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Oral morphogenesis during asexual reproduction in *Paramecium tetraurelia**

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SUMMARY

Oral morphogenesis in stock 51 of *Paramecium tetraurelia* was investigated using the techniques of Chatton-Lwoff and protargol silver impregnation. During the stomatogenesis accompanying divisional morphogenesis a new oral anlage field and endoral kinety are formed and persist throughout the interfission period in both the proter and the opisthe. This previously overlooked fact is important for understanding the developmental origins and significance of the endoral kinety and the oral anlage field. Previously, the kinetosomes constituting the oral anlage field were thought to be formed just prior to the onset of stomatogenesis, being in some way derived from the kinetosomes of the endoral kinety. The demonstration of a permanent anlage field as an integral component of the oral assemblage suggests that the earliest stages of stomatogenesis might best be viewed as temporally controlled surfacing and/or ciliation of pre-existing kinetosomes rather than their *de novo* synthesis. The endoral kinety would thus have no contributory role in the formation of the anlage field used in the immediately ensuing stomatogenesis.

1. INTRODUCTION

Transverse division of *Paramecium* into two daughter cells is accompanied by the synthesis and assembly of a new oral apparatus for the posterior fission product, the opisthe, the original ingestatory assembly being retained by the proter, the anterior fission product (Hertwig, 1889). The new oral primordium or anlage arises prior to the beginning of cell constriction from a seemingly unordered field of kinetosomes (basal bodies). This field appears on the posterior portion of the right wall of the vestibulum, a depression in the cell's ventral surface which leads to the oral apparatus. Maturation of the oral anlage consists in part of the progressive organization of this kinetosomal field into three ciliated buccal complexes: the ventral and dorsal peniculi and the quadrulus. Concomitant with the maturation of the oral anlage is its translocation posteriorly during the cortical growth accompanying fission (Plate 1, fig. 4).

Despite numerous studies investigating stomatogenesis in diverse species of *Paramecium* (von Gelei, 1934; Roque, 1956*a*, *b*, 1961; Yusa, 1957; Ehret & Powers, 1959; Porter, 1960; Ehret & de Haller, 1963; Gillies & Hanson, 1968; Ehret &

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McArdle, 1974; Kaneda & Hanson, 1974), at least two aspects of this process remain unclear. The first of these is the origin of the kinetosomes composing the aforementioned vestibular kinetosomal field. Roque (1956*a*, *b*, 1961) states that they arise by division of the kinetosomes composing the endoral kinety (EK), an additional oral structure, whose structure and disposition will be discussed later. Porter (1960) has postulated, alternatively, that the kinetosomes of the anlage field derive from the right vestibular kineties, i.e. rows of kinetosomes located within the vestibulum, with the EK acting as an organizer of stomatogenesis. Ehret & Powers (1959) and Dippell (1968), however, have shown that kinetosomes do not arise by the actual division of pre-existing kinetosomes, although in *P. tetraurelia* they do form in an intimate, topographically specific relation to the pre-existing kinetosome (Dippell, 1968).

Secondly, despite the presumed cardinal importance of the endoral kinety as either a 'stomatogenic' kinety (Roque, 1956*a*, *b*, 1961) or an 'organizer' of the kinetosomal field (Porter, 1960), the origin of this structure in the developing oral anlage has not yet been elucidated. Also unresolved is the question of the continuity of the endoral kinety in the original oral apparatus from one generation to the next.

The present study of the stomatogenesis accompanying divisional morphogenesis was undertaken to resolve the above questions and to elucidate the earliest stomatogenic events. Special attention will be paid to the question of a possible role of pre-existing structures of the oral assemblage in the formation of the new oral assemblage. The oral assemblage may be defined as both the vestibulum and its kineties as well as the buccal organelles, the latter referring to those structures derived from the oral anlage during stomatogenesis. The importance of resolving the question of the role of the pre-existing oral assemblage in the formation of the new one is obvious if one recalls that normally an oral assemblage can arise only if another such structure is already present (Sonneborn, 1963). Information garnered through such studies might reasonably be expected to provide new insights into the nature of the oral assemblage as a 'primary organizer' of the hereditary ventral pattern of kineties, a pattern which is reconstructed at each generation in both the proter and the opisthe. That the oral assemblage does indeed function in this capacity was demonstrated by Sonneborn (1963) in studies of the effects of the removal or addition of an oral assemblage upon the extant ventral pattern of kineties in double animals.

2. MATERIAL AND METHODS

(i) *Culture*

Stock tubes of *Paramecium tetraurelia*, stock 51 (Sonneborn, 1975), formerly designated as *P. aurelia*, syngen 4, were maintained at 27° C and fed for one fission per day with a baked lettuce infusion inoculated 24 h previously with *Klebsiella pneumoniae* (for details see Sonneborn, 1970). When abundant fission stages were required, a 10 ml aliquot was removed from the appropriate stock culture and grown in an excess of food for at least 48 h. Twelve hours before dividing animals were to be isolated, a 1 ml sample from the maximally growing culture was placed in a small

(60 × 15 mm) Petri dish and supplied with an excess of food. Under these conditions, which allow the maximal rate of growth, approximately 20% of the cells were observed to be in some stage of division at all times.

In order to procure cells at approximately the same stage in the cell cycle, fission forms were individually selected such that their capture and subsequent expulsion from the micropipette effected separation of the proter from the opisthe. Separation of the division products was designated as the beginning of the cell cycle. Dividing animals meeting the above criterion were collected for a 5 min period, expelled from the micropipette and all undivided, but dividing cells removed. A population of cells synchronized by this technique customarily divides within a 60 min period, at the time of the next fission, although the majority divide within 20 min of each other.

(ii) *Silver impregnation*

Cortical structure was visualized by the Chatton-Lwoff (1930) and protargol (Dragesco, 1962) techniques of silver impregnation. The former technique produces consistently excellent visualization of cortical structures which results from the deposition of silver grains on the cell surface around the base of the cilium and not from the actual staining of the kinetosomes (Dippell, 1962). One is therefore led to suspect that only cilia-bearing kinetosomes or perhaps those kinetosomes in intimate contact with the cell membrane are revealed by this procedure, as will be discussed below.

Protargol impregnation, on the other hand, actually stains the kinetosomes and should delineate both barren and ciliated kinetosomes. Practical considerations, however, severely limit the use of protargol impregnation in studies of stomatogenesis in *Paramecium*. Heavy staining of the macronucleus and trichocysts in protargol-stained cells almost invariably obscures the oral assemblage and hence the oral anlage. Furthermore, the fact that the oral anlage is not on the cell surface but within the wall of the vestibulum further reduces the probability of satisfactory visualization of this organelle. It has, however, been possible to obtain a clear visualization of the distribution of kinetosomes in the interfission oral assemblage in a limited number of cells.

Unless otherwise stated, all descriptions presented below were made from cells impregnated by the method of Chatton-Lwoff.

3. RESULTS

(i) *Interfission oral assemblage*

Since a clear conception of the three-dimensional arrangement of the components of the oral assemblage during the interfission period is requisite to understanding oral anlage development, their disposition is briefly outlined below. The present study confirms the observations of Allen (1974) and Ehret & McArdle (1974) in which more detailed accounts including ultrastructural particulars may be found. Only those structures visualized by the Chatton-Lwoff or protargol techniques of silver impregnation will be considered here. Corliss's (1955) nomenclature of ciliate

buccal organelles will be adopted throughout. In all cases left and right refer to the cell's left and right.

When a silver impregnated cell is viewed from the ventral surface the most conspicuous feature is a comma-shaped opening termed the buccal overture (Plate 1, fig. 3). This structure is approximately 15 μm in length and is located slightly posterior to a point midway between the cell's anterior and posterior poles. In *Paramecium*, however, the buccal overture is not located on the cell surface but is found at the base of a roughly oval depression termed the vestibulum. Each of the vestibular walls forms a curved surface oriented more or less perpendicularly to the ventral surface of the cell and bears a variable number of short kineties. These kineties are qualitatively indistinguishable from the kineties covering the rest of the cell surface but are distinguished by their position entirely inside the vestibulum.

Immediately dorsal, i.e. behind the buccal overture and deeper within the cell, is located a peristomal cavity part of whose walls bear the three ciliated buccal complexes: the dorsal and ventral peniculi and the quadrulus (described below). The walls of this cavity funnel deeply into the endoplasm, narrowing posteriorly. At its narrowest point is located the cytostome or true cell mouth, distal and dorsal to which food vacuoles form. The short region between the cytostome and the food vacuole forming region is designated the cytopharynx.

All of the ciliated buccal complexes contribute to the wall of the peristomal cavity. Fig. 2 represents the anterior portion of an oral assemblage viewed from the ventral surface with the vestibular kineties omitted. Beginning at the bottom right of the figure and proceeding counter-clockwise around the figure are found the ventral and dorsal peniculi, the quadrulus and the endoral kinety. As can be seen (Fig. 1 A, B), the major portions of all of these ciliary elements, except the endoral kinety, are located on the left wall of the peristomal cavity. The right wall of the peristomal cavity is mainly ribbed wall.

The penicular complex, consisting of eight ciliated rows of kinetosomes arrayed in two groups of four rows each (Fig. 1 B) has its anterior-most end on the left side of the peristomal wall and extends in a posterior and ventral direction. The left-most (relative to the cell's left and right) group of four rows, the ventral peniculus, terminates two-thirds of the way down the wall of the peristomal cavity (Fig. 1 B), each of the four rows extending farther posteriorly than the one to its left. The dorsal peniculus, located anteriorly and to the right of the ventral peniculus, parallels the course of the latter down the left side of the peristomal wall where the two left-most rows of the dorsal peniculus terminate. The two right-most rows continue across the ventral wall of the peristomal cavity at the level of the cytostome and terminate on the dorsal surface of the wall of the peristomal cavity (Fig. 1 A).

The quadrulus, composed of four rows of kinetosomes, has its anterior end just inside the buccal overture and slightly to the left of the end of the anterior suture (Fig. 2). These four rows curve down the peristomal wall, the anterior (right-most) row bisecting the peristomal wall along its anterior–posterior axis. Unlike the evenly spaced rows of the penicular complex, the two right-most rows of the quadrulus are more closely apposed than the others. As the quadrular rows extend posteriorly, the

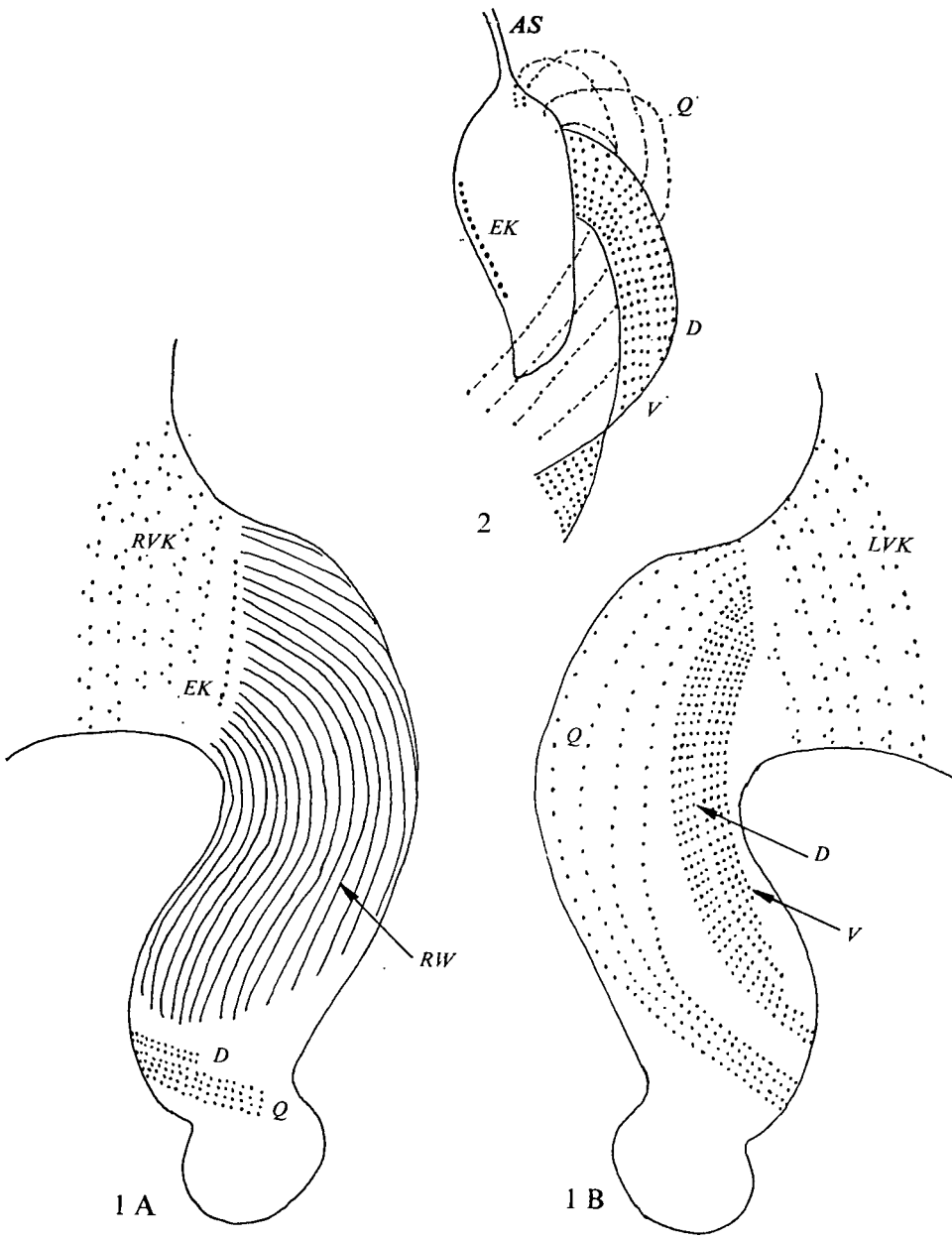

Fig. 1. Diagrammatic representation of an interfission oral assemblage in *P. tetraurelia*. Right vestibular kineties (*RVK*), left vestibular kineties (*LVK*), endoral kinety (*EK*), ribbed wall (*RW*), dorsal (*D*) and ventral (*V*) peniculi, quadrulus (*Q*). × 3100.

Fig. 2. Ventral view of the oral assemblage with the vestibular kineties omitted. Anterior suture (*AS*). × 3100.

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lateral and longitudinal spacing between adjacent kinetosomes first increases, but then rapidly decreases. At a point about two-thirds of the way down the wall of the peristomal cavity the quadrular rows bend sharply to the left and begin to run parallel to the dorsal penicular rows (Fig. 1B). All four quadrular rows continue around the cytostome, still parallel to the dorsal peniculus, onto the right wall of the peristomal cavity, and end near the dorsal surface of the peristomal cavity (Fig. 1A).

The ribbed wall is the major constituent of the peristomal wall not composed of ciliary complexes. This component rarely stains with either the Chatton-Lwoff or protargol techniques and hence is generally outside the scope of the present study. Occasionally, however, the ribbed wall can be seen to form the major portion of the right half of the wall of the peristomal cavity (Fig. 1A). By means of transmission electron microscopy of serial sections through the oral assembly, Allen (1974) has determined the exact extent and ultrastructure of the ribbed wall in *P. caudatum* and should be consulted for a detailed account.

Ventral to the vestibular terminus of the ribbed wall and between it and the buccal overture is located the endoral kinety, a linear assemblage of one or two rows of kinetosomes (Fig. 1A). Confusion concerning the multiplicity of this structure has resulted from the techniques used in its visualization. When the Chatton-Lwoff silver impregnation technique is employed, the endoral kinety appears as a short, single row of kinetosomes (Yusa, 1957; Porter, 1960; Kaneda & Hanson, 1974); electron microscopy reveals it as double (R. V. Dippell, personal communication; Sibley, personal communication; Ehret & McArdle, 1974) with only the outermost row being ciliated (Ehret & McArdle, 1974). These EM studies have neither revealed the extent of the non-ciliated component of the endoral kinety nor resolved whether all of the kinetosomes composing the outer, i.e. more ventral, row are ciliated.

The protargol visualization of the interfission oral assemblage obtained in the present study clearly resolves the above ambiguity, demonstrating the endoral kinety to be composed of two parallel rows of kinetosomes that are staggered relative to each other and which extend the full length of the right vestibular wall (Plate 5, fig. 20). Furthermore, a comparison of the images obtained with the protargol and Chatton-Lwoff impregnation leads to the conclusion that during the interfission period only those kinetosomes in the central region of the outermost kinetosomal row are ciliated.

(ii) *Divisional stomatogenesis*

The present study of oral morphogenesis is in general agreement with published descriptions of the course of events during oral anlage formation and maturation in *P. tetraurelia* (Roque, 1956*a*, *b*, 1961; Yusa, 1957; Porter, 1960; Kaneda & Hanson, 1974). Briefly, these investigators envision stomatogenesis as proceeding in the following manner.

The earliest sign of the new oral apparatus is the appearance of a field of kinetosomes between the innermost right vestibular kinety and the endoral kinety. These kinetosomes appear about three-quarters of the way through the cell cycle, their

appearance being concomitant with or slightly preceded by an apparent elongation of the endoral kinety posteriorly. The next step in the formation of the oral anlage is the ordering of these kinetosomes into several, the exact number unspecified, rows of kinetosomes oriented parallel to the endoral kinety. The number of these rows increases until 12 rows of kinetosomes are produced. At this point the full complement of ciliary rows necessary to produce the three buccal complexes is present. These 12 rows are subsequently partitioned into three groups of four rows each, representing the ventral and dorsal peniculi and the quadrulus of the new oral assemblage. As the division process continues, the new oral apparatus is translocated posteriorly, presumably by the cortical growth accompanying fission.

Many of the details of the stomatogenic process have been left occluded by the above studies. None of them has elucidated the manner in which a new endoral kinety is formed in the opisthe and all of them have assumed that the old oral assemblage retains the original endoral kinety. Furthermore, since it has always been assumed that the field of kinetosomes which gives rise to the oral anlage is produced anew just prior to the onset of the division process, no consideration has been given to the possibility that this field is an integral, permanent part of the oral assemblage and as such is formed in both proter and opisthe as part of each stomatogenesis. The following new information is based on the author's observations except where noted.

Table 1. *Increase in Chatton-Lwoff-demonstrable kinetosomes in the endoral kinety as a function of time in the cell cycle (no. of cells)*

No. of kinetosomes in EK visualized by the Chatton-Lwoff technique	Stage in cell cycle		
	0·19	0·39	0·60
11	3	—	—
12	8	1	3
13	6	5	4
14	1	6	4
15	—	5	6
16	—	1	14
17	—	—	3
18	—	—	2
19	—	—	0
20	—	—	0
21	—	—	0
22	—	—	1

Regression analysis: $df = 76$, $t = 7{\cdot}0$, $P = \ll 0{\cdot}001$.

The present study indicated that the first event presaging division is a gradual, apparent increase in the number of kinetosomes which compose the endoral kinety. This apparent increase commences much earlier in the cell cycle than Roque (1956*a*) reports. Kinetosomes previously unresolved by Chatton-Lwoff impregnation are visualized primarily at the posterior end of the endoral kinety (Table 1).

In addition, a faintly staining streak believed to represent subsurface kinetosomes is observed extending from the posterior end of the endoral kinety in a slight arc and terminating near the posterior junction of the left and right vestibular walls (Plate 1, figs. 5A, B). The extent of the endoral kinety at later stages in stomatogenesis corresponds to the position of this streak.

At approximately three-quarters of the cell cycle, a field of kinetosomes appears in an area bordered dorsally by the extended endoral kinety and the ribbed wall, and ventrally and anteriorly by the innermost right vestibular kinety. Posteriorly the field extends into an area previously devoid of visible kinetosomes formed by the termination of the three innermost right vestibular kineties two-thirds of the distance posteriorly down the vestibular wall (Fig. 1A). Since this kinetosomal field is organized into the new buccal organelles, it will henceforth be referred to as the anlage field. This field is initiated by the simultaneous appearance of widely scattered faint depositions of silver grains (Plate 1, fig. 5A, arrow). The endoral kinety is still visible as a structure distinct from the kinetosomes composing the anlage field (Plate 1, fig. 5A). There is a rapid increase in the argentophilia of these kinetosomes (Plate 1, fig. 6A, B), as well as an increase in their number, which leads to the obscuring of the endoral kinety by the anlage field. Therefore the fate of the endoral kinety cannot be stated, although Porter (1960) claims that it is incorporated into the anlage field.

From the first appearance of silver depositions in the area of the anlage field until the endoral kinety is no longer distinguishable from the anlage field will be termed stage I. The over-all shape of the anlage field at this time is that of a wedge convex on its dorsal edge and concave on its ventral edge.

Despite the assertion of Porter (1960) that the right vestibular kineties participate in the formation of the anlage field, no evidence was found for the involvement of either these kineties or any of the three buccal complexes in the formation of the anlage field.

Shortly after the appearance of the anlage field the kinetosomes composing it appear in numerous short rows of three or four kinetosomes which are oriented roughly parallel to the buccal overture (Plate 2, fig. 7). There is a concomitant narrowing of the anlage field as well as its extension posteriorly around the end of the vestibulum to its left side and then anteriorly. During this radical change in shape there is no obvious increase in the number of kinetosomes composing the anlage. Upon completion of this spatial reorganization the anlage field has become organized into three rows of evenly spaced kinetosomes (Plate 2, fig. 8). The entire oral anlage lies in a pouch which is formed concomitantly with the organization of the anlage into the aforementioned three rows and projects laterally from the right vestibular wall between the normal position of the endoral kinety and the innermost right vestibular kinety. The dorsal face of the pouch will become the left half of the new peristomal wall while the ventral face will become the right half. The overall shape of the oral anlage when viewed from the ventral surface is that of a reversed J whose top is located at the same level as the anterior end of the buccal overture and whose posterior end curves around the posterior end of the vestibule. The

number of rows in the anlage rapidly increases to six, still in the reversed J configuration (Plate 2, fig. 9). The extension of the anlage around the posterior end of the vestibulum during the three and six row stages will be designated as stage II in the development of the oral assemblage.

The six rows present in the stage II anlage subsequently increase to 12 in number, all equidistantly spaced, with the right-most rows extending towards, but not on to, the future right half of the periostomal wall. Whether each of the six new rows is formed between the pre-existing rows or all arise together at one side or the other of the six rowed anlage, parallel to the pre-existing rows, is not clear from the present study. Based on EM studies of stomatogenesis in *P. bursaria*, however, Ehret & Powers (1959) and Ehret & de Haller (1963) have ascertained that new rows are added between the pre-existing rows.

The 12-membered anlage now begins to move away from the right vestibular wall, the anterior reaches moving towards the cell's right and posteriorly (Plate 2, fig. 10). The segment of oral development from the formation of the six rowed anlage to the twelve rowed anlage will be designated as stage III.

Subsequently, the 12 rows of the stage III anlage are partitioned into 3 groups of 4 rows each (Plate 3, fig. 11), as Roque (1956*a*, *b*, 1961) and Yusa (1957) have reported. The left-most two groups, i.e. those closest to the dorsal junction of the anlage pouch and the right vestibular wall of the old oral assemblage, will become the penicular complex of the opisthe's oral assemblage. The third group of rows of kinetosomes, which will become the quadrulus, is positioned farthest to the right of the three groups, but still remains on the future left wall of the new peristomal cavity. The portion of oral development during which the 12 rows of the stage III anlage are partitioned into the three ciliated buccal complexes will be designated as stage IV.

The kinetosomes of the 12 rows in the stage IV anlage, especially those of the presumptive quadrulus, are much more closely spaced than in the interfission oral assemblage. Although an accurate count of the total number of kinetosomes is impossible to make using silver impregnated cells, it appears that the majority, if not all, of the kinetosomes necessary for the formation of the mature buccal organelles are represented in the oral anlage at this stage.

The origins and morphogenesis of the new endoral kinety and anlage field in both proter and opisthe will now be examined. As was mentioned above, the endoral kinety associated with the old oral assemblage is not discernible as a discrete structure from the end of stage I through stage II (Table 2). At the beginning of stage III, however, a largely single, somewhat irregular row of kinetosomes appears between the ventral termination of the ribbed wall and the dorsal junction of the anlage pouch with the peristomal wall, i.e. in the position normally occupied by the endoral kinety in the interfission cell (Plate 3, fig. 12, arrow). Several interpretations present themselves: (1) this row of kinetosomes represents the old endoral kinety which may have been obscured by its intimate association with the anlage field until the movement of the latter away from the vestibular wall; (2) these kinetosomes may bear some relation to the unciliated inner row of the endoral kinety,

perhaps a relation of identity, i.e. the heretofore barren kinetosomes become ciliated at this time, or, alternatively, being new kinetosomes which form in close association with the barren row, as Dippell (1968) has shown to be the case for new kinetosomes in surface kineties; and (3) these kinetosomes arise *de novo*, without pre-existing

Table 2. *Summary of stomatogenic events in proter and opisthe*

	Stomatogenic events		
Anlage stage	Proter	Opisthe	
I	Anlage field appears	—	—
	Endoral kinety no longer visible	—	—
II	Organization of anlage field	—	—
		Three-rowed anlage Six-rowed anlage	First sign of anlage field in opisthe
III	Single row of kinetosomes appears	Twelve-rowed anlage	Increase in number of kinetosomes
IV	Double row of kinetosomes Triple row of kinetosomes	Partitioning of 12-rowed anlage into three presumptive buccal complexes	New anlage field and endoral kinety present
Fission furrow present	New anlage field and endoral kinety		

kinetosomes being present in the area. It is not possible to distinguish between these alternatives at the present time, although the resolution of this point is presently being sought through EM examination of serial sections of the oral apparatus and oral anlage.

During stages III and IV this kinetosomal row expands laterally into three closely applied rows of kinetosomes which then show subsequent progressive disorganization (Figs. 13, 14, 15, 16A), becoming completely anarchic by the time the fission furrow is evident. Concomitant with the disorganization of these three rows

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PLATE 1

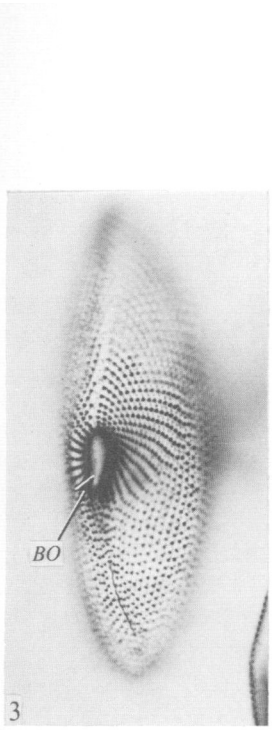
Plates. Technique used in visualization is indicated in parentheses, as Chatton-Lwoff (C & L) or protargol (P). Magnification is × 495 for Figs. 3 and 4, × 3100 for Figs. 5–20.

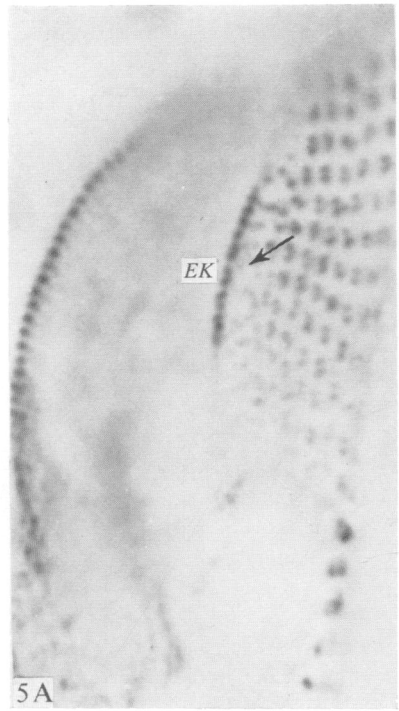
Fig. 3. Silver impregnation of an interfission cell showing the ventral pattern of kineties and the buccal overture (*BO*). × 495. (C & L.)

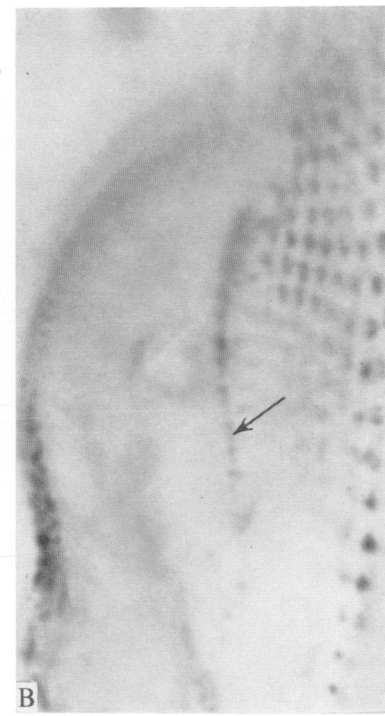
Fig. 4. Silver impregnation of a nearly divided cell showing the old (*OA*) and new oral assemblage (*OA'*). × 495. (C & L.)

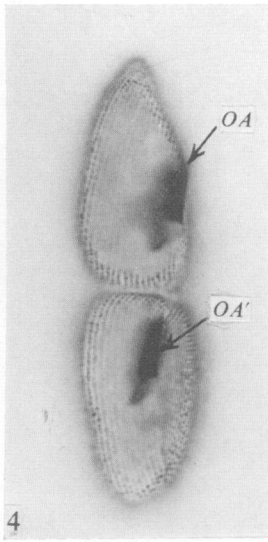
Fig. 5. Silver impregnation of an oral apparatus at 0·76 of the cell cycle showing the extended endoral kinety (*EK*). Arrow in (*A*) points out the faintly staining kinetosomes of the anlage field (*AF*), arrow in (*B*) the faint streak believed to represent near-surfaced kinetosomes. × 3100. (*C* and *L*.)

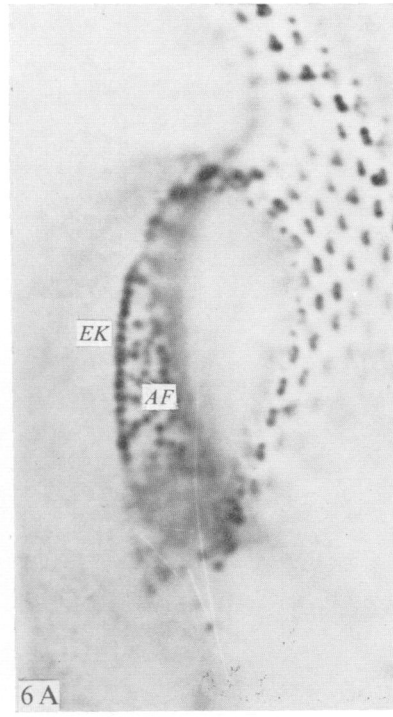
Fig. 6. Silver impregnation of an oral apparatus slightly later in the cell cycle than the cell in Fig. 5 showing the anlage field and endoral kinety. × 3100. (C & L.)

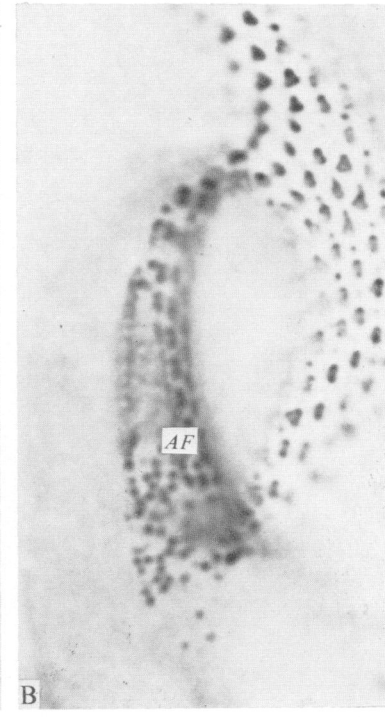












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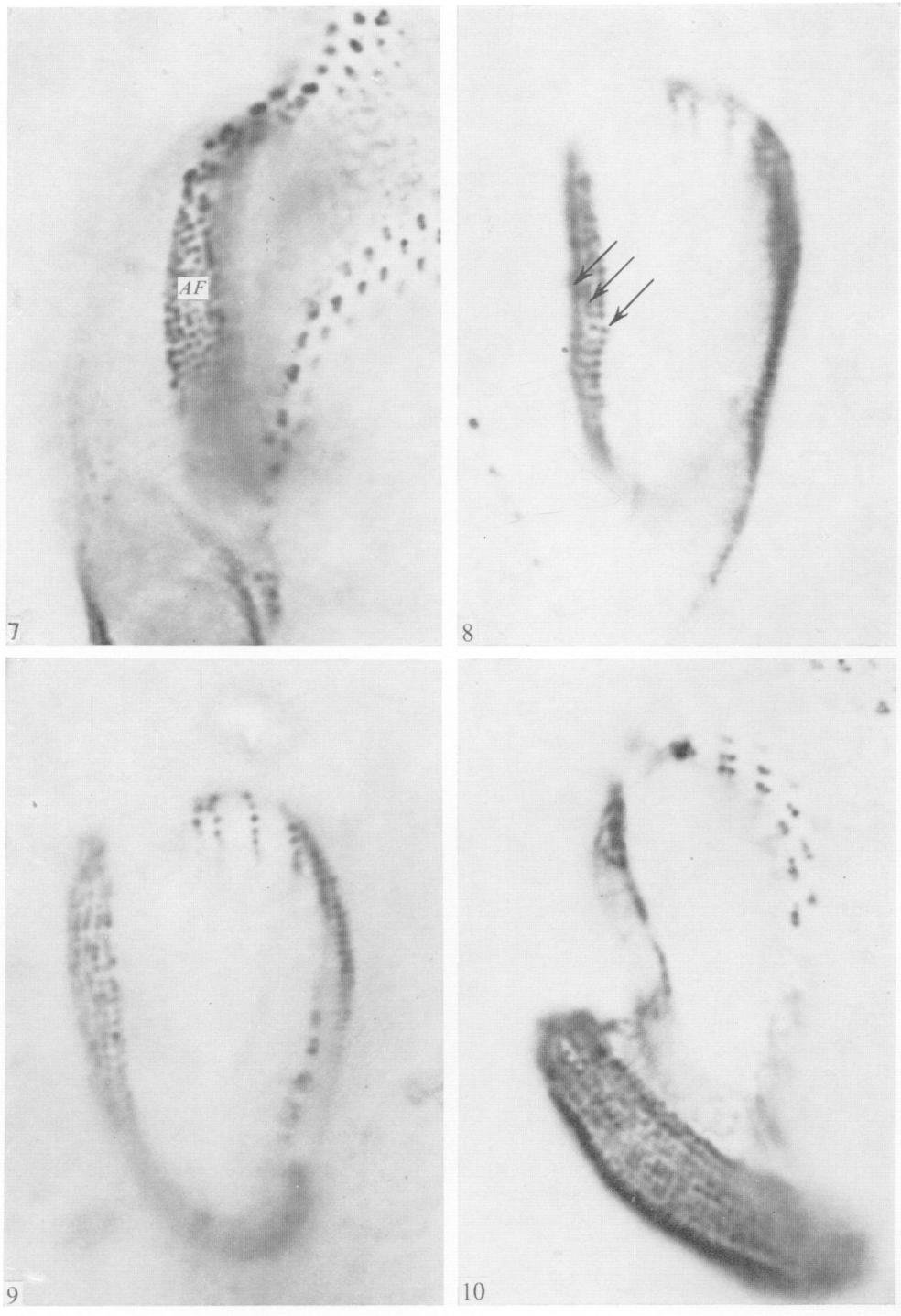

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is the appearance of a single row of kinetosomes between them and the ribbed wall (Plate 4, fig. 16 A, B). Both the disorganized field of kinetosomes and the new single row extend the entire length of the right wall of the vestibulum. It is suggested that the kinetosomes derived from the breakdown of the above triple row constitute a new anlage field in the proter and that the single row of kinetosomes represents the new endoral kinety, since their position and appearance are identical to the location and appearance of these entities in the interfission cell. It can thus be seen that far from passively inheriting the old oral assemblage at fission without undergoing any stomatogenic process, there are at least two morphogenetic events associated with the oral assemblage of the proter: the establishment of an anlage field and of an endoral kinety.

The anlage field associated with the future opisthe is first discernible during stage II of anlage formation. Scattered kinetosomes appearing to arise in close association with the presumptive quadrulus appear in transverse streaks across the ventral, i.e. future right face, of the peristomal wall (Plate 5, figs. 17, 18, arrows). By stages III and IV, a time when the triple row of kinetosomes observed in the proter is just beginning to become disorganized, these kinetosomes occupy the entire ventral face of the anlage pouch and are bounded at a slight distance along their left margin by a single row of kinetosomes, presumably the new endoral kinety (Plate 5, fig. 19).

As was previously noted, the stage IV anlage appears to possess a full complement of kinetosomes, albeit more closely packed than in an interfission oral assemblage. Maturation of the oral anlage into the interfission configuration previously described appears to be brought about by the differential growth and expansion of the non-kinetosomal components of the peristomal wall, rather than by the synthesis of any great number of new kinetosomes. Foremost among the non-kinetosomal elements which must be formed anew in the oral anlage is the ribbed wall; EM studies are now underway to elucidate the ontogeny of this structure.

Upon separation of the fission products the newly formed anlage field in both the proter and the opisthe begins to lose its affinity for silver. Between 0·15 and 0·19 of the cell cycle following fission, the anlagen fields are no longer visualized by the Chatton-Lwoff method, disappearing more or less all at once. The portion of the endoral kinety demonstrable by Chatton-Lwoff silver impregnation is reduced to the early interfission length of 12 or 13 kinetosomes. Food vacuole formation, which ceases during stage IV, is re-initiated between 10 and 15 min after separation, with both the proter and the opisthe beginning to feed at about the same time.

When cells in the interfission period are stained by the protargol procedure quite

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PLATE 2

Fig. 7. Silver impregnation of the anlage field (*AF*) showing the progressive ordering of the kinetosomes composing it. × 3100. (C & L.)

Fig. 8. Silver impregnation of a three-rowed anlage. Arrows indicate the three rows. × 3100. (C & L.)

Fig. 9. Silver impregnation of a six-rowed anlage. × 3100. (C & L.)

Fig. 10. Silver impregnation of a twelve-rowed anlage. × 3100. (C & L.)

a different image is obtained. Kinetosomes are clearly visualized (Plate 5, fig. 20) in the area between the endoral kinety and the innermost right vestibular kinety, where, it will be recalled, none are visible at this time with the Chatton-Lwoff procedure. Furthermore, these kinetosomes are not randomly arranged, but occur in linear arrays of three or four kinetosomes. As was previously mentioned, protargol staining reveals the endoral kinety to be fully double and to extend the full length of the right vestibular wall, although Chatton-Lwoff impregnation visualizes at this time but a single short row. The significance of the disparity between Chatton-Lwoff and protargol-demonstrable kinetosomes will be considered in the Discussion section.

4. DISCUSSION

With respect to the course of events during the formation and maturation of the oral anlage during divisional stomatogenesis, the present study is in general agreement, with the important exceptions regarding the morphogenetic role and the formation of the endoral kinety and the anlage field, with the findings of Roque (1956*a*, *b*, 1961), Porter (1960), Yusa (1957) and Kaneda & Hanson (1974), and thus this aspect of the present study needs little discussion.

Previous investigations of stomatogenesis in various species of *Paremecium* have confirmed the *de novo* origin of the three new buccal complexes during binary fission (Roque, 1956*a*, *b*, 1961; Yusa, 1957; Ehret & Powers, 1959; Porter, 1960; Ehret & de Haller, 1963; Gillies & Hanson, 1968; Ehret & McArdle, 1974; Kaneda & Hanson, 1974). In all cases buccal complexes are formed by the progressive ordering of a field of kinetosomes. The origin of the kinetosomes constituting this

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PLATES 3 AND 4

Fig. 11. Twelve-rowed anlage which has divided the three presumptive buccal complexes: quadrulus (*Q*), dorsal peniculus (*DP*) and ventral peniculus (*VP*). Top arrow indicates the position of the original endoral kinety which is here out of focus. × 3100. (C & L.)

Figs. 12–16. Series of photographs illustrating the development of the new anlage field (*AF'*) and endoral kinety (*EK'*) in the proter. × 3100. (C & L.)

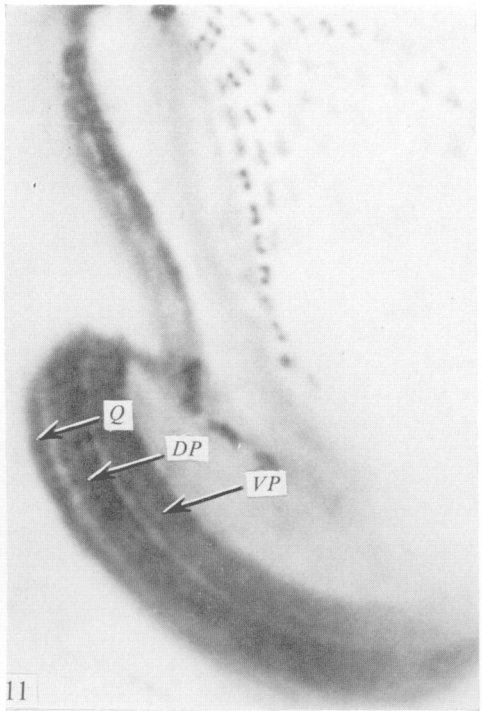
Fig. 12. Stage III anlage showing the first appearance of the row of kinetosomes which will give rise to the new anlage field (arrow). × 3100. (C & L.)

Fig. 13. Two focal levels of the double row of kinetosomes which will give rise to the new anlage field. This cell is slightly later in stomatogenesis than the cell in Fig. 10, approximately at stage IV. Right vestibular kineties (*RVK*) have divided, and the fission space is indicated by the arrow. × 3100. (C & L.)

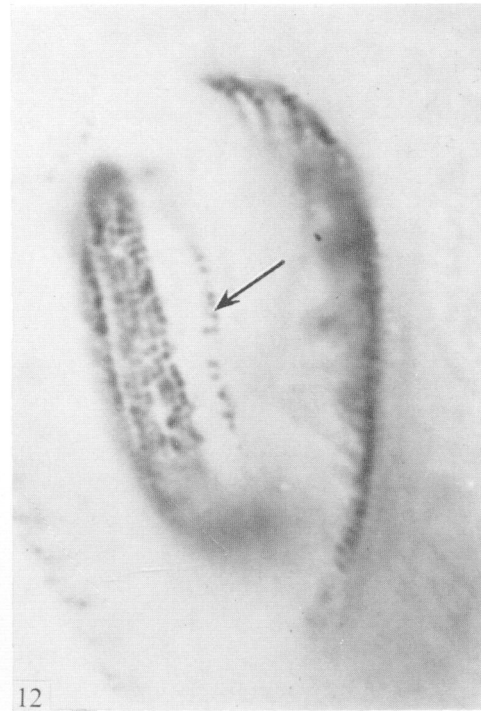
Fig. 14. Arrow indicates the mostly triple row of kinetosomes which will give rise to the anlage field. Quadrulus (*Q*), peniculus (*P*) and right vestibular kineties (*RVK*) are indicated. × 3100. (C & L.)

Fig. 15. Cell slightly later than in Fig. 14 showing the triple row of kinetosomes (arrow). × 3100. (C & L.)

Fig. 16A. Two focal levels of a cell showing the new anlage field (*AF'*) and endoral kinety (*EK'*). The positions of the ribbed wall (*RW*) and the right vestibular kineties (*RVK*) are indicated. × 3100. (C & L.)



11



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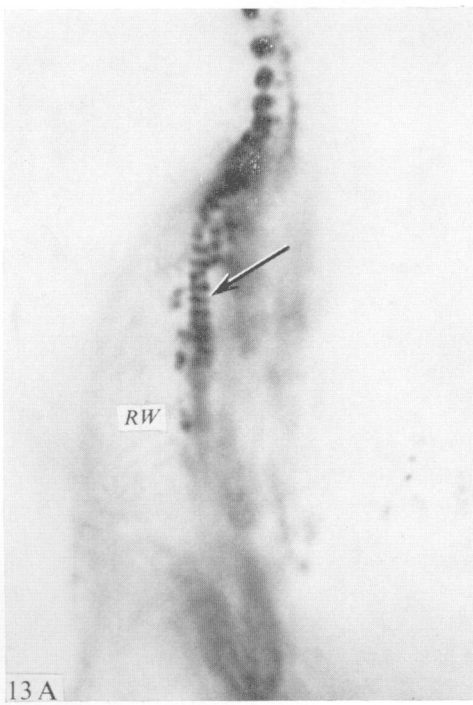
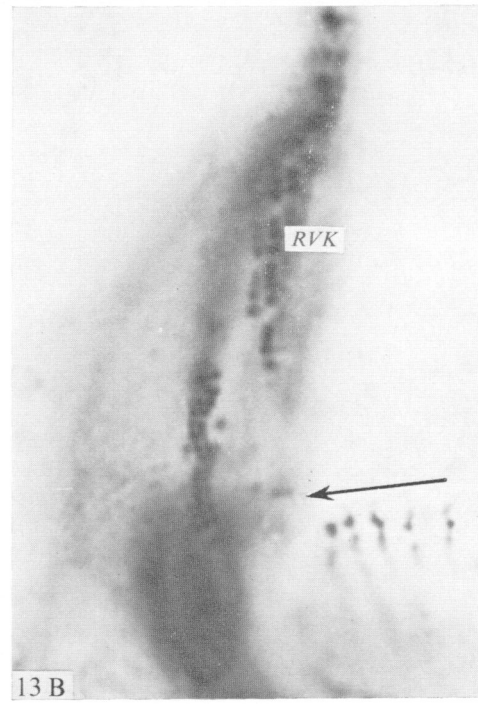


13 A



13 B

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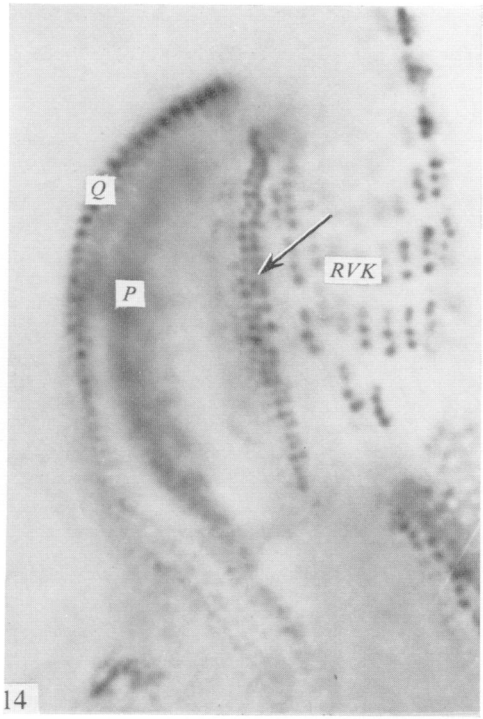

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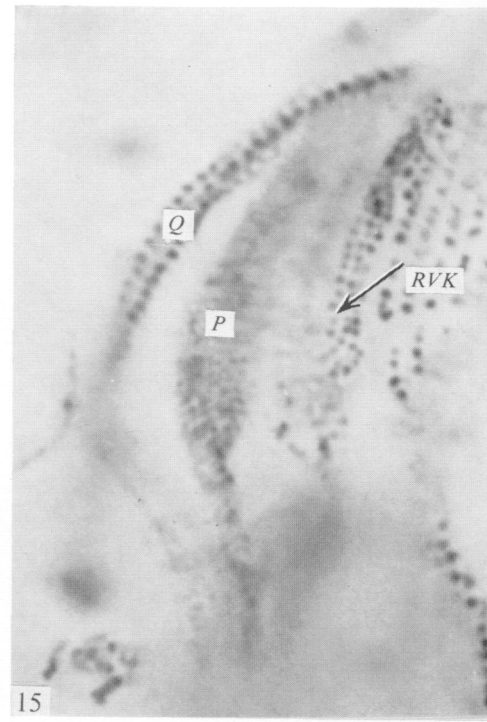

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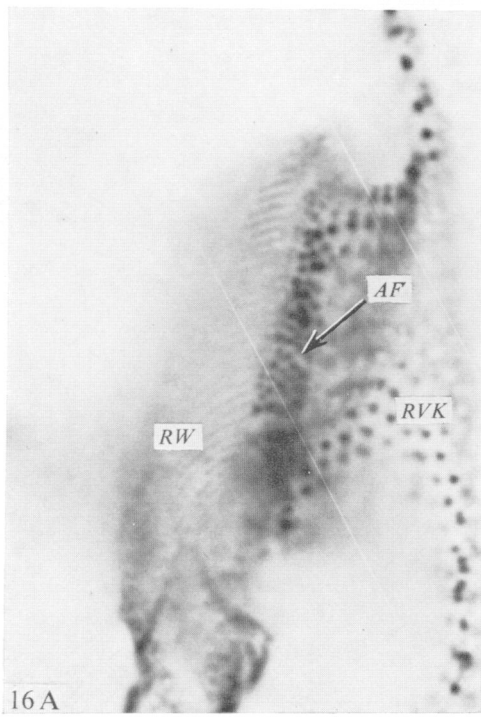

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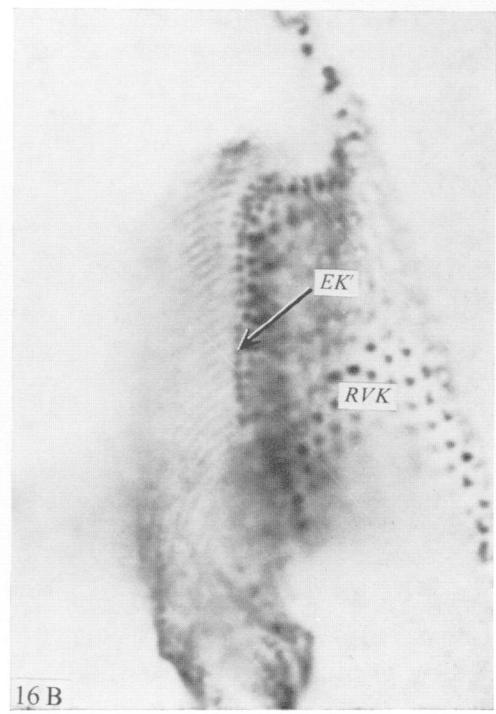

16 B

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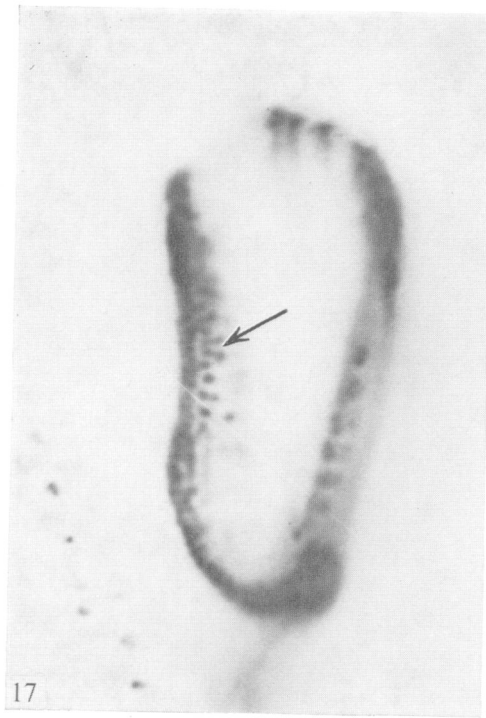
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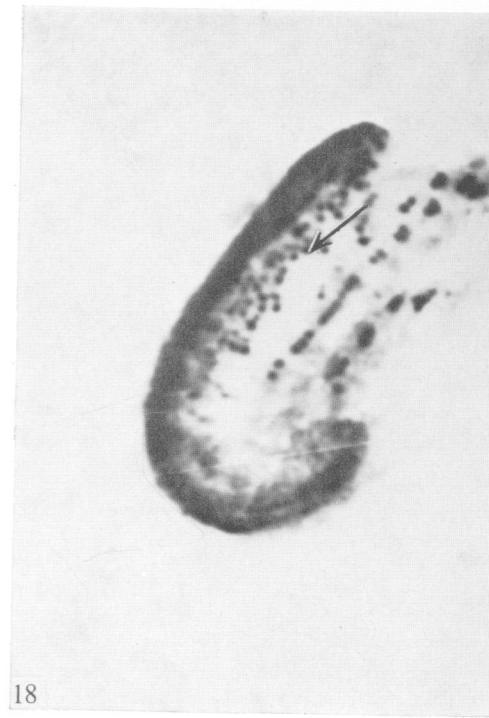
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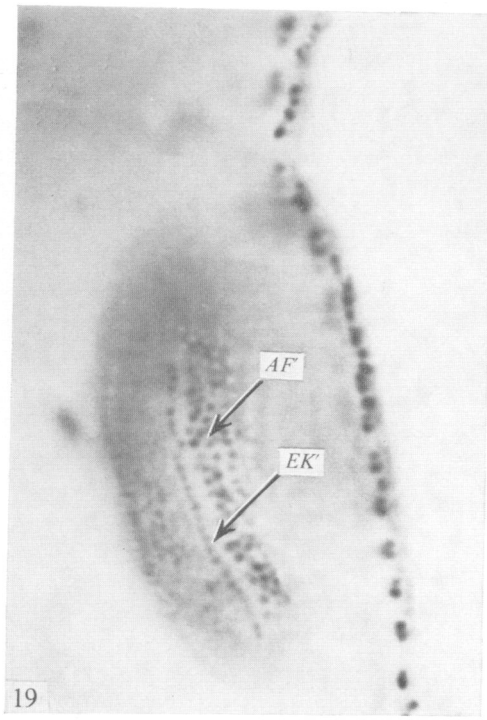
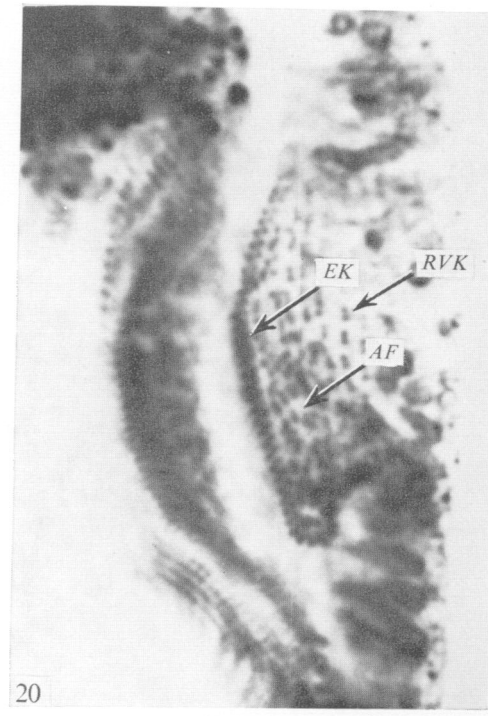


19



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field, the manner in which this field is ordered into the rigidly structured buccal elements and the possible role of the endoral kinety in either of these processes are all as yet unresolved. In no case has the series of events leading to the formation of new anlage fields and endoral kineties in either the proter or the opisthe been elucidated. The following discussion concerns these problems.

A variety of sources for the kinetosomes constituting the anlage field has been suggested. As previously mentioned, Roque (1956*a*, *b*), influenced by the Chatton-Lwoff kinetosome theory (1935) postulated their formation by division of the kinetosomes composing the endoral kinety. According to the kinetosome theory, kinetosomes arise only by division of pre-existing kinetosomes. Subsequent investigations by Ehret & Powers (1958) and Dippell (1968) have demonstrated the fallacy of this particular facet of the theory. Gillies & Hanson (1968) have observed instances during stomatogenesis in *P. trichium* which seem to indicate that a doubling of the Chatton-Lwoff-demonstrable kinetosomes in the endoral kinety precedes the appearance of the anlage field. They suggest that this supports the view that the endoral kinety actively participates in the formation of the anlage field. Alternatively, Porter (1960) suggests that the right vestibular kineties contribute kinetosomes to the formation of the anlage field.

The present study supports neither Roque's nor Porter's interpretation. It indicates that an increase in the number of Chatton-Lwoff-demonstrable kinetosomes in the endoral kinety begins very early in the cell cycle, only 0·39 of the cell cycle (1·5 h) (Table 1) after the completion of the previous cycle. This apparent increase continues up until the time the anlage field is first visualized by Chatton-Lwoff impregnation. The kinetosomes composing the anlage field appear simultaneously, at points widely separated from the endoral kinety and at first are marked by a very light deposition of silver grains, subsequently increasing in argentophilia. Two interpretations are possible. The endoral kinety, either the ciliated or the barren row, could serve as the site of formation of the kinetosomes which will constitute the anlage field, the newly formed kinetosomes migrating subcortically and surfacing at some distance from the site of their formation. As Sonneborn (1974) points out, kinetosomes are seldom used at the immediate site of their synthesis and regularly migrate before reaching their final position.

PLATE 5

Figs. 17–19. Series of photographs illustrating the formation of the anlage field and endoral kinety in the opisthe.

Fig. 17. Stage II oral anlage showing the scattered kinetosomes of the anlage field (arrow) arising in close proximity to the kinetosomes of the presumptive quadrulus. × 3100. (C & L.)

Fig. 18. Early stage III anlage showing the increase in number of the kinetosomes which will constitute the anlage field (arrow). × 3100. (C & L.)

Fig. 19. Stage IV anlage showing the new anlage field (*AF'*) and endoral kinety (*EK'*). × 3100. (C & L.)

Fig. 20. Protargol stained interfission cell showing the fully double endoral kinety (*EK*) extending the entire length of the right vestibular wall, the anlage field (*AF*) and the right vestibular kineties (*RVK*) (*P*). × 3100.

The present study, which has established the continuity of an oral anlage *field* from stages III–IV in one cell cycle to stages I–II in the next cycle, suggests another hypothesis. It is postulated that there is a direct continuity and identity of the stage III–IV *kinetosomes* with those of the next stages I–II. Additionally, one might speculate as to whether there is a positional persistence of the kinetosomes from one cell cycle to the next, i.e. do individual kinetosomes 'reappear' in the same location where they 'disappeared'? The observations made in the present study do not permit the resolution of this question, although one set of observations does bear, in a suggestive way, on the more general question of whether a kinetosome can 'remember' its former position. It will be recalled that during the formation of the anlage field in the proter three rows of kinetosomes are formed which become disorganized. At stage II of the next cell cycle the disorganized kinetosomes of the anlage field again form into three rows. Given the hypothesis of the continuity of the kinetosomes of the anlage field from one cell cycle to the next, one might postulate that the kinetosomes of the stage II oral anlage 'know' where they came from during their disorganized stage and reorganize, at the appropriate stage, into the same rows.

The disparity between the visualizations obtained with the Chatton-Lwoff and protargol impregnations, it is hypothesized, is attributable to the temporally specific surfacing and/or ciliation of the kinetosomes composing the anlage field. It is suggested that the new anlage fields which arise in the proter and the opisthe merely deciliate and submerge after the separation of the proter and the opisthe, rising and/or re-ciliating during the next stomatogenesis. That the kinetosomes of the anlage field do not appear in short rows immediately during stage I, but rather as scattered kinetosomes may be explained by the ciliation and/or surfacing of kinetosomes within each row at different times. It is further suggested that the above hypothesis may account for the difference in visualizations of the endoral kinety obtained with protargol and Chatton-Lwoff impregnations. It must be assumed that one entire row of kinetosomes and most of another are barren and/or submerged throughout the interfission period. The lengthening of the endoral kinety observed prior to the onset of stomatogenesis thus would not represent the synthesis of new kinetosomes but rather the ciliation and/or surfacing of pre-existing ones. One might therefore best view the earliest stages of stomatogenesis, i.e. stage I and the beginning of stage II, as a temporally controlled surfacing and/or ciliation of kinetosomes rather than their *de novo* synthesis.

Supporting evidence for the continuity of kinetosomes through successive cell generations in an unciliated condition is found in studies of the suctorian, *Tokophrya infusionem* (Millecchia & Rudzinska, 1972) where an unciliated anlage of from 18 to 25 kinetosomes persists in the non-ciliated adult, acting as the site of synthesis of new, ciliated kinetosomes for the developing embryo. Although the situation is not directly analogous to that which has been demonstrated to occur in *Paramecium*, it does demonstrate that unciliated kinetosomes can be maintained from one generation to the next.

What is the role of the endoral kinety in oral morphogenesis? Porter (1960)

suggests that it functions as an organizer of the newly forming oral anlage. There is no evidence which requires this interpretation. The only possibly relevant evidence comes from Hanson's (1955, 1962, 1969) studies of the effect of UV irradiation of the general area of the posterior part of the right vestibular wall. Irradiation of these areas prior to division or conjugation, two processes in which a new oral anlage is formed, results in the production of a high percentage of opisthes or exconjugants with abnormal or missing oral assemblages. Unfortunately, the technique does not allow the area of irradiation to be restricted, for example, to just the endoral kinety or just the area where the anlage field will arise. The data permit only the generalization that the posterior part of the right vestibular wall is in some way important in insuring that a new oral anlage is formed, without assigning causality to any specific part of it. Had these investigations been coupled with cytological studies to ascertain the specific effects of irradiation upon the existing oral assemblage, one might have a better idea of what sorts of visible damage produce a specific visible result in the opisthe of the irradiated cell, or in the irradiated exconjugant. It is thus as yet impossible to come to any conclusion concerning the nature of the endoral kinety as an organizer of the oral anlage during stomatogenesis.

In the previous paragraphs, arguments have been presented against the endoral kinety's causal involvement in stomatogenesis. It has been mentioned that correlated with stomatogenesis is the formation of a new oral anlage field and endoral kinety in both proter and opisthe. This is not to say that both fields originate in the same manner. In the proter, which, it should be recalled, retains the old oral assemblage, the new anlage field is formed as a replacement for the one used during formation of the oral anlage. No pre-existing anlage field existed in the oral anlage passed to the opisthe and the anlage field formed in this instance is the first ever appearing in that cell. Furthermore, the present observations indicate that the kinetosomes which will comprise these new fields originate in different locations, as will be discussed more fully below.

With the expansion of the anlage pouch and the concomitant shifting of the oral anlage to the right of the old oral assemblage a largely single row of kinetosomes appears at the site occupied in interfission cells by the endoral kinety. It subsequently doubles, then triples, and the kinetosomes become more widely spaced and finally unordered. These kinetosomes now represent a new anlage field in the proter. At a time when the above three rows are becoming unordered, it is first possible to discern the new endoral kinety, which appears more or less simultaneously at what will be the dorsal edge of the anlage field. No observation supports the interpretation that ciliated or surfaced kinetosomes of the old endoral kinety are utilized in the formation of the new. However, since in the Chatton-Lwoff technique only ciliated or, perhaps, near-surfaced kinetosomes are visualized, it is impossible to eliminate the possibility that kinetosomes arising within the new anlage field might migrate beneath the surface to arise at the site of the new endoral kinety. Additionally, one cannot eliminate the possibility that the inner, heretofore barren, row of kinetosomes of the old endoral kinety might participate in the formation of the ciliated row of the new endoral kinety.

The kinetosomes which give rise to the anlage field in the opisthe's oral assemblage have been shown to appear first in close proximity to those kinetosomes constituting the presumptive quadrulus, no ribbed wall intervening between these two structures at this time. Their rapid proliferation across the future right wall of the peristomal cavity is followed by the appearance of the new endoral kinety at the future dorsal boundary of the anlage field. Not until both the anlage field and the endoral kinety have formed is there any evidence of the development of the ribbed wall. Here again the present study does not indicate any relation between the kinetosomes forming the new endoral kinety and those of the anlage field. The physical separation of the two buccal cavities at the time of the new endoral kinety's appearance precludes inclusion of kinetosomes of the old (proter's) endoral kinety into that of the opisthe. Even at earlier stages the entire width of the oral anlage separates the site of appearance of the new endoral kinety from the old. Any continuity of kinetosomes from one endoral kinety to the next seems therefore to be most improbable.

It can thus be seen that the formation of the new anlage field in proter and opisthe has little in common. Both the location in which the kinetosomes which go to make up the anlagen fields first appear and their relation to pre-existing structures of the oral assemblage are different. The one obvious similarity between the sites in which the oral anlage of proter and opisthe arise is their ventral boundary: the innermost right vestibular kinety. That this structure is the only obvious similarity marks it as worthy of closer examination. One might envision the innermost right vestibular kinety as a visible reference boundary which in an unspecified manner transmits positional information (Wolpert, 1969, 1971) concerning the formation of the anlage field and endoral kinety in both the proter and the opisthe.

The importance of regions exhibiting juxtaposition of two widely different kinety patterns as reference boundaries has been clearly demonstrated in ciliates (see Sonneborn, 1963, 1974, and Frankel, 1974, for comprehensive reviews). The junction between the vestibular kineties and the ciliated rows of the three major buccal complexes certainly qualifies as such an area – a fact which Sonneborn (1963) notes – for these two groups of kinety systems are widely different in spacing and meet at nearly right angles. Thus *à propos* of the previous discussion concerning the endoral kinety as an organizer, it is seen that no specific structure need act as the buccal organizer. That this 'organizer' may not even be part of the visible oral assemblage was indicated by the experiments of Hanson & Ungerleider (1973) when cells which had become astomatous through UV irradiation were able to regain their oral assemblage if they underwent autogamy, a sexual process in the course of which a new oral anlage is produced, within four cell generations of having lost said structures. Their experiments further indicate that, at least in part, buccal morphogenesis depends upon a pre-existing cytoplasmic differentiation of the cell.

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