Dendrocometes paradoxus.

Part I.—Conjugation.

By

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With Plates 17 and 18.

INTRODUCTION.

There is no species of the class Acinetaria that can be obtained with greater facility at all times of the year than Dendrocometes paradoxus (Stein). This is due to the fact that it is found with unfailing regularity on the gills of our commonest fresh-water Crustacean, Gammarus pulex.

In addition to the fact that it is readily obtained, however, it presents us with the further advantage of being attached to a soft and tolerably transparent gill, which can be easily seen with the naked eye, and manipulated without difficulty on the slide and in the paraffin bath. The difficulties that have usually to be overcome in preserving and staining the Infusoria whole or in sections are, in this form, largely obviated, and it is not very difficult, after the preliminary examination of the specimens attached to any one gill, to imbed and cut them into a series of sections in any plane that may be desired. Possessing these advantages, it was obvious that a careful examination of the changes of the nuclei
during conjugation and gemmation might yield results of interest and importance.

I have had preparations of Dendrocometes under observation for a considerable number of years, but it was not until 1899 that I obtained specimens which clearly showed the mitotic figures of the division of the micronuclei, and then I determined to investigate the matter with greater thoroughness. I may explain that during the last two years I have entrusted the mounting of the preparations, as well as the staining and cutting of the sections, to Mr. J. T. Wadsworth, and that he has saved me an immense amount of time and labour in pointing out to me the slides that exhibited interesting features, and in keeping a catalogue of the preparations. The discoveries therefore that are here recorded were, in the first instance, made by him, and I have to acknowledge here his unfailing assistance and perseverance in the investigation.

The structure of Dendrocometes has been investigated by Bütschli (1), Wrzesniewski (27), Plate (21), Maupas (19), Schneider (24), and Sand (23); but, notwithstanding their excellent work, many points of interest and importance remain to be illustrated and described.

Dendrocometes paradoxus is found attached to the gills of Gammarus at all times of the year, but in the summer months there are usually fewer specimens on each gill than in the spring and autumn. We have found that by keeping about twelve or fourteen Gammarus in a shallow pie-dish containing an inch or two of water, with a little mud and waterweed at the bottom of it, the number of Dendrocometes on the gills increases, and that in a fortnight’s time a considerable number of pairs may usually be found in a state of conjugation.

Dendrocometes appears to have a wide geographical distribution. I have found it myself on Gammarus at Oxford, Cambridge, in the neighbourhood of Manchester, and on specimens of Gammarus sent to me by Mr. Bolton from Birmingham. It was observed by Prof. Lankester on Gammarus in the ponds at Hampstead. On the continent of
Europe it also appears to be generally distributed. It certainly occurs in Belgium (Sand), France (Maupas), Germany (Butschli, Plate, etc.), Russia (Wrz.). It also occurs in North America (Butschli). It is probable that the Dendrocometes which occur on the gills of Gammarus putaneus (Lachmann) may be a distinct species, but, apart from this, Dendrocometes only occurs on the gills of Gammarus pulex, although exceptionally a few specimens may be found attached to the hairs on the legs of the same host (Sand).

Dendrocometes paradoxus has a planoconvex-shaped body, and is provided with three, four, or five arms (Pl. 17, fig. 1). The morphology of these arms is a matter of some dispute, but it is not an unreasonable view to regard them as homologous with a bundle of Acinetarian tentacles. They capture, kill, and partially digest the prey in the same manner as the tentacles and suckers of other Acinetaria. The body contains a single large meganucleus, and a variable number, but usually three, micronuclei. There is a single large contractile vacuole. Reproduction is effected by an interesting process of internal gemmation, first described by Butschli (1). The single planoconvex bud that is formed is frequently called the "embryo," but I think it is more appropriate to call it the "gemmula." It escapes from the parents without arms, but provided with a girdle of three bands of cilia, and swims away.

From time to time, or perhaps, under certain conditions only, nearly the whole substance of an individual escapes from the pellicular sheath and swims away from the gill in a form which cannot be readily distinguished from a gemmula.

With this brief introductory account of the structure and reproduction of the animal I pass on to the phenomena of conjugation, to which I have paid special attention.

Part I.—Conjugation.

The phenomena of conjugation in Dendrocometes may be briefly stated as follows:
Two individuals in proximity on a gill of Gammarus send out simultaneously blunt lobe-like processes, which may be called the conjugative processes. These meet but do not completely fuse, a distinct membrane delimiting the process of each individual throughout the conjugation. This membrane does not prevent the fusion of the meganuclei nor of the conjugative micronuclei in the latter stages, nor does it prevent a certain amount of mixture of the cytoplasm of the conjugating individuals.

Stage A (Pl. 17, fig. 2).—In the initial stage one or both the meganuclei may exhibit pseudopodial processes indicating that they have some power of amoeboid movement. The micronuclei are a little but sometimes very little larger than they were before conjugation. (In this respect, as in so many others, there is considerable variability. The reader will notice that the individuals drawn in fig. 1, in which the conjugative processes have not yet met the micronuclei, are actually larger than they are in the individuals drawn in fig. 2, which are actually conjugating.)

Stage B (fig. 3).—The micronuclei increase considerably in size during this stage, the chromatin being resolved into a delicate skein. The meganuclei also increase in size, become spindle-shaped, and show an arrangement of the chromatin into roughly parallel lines. From this stage onwards until Stage K (fig. 13) is reached, the meganuclei increase in size without showing any material change in structure; and as the interest of the phenomena now centres in the micronuclei, further reference to the behaviour of the meganuclei will be for the present omitted.

Stage C (figs. 4, 5).—When the micronuclei have reached their full size the chromatin collects in the form of numerous minute chromosomes in an equatorial plane. At the same time extremely delicate, faintly staining threads (linin threads) roughly parallel with one another, forming a tub-shaped spindle, make their appearance. Neither in this nor in any other stage of mitosis is there any sign of the presence of centrosomes or similar bodies. I have found it
impossible to count the number of chromosomes with any degree of accuracy in this or in any other stage of division. The mode of division of the chromosomes is also very difficult to determine, but I have seen V-shaped appearances, Pl. 18, fig. 13, very similar to those figured by Prowazek (22) in Bursaria. I believe with him that these appearances point to longitudinal division.

The chromosomes separate into two parties, which travel to the opposite poles of the spindle (fig. 5), where they apparently fuse to form a solid irregular lump of chromatin. The spindle then elongates enormously, so that the two chromatin bodies are sometimes separated from each other by a distance equal to three fourths of the full diameter of the Dendrocometes. The spindle then becomes detached from the chromatin and dissolves in the cytoplasm (fig. 6). The stages in the division of the micronuclei are usually synchronous in the two individuals, but sometimes one set of figures is a little in advance of the other, as shown in fig. 5.

By this division six micronuclei are formed in those individuals which began the process with three.

Stage D (fig. 7).—One of the five micronuclei of each individual passes down the conjugative process to a position very close to the membrane, where it enlarges and again forms a mitotic figure. The other micronuclei degenerate and disappear.

The nuclei which are found close to the membrane give rise by their division to the germ nuclei, to use the term employed by Wilson (26). The division is always in a plane parallel with the membrane (fig. 8).

Stage E (fig. 9).—The germ nuclei take up such a position in contact with the membrane that each germ nucleus of an individual is exactly opposite one of the other individual. These nuclei consist of a clear vacuole containing a single coarse skein of chromatin. The spindle entirely disappears.

Stage F.—The germ nuclei then fuse in a manner shown in Pl. 17, fig. 10, and Pl. 18, fig. 12, giving rise to the cleavage nuclei of the two individuals.
Attention may here be directed to two important points of comparison with ciliate Infusoria. The difference between the migratory or male germ nucleus and the stationary or female germ nucleus is in Dendrocometes reduced to a minimum. It is possible that in all cases one germ nucleus traverses the membrane and the other does not, so that the distinction remains, but the two nuclei are as nearly neuters as can be. In the second place, the fusion of the germ nuclei takes place during a resting and not in a mitotic state.

According to the researches of Manpas, Hertwig, and others, the germ nuclei of the ciliate Infusoria fuse when in the form of spindles or mitotic figures.

Stage G (Pl. 17, fig. 11).—One of the cleavage nuclei passes into each of the conjugating individuals and prepares to divide again by mitosis. The early stages of this division probably occur very soon after the fusion of the germ nuclei, as the figures may be seen sometimes quite close to the membrane (cf. Pl. 18, fig. 13). This stage may be distinguished from Stage D, which it somewhat resembles, by the fact that the axes of the spindles are not parallel.

Stage H (Pl. 17, fig. 12).—The cleavage nucleus divides into two nuclei which take up a position in close proximity to the meganucleus.

Stage J (Pl. 18, fig. 1).—The nuclei formed by the division of the cleavage nucleus again divide, and almost immediately one of the four becomes a little larger than the other three.

Stage K (Pl. 17, fig. 13).—The largest of the four nuclei of the last stage becomes the new meganucleus, the other three the new micronuclei. There is some evidence to show that occasionally two of the three smaller nuclei again divide in this stage, giving rise to a condition in which there are six nuclei in all, as seen in the preparations from which Pl. 18, fig. 8, was drawn. In some cases, too, it appears that two nuclei enlarge to give rise to new meganuclear structures, as seen in Pl. 18, fig. 19. Variations of this kind at these stages have added very much to the ordinary
difficulties of the investigation, but the establishment of the fact that variations of such an important character do occur is, in my opinion, a result of considerable interest. At some time during the last three stages (H, J, K) the old meganucleus becomes very large, and is bent on itself in the form of a loop or horseshoe. One extremity of this figure passes into the conjugative process, and approaching the limiting membrane traverses it and fuses with the corresponding extremity of the meganucleus of the other individual. The exact phase at which this meganuclear conjugation takes place seems to vary considerably; all that can be said at present is that, so far as my experience goes, it usually occurs between Stages J and K. The number of cases of actual contact of meganuclei observed by Mr. Wadsworth and me is small, and this may be interpreted to mean either that the period of meganuclear conjugation is very short or that it does not always occur. Until some satisfactory method is invented of watching the nuclear phenomena of the conjugation of living Dendrocometes, it is impossible to prove that the meganuclear conjugation never fails. I am inclined to believe that it always occurs. Similarly I have no proof to offer of the length of time occupied by this process; but I am inclined to believe, on the circumstantial evidence at my disposal, that it is very brief.

Soon after the meganuclei have conjugated they separate and begin to degenerate.

The usual phenomena of nuclear change during conjugation in Dendrocometes may be represented by the following diagram, in which the circles above M represent the stages in the meganucleus, and the black dots above m, m, m, the stages in the history of the micronuclei. Conjugation of the meganuclei usually occurs in Stage J, as explained above, and in Stage K these bodies disintegrate.

In the following diagrams I have endeavoured to interpret certain phenomena which appear to be variations of the more usual stages. In Diagram B, which starts with Stage G, the important variation is that two of the micronuclei formed
by the second division of the cleavage nucleus divide again (see p. 330).

In Diagram C we have the same variation as in Diagram B,

with the additional peculiarity that two of the nuclei give rise to new meganuclei in Stage K, and I have added in Stage L the suggestion that these two new meganuclei
fuse together to form the single meganucleus of the new individual.

The phenomenon of conjugation in the Acineta has been known for a great many years. It has been observed in several species of Acineta by Claparède and Lachmann (4), Fraipont, D’Udekem, Keppen (15), and others; in Metacineta (Claparède and Lachmann and Lieberkühn), in Podophrya, Tokophrya, Stylocometes, and in Dendrocometes. In Dendrocometes it was observed by Wrzesniowski (27), but more fully described by Plate (21).
As regards the external features very little can be added to Plate's description. As he observes, conjugation usually occurs when the gill of the host is unusually crowded with the Dendrocometes. The two conjugating individuals are in most cases similar in all essential respects. Occasionally, however, a difference may be observed between the two conjugates. Plate states that sometimes one of the individuals which is clearer than the other withdraws its tentacles during the process. Sand (23) says, "Chez Dendrocometes la conjugaison a lieu souvent entre un individu amaigre et un animalcule bien nourri." Mr. Wadsworth and I have frequently observed differences between the conjugating individuals, and Mr. Wadsworth has observed the retraction of the arms of certain individuals during conjugation.

Plate observed occasionally a conjugation of three individuals. This I am able to confirm, but the occurrence is so rare that no series of nuclear changes in them have been followed.

As Plate denied the existence of micronuclei, he failed to see any of the stages of their division and conjugation which are described in this paper. In one of his figures he shows the points of the meganuclei in the bases of the conjugative processes, but he did not observe the fusion of these bodies. He gives a good figure to illustrate the fragmentation of the meganucleus at the close of conjugation. In Schneider's (24) figures of Dendrocometes in the act of conjugating the meganuclei are shown to be approaching much more closely than in Plate's figure, and in the same author's figures of the closely allied genus Stylococmutes the meganuclei are actually shown to be in contact. The statement made by Plate and Schneider that the new meganucleus is formed by a regeneration of the fragments of the old is not correct.

The mixing of the cytoplasm in the conjugative process is affirmed, and I believe correctly, by Plate, Sand, and others. I have myself observed a flow of protoplasm passing backwards and forwards through the membrane with each
Dendrocometes Paradoxus.

contraction of the vacuole for several hours in one pair, but
the period during which this occurs is limited, and during
the greater part of the forty-eight or more hours during
which the pair remain in conjugation no interchange of
particles can be seen.

An important feature of the conjugation of this genus
is that the ordinary vital processes of the individuals are
not materially affected during the act. The arms remain
fully extended—they catch and swallow particles of food,
which are digested in the ordinary course. The contractile
vacuoles continue their pulsations for some time after the
process has commenced.

Conjugative Processes.—These processes are formed
specially for conjugative purposes in Dendrocometes. In
development and in structure they differ from ordinary arms,
and I do not consider that they are rightly considered to be
homologous with arms. This view does not agree with that
expressed by Bütschli (2), who, on the strength of the
observations of A. Schneider on Stylocometes, regards them
as rudimentary arms. In Stylocometes, according to that
author, an arm of each of two neighbouring individuals
becomes abnormally thick and elongated to form the conjuga-
tive process, and that it is a true arm is proved by the fact
that it contains a canal. I have had no opportunity of
examining Stylocometes, but I do not feel that Schneider's
account of it is convincing. Until his account is confirmed,
therefore, I must agree with Plate that the conjugative pro-
cesses in Dendrocometes are not homologous with the arms.

Bütschli (2) states that in Dendrocometes one individual
 sends out a process which fuses with the body-wall of
another, but that such an occurrence is rare. I have never
seen such a phenomenon. In nearly all cases the conjugative
processes begin and grow simultaneously, so that they are
throughout approximately equal. In the preparation from
which fig. 1 was drawn, the individual to the left has a
decidedly longer process than the individual on the right, but
such a difference as this is exceptional. It is impossible to
state what stimulus there is that causes two individuals to begin the sexual proceeding. It may be that some excitement may be caused by the touching or brushing of the arms, but nothing of the kind has been observed. Judging from the development of the conjugative processes alone, which is all that we have to guide us in the initial stages, it seems probable that the sexual stimulus affects the two individuals simultaneously, and that there is no differentiation of sex.

The Micronuclei.

It is an interesting fact that, notwithstanding the careful and elaborate investigations made by several observers on the micronuclei of the Ciliata, there is at present no satisfactory account of these structures in the Acinetaria.

That micronuclei do occur in all Acinetaria is not yet proved, but nevertheless it is extremely probable that they are as constant a feature of the anatomy of this group of animals as they appear to be of the Ciliata.

In Bütschli’s great work on the Infusoria (p. 1873) the following notes will be found on the subject. Bütschli himself in 1867 discovered a micronucleus in a species of Sphaerophrya. Maupas proved with certainty the existence of a micronucleus in Tokophrya limbata, Acineta tuberosa, Podophrya fixa, and Podophrya cyclopum. He probably, but not with certainty, found them also in Acineta Jolyi, whilst certain bodies which may have been micronuclei were seen in Ephelota gemmipara. In Tokophrya limbata Möbius also proved the existence of micronuclei.

In a recent paper on the anatomy of a new species of Ephelota, Ishikawa writes with some uncertainty about the existence of micronuclei. The most satisfactory figures of the micronuclei of Acinetaria are those given by Keppen (15), but as his monograph is written in the Russian language I am unable to read it.

The authors of the ‘Traité technique de Zoologie’ (5) accept
the view that there is a micronucleus in the body of the Acinetarian.

In the recently published monograph of the Acinetaria, however, Sand (23) denies the existence of true micronuclei. He believes, however, in the existence of a body lying close to the meganucleus, which he calls the "centrosome."

"Un corps un peu plus colorable que le noyau, creusée exprès pour le recevoir. . . . Quand ce corps est vu au centre du noyau, il paraît entouré d'une petite zone claire formée par le cytoplasma qui le sépare du noyau." He further states that no Acinetarian ever contains two of these bodies, and that it is absolutely homogeneous, both in a state of rest and of division. He has proved the existence of this body in sixteen species. I have very little doubt that the "centrosome" of Sand is not a "pseudo-micronucleus," but a true micronucleus. Maupas (20) says that his series of stages of the micronuclei of Podophrya fixa is incomplete, and he would probably himself admit that the figures he has published of them are not satisfactory. To account for his unsatisfactory results in the Acinetaria, Maupas ventures upon the statement that the micronuclei of these Infusoria are smaller than they are in the Ciliata, and stain very lightly. He adds that the study of the nuclear phenomena of the Acinetaria is beset with so many difficulties that he considers it to be "une des recherches les plus pénibles qu'un micrographe puisse entreprendre."

In Dendrocometes, however, these excuses cannot be put forward, as the micronuclei are even larger than they are in many Ciliata, and their affinities for certain stains are exceedingly powerful.

The presence of micronuclei in this genus has, nevertheless, been denied.

Plate (21) described a number of small bodies in the cytoplasm which, on account of their affinities for safranin, he called the "Tinktinkörnchen," but he denied the existence of "Nebenkerne." Maupas replied to Plate's paper by stating emphatically that micronuclei do occur in Dendrocometes
and referring to Plate's own figure, pl. vi, fig. 17, as affording a convincing proof of it. Neither Butschli (1), Wrzesniowski (27), nor Schneider (24) appear to have observed them, and Sand (23) only mentions one "centrosome" as being present in this genus.

The number of micronuclei in Dendrocometes varies, but, as stated above, the usual number is three. In individuals that are neither conjugating nor preparing for gemmation it is sometimes difficult to count the micronuclei, as they are very small, and are difficult to distinguish from other bodies in the cytoplasm which have affinities for the stains that are used. However, a certain number of individuals with moderately clear cytoplasm can nearly always be found, and in these it is not difficult to recognise the micronuclei in sections stained by the iron-hæmatoxylin and iron-brazilin methods (13). There are sometimes two micronuclei, and sometimes four, and but rarely five. I do not believe that there are ever more than five or less than two.

In the state of rest the micronucleus consists of a small, irregular granule of chromatin, enclosed in a clear zone, which is invariably perfectly spherical in shape. There is no evidence of a definite membrane surrounding the clear zone. The zone is from 2—4 μ in diameter. It is very difficult to form an opinion of the nature of the substance composing the clear zone. When the nucleus is in a state of rest this zone resists the action of all the stains I have tried, and no lines nor granules of any kind or description can be seen in it. It is possible that it may be a mere artefact, due to the shrinkage of the chromatin during the process of preservation, but the regularity of its shape and its relation to the chromatin granule do not support this view. In my opinion it really represents the "achromatin" elements of the nucleus.

When preparing for division the micronuclei increase considerably in size, the solid granule of chromatin becoming converted into a coarse skein (Pl. 18, fig. 14 m). Later the skein breaks up into a much finer tangle (Pl. 17, fig. 3), which gradually fills up nearly the whole of the clear zone. In
the next stage there is a differentiation of the skein into a number of lines, which stain very faintly in iron-hæmatoxylin, but a little more deeply with iron-brazilin, and a band of deeply staining rods and granules having the usual chromatin reactions. At this stage the micronucleus is frequently 10 μ in diameter (Pl. 17, figs. 4, 11, 12). The exact determination of the nature of the equatorial band of chromatin granules is a matter of considerable difficulty. With the highest powers of the microscope the granules or minute rods appear to be connected together in the manner shown in Pl. 18, fig. 13, but still I do not feel so far convinced of this connection as to deny the proposition that they are isolated chromosomes. With lower powers of the microscope they have every appearance of being a band of rod-shaped chromosomes (Pl. 17, fig. 12). Whatever the future may reveal regarding these bodies, I think it is clear that the chromosome elements are numerous,—too numerous, in fact, to count with any degree of accuracy.

The micronucleus next becomes somewhat oval in shape (Pl. 18, fig. 16), and the band divides into two bands. The faintly staining lines, which we may call the linin threads, are arranged in a roughly parallel manner forming a somewhat tub-shaped spindle. They do not come to a point at each end of the figure, and there is never any dot or granule that can suggest the presence of a centrosome. In the next stage (Pl. 18, fig. 17) the two bands of chromosomes have passed to the extremities of the figure, and soon become aggregated together to form a single irregular lump. In some cases there is a clear vacuole present at one of the poles (the right pole in Pl. 18, fig. 17), but I have seen it so rarely that I am at a loss to understand its meaning. In the next stage (Pl. 18, fig. 18) the spindle becomes very much more elongated, so that the whole figure may be 25—30 μ in length; the chromatin is in the form of a single spherical lump surrounded by a clear zone at the points of the spindle. The spindle next becomes detached from the new micronuclei (Pl. 17, fig. 6), and gradually dissolves in the
cytoplasm. Throughout this mitosis the clear outline (membrana limitans?) is never lost.

The mitosis of the micronuclei of Dendrocometes has considerable resemblance to the mitosis of the micronuclei of Paramoecium, as described by Hertwig (8), of Bursaria, as described by Prowazek (22), and of other Ciliata.

The Meganucleus.

In specimens of Dendrocometes that are neither conjugating nor preparing for gemmation, the meganucleus is usually a spherical body 0.02 mm. in diameter. It is not, however, constant in shape, many examples being found that are oval or even spindle-shaped.

It is usually situated in the centre of the animal's body, but it is often more or less excentric. On examination in section with an oil immersion lens, there may be seen a distinct meshwork of darkly staining lines which appear to support a series of minute rounded chromatin granules (Pl. 18, fig. 14). In many cases the lines connecting these granules cannot be easily seen when the appearance is as shown in Pl. 18, fig. 10. In the meshes of the darkly staining chromatin there is a homogeneous substance, which in many iron-hæmatoxylin preparations is quite colourless, but stains faintly yellow with brazilin.

Plate states that in the nucleus there are a number of nucleoli: "Bei einem Thier meiner Präparate ist der ganze Kern dicht erfüllt von solchen Binnenkörperechchen deren jedes von einem hellen Hof umgeben ist." I have frequently seen in my preparations appearances similar to this described and figured by Plate, but my interpretation of them is different. In the first place I must state very emphatically that in my opinion there is never in the meganucleus of Dendrocometes any body or bodies which correspond with the nucleoli of Metazoan cells. The clear space round the "Binnen-körperchen" of Plate is in my opinion due entirely to the refraction of the light in passing through the preparation; and this opinion has been thoroughly tested by comparison
of whole mount preparations such as Plate studied, with the thinnest sections of the meganucleus. My opinion is that the substance in the meshes of the chromatin is quite homogeneous. The periphery of the meganucleus is not in my opinion surrounded by a definite membrana limitans, notwithstanding Plate's statement that "ein Kernmembran ist deutlich erkennbar."

When the meganucleus is spherical or oval in shape and situated at the centre of the body, or in other words, when it is at rest, no definite membrana limitans can be seen (Pl. 18 fig. 10); but in the elongated and dividing meganuclei the chromatin network at the periphery is so arranged that a limiting membrane seems to surround the whole nucleus. The limiting membrane is not a definite and peculiar structure of the nucleus, but a temporary arrangement of the substance of the chromatin-bearing network at the periphery during the nuclear movements.

During gemmation the meganucleus undergoes a simple constriction, and is divided into two parts, one of which is retained by the parent, and the other by the young bud (Pl. 18, fig. 14). There is no reason to believe that these two parts are exactly equal in size. In many preparations the part retained by the bud is apparently smaller than that retained by the parent, but, as I have no means of measuring the capacity of these bodies, I cannot make any positive statement on the subject. There is, however, no arrangement of the chromatin in rods or bars during this division which would suggest equivalent chromatin division. Nor have I been able to find after very careful search anything of the nature of anterior fibres, centrosomes, asters, or other characteristic features of karyokinesis. There can be no doubt whatever that when the meganucleus divides the process is purely amitotic.

The Fusion of the Meganuclei during Conjugation.—Whatever difficulties there may be in finding an explanation of the fact, there can be no doubt that the meganuclei do, during conjugation, meet and become
continuous. The statement of this fact was made in my preliminary communication (Hickson, 1900, 12). I am not the first, however, to maintain that the meganuclei of the Infusoria fuse. In 1867 Stein made the following statement concerning the Acinetaria (Stein, 'Der Organismus,' vol. ii, p. 139), 1867:

"Die conjugation verläuft auch bei denjenigen Acineten, bei welchen sie bisher genauer studirt wurde, im Wesentlichen auf dieselbe Weise, wie bei der gleichartigen Conjagation der Vorticellen; es verschmelzen zuerst die Körper der beiden Acineten zu einem einzigen, und dann fließen auch deren Nuclei in einem gemeinsamen Nucleus zusammen."

Bütschli (2) gives a figure (pl. lxxiii, 96) of two attached Vorticellids in which the meganuclei are in junction, but considers that this is doubtfully a case of conjugation. Schneider (24), in Stylocometes, figures the junction of the meganuclei in two individuals that are conjugating, but suggests that this also may be a case of fission. Bütschli may have been right as regards his Vorticellids, but such a method of fission as Schneider suggests for Stylocometes is extremely improbable. In a recent paper by Prowazek (22) a number of new and excellent figures are given of the nuclear phenomena during the conjugation of Bursaria, and it seems probable from these that in this ciliate Infusorian there is a junction of the meganuclei before they disintegrate. Unfortunately Prowazek's description is not very clear, and he does not attach much importance to the phenomenon.

In my preparations of Dendrocometes I have at least three cases in which the meganuclei actually touch, but a considerable number in which they approach one another very closely in the conjugative processes. That the junction is not merely casual contact, but actual organic connection, is proved by the preparation which is represented in Pl. 18, fig. 11. Here there is no sign of any boundary between the two nuclei, and the chromatin granules are fixed in such a manner as to suggest very forcibly that during life they were
DENDROCOMETES PARADOXUS.

flowing from one side into the other. Apart from this evidence, however, attention may be called to the fact, which is evident not only from my own preparations (Pl. 18, figs. 6—8), but also from Schneider's figure of Stylocometes, that when the points of the meganuclei pass down the conjugative processes they converge to the same spot on the membrane. This shows, I think, that there is some force at work which is bringing them together. Sand refers to this in Stylocometes when he says, "Les deux noyaux s'approchent et se placent dans le bras dilaté vis-à-vis l'un de l'autre séparés par une couche de plasma." But he adds, "Pourquoi, dira-t-on, les noyaux vont-ils se placer dans le pont qui réunit les deux Stylocometes ou les deux Dendrocometes? C'est peut-être pour diriger les échanges et les mouvements qui ont lieu dans ce pont."

The organic junction of the two meganuclei lasts a very little while, I believe, and it is probably followed immediately by their disintegration. Each meganucleus breaks up into a number of irregular lumps, in each of which there are at first several granules of unaltered chromatin. A large piece of darkly staining substance is frequently present in these lumps, but in many of them the central parts are simply vacuolated (Pl. 18, figs. 15 and 19).

In the next stage the cytoplasm is filled with numberless vacuoles, granules, and lumps (Pl. 18, fig. 19) of endless forms, sizes, and colourable property. In such a preparation as that from which the figure was drawn, it is almost impossible to distinguish the various remnants of the old meganuclei from food bodies and micronuclei.

The mode of formation of the new meganucleus at the close of conjugation is of great importance and interest.

I must confess that in the earlier stages of this investigation I had some hesitation in believing that the new meganucleus is formed from a product of the segmentation nucleus. The descriptions, and more particularly the figures, of the nuclei of conjugation in Ciliata by Maupas (20), Hertwig (8), Hoyer (14), and others, although unanimous
are not convincing. In none of these papers are the stages of the enlargement of the micronuclear element to form the characteristic meganuclear body very complete, and it seemed to me that there was just a possibility that these authorities were mistaken, and that the new meganucleus arises independently in the cytoplasm, or from one or more of the old meganuclear fragments. This hesitation in accepting the orthodox view was due to the fact, that in the earliest stages I had then found of the formation of the new meganucleus there was no chromatin in its centre. It was, moreover, very much larger than any of the other micronuclei, and the connection between it and a micronucleus could not be traced.

The subsequent discovery of the intermediate stages, however, removed my doubts, and now I feel that it is quite an established fact that the new meganucleus is formed from one of the four nuclei produced by the second division of the germ nucleus. Plate, Schneider, and Sand, who maintain that the new meganucleus is formed by the reconstitution of one or more fragments of the old meganucleus, are in error, and I believe that the views expressed by Maupas and Hertwig as to the origin of the new meganucleus in Ciliata are correct.

The principal stages in the formation of the new meganucleus are shown in Pl. 18, figs. 2—5. In the first stage one of the four micronuclei (figs. 1 and 2) increases in size in a manner very similar to that in which the micronuclei swell up just before mitosis in bud formation, or in the earlier stages of conjugation. In the preparation, the enlarged micronucleus was 5 μ in diameter, and the others 4 μ. This enlargement is caused by a considerable increase in the clear substance, and by the resolution of the chromatin into a coarse skein. In the next stage (fig. 3) the nucleus has still further increased in size to about 8 μ in diameter; the chromatin has become more diffused, and does not stain so deeply. It is probable that the change in staining power indicates some slight change in constitution, but there is no evidence as to the nature of this change. The greater
part of this modified chromatin is arranged in the form of a thick ring at the periphery. There are, however, some strands stretching across the nucleus, and a considerable number of rows of granules extending from the edge into the cytoplasm. I think there can be little doubt that at this stage either the whole or the greater part of the chromatin, in its modified form, passes into the surrounding cytoplasm, leaving the new meganucleus perfectly clear and homogeneous.

The elimination of chromatin from nuclei is a phenomenon of rare occurrence in animal and vegetable cells. In the maturation of the ovum of many animals a considerable amount of chromatin is ejected into the cytoplasm. Wilson (26) says, "In these cases (Asterias, Polychaeras, Thalassemia, Nereis) only a small fraction of the chromatin substance is preserved to form the chromosomes, the remainder degenerating in the cytoplasm. Some years ago I described the fragmentation of the germinal vesicle of the Stylasterid Allopora (this Journal, vol. xxix) and the distribution of its chromatin in the cytoplasm. A similar phenomenon occurs in Distichopora (10). I have recently devoted a considerable amount of attention to the ovum of Alcyonium, and in this case, too, the whole of the chromatin appears to be ejected into the cytoplasm before fertilisation takes place. In certain insects, judging from the figures given by Henking (9) and others, the amount of chromatin that takes part in the formation of the first polar figure is a very small fraction of the chromatin originally present in the germinal vesicle (cf. Cuenot, 3a).

That the elimination of chromatin is not confined to the nuclei of egg cells is clear from the discovery of Boveri's, that in those blastomeres in the early stages of development of Ascaris which are destined to produce somatic cells, "a portion of the chromatin is cast out into the cytoplasm, where it degenerates, and only in the germ cells is the sum total of the chromatin retained" (quoted from Wilson, 26). In all these cases of the elimination of chromatin from the nuclei of ova and blastomeres there
appears to be no recovery in the amount of chromatin before the next division occurs. In the history of the formation of the new meganucleus of Dendrocometes, however numerous, granules of chromatin subsequently appear at the periphery (Pl. 18, figs. 4, 7, 8), and later they invade the clearer central parts (Pl. 18, figs. 5, 15), to build up the characteristic chromatin network of the functional meganucleus. The exact meaning of this elimination and recovery of chromatin at this stage is a mystery, but taken in conjunction with the other phenomena of conjugation, it may be regarded as a part of the general process of protoplasmic reconstitution of the organism, which is the essential feature of the sexual act.

**General Considerations.**

Maupas, in his famous work on the conjugation of the Infusoria, expressed the opinion that conjugation is essentially an affair of the micronuclei; and I think that the prevailing opinion held by zoologists who have taken a special interest in this matter, is in general agreement with this view. Bütschli’s opinion, as expressed in ‘Infusoria’ (p. 1643), is that the meganucleus is of the nature of a somatic nucleus (Gewebekerne), which becomes gradually exhausted (allmählich abgenutzt wird) during somatic life, whilst the micronucleus is of the nature of the sexual nuclei of Metazoa, and does not become exhausted by the vital processes (keine solche Abnutzung erfahrt). Wilson (26) expresses very fairly the prevalent view in this sentence: “During conjugation the macronucleus degenerates and disappears, and the micronucleus alone is concerned in the essential part of the process.”

With the general proposition that the meganucleus is of the same essential nature as the nucleus of the somatic cells of the Metazoa, and that the micronucleus is essentially a sexual nucleus, I am in agreement; but there are serious objections to be raised to the further proposition, that the only
essential process in connection with conjugation is that in which the micronuclei are concerned.

Plate expressed a view that during conjugation there is a recovery of some essential substance of the nucleus from the cytoplasm (see Bütschli, 2); but, as Bütschli rightly points out, it is difficult to understand upon what grounds Plate's view is based.

Without going further into a review of the opinions of various writers on conjugation, it may be sufficient to state here the problem which is still in need of solution. Is the interchange of molecules of the cytoplasm of the two conjugates during conjugation an essential part of the process?

This question cannot be answered by direct evidence at present. Whatever interchange of molecules of the cytoplasm there may be during conjugation, no method of observation has yet been discovered by which the course of the molecules of one individual can be traced into the body of the other. It is otherwise with the micronuclear nucleoplasm, the peculiar structure and staining properties of which enable us to trace with certainty the course of one micronucleus (or germ nucleus) into the body of the other. The direct evidence which we have in the case of the micronuclear fusion is absent in the case of the cytoplasm. It is not reasonable to conclude from this alone that the cytoplasm plays no part in the process of conjugation, nor that conjugation is simply "une affaire de micronucleus." It appears to me that there is some indirect evidence, however, on this point which is worthy of considerable attention. If the micronuclei alone were concerned in the process, the act of conjugation need be of very short duration. In fact, if the germ nuclei were prepared for their transposition a mere momentary contact would be sufficient. It might also be conceived that such a momentary conjugation would be of advantage to the species in lessening the disadvantageous conditions of the conjugating phase, particularly in the free-swimming Ciliata. In all cases, however, the conjugation is a lengthy process, lasting from twelve to forty-eight hours or more. It is inconceivable that the state of
insensate, helpless, defenceless syzygy would remain so long if there were nothing else of essential importance done but the interchange of germ nuclei. The fact suggests that there is during the process some interchange of the molecules of the cytoplasm, and, indeed, that the interchange or mixing of the molecules is thorough, and not partial or local in character. If there is during conjugation an interchange of the molecules of the cytoplasm such as has been suggested, it is probable that some protoplasmic streaming movement would be noticed between the two individuals. The observations on the changes or movements of the cytoplasm during the process are, however, very limited. Maupas observed that numerous granules (zooamylum) appear in the cytoplasm during the conjugation of certain Ciliata, which he supposed to be connected in some way with the active metabolism that is going on; but I cannot find in his writings any reference to a streaming movement taking place between the two individuals. But Maupas, like Büt schli and many others, it must be remembered, regarded the micronuclear phenomena as the only essential phenomena of the process, and did not expect to find any such flow of cytoplasm.

In Dendrocometes a flow of cytoplasm between the two conjugates does certainly take place. This was observed by Plate and is confirmed by my own observations. Sand (23, p. 100) goes so far as to say that conjugation is essentially a process of plastogamy, and that there is not the least mixing of the nucleoplasm of the two individuals. But Sand’s view is, I believe, as far wrong in the one extreme as the older view is in the other.

Whether a similar streaming movement of the cytoplasm between the conjugates can actually be observed in the group of the Ciliata or not, is a question upon which I have no evidence to offer. But whether it can or cannot be observed under the microscope, the intimate contact of the two cytoplasms renders an invisible interchange of molecules

1 Delage and Hérouard say, “Les phénomènes intérieurs de la conjugaison sont surtout nucléaires.”
possible, and the "onus probandi" really rests upon those who maintain that two globules of protoplasm, such as these, can remain in junction for twenty-four hours without becoming intimately mixed.

A third point of indirect evidence bearing upon this question is afforded by the behaviour of the meganucleus of Dendrocometes during conjugation. If we regard the meganucleus as a somatic nucleus—that is to say, as a nucleus which is functionally connected with all the vital functions except the sexual functions of the body, and as a nucleus therefore which controls or is controlled by the greater part of the cytoplasm of an animal cell such as Dendrocometes is,—then the presence of the meganuclei in the conjugative processes during the interchange of the molecules of the cytoplasm is not a matter for surprise. "Pourquoi, dira-t-on," says Sand, "les noyaux vont-ils se placer dans le pont qui réunit les deux Stylocometes et les deux Dendrocometes? C'est peut-être pour diriger les échanges et les mouvements qui ont lieu dans ce pont." I am prepared, however, to go further than Sand, and regard the presence of the meganuclei in the conjugative processes (le pont) not only as evidence of their relation to the interchanges taking place in the cytoplasm, but as evidence of the necessity of the interchange of molecules of the substance of the meganucleus itself. During conjugation there is, in my opinion, a mixing or a shuffling of the molecules of all the essential plasms of the body, namely, of the micro-nucleoplasm, of the meganucleoplasm, and of the cytoplasm.

Concerning the conjugation of the meganuclear elements two or three obvious objections appear. It might be urged that the rarity of recorded observations of the fusion of the meganuclei in the Heterokaryota, the disintegration of the meganucleus during conjugation, and the origin of the new meganucleus from the micronuclei, are facts which prove that the junction of the meganuclei during conjugation in Dendrocometes is a matter of no essential importance.

It may be pointed out that in the majority of the Ciliata
the meganucleus undergoes fragmentation at an earlier stage than it does in Dendrocometes, and consequently any conjugation that takes place between meganuclear fragments might be very easily overlooked. The fact that in his most recent publication Prowazek figures (figs. 27 and 30) the extension of a fragment of the meganucleus of one conjugating Stylonychia into the body of the other, supports the suggestion that it may occur elsewhere. The second objection is fatal to the view I am putting forward, if it is true, that the meganucleus dies when it fragments. It is, however, really of the nature of an assumption to say that the meganucleus dies at the close of conjugation.

Entz, Balbiani, Gruber, Maupas, Hoyer, and Prowazek are agreed in the statement that the fragments of the meganucleus are absorbed by the cytoplasm. In some species (Chilodon cucullatus, Colpidium colpoda, etc.) the meganucleus does not even fragment, it simply gradually diminishes in volume and disappears. On the other hand, Bütchli (2, p. 1617) is of opinion that in Colpidium and Stylonychia the fragments of the meganucleus are ejected by the anus after conjugation. Having very carefully examined the process in Dendrocometes, and found no evidence of the rejection of any part of the meganucleus during or after conjugation, I am disposed to agree with those who believe that the old meganucleus is, as a rule, absorbed by the protoplasm. It is quite possible, however, that with the absorption of the greater part of the meganucleus there may be a rejection, in some species, of the remainder.

The expression "absorption" or "solution," as applied to the meganucleus at this stage, is very liable to mislead. We may hold the view that the meganucleoplasm is killed, converted into some proteid food substance, and then assimilated by the surrounding cytoplasm, and we may use the word "absorption" to express this meaning. Or we may hold the view that the meganucleoplasm becomes more fluid in consistency, and is diffused in a chemically unaltered, or very slightly altered, condition through the cytoplasm, and
we may use the word "solution" to express this meaning; but we have no evidence that it is either of these processes that actually takes place. All the information we have is that at a certain stage in conjugation certain structures, which by their form and reactions to certain stains we recognise to be meganucleoplasm, become indistinguishable from ordinary living cytoplasm. There is evidence of a certain change in chemical constitution, and perhaps this is only a very slight change, and there is evidence of a certain change in consistency. There is really no evidence that any substance actually dies. Theoretically, there is no inconsistency in the view that after the disappearance of the old meganucleus, its nucleoplasm is still living in a modified form diffused through the cytoplasm. The new meganucleus of the Dendrocometes individual is an enlarged and modified nucleus derived from one of the four micronuclei which are produced by the second division of the segmentation nucleus, as described above, or, to put the matter in few words, the meganucleus is derived from a micronucleus. The important changes which occur during the transition from a structure we call a micronucleus to a structure we call a meganucleus are these:—1st. A considerable increase in size (from 4 μ in diameter to over 12 μ in diameter in Dendrocometes). 2nd. A considerable increase in the amount of chromatin. From whence is this increase in substance derived? It must come either directly as formed nucleoplasm, or indirectly as food material from which nucleoplasm can be formed, from the surrounding cytoplasm. The evidence as to which of these two alternatives is correct is not conclusive, but there is no sign of such metabolic activity as might be expected if the material brought to the new meganucleus is unformed food material, and consequently it is very probable that the increase in size is due to formed nucleoplasm transfused from the cytoplasm to the new meganucleus. If this is the case, then the phenomenon of the conjugation of the meganuclei receives an explanation.

This view appears to me to receive considerable support from the observation made by Bütschli that the posterior
fragment of the meganucleus of *Euplotes charon* does not die, but fuses with the new meganucleus. A similar observation was made by Maupas on *Euplotes patella*.

The investigation of the conjugation of *Dendrocometes* described in this paper throws no new light on the important question of the initial stimulus to syzygy. It is well known that Maupas was able to induce conjugation in several species of Ciliata by a judicious withdrawal of food material after a certain number of binary fissions; that he was of opinion that in natural conditions it is the exhaustion of the food supply which affords the main stimulus to the epidemics of conjugation. The views of Maupas have recently received some support from the experiments of Prowazek (22), who was able to induce conjugation by hunger in *Stylonychia pustulata*. On the other hand, Joukowsky (17) failed to induce conjugation by hunger in *Pleurotricha* after experimenting for eight months and reaching the four-hundred-and-fifty-eighth generation.

I tried the experiment several times of isolating a number of *Gammarus* bearing the *Dendrocometes* in filtered water for six days or a week, and obtained in some cases sufficient evidence that the Acinetarians were affected by hunger; but there were on an average neither more nor less pairs in conjugation than in the *Dendrocometes* of the control experiment. Starvation cannot be extended for more than a week in this case, as the hosts soon die in the filtered water, and their macerating bodies afford ample food again for the epizoites.

*Dendrocometes* itself is peculiar among Infusoria in that it appears to be capable of feeding all through the process of conjugation. Mr. Wadsworth and I have observed the arms of conjugates catch food and pass it down into the body protoplasm. Judging from the food granules as seen in sections, the onset and progress of conjugation appear quite indifferent to the condition of hunger or satiety.

The following notes will illustrate this point:

(The letter F in the third and fourth columns signifies that
the conjugate contains food vacuoles, and the letter S that its cytoplasm is clear or moderately clear.)

<table>
<thead>
<tr>
<th>Number of Slide</th>
<th>Stage of Conjugation</th>
<th>Conjugate A</th>
<th>Conjugate B</th>
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<tr>
<td>146</td>
<td>B</td>
<td>F.</td>
<td>F.</td>
</tr>
<tr>
<td>83, 84</td>
<td>B</td>
<td>F.</td>
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<td>10</td>
<td>C</td>
<td>F.</td>
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<td>129</td>
<td>C</td>
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<td>127</td>
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<td>121</td>
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<td>25</td>
<td>J</td>
<td>S.</td>
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<td>127</td>
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<td>120</td>
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The General Morphology of the Heterokaryote Body.—The investigations of Maupas (20), Bütschli (2) and Keppen (15), notwithstanding the writings of Plate (21), and more recently of Sand (23), have placed beyond all reasonable doubt the zoological affinity of the classes Acinetaria and Ciliata. In these two classes alone there are two kinds of nuclei in each independent organism. In all other Protozoa, with the exception perhaps of a few forms like Pelomyxa, in which there are only scattered granules of chromatin, there is only one kind of nucleus. This fundamental distinction of the Ciliata and Acinetaria justifies us in placing them together in a subdivision of the Protozoa, which may be called the Heterokaryota (Hickson, 11).

There may be some difficulty in giving an absolute definition of what is a nucleus. It will be agreed, however, that every structure in a protoplasmic mass that contains

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1 Caenot (3) has recently discovered that in a Gregarine belonging to the genus Diplocystis, which is parasitic in the common cricket, the two forms of nuclei occur. The micronucleus, however, does not become visible until the onset of sporulation, but it then divides by a mitotic process to give rise to the nuclei of the spores, while the meganucleus disappears.
chromatin and that divides by mitosis is a nucleus. It is frequently very difficult, however, to distinguish true nuclear chromatin from substances in the cytoplasm that are not chromatin, and there are many examples of nuclei known to science that do not divide by mitosis. It may be taken, however, as a further axiom of histology that every structure originating as a daughter nucleus by mitosis of a pre-existing nucleus is itself a nucleus.

Both the meganucleus and the micronucleus of the Heterokaryote body, therefore, are true nuclei; the former on the ground that it originates from the nucleus formed by the mitotic division of a micronucleus, notwithstanding the fact that it always divides amitotically, and the latter on the ground that it divides by mitosis. These two nuclei, however, differ from each other in several important particulars. The meganucleus is very much larger in bulk during the somatic life of the individual than the micronucleus. In fission or gemmation it divides amitotically. It does not divide during conjugation, but during or at the close of this process it ceases to exist as a definite entity.

The micronucleus, on the other hand, is very much smaller than the meganucleus during somatic life. In fission and gemmation it divides by mitosis. It does divide, again by mitosis, during conjugation, and one of the products of its division gives rise to the germ-nuclei. It is not necessary to discuss further in this place the relations of these two nuclei. The reasons set forth by Bütschli with masterly ability in his great work on the Infusoria, for considering the meganucleus to be the "somatic" nucleus, and the micronucleus as the "sexual" nucleus, are sufficient for my purpose. If, however, we accept the view that in the body of the Heterokaryote there is one (or occasionally more than one) somatic nucleus and one or more than one sexual nuclei, we are led to the further inquiry whether there is also a distinction between the somatic cytoplasm and the sexual cytoplasm.

1 Apparent exceptions to this rule are afforded by the meganuclei of Opalina and Kentrochons.
There is no evidence of a positive character to show that this is the case, but the absence of any visible boundary line between the sexual cytoplasm and the surrounding somatic cytoplasm is not a definite proof that the distinction does not occur. Many instances could be quoted, both from animal and vegetable tissues, in which each nucleus of a plasmodium has its own sphere of influence in the surrounding protoplasm, even when no cell boundaries can be distinguished. It is, indeed, contrary to our general knowledge and usual conceptions of cell structures that any nucleus should be entirely independent of the cytoplasm that immediately surrounds it, just as it is that any nucleus should exist entirely free from any cell protoplasm.

There is one feature of the sexual cells of the Metazoa which at this point in the argument I should like to call attention to. When ova and spermatozoa are ripe, that is to say, when they are ready to perform the only function they possess, they are entirely free from surrounding cell structures. There is no reason to believe that in any case I can call to mind the individual ovum or spermatozoon is in protoplasmic continuity or even contact with other cells. There are no other cells of the animal body, except the white blood-corpuscles, of which the same statement can be made, and it is a feature of some interest and importance that in the Metazoa these cells are in their mature condition independent entities. Now in the Heterokaryota the sexual cytoplasm must be in contact with, and in all probability is in continuity with, the somatic cytoplasm at the time of maturity, and even after the fertilisation has been effected. In this respect then, there is an essential difference between the Metazoa and the Heterokaryota. In the Metazoa a conjugation of the somatic cells and of the somatic nuclei could have no possible effect upon the sexual cells, either before or after fertilisation. In the Heterokaryota, on the other hand, whatever effect the conjugation of the meganuclei and the somatic cytoplasm may have, it must be felt by the sexual nuclei and the sexual cytoplasm with which they are in contact. This consideration
throws some light on the phenomenon of the conjugation of the meganuclei in the Infusoria, a phenomenon which has no parallel in the Metazoa.

In the recent discovery of the phenomenon called "Xenia" by the botanists in plants, we find a parallel although not strictly homologous case. The ripe ovum of the angiosperm is not an isolated cell. Its germinal cytoplasm is continuous with the general cytoplasm of the embryo sac, in just the same way as, according to my views, the germinal cytoplasm of a Dendrocometes is in continuity with the somatic cytoplasm. It is quite possible, therefore, that anything which influences the polar nuclei or the general cytoplasm of the embryo sac would influence also the ovum (oosphere) before or after fertilisation is effected. Nawaschin and Guignard (7) have shown that in Lilium and some other Angiosperms the second nucleus of the pollen grain does pass down the tube, and conjugates with one of the polar nuclei to form the mother nucleus of the endosperm nuclei. The second nucleus of the pollen grain and the polar nuclei of the embryo may be compared with the meganuclei of the Heterokaryote body. Like these nuclei they conjugate at the time of the true sexual conjugation of the germinal nuclei, and, moreover, they do not by subsequent division give rise to the nuclei of the new individual. It is true that there are important differences between the two cases. In the plant the conjugation of these nuclei is not temporary as it is in Dendrocometes, but permanent, and the product of the conjugation gives rise to a considerable progeny of well-defined nuclei in the endosperm before their history is closed. But such differences as these are not surprising in organisms so widely separated as the Infusorian and the Angiosperm plant. Detailed comparison of the two phenomena would probably not be profitable, and might, indeed, be misleading. All that the comparison can do for us at present is to confirm the impression that the temporary fusion of the meganuclei of Dendrocometes that has just been described is an important and essential part of the process of conjugation, and not an exceptional or accidental
juxtaposition of the nuclei in the individual cases examined. It may also lead to the discovery of other cases of the conjugation of meganuclei in the Acinetaria and in the Ciliata.

As a general result of these considerations, it seems to me that we must either abandon the use of the expression "unicellular organisms" in our definition of the Protozoa, or else very largely extend the meaning of the term "cell." In the recent text-books published by Sedgwick and by Shipley and Macbride, the former course is adopted; but Lang, in his "Lehrbuch der vergleichenden Anatomie," 2nd edition, 1901, says, "Die einfachsten Organismen, die einfachsten Thiere (Protozoa), und die einfachsten Pflanzen (Protophyta) sind weiter nichts als selbständig und unabhängig lebende Zellen."

The body of a Paramaecium or of a Dendrocometes is no more a single independent cell than is the embryo sac of an Angiosperm plant.

If we are prepared to extend the use of the term cell so as to include all structures that are bounded by an undivided cell wall or cell boundary, then the expression "unicellular" may still be applied to the Protozoa; but, in my opinion, the inconvenience of such a course would far exceed the advantages it might present.

List of the Principal Papers referred to in this Memoir.

2. O. BEUTSCHL.—"Infusoria" in Bronn's 'Thierreich,' 1859.
3a. L. CUNOT.—'L'Épuration nucléaire au début de l'Ontogenèse,' t. c., p. 190.
EXPLANATION OF PLATES 17 & 18,

Illustrating Mr. Sydney J. Hickson's paper on "Dendrocometes paradoxus."

PLATE 17.

The figures in this plate, with the exception of 1, 2, and 5, are constructed from a series of drawings of the actual sections of the Dendrocometes. The micronuclei do not all occur in the same plane as represented, and it is very rarely that the whole of the meganucleus can be seen in one section. The structures have been represented as nearly as possible in their true relative positions. Throughout, M. refers to the meganucleus; m., the micronuclei; A., the arms; P., the conjugating process; L. M., limiting membrane. The number at the end of the description of each figure refers to the permanent preparation from which the figure was drawn. These preparations are preserved in the Zoological Laboratory at the Owens College, and may be inspected by qualified zoologists.

Fig. 1.—Two individuals of Dendrocometes about to conjugate. Each one is protruding a conjugating process (P. P.) and these ultimately meet. In these two individuals the arms are of approximately the same size and degree of branching. Whole mount, No. 14.

Fig. 2.—Two individuals which have just joined together in conjugation (Stage A). One of them (to the right) has one short arm and two very rudimentary arms (A. 2, A. 3). The other has one large branched arm and two shorter simple arms (see p. 328). The micronuclei are very small, and the meganuclei have undergone very little change (see p. 331). Whole mount, No. 29.
Fig. 3. Stage B.—The meganuclei have become spindle-shaped and are slightly enlarged. The micronuclei (three in each individual) are considerably enlarged, the chromatin forming a loose meshwork. Section No. 139.

In this and the following figures details of the arms are omitted.

Fig. 4. Stage B (later).—The micronuclei now show chromosomes arranged in equatorial planes and linin fibrils running through them to the poles. In this preparation only two micronuclei can be seen in each individual. Sections No. 129.

Fig. 5. Stage B (close).—The micronuclei are in later stages of their mitosis. In the individual on the right the chromosomes of the micronuclei have separated into two parties travelling toward the poles. In the individuals on the left the chromosomes have reached the poles and fused into a compact mass. Whole mount, No. 14.

Fig. 6. Stage C.—There are now six micronuclei in each individual, and the undissolved remnants of some of the spindles (Sp.) may be seen in the cytoplasm. The meganuclei have been omitted from the drawings to render the positions of the nuclei clear. Section No. 139.

Fig. 7. Stage D.—Five of the micronuclei in each individual are now undergoing degeneration, but one in each (m.c.) travels down the conjugative process and approaches the membrane of separation. The meganucleus is omitted in the figure from the individual on the right. Sections No. 126.

Fig. 8. Stage D.—The conjugative micronuclei (mc., mc.) are now dividing by mitosis in the conjugative process of each individual. Sections No. 134.

Fig. 9. Stage E.—The conjugative micronuclei have now divided into two separate nuclei,—the "germ nuclei." Sections No. 138.

Fig. 10. Stage F.—The germ nuclei of the two individuals have now fused or are fusing (upper one). Sections No. 140.

Fig. 11. Stage G.—The cleavage nuclei (S.m.) formed by the fusion of the halves of the conjugative nucleus in the last stage now travel towards the centre of each individual and again show mitosis. During the preceding stages the meganuclei have been gradually enlarging and have now reached a considerable size. Degenerate remnants of the other micronuclei may still be seen in the cytoplasm. Sections No. 141.

Fig. 12. Stage H.—The segmentation nuclei having divided once more show mitosis. Sections No. 107.

For illustrations of Stage J see Plate 17, figs. 1, 7, 8.

Fig. 13. Stage K.—The segmentation nuclei have now divided into four nuclei in each individual, three of which become reduced in size and the chromatin concentrated into a single granule, and one in each becomes enlarged to form the new meganucleus (n. M.). The old meganucleus is beginning to disintegrate. Sections No. 25.
PLATE 18.

1. Section through one of a pair of conjugates after the second division of the segmentation nuclei (Stage J), showing one nucleus (n. M.) slightly larger (5 μ) than the other three (4 μ). This larger nucleus becomes the new meganucleus. No. 122.

2. The new meganucleus from the last figure enlarged to show that the chromatin is at this stage in the form of a coarse skein lying in the centre of a clear space.

3. The new meganucleus at a later stage. It is now about 8 μ in diameter. A considerable quantity of the chromatin has now collected at the periphery, and some of it appears to be escaping into the cytoplasm. No. 145.

4. New meganucleus at a still later stage, 10 μ in diameter. Darkly staining granules of chromatin are now seen at the periphery, one or two within the periphery, but the central parts stain very faintly indeed. Series 131.

5. New meganucleus at a still later stage, 12 μ in diameter, containing numerous evenly distributed granules of chromatin. Slide 131.

6. Section through a pair of conjugates showing the approach of the old meganuclei (M.) to each other at the bar of junction. No. 52. (The micronuclei were not clearly stained in this preparation, and are consequently entirely omitted from the drawing.)

7. Section through a pair of conjugates (Stage J), showing one meganucleus at the limiting membrane, the other pointing towards it but not reaching it. There is one new meganucleus and three micronuclei represented in each. (In the preparation, owing to an unfortunate tear, only one micronucleus can be actually seen in the lower conjugate.) No. 141.

8. Section through a pair of conjugates (Stage J), showing the approach of the old meganuclei to each other at the limiting membrane. In each of these there is one new meganucleus and five micronuclei (an exceptional condition). In the cytoplasm of the lower conjugate there may be seen three granules of chromatin (?). These may be the remnants of the polar nuclei. Slide 131.

9. A small portion of the old meganucleus of one of the conjugates of the last preparation more highly magnified, showing the chromatin arranged in irregular parallel lines with thickened nodes and lumps.

10. Section through a resting meganucleus, stained by iron-hæmatoxylin. No membrana limitans can be seen. No. 255.

11. Section through the conjugative processes of a pair of Dendrocometes, showing the organic fusion of the two meganuclei in Stage J. No. 98 S.

12. Section through the conjugative processes of a pair of Dendrocometes
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(Stage F), showing the fusion of the germ nuclei (G. m.). On the left the two nuclei have not completely joined; the chromatin is in the form of a coarse skein with thickened nodes. On the right the pair have fused, and the chromatin has assumed an irregular asterid form. No. 140.

13. Section through the conjugative processes after the fusion of the germ nuclei to form the segmentation nuclei (S. m.). The segmentation nuclei become mitotic soon after their formation, but the axes of the figures are not parallel with the membrane nor with one another. In this case the upper figure is seen in longitudinal section and the lower in transverse section. No. 117.

14. Section through a Dendrocometes at an early stage in the formation of a gemmula to show the normal mode of division of the meganucleus. B. B. Bands of concentric modified cytoplasm which form the peritrichous bands of cilia of the gemmula. No. 34 s.

15. Section through one of a pair of conjugates in Stage K, showing the fragments of the old meganucleus and the new meganucleus. m., One of the micronuclei? No. 92.

16—18. Three stages in the division of the micronuclei. 16. Immediately after the division of the chromosomes. 17. The chromosomes separated to the poles of the figure. 18. The chromosomes collected into a granule of chromatin at each of the poles, and the achromatin in the form of an elongated spindle. 16 and 17, No. 129. 18, composition drawing from several preparations.

19. Section through a Dendrocometes at the close of conjugation. It shows the rare condition of two new meganuclei. The old meganucleus has almost completely disintegrated. No. 74 E.

NOTE.—In Stage K, when the old meganucleus has fragmented, it is extremely difficult to distinguish the micronuclei from fragments of the old meganucleus. I have therefore made no attempt to reconstruct Fig. 15 and Fig. 19, so as to show all the micronuclei in their correct relative positions. These two figures were drawn with the assistance of the camera lucida from one section only of each series of sections.