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THE SYMBIOTE OF THE MEMBRACID TRICENTRUS ASSAMENSIS VERSUS PATHOLOGICAL CELL-INCLUSIONS

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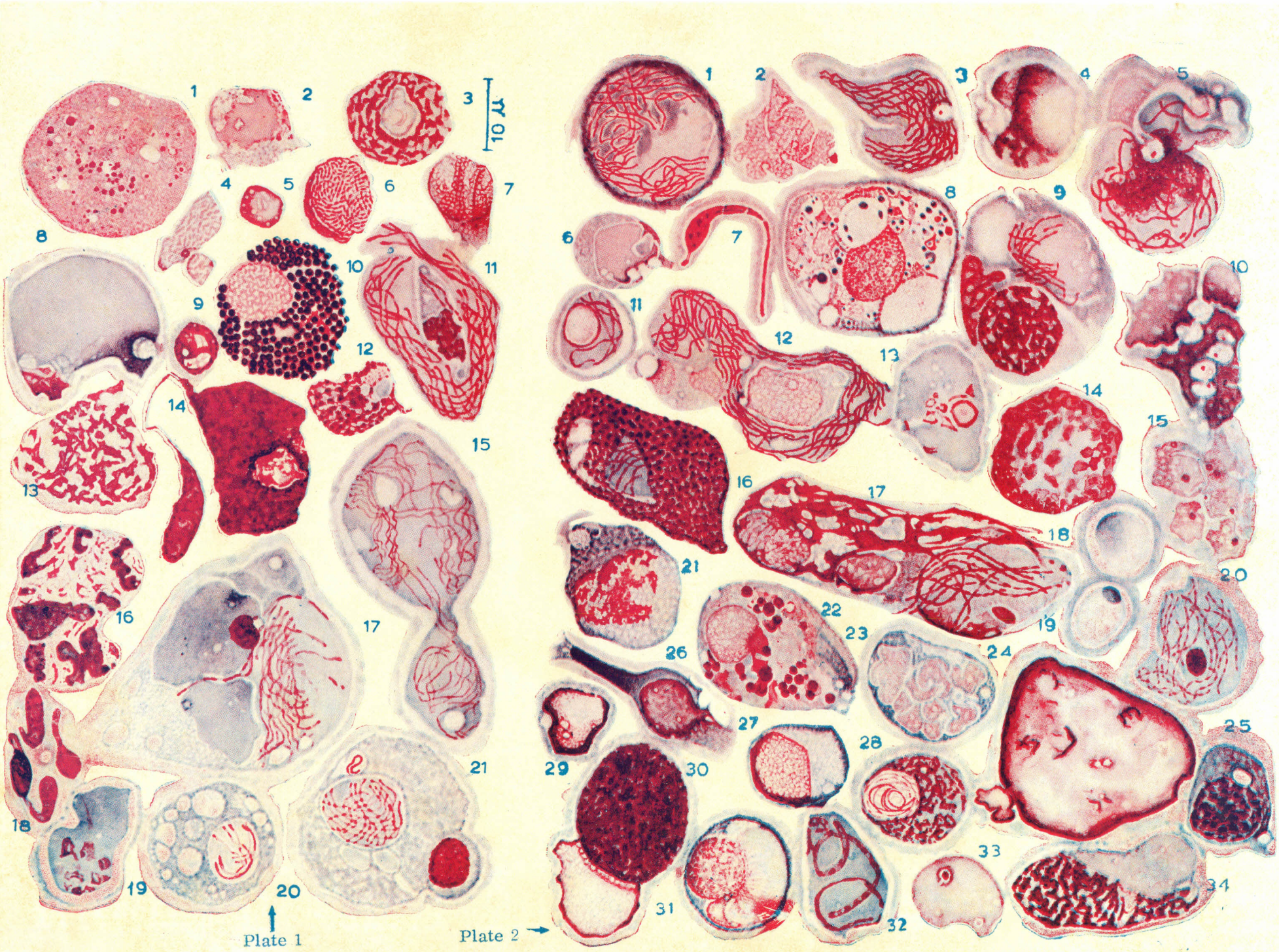
Abstract. *Tricentrus assamensis* can be considered a classical object for the study of symbiosis. Its symbiotic bacterium is the longest so far recorded. Whereas other germs occur solely as independent cell-inclusions here protoplasmic pieces can also contain the germ. As a result of autolysis both protoplasm and nucleus give rise to disintegration bodies. The nucleus with its nucleic acids, losing gelling property, gives rise to various forms of nuclear debris, which as cell-inclusions, have been mistaken for microorganisms. Thus there have been two types of pseudosymbiotes, mistaken as Cicadomyces, protoplasmic and nuclear disintegration bodies.

The one problem in sysmbiosis still awaiting solution is the identity of the symbiote alongwith other cell-inclusions which are being regularly ignored. When a tissue cell is infected pathological changes are bound to occur giving rise to cellular debris, both protoplasmic and nuclear in origin. Some of these artefacts have been mistaken for mysterious microorganisms, designated Cicadomyces, a class of germs found only among insects, and the tumour containing them, Mycetome. The following points speak against their being living organisms. In the first instance no Cicadomyces has been cultivated, whereas symbiotic yeasts and bacteria have been. As pathological artefacts they are common to many insects. On the contrary it was shown that insect species can be differentiated by means of their symbiotes.[2] Then there are two types of Cicadomyces though not recognized as such. The protoplasmic degradation bodies stain blue with Giemsa and in smears treated with pepsin, are easily digested. The other kind of Cicadomyces stains red with Giemsa and appears sponge-shaped or honeycombed. However, no attempt has been made to establish how these cellular debris originate. This really becomes the work of comparative pathology which was never undertaken before. In the entire literature there is the single case of *Cicadella viridis* which has been thoroughly studied. Buchner[2] studying it cytologically, established two symbiotes, one as bacterium and the other as Cicadomyces. On cultivation it was found that there were two becteria instead of one.[3] Their identity could be confirmed for they produced green and yellow colours *in vitro* which were identical with the colours of the insect. This work was further confirmed[4] later on. The Cicadomyces as expected, could not be grown.

I was on the look out for symbiote which would be long enough not to be mistaken for normal cell granules. Moreover, it was to reveal interplasmic infection so that such a body, taking the blue stain, with long red bacterial filaments, could never be accepted for Cicadomyces. The membracid insect, *Tricentrus assamensis*, has become a classical object for such a study. My colleague, Dr. Mohan Babu Naidu,[5] has published a short note to show specificity of symbiosis, taking three membracids, including *T. assamensis*. The technique adopted here is that of pathologists and microbiologists, of making tissue smears and staining with Giemsa. Pl. I Fig. 17 shows a giant cell, already a pathological condition. Its nucleus is exceptionally small and wanting in dot-shaped chromatin granules. Its protoplasm is divided into partitions, one pale blue, full of red-stained bacterial filaments, and protoplasmic pieces which do not support the bacterium, are stained deeper as purple. Fig. 17, at the lower corner, shows a grey region, with three large vacuoles, revealing poverty of protoplasm. When another mammoth cell disintegrated it released a protoplasmic body (Fig. 15). It is shaped like a large yeast, but full of red bacterial filaments. Fig. 21 shows a large cell with poor, grey-stained, protoplasm. In the centre there is a round piece of blue-stained protoplasm, full of bacterial threads, which, however, are granular, showing a starving condition. Close to the central protoplasmic piece lies a vacuole, with a loop of bacterium deeply stained. The vacuole was created on the bacterium exhausting the protoplasm and subsequently dying and taking the deep stain. In Fig. 21 the nucleus is flat, without chromatin granules, and lies at a corner as about to be discharged from the cell (Fig. 20). The protoplasm is again grey and further full of vacuoles, with a round blue protoplasmic piece, similar to that of Fig. 21. An actual disrupted cell is illustrated in Fig. 11. The protoplasmic piece, all around is pale blue, with red bacterial filaments, well-grown and intertwined. In the centre is the residual nucleus, with its chromatin degenerated, offering a sponge-like or honey-combed appearance but stained deep red. Adjoining it is a triangular piece of thin greyish protoplasm with two vacuoles. When protoplasmic disintegration pieces are small the germs they contain are also short. Two such pieces are shown in Fig. 4. In Fig. 21 a bacterial colony was seen in a round protoplasmic piece with the bacterial filaments as granular. A free bacterial colony, is seen in Fig. 6, the filaments are all granular, unlike the healthy filaments seen in Fig. 11.


10 μ
Plate 1
Plate 2

We now turn to bodies of nuclear origin. A normal nucleus is round with chromatin granules dot-shaped. Fig. 12 shows the chromatin granules to be normal but not the nucleus. It reveals the early stage of autolysis when the nucleic acids of the nucleus start losing their gelling property and chromatin bodies separate from one another and which later flatten themselves. In Fig. 12 chromatin granules are round or dot-shaped but in Fig. 3, further separated, and flattened in shape. In the centre of Fig. 3 a piece of protoplasm is stained bluish and contains a couple of bacterial threads faintly stained. A much larger nucleus in an advanced stage of degeneration, has its chromatin bodies, separated much further, and transformed into bizarre forms, together as a typical unit, seen in Fig. 13. Such bodies have actually been mistaken for Cicadomyces when stained with iron haematoxylin whereas Giemsa stains them red being chromatinous in nature. When degeneration proceeds further nucleic acids become more fluid and chromatin granules fuse into one mass from which a club-shaped portion has extended, connected by a thread of liquefied nuclear material (Fig. 14). At one spot the liquefication has created a vacuole-like space. Such bodies are independently seen in Figs. 5 and 9, which have resulted from intense nuclear disintegration. A stage between what is seen in Figs. 13 and 14, with their characteristic features, is observed in Fig. 16. The longer bizarre-shaped bodies of Fig. 13, are further reduced almost to red fibres, whereas portions similar to the club (Fig. 14), are incorporated in Fig. 16. The entire complex illustrated in Fig. 16 is chromatinous or nuclear in nature. It was originally a giant nucleus, like Fig. 14. Fig. 18 shows a cell with blue protoplasm and two vacuoles, but its nucleus disintegrated into four club-like pieces. Fig. 19 is similar but the chromatin materials is seen like chromosome bodies splitting into pairs. We have to remember that disintegration can assume many ways which explains the different forms of nuclear debris. A large cell, with a small nucleus, already showing autolysis, gave a smear (Fig. 8). Its blue protoplasm is obvious. To the left a red-stained body is the degenerated nucleus which, on account of its fluidity, has assumed the flat shape it presents. A nucleus, reduced to a thin sieve-like structure, is seen in Fig. 7. Another where the nucleus had become quite fluid is illustrated in Fig. 2. Since I wished to record all types of bodies that were observed in the smear of a bacteriotome I did not miss Fig. 10. It recalls a phagocytic cell of the insect; the point to note is its thin nucleus, similar to the nucleus (Fig. 7). Finally Fig. 1 represents an Oenocyte, normal to the insect.

In pl. II, Fig. 9 shows a large cell with an equally large nucleus. The chromatin bodies are not dot-shaped, as in Fig. 12, pl. I, but already flattened, approaching the more advanced condition shown in Fig. 3, pl. I. In Fig. 9. pl. II there is a protoplasmic piece, with bacterial thread similar to that of Fig. 17, pl. I. Close to the nucleus in Fig. 9, pl. II there is a red, sponge-like honey-combed body, representing the other type of Cicadomyces. This red cell-inclusion, of Fig. 9, pl. II is identical in structure and in staining with the residual triangular nucleus of cell (Fig. 11, pl. I). The red cell-inclusion of Fig. 9, pl. II has resulted from phagocytosis. This is quite intense which explains how almost all the cells of bacteriotome carry germs, and also other cellular debris, whereas the tissue cells in other parts of the body do not. To be plain I emphasize phagocytosis leading to harbouring infection more than the power of the germ to spread vigorously, imparting a sort of predisposition on the part of some tissue cells and thereby a limitation in the spread of infection. Fig. 16, pl. II is a mammoth nucleus but the chromatin granules are normal. There is an empty space within, as was also the case in Fig. 3, pl. I. Hence Fig. 16, pl. II, also contains a piece of protoplasm, stained deep blue, with red bacterial filaments. I have found nothing so characteristic as shown in Fig. 17. It is a long giant cell. Its nucleus, having been liquefied, has stretched out into strands forming a sort of network and presenting a case for the pathologist rather than for a normal cytologist. Right below is a piece of protoplasm with long bacterial threads and a piece of chromatinous body or a small nucleus. To the left below are two bodies mainly protoplasmic but superimposed by thin strands nuclear disintegration material. When such bodies, of mixed nature, are liberated on the cell breaking down, they would be mistaken for free Cicadomyces. Another excellent case is presented in Fig. 12. The protoplasm all around the cell is blue and contains vacuoles and is full of red filaments of bacterium obviously well grown. In the centre is a large and thin, red body like the nucleus of cell (Fig. 10, pl. I), and like the degenerated nucleus (Fig. 7, pl. 1). Moreover, identical two smaller red bodies lie on the left below in Fig. 12, pl. II. Bacteria are obvious but a typical nucleus is absent. Instead we have nuclear disintegration bodies staining red otherwise mistaken as the other type of Cicadomyces. Fig. 5 offers a clue to the origin of nuclear disintegration bodies. There is a bluish protoplasmic piece with well stained bacterial threads. There is no nucleus, as such, since it has become fluid enough to spread itself as a red patch. Some liquefied nucleic acid penetrating a portion of protoplasm together form a piece on the top left in Fig. 5. The liquefaction of the nucleus has also been illustrated in Fig. 2, pl. I. A small cell (Fig. 11, pl. II) having suffered complete nuclear autolysis, shows, instead of a nucleus, a vacuole, but the protoplasm contains bacterial threads. A large cell, having similarly ejected its nucleus, is now a mass only of protoplasm, full of bacterial filaments (Fig. 1). Whatever, may be its origin there is no nucleus or any red-stained chromatinous residue, also seen before in Fig. 20, pl. I. Now imagine cell (Fig. 17, pl. II) disintegrating and liberating the protoplasmic piece to its right. It would give rise to body Fig. 20, where the nucleus is its own, reduced in size as in Fig. 17. The protoplasm is full of bacterial threads, however, beaded or granular. The symbiotic bacteria do not secrete nucleases and as such do not

enter the nucleus. Nuclear disintegration results from autolysis and this from the slow death of the cell due to infection. Hence we get nuclear debris always free from germs but protoplasmic pieces, with and without germs, in fact with germs also in exceptional cases, *T. assamensis* being the best so far known. Thus we get two types of cellular debris, nuclear which is free from germs, and protoplasmic which do contain bacteria. Figs. 2 and 3 pl. II represent the two kinds of cellular debris or the two kinds of Cicadomyces. The nuclear or chromatinous nature of the object (Fig. 2), stained red, is comparable with the red, thin, flat bodies of Fig. 12, and with the nucleus of Fig. 27, both of pl. II and with the nucleus (Fig. 7, pl. I). Fig. 25, pl. II, resembles a leucocyte or is perhaps actually one. No one has studied the phagocytic cells of this insect. When the cell (Fig.25), degenerates the chromatin bodies disintegrate and the vacuole enlarges. Such a case is seen in Fig. 28, where the protoplasm is full of chromatinous residues, and the vacuole has become large and contains coiled bacterial threads. The phenomenon of giant cell formation, though pathological, is not confined to tissue cells. Prof. P. Lindner, who had studied yeasts as much as any one can expect to have done, discovered giant yeast cells. Fig. 32 shows a protoplasmic infection by a giant bacterial filament. Fig. 7 is also a free giant filament of the same bacterium.

We now turn to nuclear degradation with liquefaction of nucleic acids already referred to. In Fig. 16, pl. II, the chromatin bodies are dot-shaped but in Fig. 30 they have fused together into a mass. Attached to the nucleus is a piece of protoplasm, the connection being brought out with all subtle details. In Fig. 3, pl. I the chromatin granules of the nucleus had flattend out. Fig. 14, pl. II shows a giant nucleus not so large so that of Fig. 30, but the chromatin granules are separating from one another and have flattened themselves. We may consider Fig. 25, rich in protoplasm and having well stained chromatin granules of the nucleus. Now if the cell lengthens and its chromatin bodies elongate themselves, though not so long as seen in Fig. 13, pl. 1, we get a case like Fig. 34, half and half nuclear and protoplasmic. In Fig. 30 the fusion of chromatin particles is due to liquefaction of the nucleic acids. In Fig. 26 the nucleus has its chromatin granules fused into a compact mass like Fig. 30. Even a superficial examination of Fig. 27 will show it has a thin flat nucleus and no chromatin granules thereby resembling Fig. 10, pl. I, and with a thin grey protoplasm. With further autolysis of the nucleus and penetration of nucleic acids into the protoplasm we shall get a case as depicted in Fig. 31. Here the diffusion of liberated nucleic acids is clearly observed. Fig. 21 shows a cell where the disintegrated nucleus is seen as residual red patches for what used to be chromatin granules. It clearly reveals a vacuolated or sieve-like background, having a sponge-like or honey-combed appearance. The protoplasmic residue has taken a brownish stain. In Fig. 21 the nucleus was a large one but has left patches of red staining nucleic acids. In Fig. 23 we have a typical case of nuclear disintegration. The cell was poor in protoplasm, which is seen as deep grey-stained body. The nuclear debris has taken pink stain and is vacuolar in appearance like pieces of sponge. Such cell-inclusions have been illustrated as Cicadomyces, or actual symbiotes. When the nuclear disintegration bodies as in Fig.23 flatten out or become thin we see them as illustrated in Fig. 15. The vacuolated or sponge-like appearance of bodies in Fig. 15 resemble Fig. 2. We have shown in Fig. 9, a red cell-inclusion, close to the nucleus. This body was foreign to the cell. Such a piece is seen along with others in Fig. 10. Figs. 10 and 15 are similar, Fig. 15 is as thin as possible whereas the bodies in Fig. 10 are very dense, due to nuclear and protoplasmic disintegration proceeding together. Fig. 5, pl. II, has been explained as nucleus losing its gelling property and leaving a red patch. Fig. 4 shows the same but stained deep grey, due to protoplasm having lost its freshness long before and subsequently oxidized. I must emphasize the similarity in structure of the red cell-inclusion in Fig. 9, and the bodies seen in Fig. 10, both with the nuclear residue of Fig. 11, pl. I. Fig. 6 represents a cell, with grey protoplasm, and nucleus pink and flat, its nucleic acid having become fluid, as in Fig. 2, pl. I. The staining of the protoplasm and the liquefied nucleus of Fig. 6, pl. II, represent what may be called postmortem staining, whereas in Fig. 2, pl. I, the nucleus was still fresh. A small nucleus, that had completely liquefied sometime ago, is seen in Fig. 33, which has not taken a bright stain but its nucleolus can be seen as deep red spot. A giant nucleus, like Fig. 30, completely liquefied gave a picture like Fig. 24. In Fig. 13 the protoplasm is grey and nucleus degenerated into red-stained bodies. The sharp margin of Fig. 13 clearly speaks of the object having been a cell. We may refer to the protoplasmic piece attached to the giant nucleus in Fig. 30; when this separates and happens to be small it will have a form as illustrated in Fig. 29. This is protoplasmic in origin, resembling the protoplasm of Fig. 30. Bodies shown as Figs. 5 and 9, in pl. I, are nuclear debris, and can be distinguished as such on careful examination. When cell disintegrates and protoplasmic pieces are subjected to oxidases they stain grey as has been noticed in cells Figs. 6 and 21. Figs. 18 and 19 are such protoplasmic pieces and would appear as though they were drawn with dilute Indian ink. A normal cell which had phagocytized red staining granules is seen in Fig. 22; its thin nucleus has to be noticed as similar in colour to the nuclear disintegration body (Fig. 2, pl. II). Another normal cell with heteroegneous cell-inclusions is shown in Fig. 8.

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