The Fine Structure of the Pellicle in the Contact Area of Conjugating *Tetrahymena pyriformis*.*

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Within the limits of the light microscope the sequence of events occurring during conjugation in *Tetrahymena pyriformis* is well known (2, 4, 5). However, certain questions regarding the finer morphological details can be resolved only with the electron microscope. One such question is concerned with the interchange of cytoplasmic particulates between mates during conjugation. Silver-line studies of single vegetative cells show no pellicular modifications in the preoral region that would indicate the presence of a special attachment organelle (1). Light microscope studies of conjugating cells give no hint of pellicular differentiation in the region of contact, between the mates. It, therefore, came as somewhat of a surprise to find a well defined region of differentiated pellicle which may serve the function of permitting a flow of some cytoplasmic particulates from one cell to the other during conjugation. The purpose of this report is to describe this observation.

Materials and Methods

The strains of *T. pyriformis* used in this investigation were the following: strains WH 6 and WH 14, mating types I and II respectively of variety 1; strains TC 110 and TC 89, mating types I and V respectively of variety 9. Representatives of two varieties were employed for comparative purposes.

The cells were grown in 500 ml. flasks in stock 2 per cent proteose–peptone–tryptone media at pH 7.2. Toward the end of logarithmic growth, they were washed twice by centrifugation in double-glass-distilled water and stored for 12 hours. The cells were then mated in equal numbers in standard ten depression slides and incubated at 25°C. Pairs accumulating at the bottom of the depression were removed with a micropipette at specified time intervals and transferred to centrifuge tubes in which they were fixed, dehydrated, and embedded in plastic.

To one part of a concentrated suspension of *T. pyriformis* were added, in succession, one part of McIlvaine buffer pH 7.4 (0.9 ml. of 0.1 M citric acid and 9.1 ml. of 0.2 M Na_2HPO_4) and two parts of 2 per cent OsO_4. The final concentration of OsO_4 was therefore 1 per cent. The period of fixation was 1 hour at room temperature. Dehydration at 15 minute intervals through changes of ethanol was carried out in centrifuge tubes. Infiltration was accomplished by three changes of a mixture of 40 per cent ethyl methacrylate and 60 per cent *n*-butyl methacrylate. The pellets were then embedded in No. 00 gelatin capsules containing the above methacrylate mixture plus 1 per cent luperco CDB. Polymerization was accomplished by heating at 60°C. for 24 hours.

Sections were cut with glass knives on a Porter-Blum microtome set to cut at 1/40 μ. The sections were then floated on 20 per cent ethyl alcohol from which they were mounted on formvar-covered grids and then studied under the electron microscope (RCA model EMU-2A). The micrographs were taken at original magnifications of 5600 to 9200 and then enlarged protographically as desired.

OBSERVATIONS

Sections through the region of attachment of mating cells show that the dense apposed pellicles are interrupted in a number of places. These interruptions constitute tiny tubular pores which lead from one cell to the other (Figs. 1 to 4). The higher magnifications (Figs. 1, 3, and 4) show continuity of the cytoplasm within the boundaries of the pores which suggests that particulates might pass readily between the cells. The diameter of the pores approximates that of the ciliary openings (0.2 μ). The pores make their appearance shortly after the two cells unite and remain until the macronuclear anlagen are fully formed; that is, until just prior to separation of the conjugants.

Metz and Westfall (3) have shown that the pellicle of *T. pyriformis* is a complex structure. The present studies demonstrate that in the region of contact between mating cells the pellicle shows considerable modification. A careful examination of the peripheral regions (Fig. 4) of contact between the cells reveals that the double-membraned surface pellicle becomes a single membrane in the area of contact, apparently as a result of fusion of the two. It may be assumed that the original pores in the pellicle through which the cilia pass have become modified into pores connecting the two cells. Whether the pore is the result of the fusion of two oppositely placed ciliary openings, one from each animal, or whether each cilium is

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capable of making its own pore, cannot be determined from the evidence so far obtained. The electron-opaque material which appears to adhere to the inner surfaces of the apposed membranes gives them a dense appearance and enhances their thickness. Just what this material is or what it means is not at all clear.

The infraciliature consisting of the kinetodesmal fibrils and the kinetosome, fragments of which are discernible in Fig. 4, has dedifferentiated completely in the region where the two membranes are in contact. There is no evidence of ciliary fragments indicating that perhaps the entire infraciliature has disappeared.

DISCUSSION

The question of whether or not cytoplasmic interchange between conjugating individuals of *Tetrahymena* is possible can now be answered in the affirmative. Particulate material of 0.2 micron or less in size can readily flow from one mate to the other through the numerous pores. The existence of these pores is probably not unique with *Tetrahymena*, although to the authors' knowledge, no reports have been made concerning other ciliates. From the frequency of the pores that are present in each thin section, it is estimated that 200 to 300 exist between the mates. With this much area of communication it seems likely that a relatively extensive mixing of protoplasmic constituents is possible during the mating process. Therefore, it is possible that whatever influence the cytoplasm may have on the nucleus and other parts of the cell, it is pooled during conjugation, affecting both cells more or less simultaneously.

The morphological dedifferentiation of the entire kinety system, as well as the buccal apparatus, during conjugation and their subsequent redifferentiation when the conjugants separate involve a remarkable series of events which require much more study before even a most elementary explanation can be forthcoming.

SUMMARY

Well defined tubules exist between mating *T. pyriformis* throughout the period of conjugation. They exist in sufficient numbers and size to permit the exchange of cytoplasmic particulates of dimensions up to 0.2 μ. The infraciliature is completely absent in the region of contact and is regenerated only after separation of the conjugants.

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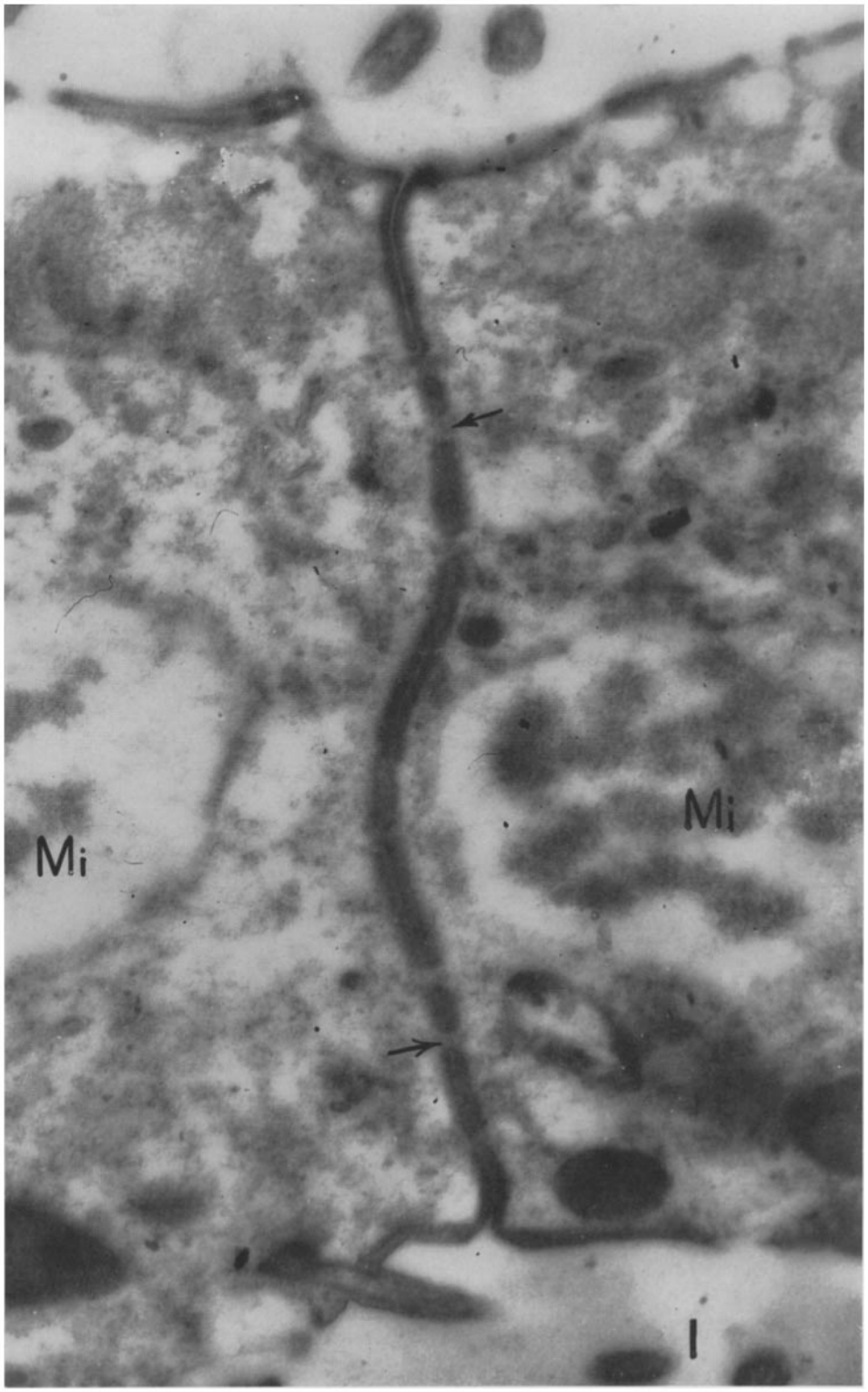
EXPLANATION OF PLATES

PLATE 422

M, mitochondria.
Mi, micronucleus.
MaA, macronucleus—anlagen stage.
MaD, macronucleus—degenerating.
P, pronucleus.

FIG. 1. Electron micrograph of a section through the plane of contact of two conjugants following the third prezygotic division. The two haploid migrating pronuclei are lying near the membranes. The pores in both membranes are indicated by arrows. Across some of these pores, the cytoplasms of the two conjugants appear to be in continuity. Note also that the size of the openings approximates that of the ciliary opening shown at the bottom of the micrograph. × 20,000.

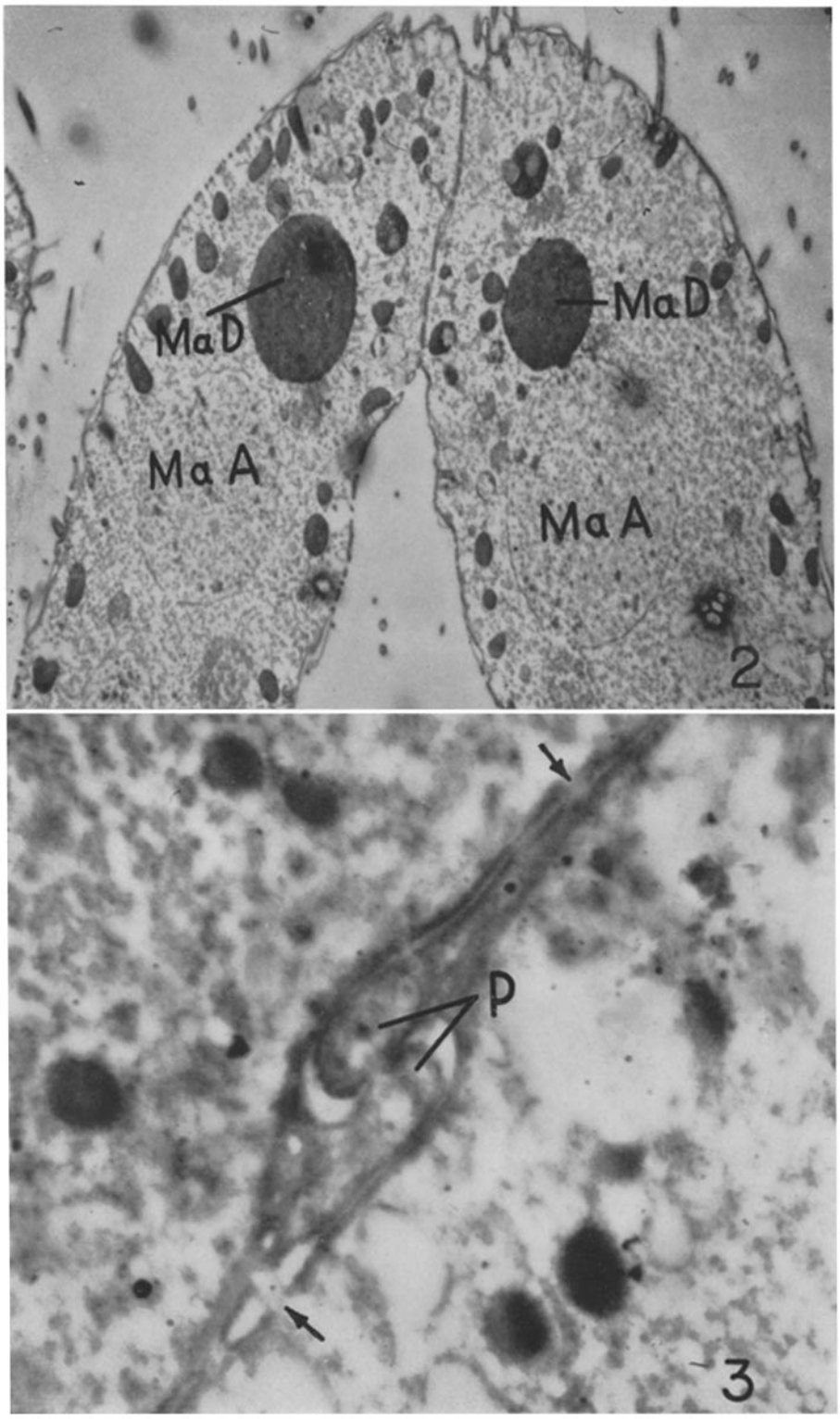


(Elliott and Tremor: Pellicle of conjugating *Tetrahymena*)

PLATE 423

FIG. 2. A low power electron micrograph of a medial section through two conjugants. The connecting pores are visible even at this magnification. The macronuclear anlagen are faintly visible showing the structure typical of this stage. The degenerating old macronuclei appear homogeneous in structure. They are located in the anterior end of the cells which is peculiar to variety 9. Note the peripheral distribution of the mitochondria which show up as dark oval-shaped bodies. × 2200.

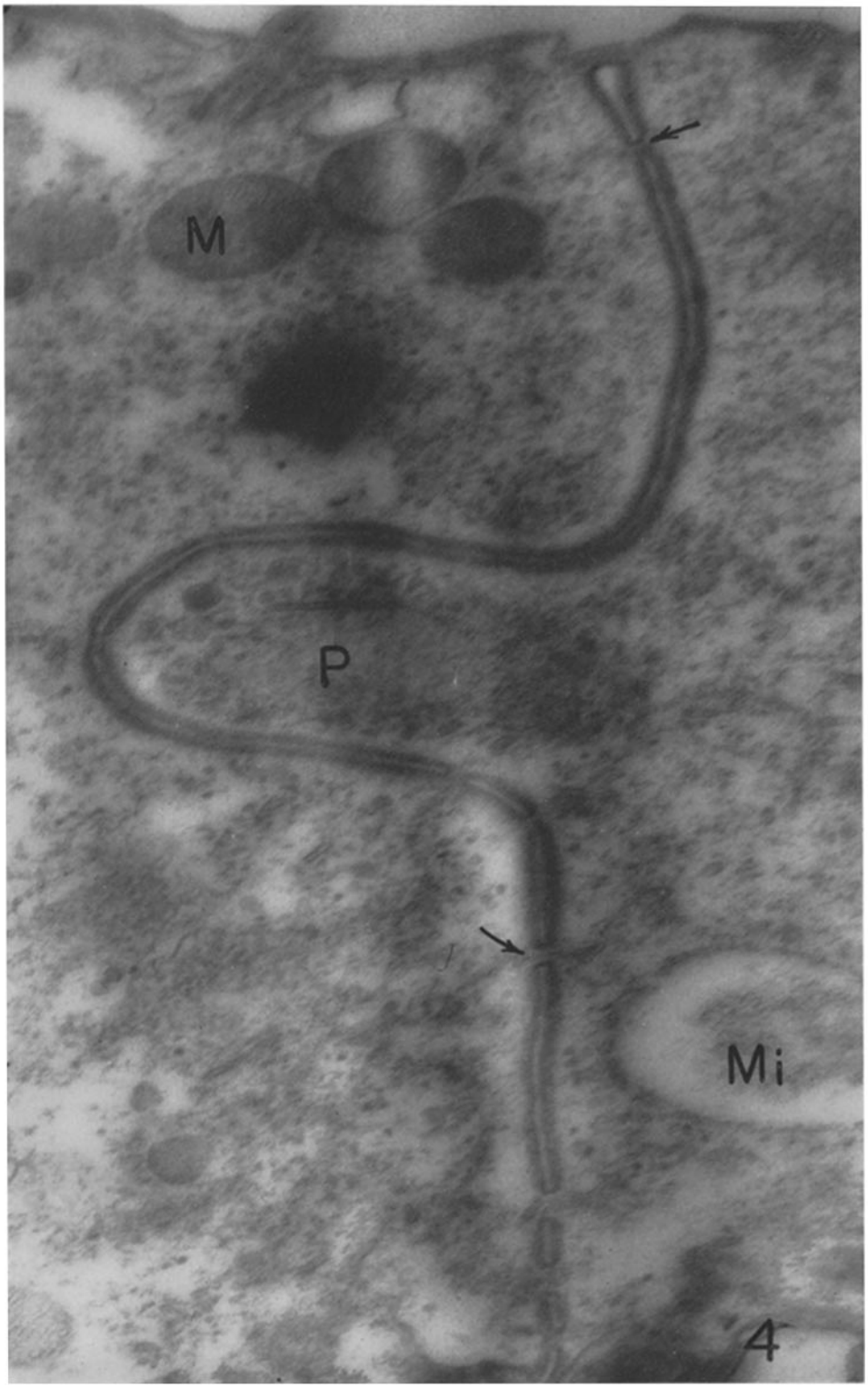
FIG. 3. This section is taken in such a way as to show the migrating haploid pronuclei passing one another on their way to the opposite mate. Note the well defined tubules (arrows) connecting the conjugants. × 11,500.



(Elliott and Tremor: Pellicle of conjugating *Tetrahymena*)

PLATE 424

FIG. 4. An electron micrograph taken through the plane of contact of conjugating cells in which the haploid pronucleus of the right hand member has made its way deep into the cytoplasm of the mate. Here again the pores and cytoplasmic continuity are clearly visible. The micronucleus visible in this section is a relict nucleus which is degenerating. × 20,000.



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