On the Life-History of the Sporozoa of Spatangoids, with Observations on some Allied Forms.

By

Helen L. M. Pixell-Goodrich, B.Sc.,
Beit Memorial Research Fellow.

With Plate 8.

Certain Spatangoids harbour peculiarly interesting parasites which were named by Giard in 1876 (**8**) Lithocystis, owing to the fact that their cysts invariably contain crystals. He interpreted the tail with which the spores are provided as two filaments which approached one another, and consequently did not realise the close affinities of this genus with Urospora, established by Schneider in the preceding year (**23**).

These gregarines were further studied by Léger in 1896, 1897 (**13**, **14**), and he also allowed the genus Lithocystis to stand distinct from Urospora, owing to their possessing "des productions cristallines caractéristiques" (**14**, p. 145).

After an extensive study of different stages of these parasites I am quite convinced that such crystals are in no way characteristic of any one genus of gregarines, but more probably common to any parasites living in the highly mineralised cœlomic fluid[1] of Spatangoids; at all events, they are present in Urospora under these conditions.

[1] Presumably this cœlomic fluid has much the same composition as that of the Echinoids, Toxopneustes lividus and Strongylocentrotus lividus, analysed by Mourson and Schlagden (**19**, p. 792). There may, however, be slight differences, for, as Léger points out, true

In fact, no satisfactory characteristic has so far been put forward for distinguishing Lithocystis from Urospora, and it seemed at first advisable to include all the Spatangoid gregarines, of which there are certainly several distinct species, in the latter genus. However, on investigating their early stages, it was discovered that certain forms have not only flagellated gametes (Pl. 8, fig. 19) now for the first time, as far as I am aware, recorded with certainty in monocystid Gregarines, but also flagellated and therefore actively moving zygotes (Pl. 8, figs. 24–26). This characteristic appears to be constant for true Urospora (those with filiform tails to the spores). It is therefore suggested that on this account the genus Lithocystis, established by Giard (**8**) in 1876, be retained for the present distinct from Schneider's genus Urospora, established in 1875 (**23**).

I am much indebted to the British Association for the use of their tables, both at Naples and Plymouth, where I have studied these parasites. The investigation of Lithocystis was undertaken at Prof. Minchin's kind suggestion in the hope of being able to clear up the question as to the occurrence of solitary encystment. My observations on this subject are recorded on page 87. Through the kindness of Prof. G. C. Bourne, much of the work has been done in the Department of Comparative Anatomy, Oxford. My husband, Mr. E. S. Goodrich, has drawn some of the living specimens and given me other valuable assistance, especially with the figures. I take this opportunity also for expressing my thanks to Mr. A. T. Watson for most generously sending me many living specimens of Pectinaria from Llanfairfechan and from his collections in Sheffield.

Echinoids in general are free from Gregarines. It may be well to mention here that, although some authors have included Strongylocentrotus as a possible host of Lithocystis, there seems to be no evidence in favour of such a procedure. Many specimens examined at Naples showed no Gregarines at all, but numerous Ciliates.

Material and Methods.

Echinocardium cordatum from Naples, Plymouth, and Port Erin have always been found to be well infected; also the deep-water species of Echinocardium and Spatangus purpureus from Plymouth.

In examining them it has been found best, after rubbing off the spines from a small area, to make a little hole in each side of the test, taking care, of course, to avoid the coils of the intestine. The cœlomic fluid, with the contents, can then be poured out into a suitable vessel and examined with a binocular microscope. Afterwards, the inside of the test is carefully washed out with sea-water introduced by a pipette through one of the holes and the washing collected and examined in a similar way. After this has been done it is rare, on cutting round the test, to find that any parasites remain except those ripe cysts generally fixed in masses to the oral surface of the host.

The cysts containing early stages are generally free in the cavity and readily distinguished by their opacity. The cyst walls of those with ripe spores, where not covered with amœbocytes, are so translucent that the spherical mass of crystals shows up with great clearness in the interior. Nearly all my work has been done on the living parasites, though films showing most of the stages have also been made, as well as sections where possible.

Hot corrosive sublimate and acetic acid mixture have been found satisfactory for fixing the sporozoite nuclei of the ripe spore. In studying differences in the shapes of the tails it has been found best to overstain with iron hæmatoxylin or hæmatein, which are fairly readily taken up by the epispore, but very readily lost again on differentiating with iron alum. Orange G and nigrosene also stain the epispore, but not very easily. Unless well stained the tails are of course practically invisible when mounted in Canada balsam. For rough comparison I have found ordinary ink (Stephens') very convenient for staining the tails of fresh spores.

Genus Lithocystis.

Trophozoites and Associates.—The long narrow trophozoite of Lithocystis has already been drawn by Léger (**14**, Pl. 11) and some of his excellent figures are reproduced in most text-books of Protozoology (Minchin (**17**), Doflein (**7**)). Even at an early stage there is a tendency in the myocyte of opposite sides to contract alternately, thus forming right-angled bends in the middle of the parasite, where association is later effected; at times the more pointed end is used for temporary fixation. The extraordinary movements and alterations of shape that are undergone by specimens, either singly or in association, could only be portrayed by the cinematograph. It appears that the protoplasm streams at a great rate down the centre of the parasite and its direction is suddenly reversed at regular intervals. All the time there is an eddy at the sides flowing in the opposite direction to the main stream, so that when one end is just empty there is a thin stream of fine granules into it down each side, often producing a kind of dagger-like point before the main stream of protoplasm rushes back. Léger has described the structure of the trophozoite and its movements, so that there is little to add. He figures the nucleus with a single karyosome, whereas I have constantly found a large one at either side of the elongated nucleus; in a Lithocystis of 2 mm. in length the nucleus was 90 μ by 40 μ, and each karyosome 20 μ in diameter. The nuclear membrane is resistant and elastic, enabling the nucleus to be squeezed quite out of its normal shape in passing the bend, where the gregarine is at times very narrow. It is carried in the main stream of protoplasm, as are also the crystals, which begin to appear at an early stage. These large calcium oxalate crystals are presumably formed by the action of the soluble calcium salts absorbed from the host's cœlomic fluid on oxalic acid secreted by the parasite. They are not very noticeable at this stage when mixed up with the dense protoplasm. It is only after encystment that they collect into the

remarkable spherical mass, in which form they are no doubt less liable to interfere with the increased activities of the protoplasm.

Trophozoites are very frequently to be seen in association. Union, which is only slight for a long time, is effected at the bend, and for hours both associates keep up their extraordinary movements, having the appearance of a large writhing X (Léger (**14**), Pl. 11, Minchin (**17**), p. 195, fig. 29*a*). Selenidia have been seen to go through somewhat similar movements during association, but owing to the greater rigidity of their ectoplasmic layers, their movements are not accompanied, as in Lithocystis, with such marked changes in outline.

By keeping the associates in cœlomic fluid in a dark place it was hoped to induce them to continue their development, but sometimes, after twenty-four hours and more, they were found to have exactly the same appearance, though with slower movements.

Occasionally forms were to be seen which had become more or less rounded off, and in which movement had almost stopped, so that their surfaces were disturbed by a rhythmical wave-like movement only (fig. 1). In this condition the parasite is an easy prey to the numerous phagocytes of the host. These arrange themselves all over the surface, uniting by their pseudopodia to form a regular network. These stages have been beautifully figured by Léger (**14**, Pl. 12), so that it is unnecessary to draw them again. Especially long pseudopodia project outwards, giving the parasites a spiny appearance. The protoplasm also takes on a very characteristic vacuolar structure, with crystals lying in the vacuoles. In many all sign of movement has stopped, the nucleus has more or less broken down, and evidently general disintegration is about to follow. At any rate, no real cyst is formed, and no further developmental stages ever occur, so that there can be no doubt that such spiny forms are in an advanced pathological condition. Exactly the same fate may happen to a pair after association, as figured by Léger (**14**, Pl. 12, figs. 3 and 4),

and reproduced by Minchin (**17**, p. 195, fig. 39*b*). That this is no ordinary encystment, as is generally stated, is made quite clear by comparison with normal stages. Immediately after normal association is effected a definite cyst is formed, on the outside of which amœbocytes can accumulate without harm to the parasites.

These necrotic forms were specially plentiful in the Channel during October and the end of September of this year (1914). One specimen of Echinocardium contained as many as thirteen, and in only three out of about fifty infected Spatangoids examined at that time was an ordinary trophozoite of either Lithocystis or Urospora found.

One must conclude from this that there is a loss of vitality among the parasites at this time of year which enables the phagocytes of the host to attack them more easily. Whether the loss of vitality results only in loss of movement, or also in the cessation of some secretion, it is difficult to say. There seems good reason, however, to think that during the healthy life of trophozoites there may be a secretion to which the amœbocytes are negatively chemiotatic, as is maintained by Cuénot (**6**). An occasional specimen has been seen, for instance, which had nearly lost all movement, and rounded off without being submitted to attack (fig. 1.) The presence of such a secretion would account also for the fact that normal associates can keep themselves free from attack during loss of movement. However, this is a difficult problem, on which there has already been considerable discussion, Siedlecki (**25**, p. 437) taking the view that movement of the parasite alone is sufficient to prevent the attack by amœbocytes in the case of annelids.

Another observation of some interest in connection with these degenerate forms was provided in October by two associates which had managed to form a cyst round themselves for protection against phagocytes, but had evidently no vitality left to proceed further, for they were lying shrunken and obviously moribund at one side of their cyst. Now, in these the protoplasm had assumed that characteristic

vacuolar appearance with crystals in the vacuoles which, I maintain, is a sign of necrosis.

Sufficient proof has now been brought forward to show that the above-described forms, with amœbocytes forming a spiny covering, are undoubtedly in a pathological condition. These were, however, the forms referred to by Léger (**14**, p. 252) as cases of normal encystment and solitary encystment. Lithocystis has consequently been quoted in text-books as a Gregarine in which a single sporont can encyst by itself and proceed to form spores. It is clear, therefore, that there is now no reason in the case of Lithocystis at any rate for thinking that solitary encystment does, or can, take place as a normal stage of development.

As will be explained below, there are constant differences in the zygotes and spores, which make it quite clear that there is more than one species of Lithocystic parasitic in Spatangoids. To prevent confusion, therefore, it has been necessary to introduce new names by which to distinguish these species. These have been made as few as possible, and several forms have been mentioned as varieties, which may eventually have to be separated as distinct species. The trophozoites, as one would expect, show no important differences, and it is only by careful comparison that they can be distinguished. The original Lithocystis schneideri, described by Giard (**8**) from specimens of Echinocardium cordatum from Wimereux, is apparently the largest form. This is a species which I have found very commonly in Echinocardium from Plymouth and Naples. In the spore (Pl. 8, figs. 12 and 14) the two edges of the tail have somewhat the appearance, before being stained, of two flagella, and this, no doubt, accounts for Giard's interpretation referred to above. When full grown the trophozoites are 3 to 4 mm. long, and the cysts formed by an associated pair are the largest to be met with, being sometimes as much as 2 mm. in diameter. L. foliacea, n. sp., has a considerably smaller tropozoite, and its cyst seldom exceeds ·6 mm. in diameter. This species is also found in Echinocardium, both in the Channel and Mediterranean.

The third form, L. microspora, n. sp., is found in Spatangus from the Channel. It is much smaller than the other species, having a tropozoite less than 1 mm. in length, and its cysts only ·1 to ·3 mm. in diameter.

With experience it becomes fairly easy to predict which species will be found in any one cyst by its size, but some of the Urospora cysts described below are exactly the same size as the small Lithocystis species (L. microspora). Generally the cysts containing adult spores of all species and both genera are massed together on the oral side of the host. The cysts are held in masses by, and more or less covered with, amœbocytes together with a quantity of dark purple or black pigment. Presumably these masses may sometimes represent an accumulation of years ; at any rate, the number of cysts varies greatly in different individual hosts.

Gametes and Syngamy.

Parasites which had recently associated and safely encysted formed spheres which were generally found floating freely in the cœlomic fluid. They could readily be distinguished from old cysts containing ripe spores by their greater opacity. By bursting cysts containing full grown gametes syngamy has been seen taking place on the slide. There is a slight sexual dimorphism, but apparently no constant difference in the size of the nuclei at this stage. The gamete which, as explained below on p. 94, must be considered as the female has its nucleus close to or extending into the small conical projection at the apex of which is the centrosome (Pl. 8, fig. 2). In the other gamete (fig. 3) a corresponding process has not been seen; it is probably lost at an early stage, the centrosome having probably travelled inwards with the nucleus, which tends to be central in position. Union between such a pair of gametes takes place along definite surfaces, producing a combination of very constant form in which the nuclei always occupy the relative positions shown in fig. 4. Exactly similar forms have been obtained directly, together with the slightly later

stages represented in fig. 5, by bursting other cysts. Consequently there seems to be no doubt that they are normal and that the gametes and zygotes do not possess at any time a flagellum such as is described below for Urospora.

Soon after this union fusion of the cytoplasm of the two gametes takes place, and the "male" nucleus approaches the "female," although they do not at once combine (fig. 5). This delay in the formation of the synkaryon is quite remarkable. The term zygote should, strictly speaking, only be used for the body formed by the complete fusion of the gametes—nuclei as well as cytoplasm. It therefore seems necessary to have another term to denote such stages as those represented in figs. 5, 11*a*, 26, etc., where the cytoplasm is completely fused but the gamete nuclei are still separate. I therefore venture to introduce the term prozygote to denote the body formed by fusion of the gametes prior to the fusion of their nuclei to form the the synkaryon (figs. 5 and 26). It will be observed that in the living (figs. 9, 10, 24, and 25) it is difficult, if not impossible, to distinguish whether the nuclei have fused or not.

The tail of the spore develops at the position of the cone and the centrosome of the female gamete, and is sometimes quite well developed before the synkaryon is formed.

Zygotes and Spores.

Even before this stage is reached it is quite easy to distinguish constant differences which make it quite clear that one is dealing with different species of Lithocystis. The adult spores are also very characteristically different as regards the shapes of the tails. Both Giard (**8**) and Léger (**14**) noticed the different sizes of the spores found in Spatangoids and referred to them as normal spores, microspores, etc. Léger also noticed the different shapes of the tails, but attributed them to stages in growth (**14**, p. 260). It seems, in the light of the present knowledge of spore-formation, very improbable that a passive organ like the tail of L. schneideri (fig. 14) corresponding to Léger's fig. 1, Pl. 13, could change its shape

to that of fig. 23*b* (Léger's fig. 2, Pl. 13) during a brief space of time. It is now proved that such is not the case by following the stages passed through from the zygote to the adult spore.

The tails in all Lithocystis are fundamentally hollow extensions of the episporé, but the walls of these tubular structures are thin and easily collapse, especially at the distal end. In L. foliata the whole tail becomes flattened at a very early stage, but specimens of L. schneideri are often to be seen in which the distal half or third of the tail is flattened while the rest remains tubular.

After the rounding off of the young spore into its final oval shape (Pl. 8, figs. 7 and 12) and the completion of the tail, the nucleus moves towards the centre of the spore and proceeds to divide into two, then into four. At this stage the epispore becomes considerably thickened. In order, therefore, to obtain good preparations of the spore with eight nuclei it has been found necessary to fix in hot corrosive acetic mixture. When the sporozoites become separated off there is a highly refringent granular residue left in the middle of the spore (fig. 13). With regard to the sporozoites themselves, there is little to say—the nucleus is about midway between the blunt and somewhat pointed extremities. Escape is effected through the funnel of the spore at the opposite end from the tail. If destined to develop, the spores, after being liberated by the rupture of their cysts in the sea water, are presumably taken in by fresh hosts with their food.

In L. schneideri the prozygote remains two-lobed for some time (Pl. 8, figs. 9–12), and the tail early assumes its normal tubular character. Apparently fusion of the nuclei does not take place until the prozygote has assumed the shape shown in (fig. 11*a*); the zygote (fig. 11*b*) then rounds itself off (fig. 12), but the synkaron remains near the tail until it begins to divide to form the sporozoite nuclei. The characteristic way in which young spores arrange themselves in groups inside the cysts with their tails towards the centre, and the later arrangement of the ripe spores in rosettes with their

funnels towards the centre and their tails projecting straight outwards, have been described by Léger (**14**). This characteristic rosette-formation is more marked in L. schneideri than in any other species. In some others there is often an arrangement in elongated groups.

In Spatangus purpureus from Plymouth the spore of a form, otherwise very similar to L. schneideri, often shows markings which give the epispore a somewhat papillated appearance. The tail of both spore and prozygote (fig. 10) is slightly longer than that of Echinocardium forms so that possibly it should constitute a distinct species.

In the case of L. foliacea, n. sp., the prozygote is top-shaped (Pl. 8, fig. 5) and soon passes into the true zygote (fig. 6); both of these forms have been obtained in the same cyst. Forms represented by figs. 4 and 5 have also been obtained in one and the same cyst, and as it is usual for all the contents of an individual cyst to reach any one stage almost simultaneously, it is concluded that in the case of this species, at any rate, the prozygote stage is very brief. After the formation of the synkaryon the characteristic tail grows apace; this, instead of remaining straight as in L. schneideri, expands near its distal end to form a flat leaf-like expansion considerably wider than the spore (Pl. 8, figs. 7 and 8). These tails are very thin and liable to twist, so that in unstained preparations it is difficult to make out their true shape.

In Lithocystis microspora, *n. sp.*, both zygotes and spores are distinctly smaller than in the other two species of Lithocystis and are in consequence easily distinguished. The zygote has the form shown in Pl. 8, fig. 16 with a small triangular tail. The adult spore has a narrow tubular tail which is generally flattened and two to three times the length of the actual spore (Pl. 8, fig. 15).

Genus Urospora.

Trophozoites and Associates.

The trophozoites of Urospora are smaller than Lithocystis rarely attaining a length of half a millimetre (Pl. 8, fig. 17).

They are elongated with coarsely granular protoplasm containing crystals as in Lithocystis. A clear epimerite may generally be distinguished at one end and by this temporary attachment may be effected. The parasite undergoes only sluggish sinuous movements with slight change of outline. The nucleus is nearly round and has often only a single karyosome. The trophozoites, either before or after association, may be attacked by amœbocytes in the same way as Lithocystis, and after studying many instances I feel confident that the same explanation should be given. After normal association encystment takes place as usual and the crystals collect into a spherical mass.

Gametes and Zygotes.

The cysts are, as a rule, considerably smaller than those of Lithocystis except L. microspora. They have fairly transparent walls, through which stages in nuclear division may be seen. At the stage represented in fig. 18 there is a distinct sexual difference in the nuclei—the upper associate with larger and less chromatic nuclei than the other, being presumably the female.

Any movement inside the cyst may be easily detected, and by breaking the wall syngamy has been detected taking place on the slide. One gamete, presumably the "male," has a long flagellum (Pl. 8, fig. 19), by the lashing of which it moves actively and seeks out the "female," in which no flagellum has been seen. Here, again, as in Lithocystis, union takes place along definite surfaces, so that a prozygote of characteristic shape is formed (figs. 20, 24–26). This retains its single flagellum, by the lashing of which it maintains a twirling movement about the rudimentary tail. This motion has been clearly seen through the cyst wall before rupture.

When Siedlecki (**24**) in 1889 described the true gametes of Gregarines and recorded for the first time the method of fertilisation, he quite realised that in the so-called "dance of the sporoblasts" the movements appeared to be due to flagella, although he could not distinguish them either in the

living or in fixed preparations. Other authors have also suggested their occurrence, but as far as I can make out this is the first genus of monocystid Gregarines in which they have been seen with certainty. Further, since here only the "male" gamete appears to be flagellated, the Urospora form a connecting link between those monocystid Gregarines in which isogamy is nearly perfect and such forms as the polycystid Stylorhynchus, in which, as shown by Léger (**15**), the "male" gamete is not only flagellated, but so very different from the "female."

In Urospora, as in Lithocystis, fusion of nuclei does not take place at once (fig. 26), so that there is a more or less prolonged prozygote stage during which the "female" nucleus is situated in the developing tail, towards which the "male" nucleus slowly moves from its original position near the flagellum.

The prozygote of the Mediterranean species Urospora neapolitana, n. sp., has a most striking appearance with its peculiar corkscrew-like tail (Pl. 8, fig. 20). This species has not been met with in the Echinocardium or Spatangus of the Channel.

The prozygote of Urospora echinocardii, n. sp., the form found commonly in the Channel Spatangoids, is somewhat larger and has a more or less straight and pointed tail (fig. 24). It only differs very slightly, if at all constantly, from the prozygote of the Urospora occurring in Spatangus (figs. 25 and 26). The spores into which they develop are also so similar that there seems to be no need at present, at all events, to establish another species for the Spatangus parasite.

After fusion of the nuclei, the oval shape of the adult spore is soon assumed (figs. 21, 22, and 28). The synkaryon divides by a simple process into two, four (fig. 29), then eight. Meanwhile, the epispore thickens considerably, and inside the spore fine protoplasm collects round the nuclei to form the eight sporozoites, leaving a coarsely granular and refringent residuum in the centre.

Spores.

U. neapolitana is also easily distinguished from the Channel species by means of its spore. This is almost round, and it has a very long filamentous tail about twenty times the length of the spore itself. It remains very tightly coiled (fig. 23*a*) even during fixation, except in rare cases, when it unwinds (fig. 23*b*). In U. echinocardii, on the other hand, the loosely-coiled filamentous tail with which the spore emerges from the cyst easily unwinds (fig. 27). It is at most seven times the length of the spore itself and the proximal tubular region of the tail tapers much more gradually into the filament. It may be mentioned here that the filament is difficult to see in the living, and if unstained and mounted in Canada balsam it is practically invisible (fig. 29), so that spores appear under these conditions to have a short, tapering tail only.

Homology of the Gametes and Zygotes.

In Urospora it seems clear that the flagellated gamete represents the "male" and the non-flagellated the "female." The tail therefore develops from that part of the prozygote which is derived from the "female." It is reasonable to suppose that the disposition of the gametes in these early zygotes of Urospora and Lithocystis is the same. Consequently, in Lithocystis the "female" gamete gives rise to that region of the prozygote from which the tail develops. In fact, the tail appears to be produced by the further growth of the conical projection bearing the centrosome of the original "female" gamete. It may even be suggested that the characteristic tail of these Lithocystis and Urospora spores has been phylogenetically derived from the flagellum originally possessed by the "female" gamete, as it corresponds in position with the flagellum of the "male" gamete.

In Urospora the flagellum of the "male" gamete arises from the region of the centrosome at the apex of the cone. Similar conical projections, with apical centrosomes, have

been described in the formation of the gametes of both sexes of various other Sporozoa, e. g. Aggregata (**21b**). The "male" gamete of Lithocystis, in which apparently the flagellum does not develop, has no doubt lost its conical process at an early stage of development, and its centrosome has possibly passed inwards with the nucleus.

Systematic.

Below are given the characteristics on which these genera were originally established. It will be noticed that in neither case is any mention made of the movements of the parasites. Other features of the trophozoites of these monocystid Gregarines are of very little value from a systematic point of view. The only given characteristic which is of any use for this purpose is, I think, the filiform tail of the Urospora spores in contradistinction to the tubular ones of Lithocystis. The latter tend to become flattened and easily recognisable, though even here the narrow tubular tail presented by L. microspora approaches that of Urospora, in which the apparently filiform tail has presumably a tubular origin.

It is with a considerable amount of hesitation that I formulate the flagellated nature of the gametes and zygotes among the characteristics of the genus Urospora because I have not had as yet the opportunity of investigating certain forms which have been ascribed to the genus. Some of the results of my search after these are recorded below, and I fully realise that it may be years before gametes and zygotes of all these elusive forms can be found and studied. However, should such flagellated gametes and zygotes be found later not to be constantly present in Urospora and absent in Lithocystis, it seems to me that the latter genus would be superfluous, and that all the five forms described should be included in the older genus Urospora.

Genus Lithocytis, Giard, 1876 (Characteristics given by Labbé) (**12a**, p. 42).—"Individus de grande taille, ovoïdes ou cylindriques avec entoplasm remplie de cristaux clino-

rhombiques d'oxalate de chaux. Kystes sphériques, spores, longuement ovoides, tronquées à une des extrémités : épispore formant un tube à parois délicates, très allongé et sineux: Toutes les spores sont rangées dans le kyste en groupes, radiairement autour de centres communs (sporozoites 8 ?)."

Emended Characteristics.—Moncystid Engregarines, with elongated trophozoites continually changing their shape, and containing rapidly flowing protoplasm; cysts spherical, gametes non-motile, slightly anisogamous. Prozygotes motionless, with short tubular tails. Spores when ripe tend to form rosettes. Epispore produced into a funnel at one end, through which the eight sporozoites escape, and into a tubular, but generally flattened, tail at the other.

(1) Lithocystis schneideri, Giard.—Trophozoites may attain a length of 4 or 5 mm. Cysts 1–2 mm. in diameter. Prozygote bi-lobed. Spores generally 22–24 μ long and 8–9 μ wide, with tail about four times this length, but narrower than the spore.

Hosts.—Echinocardium, Naples and Channel, and Spatangus purpureus from the Channel.

(2) Lithcystis foliacea, n. sp.—Trophozoites smaller than L. schneideri; maximum length 2–3 mm. Cysts generally about ·6 mm. in diameter. Zygote top-shaped. Spores about 24 × 9 μ, but their tails are only three times as long, and widen out towards their distal end to form a leaf-like expansion, which may be as much as 15 μ wide.

Hosts.—Echinocardium cordatum, Naples, Plymouth.

(3) Lithocystis microspora, n. sp.—Trophozoites 1 mm., or less, in length. Cysts ·1–·3 mm. in diameter. Zygotes small; spores generally 12–13 μ long and 6–7 μ wide, with tail two to three times this length, narrow, and tapering.

Host.—Spatangus purpureus, Channel.

Genus Urospora (Aimé Schneider), 1875 (**23**).—"Monocystidée de forme allongée, terminée en pointe aiguë en arrière, arrondie en avant et légèrement micronée au pôle supérieur Epicyte à simple contour. Entocyte à grains très-fins.

Kystes à sporulation complète, déhiscents par simple rupture. Spores pourvues d'un appendice immobile, filiform, environ de la longeur de la spore, et inséré sur son extrémité la plus large. Cette spore contenant, à l'état de maturité, six ou sept corpuscles falciformes très-allongés et diversement groupés à son intérieur, offrant en outre un nucleus de reliquat au centre ou à la base des corpuscles."

Emended Characteristics.—Monocystid Eugregarines. Trophozoites elongated, and coarsely granular. Somewhat sinuous movements with slight change of outline. Anisogamy, ♂ gametes with a long flagellum, ♀ non-motile. Early zygotes (Prozygotes) motile retaining flagellum until after beginning of the formation of the tail. Spores with eight sporozoites. The epispore produced into a funnel for the escape of the sporozoites at one end, and at the other into a filamentous tail of varying length.

(1) Urospora neopolitana, n. sp.—Trophozoite small, about 200–300 μ long, and 40 μ wide. Cysts 100–200 μ in diameter. Prozygotes motile retaining flagellum of ♂ gamete until corkscrew-like tail is well developed (fig. 20). Adult spores about 12 μ long and 7 μ wide. Tails about twenty times the length of the spores, but generally tightly coiled (fig. 23).

Host.—Echinocardium cordatum, Naples.

(2) Urospora echinocardii, n. sp.—Trophozoites and cysts indistinguishable from those of Urospora neapolitana. Prozygote flagellated and with a pointed or fusiform tail (figs. 24–26). Spores may be as long as 19 μ. Their tails are six or seven times this length and never form a tight coil as in Urospora neapolitana (fig. 27).

Hosts.—Echinocardium and Spatangus, Plymouth.

Observations on Certain Parasites Included by Labbé and others as Urospora.

(1) On the so-called "Urospora sænuridis" (Köll.) from Tubifex. References taken from Labbé (**12a**, p. 43).

1843. Gregarina s., Kölliker (**11** (*a*), p. 12).

1882. Urospora s., Nasse (**21a**, p. 26).
1882. „ „ Bütschli (**3**, p. 557).
1872. „ „ Lankester (**12b**, p. 348).

After studying Kölliker's description and figures I quite agree with Hesse (**10**, p. 43, footnote) that the gregarine there described has no connection with Urospora, but is a Monocystis.

Schneider was apparently the first to suggest that the parasite described by Lankester in 1872 should be included in his new genus Urospora, and Labbé definitely included this form among Urospora without expressing any doubt, and so have other modern authors. Lankester recorded (**12b**, p. 348) that he only found one specimen of Tubifex infected with ripe spores and no doubt purposely refrained from giving any name to the parasite. He, however, pointed out the resemblance to the psorosperms of fish and to this group of Sporozoa (Neosporidia) it undoubtedly belongs. The presence of a tail to the spore probably induced some observers to rush to the conclusion that it was a species of Urospora, but I have no doubt that this rather rare parasite is identical with Myxocystis ciliata described by Mrazek (**20**) from Limnodrilus. The results of studying this parasite, which presents some interesting problems, I hope to publish shortly.

The other two supposed references to this hypothetical species, Urospora sænuridis, can be rapidly dealt with. Nasse's observations (**21a**, p. 26) undoubtedly refer to the neosporidian Myxocystis ciliata, and Bütschli mentioned (**3**, p. 55, footnote) that he had not seen the parasite, but only judged it to be Urospora from Nasse's figures.

Thus this "species" of Urospora may be considered as truly a myth!

(2) Urospora nemertis (Köll.). References taken from Labbé (**12a**, p. 43).

1845. Gregarina n., Kölliker (**11** (*b*), p. 100).
1848. „ „ „ (**11** (*a*), p, 1).
1867. „ „ McIntosh (**16**, p. 38).
1875. Urospora n., Schneider (**23**, p. 597)
? 1893. „ „ Bürger (**1**, p. 208).

So far I have searched without success for this parasite which Schneider said was rare in Valenciennia at Roscoff. Kölliker's gregarine came from Nemertes delineatus (Polia delineata) from Naples. It seems somewhat doubtful, however, judging from his description (**11** (*a*), p. 1) whether he was dealing with Urospora. I have been more fortunate in finding the parasite in Lineus gesserensis (Borlasia olivacea) described by McIntosh in 1867. From this early description alone it is quite clear that it is a very different parasite from Urospora with which, so far as I know, McIntosh has never suggested its affinities. The thick gelatinous cyst (staining bright blue with Nigrosene) separates it clearly, but I have not yet been able to study it fully owing to its rarity. Bürger's parasite, briefly recorded in 1893 (**1**), and again in his monograph of the Nemerteans, 1895 (**2**), is likely to be the same as McIntosh's parasite. In 1896 Gravier (**9**, p. 307) recorded a parasite from a Phyllodocid (Eulalia punctifera, Grube), and this has also been included among the Urospora. It may be of interest to mention in this connection that at Plymouth in September I found a Lineus which had swallowed a whole Phyllodoce nearly as big as itself. It seems to be so very important that records of the natural food of all hosts should be kept if progress with the life-histories of many parasitic protozoa is to be made.

When the spores of U. nemertis are again found it will be interesting to see whether their tails are as short as described and figured. The thin filamentous ends of the tails in Urospora are difficult to see in the living and practically invisible when mounted in Canada balsam unless they have previously been deeply stained. Consequently, spores which have been differentiated to show the contained sporozoite nuclei have the appearance shown in Pl. 8, fig. 29 with only the thick proximal part of the tail at all visible, and this is very similar to Scheider's figure of U. nemertis (**23**, Pl. 21, fig. 4 *a–c*).

(3) U. sipunculi from Sipunculus nudus and U. synaptæ from Synapta inhærens, and S. digitata

are well established species that require much further study.

(4) Urospora lagidis, St. Joseph (**22**); Brasil (**4** and **5**).

Host.—Pectinaria (Lagis) koreni.

From Brasil's interesting description of Urospora lagidis, the gametes seemed to have a great resemblance to those of Lithocystis and as he did not figure the spore I made strenuous efforts to re-obtain the parasite. Six specimens of Pectinaria that were procured from the only collecting ground at Plymouth proved to be uninfected, but nearly all the specimens from Llanfairfechan had a good infection and from them I have been able to obtain other stages besides spores. These have been studied chiefly in a living condition, whereas Brasil studied fixed material.

The milk-white trophozoites can be seen through the body-wall of living Pectinaria roaming about in the cœlom and encysted specimens form spheres about 1 mm. in diameter which are generally carried forwards towards the oral end of the host. Here, as they ripen, they gradually become massed together connected by the amœbocytes on their surfaces and often suspended from some of the viscera or the body wall.

The spore (Pl. 8, fig. 30) proved to have a tail much more like that of Lithocystis than Urospora. At its other end there is a deep funnel (not two spines as previously described). I have not, so far, been fortunate enough to obtain living gametes, but the zygotes that have been met with several times have been quite motionless.

All these details point to the fact that the Pectinaria parasite should be regarded as Lithocystis rather than Urospora. I would not venture, however, to make this alteration without the most conclusive evidence, and for this, as pointed out on p. 95, it will be necessary to study not only the living gametes of this species, but also those of U. nemertis, U. sipunculi, and U. synaptæ.

Summary.

(1) The sporozoa of Spatangoids, previously referred to Lithocystis schneideri, Giard, include at least five species. In addition to Giard's original species of Lithocystis it has been necessary to establish two others, namely, L. foliacea, n. sp., and L. microspora, n. sp. Further, the genus Urospora is also represented by two species which have not been recorded before, namely, U. neapolitana, *n. sp.*, from Naples, and U. echinocardii, n. sp., from Plymouth. On the other hand, it has been shown that there is no such species as "Urospora sænuridis," which has been ascribed to Tubifex by some authors.

(2) The instances of so-called "solitary encystment," of which Lithocystis is quoted in text-books as furnishing a clear case, are shown not to be normal stages at all, but necrotic specimens attacked by the phagocytes of the host.

(3) In both Lithocystis and Urospora there is intercalated a stage—Prozygote—in which the cytoplasm of the gametes has fused and the tail of the spore has appeared, but the nuclei have not yet combined to form the synkaryon of the true zygote.

(4) In Urospora, both the "male" gamete and the prozygote are flagellated and motile.

October, 1914.

References.

1. Bürger, Otto.—"Sudgeorgische und andere exotische Nemertinen," 'Zool. Jahrb. Syst.,' vii, 1893, p. 208, t. 9, figs. 17, 17*a*.
2. ——— "Nemertinen," 'Fauna und Flora des Golfes von Neapel Monographie,' xxii, 1895.
3. Bütschli, O.—"Sarkodina und Sporozoa," Bronn's 'Klassen und Ordnungen des Thier-reichs: I Protozoa,' 1880–82, p. 557, note.
4. Brasil, L.—"Contrib. à la Connaissance de l'appareil digestif des Annélides Polychètes (l'epithélium intestinal de la Pectinaire)," 'Arch. Zool. Expér.,' (4), ii, 1904, pp. 215–225.

5. Brasil, L.—"Recherches sur la Reproduction des Grégarines Monocystidées," 'Arch. Zool. Expér.," (4), iii, 1905, pp. 17–38.

6. Cuénot, L.—"Recherches sur l'évolution et la conjugaison des Grégarines," 'Arch. de Biol.,' xvii, 1901.

7. Doflein, F.—'Lehrbuch der Protozoenkunde,' Jena, 1911.

8. Giard, A.—"Sur une nouvelle espèce de Psorospermie (Lithocystis Schneideri) parasite de l'Echinocardium cordatum," 'C.R. Ac. Sc.,' lxxxii, 1876, pp. 1208–1210.

9. Gravier, C.—"Recherches sur les Phyllodociens," 'Bull. Sc. France et Belg.,' xxix, 1896, p. 307, t. 25, figs. 18 and 19.

10. Hesse, E.—"Contribution à l'étude des Monocystidées des Oligochètes," 'Arch. Zool. Expér. et Gen.,' iii, 1909, pp. 27–301.

11. Kölliker, A.—"Beitrage zur Kentniss niedere Thiere," (*a*) 'Zeits f. wiss. Zool.,' i, 1848. p. 1, t. 1, fig. 4*b*; (*b*) 'Zeits. f. wiss. Botanik,' i, 1845, pp. 46–103, no figures.

12a. Labbé, A.—'Sporozoa' in 'Das Thierreich,' lief 5, Berlin (Friedlander), 1899.

12b. Lankester, E. Ray.—"Remarks on the Structure of the Gregarinæ and on the Development of G. (Monocytis) Sipunculi (Köll.)," 'Quart. Journ. Micr. Sci.,' 12, 1872, pp. 342–351.

13. Léger, L.—"Lithocystis schneideri," 'C.R. Ac. Sc.,' cxxiii, 1896, pp. 702–705.

14. ——— "Contribution à la Connaissance des Sporozoaires . . .," 'Bull. Sci. Fr.-Belgique,' xxx, 1897, pp. 240–264.

15. ——— "La Reproduction sexuée chez les Stylorhynchus," 'Arch. Protistenkunde,' iii, 1904, p. 303.

16. McIntosh, W. C.—"On the Gregariniform Parasite of Borlasia," 'Trans. Micros. Soc. Lond.,' xv, 1867, pp. 38–41.

17. Minchin, E. A.—'The Sporozoa : a Treatise on Zoology' (Lankester). London : A. & C. Black, 1903.

18. ———'An Introduction to the Study of the Protozoa.' London : Arnold, 1912.

19. Mourson, J., and Schlagdenhauffen, F.—"Sur la Composition du Liquide Cavitaire des Oursins," C.R. Ac. Sc., xcv, 1882, p. 791.

20. Mrazek, A.—"Sporozoenstudien. Zur Auffassung der Myxocystiden," Arch. Protistenkunde, xviii, pp. 245–259.

21a. Nasse, D.—"Beitrage sur Anatomie der Tubificiden," Inaug. Diss. Bonn., 1882.

21b. Pixell-Goodrich, H. L. M.—"The Sporogony and Systematic Position of the Aggregatidæ," 'Quart. Journ. Micr. Sci.,' 60, pt. 1, pp. 159–174, 1914.

22. St. Joseph, Baron de.—"Les Annélides polychètes des côtes de la France," 'Ann. Sci. Nat. Zool. and Paléont.' vii, 1898.

23. Schneider, Aimé.—"Contributions à l'histoire des Grégarines des Invertébrés de Paris et de Roscoff," 'Arch. de Zool. expér.,' iv, 1875, pp. 493-604.

24. Siedlecki, M.—"Ueber die Geschlechtliche Vermehrung der Monocystis ascidiæ," 'Anz. Akad. Wiss. Krakau,' 1899.

25. ———"Quelques observations sur le rôle des Amibocytes dans le cœlome d'un Annélide," 'Ann. l'Instit. Pasteur,' xvii, 1903, pp. 449–460.

EXPLANATION OF PLATE 8,

Illustrating Mrs. Helen L. M. Pixell-Goodrich's paper on "The Life History of the Sporozoa of Spatangoids with Observations on some Allied Forms."

[Unless otherwise stated, the parasites figured came from Echinocardium cordatum, Naples. Preparations were stained with iron-hæmatoxylin and drawn at an approximate magnification of 2000, except where otherwise indicated.]

Fig. 1.—Lithocystis schneideri rounding itself off and still unattacked by amœbocytes. Drawn from the living. × 16.

Fig. 2.—Female gamete of L. foliacea stained Carm Alum.

Fig. 3.—Male gamete of L. foliacea stained Carm Alum.

Fig. 4.—Uniting gametes of L. foliacea stained Carm. Alum.

Fig. 5.—Prozygote of L. foliacea.

Fig. 6.—True zygote of L. foliacea after the formation of the Synkaryon.

Fig. 7.—Uninucleate sporoblast of L. foliacea. × 1000.

Fig. 8.—Adult spore of L. foliacea. × 1000.

Fig. 9.—Prozygote of L. schneider, living specimen.

Fig. 10.—Prozygote of L. schneideri from Spatangus purpureus, Plymouth. Drawn from the living.

Fig. 11.—Later stages of L. schneideri showing the proximal ends only of tails; (*a*) Prozygote and (*b*) true zygote, after the formation of the synkaryon. × 1000

Fig. 12.—Sporoblast of L. schneideri. × 1000.

Fig. 13.—Spore head with sporozoites and refringent residuum. Drawn from the living. × 1500.

Fig. 14.—Adult spore of L. schneideri. × 1000.

Fig. 15.—Adult spore of L. microspora from Spatangus, Plymouth.

Fig. 16.—Zygote of L. microspora from Spatangus, Plymouth.

Fig. 17.—Trophozoite of U. echinocardii from Spatangus, Plymouth. × 160.

Fig. 18.—Encysted associates of U. neapolitana (*a*) ♀ with large nuclei; (*b*) ♂ with small, more chromatic nuclei. × 260.

Fig. 19.—Flagellated gamete (♂) of U. neapolitana. Drawn free-hand from the living by E. S. G. Magnification slightly over 2000.

Fig. 20.—Flagellated Prozygote of U. neapolitana. Drawn similarly to fig. 19.

Fig. 21.—Zygote of U. neapolitana after loss of flagellum.

Fig. 22.—Uninucleate sporoblast of U. neapolitana.

Fig. 23.—Adult spora of U. neapolitana (*a*) with tail tightly coiled; (*b*) with tail partly uncoiled.

Fig. 24.—Living Prozygote of U. echinocardii from Echinocardium, Plymouth.

Fig. 25.—Living prozgote of U. echinocardii (?) Spatangus, Plymouth.

Fig. 26.—The same Prozygote, showing nuclei not fused; stained iron hæmatoxylin and Orange G.

Fig. 27.—Adult spore of U. echinocardii from Echinocardium, Plymouth.

Fig. 28.—Sporoblast of U. echinocardii from Spatangus, Plymouth, stained iron hæmatoxylin and Orange G.

Fig. 29.—Young spore of U. echinocardii with four nuclei; from Spatangus, Plymouth.

Fig. 30.—Ripe spore of Urospora lagidis from Pectinaria (Lagis) koreni.