \times 98{\cdot}7$ μ. The protomerite of *S. bahli* is broader than long and is conical or sub-conical in shape, hence it differs from others in this respect. Further the nucleus of *S. gladiator* is ovoidal and contains a single karyosome, whereas the nucleus of *S. bahli* is ellipsoidal and contains several karyosomes. Thus it resembles, in this respect, *S. oblongatus* and *S. longicollis*, but differs from them in other respects, *e.g.*, in the shape of the protomerite, the shape of the cyst, the size of the spores, etc.

The following table (on pp. 62 and 63) shows the various points of resemblance and difference between *S. bahli* and other species of this genus which have been previously described.

To sum up, the specific characters of *S. bahli* are as follows :—Sporonts solitary, elongate, maximum size 2000 $\mu \times 98{\cdot}7$ μ ; L. P. : L. T. : : 1 : 37·5 in maximum-sized individual ; epimerite elongated, hollow and tubular consisting of a retractile proximal and a tongue-like distal portion ; protomerite conical or sub-conical ; L. P. : L. T. : : 1 : 18·63 ; W. P. : W. D. : : 1 : 1·38 ; deutomerite broadest behind the septum and gradually tapering posteriorly ; pellicle 2·5 μ thick ; endocyte brown in cephalonts, dark-bluish in sporonts ; nucleus ellipsoidal with several karyosomes ; cysts spherical, sub-spherical or egg-shaped ; dehiscence by pseudocyst or simple rupture ; spores hat-shaped, dark-brown or black, $11 \times 7{\cdot}5$ μ.

Characters.	*S. oblongatus.*	*S. longicollis.*	*S. brevirostris.*	*S. gladiator.*
1. Sporonts	Solitary, elongate, maximum length 3000 μ.	Solitary, elongate, measurements not mentioned.	Solitary, stout bodied, maximum size not mentioned.	Solitary, elongate; maximum size 720 μ.
2. Epimerite	A thick cylindrical neck with a terminal dilated portion with papilla on extremity; whole epimerite being one-and-a-half to twice the length of the protomerite alone.	A long slender cylindrical neck terminating in a slightly dilated papillate anterior end; the whole being three or four times the length of the protomerite alone.	A small xiphoid coinoidal tongue projecting upward from the centre of the protomerite, whole length being equal to half that of the protomerite.	Consists of two parts: (i) a very long slender neck and (ii) a dilated xiphoid-shaped apical portion, often longer than the whole body.
3. Protomerite	Globular, constriction at septum.	Pentagonal in lateral optical view, truncate at apex; slight constriction at septum; width equal to length.	Cylindrical, of nearly equal width throughout, corners rounded at anterior end; no constriction at septum; width equal to length.	Short and globular.
4. Deutomerite .	Cylindrical, tapering slightly from middle, ending in a rather slender blunt posterior extremity.	Elongate, cylindrical, tapering in posterior two-thirds and ending in a rather blunt point.	Just below the septum it is a little wider than the protomerite and tapers to a rather sharp point.	Elongate, cylindrical, with a slender attenuated extremity, bluntly pointed.
5. L. P. : L. T.	1 : 6 to 1 : 8	1 : 10	1 : 4	×
6. W.P. : W.D.	1 : 2	1 : 1·1	1 : 1·2	×
7. Nucleus	Ellipsoidal with several karyosomes.	Ellipsoidal with several karyosomes.	Spherical with 6 to 9 small karyosomes.	Ovoidal with one karyosome.
8. Endocyte	Yellowish in cephalonts becoming black in sporonts.	Dense	Not described	Not described.
9. Cyst	Irregularly spherical, with slight depressions and protuberances.	Irregularly spherical, surface covered with indentations and papillae.	Unknown	Unknown.
10. Spores	Brown, united in chains; 7 μ in length.	Same as in *S. oblongatus.*	Ditto	Ditto.
11. Host	*Opatrum sabulosum* (L), & *Ascida grisea* (F).	*Blaps mortisaga*	*Hydrophilus* sp., larva	*Helenophorus collaris* L.
12. Habitat	Intestine	Intestine	Intestine	Intestine.
13. Locality	Paris and Poitiers, France.	Paris	Germany	Grenoble, France.

Characters.	*S. gigenteus.*	*S. insignis.*	*S. eledonae.*	*S. bahli.*
1. Sporonts	Solitary, elongate; maximum size 1800 .μ	Solitary, very elongate; length 1000 μ.	Solitary, maximum length 300 μ.	Solitary, elongate; maximum length 2000 μ.
2. Epimerite	A long pointed cone, situated upon a conoidal projection of the protomerite.	A large flattened disc, depressed slightly in centre crenulate on periphery, longitudinally striated and carrying at base a circle of very many short upwardly directed digitiform processes.	Long and thick style with a small knob at its extremity.	An elongated, hollow, tubular structure, consisting of two parts (i) a proximal neck which is hyaline and retractile and (ii) a distal tongue-like portion. Length on an average two-and-a-half times than that of the protomerite but it never exceeds the total length of the adult gregarine.
3. Protomerite.	Dome-shaped, dilated above the base, and flattened anteriorly; constriction at the septum.	Sub-globose, flattened, twice as wide as high; constriction at the septum.	Hood-shaped or obtuse, cone-like in appearance; stuffed with small reserve granules; constriction at the septum.	Usually broader than long, conical or subconical; constriction at the septum.
4. Deutomerite	Cylindrical, widest at the septum, terminating in an abrupt but sharply pointed cone.	Cylindrical, widest at end of anterior third, flattened at posterior extremity.	Widest shortly behind the septum, tapering to a fully stumpy hinder end posteriorly.	Widest at the septum, tapering gracefully posteriorly, but never ending in a sharp point.
5. L.P. : L.T.	1 : 9 to 1 : 18	1 : 15	1 : 6 to 1 : 7	1 : 18 to 1 : 63.
6. W.P. : W.D.	1 : 1 to 1 : 1·5	1 : 1·3	1 : 1·2 to 1 : 1·4	1 : 1·38.
7. Nucleus	Not described	Spherical with one karyosome.	Relatively small	Ellipsoidal often with several karyosomes.
8. Endocyte	Dense	Not described	Conspicuously big granules present.	Brown in cephalonts becoming dark-blue in sporonts.
9. Cyst	Spherical, diam. 450 μ; entire surface papillated; dehiscence by pseudocyst.	Sub-spherical or sub-ovoidal, diam. 430 × 330 μ; dehiscence by pseudocyst.	Unknown	Spherical, subspherical or egg-shaped; entire surface papillated; diam. 208 μ-352 μ × 80 μ × 320 μ; dehiscence by pseudocyst or simple rupture.
10. Spores	Irregularly sub-spherical, black and measure 11 μ × 7 μ united in chains.	Irregularly hat-shaped, 10 μ long, extruded in chains.	Unknown	Irregularly hat-shaped, dark-brown or black and measure 11 μ × 7·5, μ; united in chains.
11. Host	*Eleodes* sp.; *Asida apaca* Say; *Ascida* sp., and *Eusattus* sp.	*Helops striatus*	(i) *Eledona agaricola*, Herbst. (ii) *Pentaphyllus testaceus*, Hellw. (iii) *Myceto-phagus piceus* Fabr.	*Gonocephalum helopioides* Frm.
12. Habitat	Intestine	Intestine	Intestine	Mld. gut and intestine.
13. Locality	Boulder and Denver, Colo.	Indre-et-Loire, France	(i) Fundort : By Sibyllenort. (ii) Fundort : Militsch. (iii) Fundort : Militsch.	Lucknow, U. P. India.

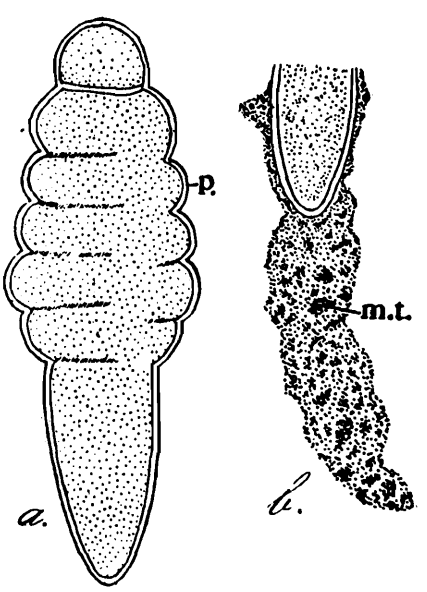
MOVEMENT.

(*a*) *Observations on the movement of* S. bahli, *sp. nov.*

When a piece of infected gut is teased on a slide and the gregarine examined under the microscope it does not move at all in the gut-fluid of the host. In distilled water slow movement can be observed, but only for a short duration. Normal saline is a suitable medium for studying the movements of gregarines, as they can live in it for a longer duration, and for this particular gregarine 0·9 per cent. saline solution proved a better fluid than the usual physiological saline solution.

The epimerite of *S. bahli* is retractile and shows active bending movements, as well as slight longitudinal contortions. When not retracted it moves to and fro and then curls up to form a coil which opens out with a jerk. More often complete bending is not effected and the epimerite moves to the right and and then to the left, as if it is searching for something. The protomerite also moves to and fro but with a greater activity than the epimerite. Sometimes it was noted that it could withdraw partially into the deutomerite and then suddenly sprang out to withdraw again, and this process was repeated several times. These movements can be compared with those of the neck of a turtle which is being partially withdrawn and extruded alternately. Occasionally, the septum was pushed into the protomerite by the onward flow of the cytoplasm of the deutomerite. These movements are, however, not necessarily seen at all times.

Sometimes, neither the epimerite nor the protomerite shows any active movements and the gregarine as a whole glides along passively. Usually *S. bahli* moves forward both by movements of epi-and protomerites and by gliding movements at the same time. When it comes in contact with an obstacle it pauses a little, changes its direction and



TEXT-FIG. 15.—*a*. A specimen of *S. bahli* showing cap-like protuberances (*p.*) : × *ca.* 200. *b*. An individual showing the mucus-tail (*m.t.*) : × 200.

continues forward. In its attempt to put aside obstacles in its way several cap-like projections, (2-12), are formed on the deutomerite

(text-fig. 15 *a*), these protuberances are produced as a result of the pressing of the body against the obstacle lying in front, and when the organism is unable to push that obstacle away, it recoils with a backward jerk or takes a slight turn and makes its way onwards. Probably, it is this backward jerk which certain authors have mistaken for a backward movement. I have never been able to detect in this gregarine a backward movement similar to its forward movements. I have also noted that an individual at times, while gliding, forms a slight curve on its body and then instantaneously straightens out, in which act the body, instead of moving forwards moves at right angles to its long axis; it is this lateral flexion, probably, which Crawley has named " transverse " movement[1].

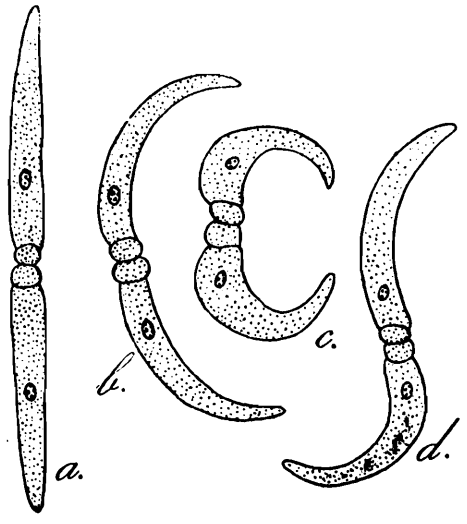
In order to test whether this gregarine could move only when in contact with a surface, half a dozen specimens were kept in saline solution under a wax-legged cover-glass and examined under high power. By changing the focus of the microscope it could be seen that they remained attached to the slide on which they moved. I was unable to see any of the gregarines leaving the slide to move upwards and reach the coverslip, as has been recorded by Crawley (*vide infra*).

Besides these facts, one important observation needs mention here. A fresh preparation in saline after five minutes showed a sticky and elastic tail being formed, presumably by the exudation of a mucoid substance from the body of the gregarine and its subsequent accumulation at the posterior end forming a " tail " To obtain a clear conception of this phenomenon about half a dozen gregarines were washed several times in saline solution, to get rid of the gut-fluid of the host and were kept in saline mixed with carmine suspension. It was observed that as the gregarines moved forward the carmine particles collected at their posterior ends and formed a " tail " (Pl. III, fig. 3, *m. t.*; text-fig. 15 *b*, *m. t.*). The " tail " may not be continuous. This suggests that there is a variation in the quantity of the exudate. It may be mentioned here that the " tail " actually retards the progress of the animal and when it becomes fairly big the organism, inspite of its best efforts to escape from it, succumbs at last. A definite trail or tract is often left behind each individual as it passes onwards.

In biassociative forms in which syzygy takes place by the union of the anterior end of the satellite with the posterior end of the primite, " tail-formation " is clearly visible at the hind end of the satellite, but a change in the direction during progression is steered by the primite; the satellite either helps the primite by moving in the same direction or just follows passively. In case the " tail " grows enormously big, cap-formation occurs in the bodies of the pair or the association snaps, in which case the primite escapes, leaving the satellite doomed to death. When the contact of the two associates takes place by their anterior ends only, forward progression comes to an end sooner or later and a rotatory movement is set up by the two individuals exerting forces in opposite directions. Their posterior ends approach closer and closer

[1] Personally, I consider that additions of such names should be avoided. For instance, " transverse " movements would be inconvenient on the part of an organism having the antero-posterior axis of its body longer than its transverse axis.

(text-fig. 16 *a*, *b*, *c*), and ultimately the pair rounds itself and becomes encysted in a common cyst. The rotatory movements may lead the



TEXT-FIG. 16.—*a*. Two sporonts of *S. bahli* in association; *b*, *c*., same deflected due to opposite forces during progression; *d*., showing an S-shaped deflection due to opposite forces not acting on the same side: × 55.

two individuals in opposite directions, whereby an S-shaped figure (text-fig. 16 *d*) is formed. In such cases it was noticed that after sometime their contact gave way and the organisms became free.

Albuminated saline or diluted glycerine inhibits progression with great rapidity. The action of certain acids, *e.g.*, 0·5 per cent. hydrochloric acid, nitric acid, sulphuric acid and acetic acid, as well as of certain alkalis, *e.g.*, 0·5 per cent. potassium hydroxide, sodium hydroxide, sodium carbonate, etc., proved in every case to be detrimental to progression, and caused death.

(*b*) *Discussion.*

The gregarines can move in a medium different from that of their natural environment, but the various factors bringing about their locomotion have formed a bone of contention amongst workers since the time the gregarines came to be known. Kölliker (1848) was the first to record the gliding and bending movements in gregarines, but he did not offer any explanation as to the cause of these movements. Leidy (1853) discovered the longitudinal striations of the epicyte and suggested their muscular function. Van Beneden detected the net work of transverse fibrillae—the so-called myocytes as named by Schneider (1873)—which are contractile and have been held responsible for the bending movements. Lankester (1872) reported upon the active movements of *Monocystis sipunculi* caused by the undulations of their lateral margins and suggested that they were like those of a planarian. Frenzel (1892) suggested that progression was due to a chemotactic affinity between the gregarines and their food, but this suggestion seems to be inadequate, as the gregarines do not show any movement on a slide with food materials on it. Following Lauterborn's observations on diatoms which move by the extrusion of gelatinous threads, Schewiakoff (1894) from his studies on *Clepsidrina muneri*, concluded that the same

phenomenon occurred in gregarines. According to him gelatinous threads exude through minute pores lying in between the ridges of the gregarines and accumulate at the posterior end, where they harden into a tough stalk, new additions to which push the animals forwards. Mühl (1921) demonstrated the presence of minute pores on the body by means of carbon tetrachloride. Lang and Doflein supported Schewiakoff's theory, while Calkins (1910) stated, " although very improbable at first sight, it is the only one thus far that fits the case " Schaudinn (1900) also supported Schewiakoff's observations by demonstrating the secretion of a gelatinous substance from the sporozoite of *Coccidium schubergi.* Although Schewiakoff worked out elaborately the mechanism of locomotion in gregarines for the first time, his emphasis upon the " mucus-tail " as a pushing element has led to a good deal of criticism. Crawley (1902, 1905) from his observations on *Stenophora juli* and *Echinomera hispida* concluded that myonemic contractions were entirely responsible for bringing about locomotion, and as mucus was merely dragged passively at the posterior end, tail-formation is an effect rather than a cause of locomotion. He says " It is an intrinsic weakness of Schewiakoff's explanation that it gives no reason why the gelatinous substance should pass backwards, instead of forwards or radially " In support of his view he has asserted that throughout the whole group of Sporozoa movement is exhibited only by those organisms which possess a muscular system. For instance, a gregarine even in its intra-cellular stage would exhibit movement, if detached from its moorings, but an adult coccidium is unable to move, as it possesses no muscular system. Further, movement is exhibited by Haemosporidia and Myxosporidia on account of the presence of a muscular system, whereas Amoebosporidia (Schizogregarines) are devoid of muscles, and are, therefore, non-motile. Thus according to Crawley, it seems strange why nature could have developed in the Polycystid gregarines a unique method of progression (as described by Schewiakoff) caused by the exudation of mucus when the muscular system is already present. Watson (1916) has also opposed Schewiakoff's theory and holds that the tail inhibits rather than promotes progression. In *S. bahli* also, it was clearly noted that with the increasing length and weight of the mucus-tail the speed of movement became slower and slower. If Schewiakoff's view is accepted, it is not understandable as to how the organisms would move when not even a trace of tail is noticed. It is, however, equally inexplicable, in actual observation, as to how the animal is able to drag forwards and cover a distance several times the total length of the tail formed by that time. It appears cogent that the mucus tail is not an aid but a definite impediment in the course of progression and is formed as an effect of locomotion rather than its cause.

According to Awerinzew (1910) both Schewiakoff's and Crawley's theories are objectionable.

Porter (1897), working on *Rhyncobolus americanus,* concluded, " It (locomotion) is probably caused by a very slight undulatory motion of the under surface of the animal " Lühe (1904), Paehler (1904), Schellack (1907), Voss (1922), Berlin (1924), Cognetti De Martiis (1927),

and others have supported Porter's theory. Roskin and Levinson (1929) could not observe slime exudation in *Nematocystis* sp. and *Polycystis* sp., and held that the contractions of the circular and longitudinal myonemes bring about locomotion in the same way as an earthworm moves through contractions of its longitudinal and circular musculature. Bowling has observed the thickening of the remarkable threads of *Zygocystis zonata*, both in the living and fixed material, but whether this indicates a cause or a result of movement is not clear. Sokolow (1912) believes, on the principle of a skyrocket, that locomotion is caused by the forceful expulsion of the fluid and contradicts Crawley's explanation of myonemic contractions. Watson (1916), from her studies on *Leidyana erratica*, has made a compromise between the two rival theories of Crawley and Sokolow by suggesting that the locomotion is caused by the myonemic contractions of that side of the animal which happens to be ventral at that time, mucus exudation merely creating friction as in the locomotion of *Limax*. Ray (1933) has confirmed Watson's explanation, excepting that he could not detect the continuity of the mucus-tail in *Stenophora khagendrae*. I agree with Watson's explanation but only for those gregarines which have developed a muscular system and not for forms like *Cephaloidophora communis* and *Chlamydocystis captiva*, which also move but possess a very feebly developed myonemic layer. In such cases undoubtedly, it is the forceful expulsion of the jelly-like substance which would take the leading part. In conclusion, it may be added that the phenomenon of movement cannot be attributed to a single cause : the type of movement, its speed and moving capacity are dependent upon the inter-action of several factors between the organism and its environment.

As regards the movements of gregarines within the body of the host certain authors have expressed the opinion that they do not move, as is evidenced by their dormant condition when an infected gut is teased and examined fresh. My observations also confirm the fact that they remain inactive in the gut-fluid even outside the body, but it is difficult to understand as to why there should have been a mechanism for locomotion at all. The probable cause of their inactivity in the exposed gut-fluid is due to the fluid becoming instantaneously viscid in the air, inhibiting the movements of the gregarines contained therein. It is probable, that the gregarines do move after detachment from the parasitised cells inside the body of the host in order to avail themselves of a greater range of nutrient material, and also to save themselves from being swept along with the food currents before encystment and that the bending movements of parasites chiefly help them in the formation of cysts.

Whether gregarines creep or swim different authors have expressed different opinions. For example, Crawley says, " Gregarines either lie against the under surface of the coverslip or upon the slide, which can be shown by raising or lowering the tube of the microscope. This shows that all studies on progression have been made on animals which are in contact with a surface." He has shown that a gregarine may be seen leaving the slide and coming upwards towards the coverslip : movement in this case having been effected by a contact of surface

(to creep upon) as offered by the extraneous particles present in the fluid. Mühl (1921) mentions that gregarines can crawl as well swim, depending upon the medium in which they are kept. I agree with Crawley's interpretation and it appears to me that in those gregarines in which the myonemes are well developed and mucus also exudes, creeping would be easier than swimming.

SUMMARY.

(1) A new record of the genus *Stylocephalus* (*Stylorhynchus*) from India has been made, and an account of the life-history of *S. bahli*, sp. nov., found in *Gonocephalum helopioides* Frm. has been given in detail.

(2) This gregarine passes all its developmental stages outside the epithelial cells of the host, as no intra-cellular stage was encountered.

(3) Sporonts are solitary and associate by their anterior ends. Gametes are anisogamous. Spores are hat-shaped and are arranged in chains, each containing eight spindle-shaped or fusiform sporozoites.

(4) Dehiscence is either by pseudocyst or by a simple rupture.

(5) Infection is purely accidental, and there is evidence of seasonal intensity of infection.

(6) The phenomenon of polynuclearism—a rare occurrence in gregarines—has been observed in *S. bahli.*

(7) This gregarine seems to be susceptible to attacks of certain fungi belonging to the family Chytridiaceae which hyperparasitize its cytoplasm as well as its nucleus.

(8) The mechanism of movement in gregarines has been discussed and an account of the observations made upon the movement of *S. bahli* has been included.

(9) A comparison of the various species of *Stylocephalus* has been given in a tabulated form.

ACKNOWLEDGMENTS.

This work has been conducted under the direct guidance of Prof. K. N. Bahl to whom I wish to submit my most respectful thanks for his encouragement, helpful suggestions and correction of this manuscript. My thanks are also due to Dr. H. N. Ray, Systematic Protozoologist, Imperial Veterinary Research Institute, Mukteswar-Kumaun, U. P., for critically examining my slides, confirming my observations, and reading through the manuscript. Dr. M. L. Bhatia has very kindly helped me in improving some of the sketches for which I am obliged to him.

REFERENCES.

Awerinzew, S., 1910.—Studien über parasitische Protozoen.—IV. Beobachtungen uber die Entwicklungsgeschichte von Coccidien aus dem Darme von *Cerebratulus* sp. *Arch. f. Protistenk.* XVIII.

Bastin, A., 1919.—Contribution a l'étude des Grégarines Monocystidées. Le cycle d'evolution de *Monocystis agilis* Hesse. *Bull. Biol. France et Belgique* LIII.

Berlin, H., 1924.—Untersuchungen uber Monocystideen in den Vesiculae seminalis der schweidischen Oligochäten. *Arch. f. Protistenk.* XLVIII.

Berndt, A., 1902.—Beitrag zur Kenntnis der im Darme der Larve, *Tenebrio molitor* lebenden Gregarinen. *Ibid.* I.

Bhatia, B. L., 1938.—*Protozoa* : *Sporozoa*, London.

Bhatia, B. L. and Setna, S. B., 1926.—On some more gregarine parasites of Indian Earthworms. *Arch. f. Protistenk.* LIII.

Brasil, L., 1905.—Nouvelles recherches sur la reproduction des Grégarines Monocystidées. *Arch. Zool. exper.* (4) IV.

Cognetti De Martiis, L., 1927.—Sul miocito e sui movimenti delle Gregarine Monocistidee. *Arch. f. Protistenk.* LVIII.

Comes, S., 1907.—Untersuchungen über den Chromidialapparat der Gregarinen. *Ibid.* X.

Crawley, H., 1902.—The progressive movement of Gregarines. *Proc. Acad. Nat. Sci. Philadelphia.* LIV

Crawley, H., 1905.—The movements of gregarines. *Ibid.* LVII.

Cuénot, L., 1901.—Recherches sur l'évolution et la conjugaison des Grégarines. *Arch. Biol.* XVII.

Cunningham, J. T., 1907.—On *Kalpidorhynchus arenicolae* a new Gregarine, parasitic in *Arenicola ecaudata*. *Arch. f. Protistenk.* X.

Duke, H. L., 1910.—Some Observations on a New Gregarine (*Metamera schubergi* nov. gen., nov. spec.). *Quart Journ. Micros. Sci.* LV.

Foerster, H., 1939.—Gregarinen in schleschen Insekten. *Zeit. Parasittenk.* X.

Frenzel, J., 1892.—Über einige argentinische Gregarinen. *Jen. Zeitchr. f. Naturw.* XXVII.

Kölliker, A., 1848.—Beitrage zur Kenntnis niederer Thiere. Über die Gattung *Gregarina*. *Zeitchr. f. wiss. Zool.* I.

Kunstler, J., 1892.—*Diplocystis schneideri*, n.g., n.sp. *Tabl. Zool.* II.

Lankester, E. Ray., 1872.—Remarks on the structure of the Gregarinae and on the Development of G. (*Monocystis*) *sipunculi*, Köll. *Quart. Journ. Micros. Sci.* (N. S.) XII.

Léger, L., 1904.—La reproduction sexuée chez les *Stylorhynchus*. *Arch. f. Protistenk.* III.

Léger, L. & Duboscq, O., 1902.—Les grégarines et l'épithelium intestinal chez les Trachéates. *Arch. Parasitol.* VI.

Léger, L. & Duboscq, O., 1903.—Note sur les developpement des Gregarines Stylorhynchides et. Stenophorides. *Arch. Zool. exper., Notes et Revue* (4) I.

Léger, L. & Duboscq., O., 1909.—Études sur la sexualitée des Gregarines. *Arch. f. Protistenk.* XVII.

Leidy, J., 1853.—On the organisation of the genus *Gregarina* of Dufour. *Trans. Amer. Phil. Soc.* X.

Lühe, M., 1904.—Bau und Entwicklung der Gregarinen. I. Teil. Die Sporozoiten, die Wachstumsperiode und die ausgebildeten Gregarinen. *Arch. f. Protistenk.* IV.

Mackinnon, D. L. & Ray, H. N., 1933.—The life-cycle of two species of "*Selenidium*" from the Polychaete worm *Potamilla reniformis*. *Parasitol* XXV.

Mühl, D., 1921.—Beitrag zur kenntnis der Morphologie und Physiologie der Mehlwurmgregarinen. *Arch. f. Protistenk.* XLIII.

Paehler, F., 1904.—Uber die Morphologie, Fortpflanzung und Entwickelung von *Gregarina ovata*. *Ibid.* IV.

Pfeffer, T., 1910.—Untersuchungen über die Gregarinen im Darm der Larve von *Tenebrio molitor*. *Ibid.* XIX.

Porter, J., 1897.—Two new Gregarinida. *Journ. Morph.* XIV.

Ray, H. N., 1933.—Studies on Sporozoa from Indian millipedes. I. *Stenophora khagendrae* n.sp., with a note on its Method of Progression. *Arch. f. Protistenk.* LXXXI.

Ray, H. N. & Chakravarty, M., 1933.—Studies on Sporozoa from Indian millipedes. II. The life-history of a cephaline gregarine, *Monoductus lunatus* n. gen., n.sp. *Ibid.* LXXXI.

Robinson, M., 1910.—On the reproduction of *Kalpidorhynchus arenicolae* (Cnghm.) *Quart. Journ. Micros. Sci.* LIV.

Roskin, G. & Levinson, L. B., 1929.—Die Kontraktilen und die Skelettelemente der Protozoen.—I. Der Kontraktile und der Skelettapparat der Gregarinen (Monocystidae). *Arch. f. Protistenk.* LXVI.

Schaudinn, F., 1900.—Untersuchungen uber den Generationswechsel bei Coccidien. *Zool. Jahrb. Abt. f. Anat. Ontog.* XIII.

Schellack, C., 1907.—Über die Entwicklung und Fortpflanzung von *Echinomera hispida* (A. Schn.). *Arch. f. Protistenk.* IX.

Schewiakoff, W., 1894.—Über de Urasche der fortschreitenden Bewegung der Gregarinen. *Zeitschr. f. wiss. Zool.* LVIII.

Schneider, A. C. J., 1873.—Sur quelques points de l'histoire du genre Gregarina. *Arch. Zool. exper.* II.

Setna, S. B. & Bhatia, B. L., 1934.—On some Gregarines from the Prawn, *Parapeneopsis sculptilis* (Heller). *Parasitol.* XXVI.

Sokolow, B., 1912.—Studien über Physiologie der Gregarinen. *Arch. f. Protistenk.* XXVII.

Vincent, Mary, 1922.—On the life-history of a new Gregarine, *Pyxinia anobii* n.sp., intestinal parasite of *Anobium paniceum* L. (Coleoptera). *Parasitol.* XIV

Voss, H. von, 1922.—Zur Kenntnis von *Monocystis naidis*. *Arch. f. Protistenk.* XLIV

Watson, M. E., 1916.—Studies on gregarines, including descriptions of twenty-one new species and a synopsis of the Eugregarines records from the Myriapoda, Coleoptera and Orthoptera of the World. *Ill. Biol. Monog.* II.

Watson, M. E., 1917.—The development of Gregarines and their relation to the host tissues in *Stenophora lacteria* Watson. *Journ. Parasitol.* III.

Woodcock, H. M., 1906.—The life-cycle of "*Cystobia irregularis*" (Minch.), together with Observations on other "Neogamous" Gregarines. *Quart. Journ. Micros. Sci.* L.