A CONSIDERATION OF THE INFECTIVE GRANULE IN THE LIFE-HISTORY OF PROTIST ORGAN- ISMS.¹

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"Harum sententiarum, quae vera sūt, Deus aliquis viderit."

DURING recent years there have been recorded observations relative to a granule-shedding phase in the life-history of certain Protist organisms. With regard to the significance of such a process there are at the present time two diametrically opposite opinions.

On the one hand, there are those who hold that granule shedding is a purely degenerative phenomenon; that, therefore, it is a condition of little consequence and one that can be conveniently ignored. On the other hand, there are those who maintain that granule shedding is a vital phenomenon, that the so-called "infective granule" is in fact a propagative unit, and, as such, is the starting-point in the life-history of one individual or indeed of a whole series of individuals.

The main argument in support of this second view is furnished by the behaviour of certain blood-inhabiting spirochaetes, and in bringing this forward as evidence of the vital nature of granule shedding, one is aware that one is approaching the subject from its most difficult standpoint, for the spirochaetes, more particularly the blood-inhabiting spirochaetes, have been assigned no very definite or secure position by the systematist. It is the tendency of all men, inherited perhaps, as Minchin (1911¹⁷) has suggested, from an original father in Eden, to classify all the living objects met with in daily life into two main types—the animal type and the vegetable type. Such a classification is obvious when one has to deal with the more highly organised forms of life, and it is only when one comes to consider the more primitive forms that difficulties in the way of differentiation present themselves. In the case of no group of organisms have these difficulties been so great as in the case of the blood-inhabiting spirochaetes. At first these organisms were looked upon as bacteria of the genus *spirillum*. Then came the

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classical work of Schaudinn (1904²³), in which spirochaetes were described as being possessed of kinetonuclear and trophonuclear apparatus together with flagellum and undulating membrane, a finding which naturally resulted in the transference of the blood-inhabiting spirochaetes from the Bacteria to the Protozoa. However, with further investigations and the refutation of certain of Schaudinn's findings, there has developed a tendency to expel the spirochaetes from the sheep-fold of the Protozoa. Such an attitude springs, obviously, from the fact that the microbiologist has fallen into the primitive principle of classification above mentioned, it being assumed that if a spirochaete is not a bacterium it must be a protozoon, and *vice versa*. To obviate the difficulty in this case, certain investigators have placed these hapless organisms in an intermediate position, and in thus seeking to establish a compromise have very rightly refused to define a hard-and-fast boundary line between the Bacteria and Protozoa.

It is for this very reason that one has made use of the term Protista, for the term is one which would include all the Bacteria and the Protophyta on the one hand, all the Protozoa on the other hand, and also all intermediate organisms, such as the blood-inhabiting spirochaetes and the Chlamydozoa.

The breaking up of the axial chromatic core of a spirochaete into granules was probably first described by Dutton and Todd (1907⁷) in the case of *Spirochaeta duttoni*. "Sometimes, especially in preparations taken just as the parasites are about to disappear from the blood, this fragmentation proceeds until the whole chromatic core is broken up into granules." Again, in regard to the phases of the organism as they occur in the tick, *Ornithodoros moubata*, they say: "The most marked feature of the spirochaetæ in the tick is, however, the extraordinary number of ways in which their chromatin may be fragmented." "The granules may be either coarse or fine. In one or two instances the granules of a finely fragmented extracellular parasite are divided transversely so that its chromatin is represented by a row of *paired* granules. The granules of the encysted parasites are usually single. In others, perhaps at a more advanced stage, they are double, and the parasite then consists of a finely blue or reddish spherical matrix, about 5 to 7 μ in diameter, in which are embedded perhaps a score of pairs of deep red granules. Many such bodies have been seen in preparations from the Malpighian tubules of an infected tick. The further development of these bodies is problematical; it is certain that some of them burst, and that their granules are thus freed. In stained specimens it is apparently possible to trace the development of each single granule to a comma-shaped mass of chromatin, and these comma forms to small spirochaetæ."

The statements of Leishman (1910¹⁶) are practically identical with those of Dutton and Todd, the spirochaetes breaking up into "coccoid granules" in the ova and tissue cells of the tick.

Again, while investigating the spirochaetosis of Sudanese fowls, Balfour of Khartoum (1908²) discovered that there occurred, from time to time, during the disease, a period which he has called the "after-phase," when the causal organism as such disappeared from the peripheral circulation, and when, coincident with this disappearance, the erythroblasts were found to contain curious intracorporeal bodies which he maintained were a stage in the life-history of the spirochæte in question. Such an interpretation was much disputed at the time, and still meets with opposition, but Balfour himself has since his original communications done much to throw light on the exact significance of these bodies (1911³). Working with infected chickens, in which a natural crisis has occurred or in which an artificial crisis has been induced by salvarsan, he found by using dark ground illumination that the spirochætes break up into granules in the internal organs. These granules, to which has been given the name "infective granules," "are discharged by the still living spirochæte from one or other end of its periplastic sheath or cell membrane, and would appear to constitute a stage in the non-sexual life-cycle of the parasite, for they seemingly enter the red cells, develop in them, undergo a process akin to schizogony, and regain the liquor sanguinis as tiny merozoites, which vary in number and slightly also in size."

The present position, then, with regard to granule shedding in the case of spirochætes, is this. Certain spirochætes do undergo a process of fragmentation into granules, but that these granules are capable of initiating a further life-cycle is highly disputed. In discussing this question Minchin (1912¹⁸) remarks: "A development of this type suggests, very strongly, affinities with bacteria, but none whatever with Protozoa of any class. The coccoid grains may be compared with the spore formation in bacteria, and with that described by Gross in *Saprospira grandis*. In all cases throughout the series of living beings, whenever an organism presents, in its fully developed adult stage, peculiarities of a special kind, it is above all to the early developmental forms that the naturalist turns for indication of the true affinities of the organism in question."

When one comes to consider the question of granule shedding in the case of trypanosomes, one finds that the published observations are much scantier. Fry (1911¹⁰) has stated that *Trypanosoma brucei* may throw off granules, and asserts that the liberated granules are distinctly motile. He maintains, moreover, that the process is of a vital and not of a degenerative character. The same is true too, according to Ranken (1912²¹), of *Trypanosoma gambiense*. But, as far as one is aware, no further development of the shed granules has been described in either instance. Here again Minchin (1912¹⁹) refuses to recognise that these granules are other than products of degeneration, for he says: "That a trypanosome or any other living cell might excrete grains which when set free could exhibit movements

due to molecular or other causes is highly probable; but, that such grains represent a stage in the life-history of a trypanosome is far from being so; nor can analogy with spirochaetes be considered as a legitimate argument in favour of any such conclusion."

On the other hand, Buchanan (1911⁶), in describing certain cultural phases of *Trypanosoma brucei*, ends with a remark of considerable significance. "A marked feature, perhaps worthy of note, was the constant presence of free chromatin granules in nearly all the smears from the second day onwards. These were apparently derived from the nuclei of disintegrated trypanosomes and possibly represented a first phase in development."

In dealing with the hæmoflagellates one must not overlook Balfour's assertion (1912⁴) that Archibald has seen definite granule shedding in flagellates from cultures of *Leishmania tropica* by using a dark field method of examination.

The granule shedding described in the previous communication in the case of *H. simondi* (1913¹⁴) is, as far as one is aware, the only observation on the occurrence of such a phenomenon in a hæmosporidian, or, rather, it is the only observation of such a phenomenon which has been interpreted as being of a vital nature.

If the intracorpuseular bodies described are indeed phases in the life-history of the hæmosporidian, then they may legitimately be taken as representing stages in the evolution of the adult parasite, *i.e.*, as being recapitulative in the phylogenetic sense (Minchin, 1912¹⁸). Now, it is an established zoological maxim in the case of highly organised animals that community in embryonic structure reveals community in descent, and, further, that the embryonic stages reveal more or less completely not only the condition of the progenitor of the whole group, but also the condition of that progenitor in its adult state. If the same maxim be applied to the Protista, then the primitive protist organism, the progenitor so to speak of either a bacterium or a protozoon or a spirochaete, would consist of a minute granule of chromatin, which, so far as one can say, is quite devoid of protoplasm. A somewhat similar conclusion has of course already been reached by others on purely speculative grounds, and for this reason one is much at a loss to understand the mental attitude of those who seek on theoretical grounds to establish the existence of a chromatinic *primum vivens* and who yet refuse to accept the infective granule as concrete laboratory evidence of the same.

What appears to me to be a strong argument in favour of the interpretation I have put on the corpuscle inclusions found in *Solea* is afforded by the fact that there are already known to exist separate and distinct hæmosporidia which conform morphologically to the phases I have described.

1. The coccoid bodies are represented by Theiler's *Anaplasma marginale* (1910²⁵). The first description of such an organism

was probably given by Smith and Kilborne in their monograph on Texas fever (1893²⁴). In the acute form of the disease large pyriform and spindle-shaped piroplasms were found in the corpuscles, whereas in subacute or chronic cases only "peripheral coccus-like bodies" were present. The view that these coccoid bodies were but phases in the life-history of the piroplasm was supported by subsequent workers, and such a view was more or less accepted until Theiler in the course of investigations on Piroplasmosis in South Africa came to the conclusion that the "marginal points" had no connection whatever with a piroplasm, but were in fact a new and distinct parasite to which he gave the name *Anaplasma marginale*. Since then certain workers have supported Theiler, but there are others who have reverted to the older opinion and who assert their belief that the parasite represents none other than the young form of one or other of the piroplasms. Certainly Theiler's parasite has been described so frequently as existing side by side with a piroplasm, that this fact alone furnishes strong presumptive evidence of a possible relationship.

2. The bacillary phase is represented by the intracorpuseular bodies first described by Graham-Smith in the blood of *Talpa europæa*, the common mole (1905¹¹). Similar bodies were found in the Soudan by Balfour (1906¹) in *Jaculus gordonii* in association with a hæmogregarine; and França (1911⁹) has recorded their presence in Portugal, not only in *Talpa* but also in *Microtus incertus* and in *Eliomys quercinus*. Laveran, who saw preparations sent by Graham-Smith, by Balfour, and by França, in each case gave the opinion that the infected corpuscles were "hématies mouchetées," i.e., that the corpuscles showed the granular basophilia which pathologists are accustomed to look for in cases of severe human anæmia, and particularly in cases of plumbism. França, being unwilling apparently to commit himself to any very definite opinion, described the bodies as "pseudo-hématozoaires endoglobulaires." More recently these bodies have been the subject of study by Brumpt (1911⁵), who has come to the conclusion that they cannot be basophilic granules inasmuch as these latter are easily decolorised by Unna's orange tannin. He suggests that they are protozoan in nature, and has given them the generic name, *Grahamella*. I have found similar bodies in *Crossopus fodiens* and in *Microtus agrestis*, in each case in association with a trypanosome. The following table in regard to the occurrence of *Grahamella* brings out a point of considerable interest in connection with the present discussion, and it is this, in every case in which *Grahamella* has been recorded it is associated with either a hæmosporidian or a trypanosome. Thomson's parasite in the mole is the subject of a communication which is, at present, in process of preparation. A study of the organism shows it to conform more closely to the hæmogregarine type than to any other hæmosporidian type. The adult trophozoite is found to occur in two phases in the blood, a normal phase with no extranuclear

chromatin granules, and a granule - shedding phase comparable to *H. simondi*.

TABLE.—*Graham-Smith's Intracorpuseular Bodies, which have been described by França as "pseudo-hématozoaires endoglobulaires," and again by Brumpt as "Grahamella," have been found to occur in—*

1. *Talpa europæa*, the common mole, along with
 - (a) Thomson's intracorpuseular parasite (1906²⁶), which has been named by França, *Elleipsisoma thomsoni* (1911⁹). It is nearly related to the hæmogregarines.
 - (b) A trypanosome, first seen by Gros in Russia (1845¹²), then described in turn by Petrie in England (1905²⁰), by França in Portugal (1911⁹), and by Wrublewski in Russia (1912²⁷).
2. *Jaculus jaculus*, the jerboa or desert rat, along with a hæmogregarine, first described by Balfour (1906¹), and then by Laveran, who has named it *H. balfouri* (1905¹⁵).
3. *Microtus incertus*, a vole, along with a piroplasm which occurs in Portugal and which has been named *Smithia microti* by França (1910⁸).
4. *Microtus agrestis*, the ordinary field vole. Specimens taken in the neighbourhood of Sheffield have been found to harbour *Grahamella* and a trypanosome, which latter shows a resemblance to but is larger than *Tr. lewisi*.
5. *Crossopus fodians*, the water shrew. Only two specimens of this little animal have been obtained. One of these showed the presence of *Grahamella* in large numbers, the other showing a scanty infection with a trypanosome which closely resembles that found in *Talpa*.

Are we to assume, then, that *Grahamella* has nothing whatever to do with the parasites along with which it has been found to exist? A consideration of the above table, I believe, goes far to dispel such an assumption, and I venture to suggest that Graham-Smith's bacillary bodies are none other than the infective granule stage of a hæmosporidian.

3. The peripheral chromatin phase is found in

- (a) The parasite which occurs in *Cottus* and which is described in the present Journal (1913¹³);
- (b) A piroplasm described by França, *Smithia microti* (1910⁸). He says of it:

"La forme jeune de cet Hématozoaire est ovulaire et a 1.5 μ de long. Sa chromatine est disposée en calotte à l'extrémité grossie.

"La forme la plus commune est ronde ou ovulaire, de 2 à 3 μ de diamètre. La chromatine des formes rondes est réduite à une petite granulation à la périphérie. Dans les formes ovalaires, elle se dispose comme un capuchon à l'un des pôles du parasite. Du reste la disposition de la chromatine est très variable, même dans des formes identiques, comme on le voit bien dans les figures qui accompagnent ce travail.

"On observe aussi, très souvent, des parasites présentant la forme d'une ellipse très allongée, ayant sa chromatine en filament le long de l'un des bords de l'Hématozoaire et dans une certaine étendue.

"On peut, enfin, observer des éléments piriformes, très intéressants, occupant les uns seulement une partie de l'hématie . . . les autres toute le diamètre de l'hématie.

"La chromatine des parasites piriformes, plus petits, se présente comme une granulation placée dans la partie plus

volumineuse ou accolée à l'un des bords. Les poires les plus grandes ont le plus souvent la chromatine condensée en un grumeau situé à la partie étroite du corps; dans un grand nombre d'exemplaires cependant elle se trouve située le long de l'un des bords du parasite, soit comme un filament, soit comme une série de granulations. Parmi les formes en poire nous en avons trouvée une où la chromatine se montre en deux filaments, un de chaque côté du parasite."

Certain parts of this description of *Smithia microti* might very well be applied to the *Cottus* parasite, and indeed a reference to França's plate of the former and to my own figures of the latter will show the close morphological resemblance between these two organisms.

It is not improbable that a recognition of the coccoid, bacillary, and peripheral chromatin types of parasite as being evolutionary phases in the fashion I have suggested, will ultimately help to dispel many difficulties in the way of a proper classification of the Hæmosporidia. If one accepts the existence of a primordial chromatin granule in the case of the hæmosporidia and its development along the lines I have indicated, then one is, I believe, furnished with a simple classification founded on phylogenetic principles. The most primitive hæmosporidian organism would be represented by such a parasite as Theiler's *Anaplasma marginale*, if indeed this is to be considered as distinct from some piroplasm. In the same category would come the bacillary bodies of Graham-Smith. The acquisition of protoplasm by the coccoid or bacillary type would be a further stage in evolution, such bodies being represented by the piroplasms. Again, the peripheral chromatin phase, as seen in *Smithia microti*, would represent an intermediate or transition phase between the piroplasms and the hæmogregarines, or indeed any other adult hæmosporidian of the cellular type. The coccoid, bacillary, and peripheral chromatin types then would be but phases in the upward evolution of a cellular individual. In this connection a question which naturally presents itself is this, why does development become retarded at any one given stage? or in other words, why does the granule in one case proceed through the bacillary and peripheral chromatin phases to the cellular type, whereas in another case it stops at an earlier stage, as for example in the case of a piroplasm? The obvious answer to this question is—that retardation of development is likely to occur just in that stage where reproduction comes possible. Further, one must bear in mind this possibility, that if a parasite becomes capable of reproduction at an early age, before acquiring adult characters, then development tends to become arrested at this adolescent stage, and the adult stage would in consequence, sooner or later, become lost. In other words, the power of reproduction of a parasite is the determinant of its stability in any given phase.

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