RESEARCH IN PROTOZOOLOGY

In Four Volumes

EDITED BY

TZE-TUAN CHEN

VOLUME 2

RESEARCH IN PROTOZOOLOGY

In Four Volumes

EDITED BY

TZE-TUAN CHEN

Professor of Zoology
University of Southern California
Los Angeles, California

VOLUME 2

PERGAMON PRESS
OXFORD · LONDON · EDINBURGH · NEW YORK
TORONTO · SYDNEY · PARIS · BRAUNSCHWEIG

Pergamon Press Ltd., Headington Hill Hall, Oxford
4 & 5 Fitzroy Square, London W.1

Pergamon Press (Scotland) Ltd., 2 & 3 Teviot Place, Edinburgh 1

Pergamon Press Inc., 44–01 21st Street, Long Island City, New York 11101

Pergamon of Canada, Ltd., 6 Adelaide Street East, Toronto, Ontario

Pergamon Press (Aust.) Pty. Ltd., 20–22 Margaret Street, Sydney, N.S.W.

Pergamon Press S.A.R.L., 24 rue des Écoles, Paris 5e

Vieweg & Sohn GmbH, Burgplatz 1, Braunschweig

First edition 1967

Library of Congress Catalog Card No. 66–22364

2818/67

PREFACE

VOLUME 1 of this series of reviews, recently published, ranged over a variety of subjects. So does this Volume 2. To include all related chapters in one volume was impractical; chapter lengths and their dates of submission were the deciding factors.

The editor is grateful to the contributors for the time and effort which they obviously put into the preparation of their chapters. Without their dedicated cooperation and their patience this volume could not be the useful compendium of protozoological information which we think it is.

The editor again thanks Dr. Ruth Stocking Lynch for her indispensable help in the editing of these chapters. The Board of Consultants, including Drs. William Balamuth, A. C. Giese, R. F. Kimball, Norman D. Levine, William Trager, and D. H. Wenrich, has again proved most helpful to the editor in the making of certain decisions on matters of importance. And to the staff of Pergamon Press in New York and in Oxford, England, the editor is deeply grateful.

Los Angeles, California T. T. CHEN

CONTENTS

MORPHOGENESIS IN PROTOZOA

VANCE TARTAR

Department of Zoology, University of Washington, Seattle

CONTENTS

I. INTRODUCTION

Morphogenesis may be roughly defined as the coordinated elaboration of visible parts by which the construction or reconstruction of individual organisms of specific form is accomplished. In protozoa all the parts elaborated by the organism are cell-parts or organelles. This restriction, excluding cellular partitioning and intercellular reactions, may provide a simpler expression of morphogenesis from which essential insights into the general enigma may be gained. As the study of bacteria and viruses has given the clue to the genetic code, so the simplest organisms with appreciable form and morphogenesis may be the most appropriate types in which to extend or complement this approach in attempting to find out how the molecular building blocks synthesized directly or indirectly by nucleoproteins are assembled into unit organelles such as a cilium, what determines how many and where these will be formed, how they are in turn organized into organelle sets such as ingestive structures, and how all these cell-parts formations (and resorptions) are integrated into the life of, first, a unicellular organism.

Thus might the morphogenesis of protozoa be viewed in a contemporary context, but our subject has a long history in gathering a wealth of observations and experimental findings, ever renewable by fresh orientations and further penetration into the implications of the phenomena described. Previous reviews and surveys should be consulted, both for the particular approach of each author and as supplementing the present account: Sokoloff,[417] Balamuth,[17] Summers,[434] Tartar,[443] Fauré-Fremiet,[152, 156, 160, 164, 167] Lwoff,[312] Gelei,[189] Weisz,[504] Grimstone,[203] Grell,[202] Trager,[476] Picken,[363] and Schwartz.[412] To review the subject today poses a dilemma: the literature has become so extensive and various that its coordination is more essential than ever, yet one person now can scarcely hope to do this well.

II. MANIFESTATIONS OF MORPHOGENESIS

Orderly elaboration of visible parts in protozoa occurs under a variety of circumstances, comparable to embryogenesis, budding, regeneration, reorganization, and metamorphosis in metazoa. In both groups, any morphogenetic sequence chosen for study will have an arbitrary beginning and end. The starting points and the tasks of morphogenesis are often different, though the underlying processes may be similar.

1. Polymorphism and Cyclical Transformations

Members of the same clone of protozoa, or even the same individual at different times, may have different forms, varying from minor modifications to extreme alternatives in the type of cell-parts elaborated. The possibility of multiple forms and how the switch from one to another is accomplished may be considered as much within the subject of morphogenesis as how any one form is produced. Indeed, this polymorphism might provide a fruitful comparison to cell differentiation in metazoa, in which it may also be assumed that descendants of one cell have the same genome, yet produce the different kinds of cell-parts characteristic of different tissues. In protozoa, all the population may transform, or only certain members; and transformation may be obligatory in a life cycle, or adventitious, depending on environmental conditions. Within a group the tendency to or capacity for polymorphism may vary, as if subject to evolutionary expansion or restriction (Corliss;[101] Williams [520]). This aspect of protozoan morphogenesis has been previously reviewed by Fauré-Fremiet.[164]

A. Dwarf and Giant Forms

Size extremes demonstrate that morphogenesis is relational, that the same form and pattern can be manifested in varying dimensions. As flatworms deprived of food can become smaller than when hatched from the egg and yet retain normal morphology, starved ciliates may have forms complete in all details but only one-hundredth the size of normal feeding individuals (see Adolph [7]). Even without starvation *Stylonychia* grown at pH 6.1 becomes progressively smaller (Darby [109]). Allescher[5] studied size reduction by starvation in several forms and came to the conclusion that the possibility of dwarfism is correlated with the form of the nucleus: *Dileptus* and many other ciliates with multiple or beaded macronuclei can become minute, but *Paramecium* with its single compact macronucleus only becomes thinner. A starved *Stylonychia* repeatedly reorganizes on a smaller scale (Dembowska [121]), but in *Blepharisma* the cells continue to divide, eventually becoming very small (Eberhardt [135]). Perfect individuals smaller than any normally occurring are also produced by regeneration of tiny fragments. In *Stylonychia* for example, Dembowska[120] said that repeated cutting gave complete animals only one-thousandth the original volume. The limit of minuteness seems to be determined by a fixity in size of the unit organelles—cilia, cirri, membranelles. With decreasing cell size the point is eventually reached at which functional feeding organelles cannot be assembled from parts not correspondingly miniaturized (Tartar,[458] p. 120).

Blepharisma may be induced to produce giants[190, 357, 77] as may *Stylonychia*[191, 6] and *Euplotes*,[480] usually by changing their food from bacteria to ciliates of increasing size until they become large enough to

ingest their own kind, for cannibals have proportionately larger feeding structures. Enlargement of the macronucleus is not due to taking over as functional nuclear material the macronuclei of the prey because these remain within the food vacuole and are digested (Tulchin and Hirshfield [482]), though there may be exceptions (Weyer [511]). Gigantism is not from suppressed division because giants can divide more rapidly than controls on a bacterial diet. Rather it is the result of "big meals", of highly assimilable food in big chunks, as when *Dileptus* feeds on planaria (Janovy [244]); and the prey may vary in promoting gigantism. *Blepharisma* giants were produced with *Khawkinea* and *Chilomonas* as food but not by *Paramecium* alone (Hirshfield and Pecora [228]). That the giant is not an equilibrium form was indicated by the incidence of abnormal fissions and monster formations observed by Giese and Janovy. Though giants reproduce as giants, in the absence of large prey they return to normal size through several rapid divisions. The same reversion to normal size is shown in *Tetrahymena* (Zeuthen and Scherbaum,[546] Zeuthen [545]). Repeated heat shocks suppress division but not growth to yield unusually large animals which return to normal size by division after treatment stops.

Among other cases are *Minodinium vorax* (Fauré-Fremiet[153]) which in an excess of its food organism attains a volume eight times the normal. Repletes undergo regression of the probosis and encyst. Cannibalizing *Oxytricha* were described by Dawson[113] and large forms of *Enchelys* have been found in nature (Fauré-Fremiet[152]). Giant *Euplotes* were produced when one member of a doublet pair was absorbed by the other (Katashima[258]). Differing from nutritional giants were the large tetraploid forms of *Chilodon* produced by MacDougall.[314]

Cannibalism in *Stentor* does not lead to gigantism (Gelei;[184] Tartar[458]) and this poses the interesting problem of why this form responds differently from *Blepharisma* which it otherwise closely resembles.

Hypotrichs have so fixed a pattern of ventral cirri that every cirrus can be uniquely designated, yet it has recently been reported (Alonso and Pérez-Silva[6]) that in giant *Stylonychia* the number and arrangement of cirri are consistently somewhat different from the normal.

Tuffrau's[480] study of gigantism in a marine species of *Euplotes* showed how the change in size may be accomplished. Bacteria-fed euplotids when supplied with a ciliate food organism undergo unequal divisions, producing larger opisthes with larger ingestive structures, leading to giants many times the former size which divide equally. Returned to bacterial food only, the reverse occurs, unequal fission now producing smaller opisthes and return to the original size.

An important question, already indicated, is whether flagella, cilia, cirri, membranelles and other units of structure are always proportionate in size to that of the individual; do they grow with the cell, or are they the same size in normal animals, tiny regenerants and giant forms? It has been assumed

that cilia and cirri are always proportionate to cell size (Dembowska;[121] Reynolds;[386] Bishop[34]) but this would imply an additional morphogenetic system, one not only for elaborating a cilium, say, but also another for controlling its later growth. There is in fact good evidence that ciliary organelles of any one type are constant in size though varying in number (Tartar;[443] Bonner[35]). For example, in the largest stentors and smallest regenerated fragments the length and width of the membranelles is the same though their number, and hence the lengths of the peristomes, varies enormously (Tartar[458]).

B. Microstomes and Macrostomes

Large and small mouthed forms of the same ciliate imply that morphogenetic regulations governing the size of the oral primordium are alterable. Thus in the transformation of *Tetrahymena patula* from a micro- to a macrostome, large ingestive structures are produced and the body then may increase in size to give the carnivorous, cannibalizing form (Corliss[101]). Usually the difference in mouthparts is in size only and not in pattern (Miller and Stone[332]) but, as Corliss noted, in microstomes the mouthparts may temporarily be reduced to a narrow slit.

Microstomes regularly feed on bacteria while macrostomes can ingest larger prey and even cannibalize. The two forms therefore seemed to be adaptations to the size of food organisms available. When *Tetrahymena vorax* was supplied with *Colpidium* they developed enlarged mouthparts by an amazing extension of the gullet, according to Kidder, Lilly and Claff.[266] When supplied with *T. pyriformis*, macrostomes were also produced, even when prey and predator were separated by a filter (Buhse,[47] see also Claff[78]). Then it was found that the same transformations occur in axenic culture in which the ciliates are living on soluble nutrients; and in this circumstance at least, the changes are related to the logarithmic growth curve (Williams;[520] Stone[426]).

At the start of a culture macrostomes convert to microstomes, proliferating and even reorganizing[518] as such during the log phase, reverting to macrostomes in the culminating stationary phase. If such forms were cannabilistic the conversion would have adaptive value in maintaining the culture after exhaustion of nutrients, with some lucky animals living at the expense of their fellows. Therefore any one of a variety of agents and nutrients favoring multiplication could likewise promote conversion to microstomes. During the stationary phase the effect on the reverse conversion to macrostomes could be more specifically tested; for instance, lowering the temperature then resulted in a higher percentage of macrostomes (Stone[426]). Earlier studies, specifically Prowazek's[378] quinine treatment promoting large forms and Fauré-Fremiet's[158] finding that lithium favors microstome formation by some action resembling the vegetalizing suppression of ciliary

proliferation in sea urchin embryos, should be retested, apart from cell proliferation, on ciliates in the stationary growth phase.

When tetrahymenids also produce cysts there are three forms (Fig. 1) all interconvertible.[520] But the transformations are by different routes. Microstomes cannot become macrostomes except by forming new mouths (Stone[426]) but a macrostome can be partially resorbed and remodeled to form a microstome (Buhse[48]). This is in accord with studies on *Stentor* in

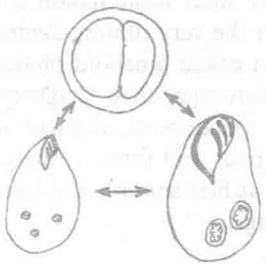

FIG. 1. Three interconvertible forms of *Tetrahymena*. Top: the cyst, undergoing first division. Left, microstome form. Right, macrostome with larger ingesta (after Williams[519]).

which it was shown that the oral structures can become reduced in size *in situ* (Tartar,[458] p. 125) but increase only through oral primordium formation (Schwartz[409]).

C. Adventitious Polymorphism

In describing the new species *Paramecium arcticum* Doroszewski[133] reported two forms with different cell shapes in the same pond. They were interconvertible yet tended to reproduce true to type.

When a species of infective *Tetrahymena* was cultured outside the host, one-fifth of the ciliary meridians were lost, to be regained on return to the host (Kozloff[296]). In a free-living species of the same genus the number of meridians was remarkably constant throughout all size changes and macrostome–microstome transformations (Williams[519]).

Populations of a species of *Diplodinium* usually bearing a uniform crown of spines sometimes showed one spine greatly elongated, as if the anlagen of two spines had fused to form the larger one. Another variation was that a spine might develop a spur and this was always the spine nearest the macronucleus—a unique case of a spatial relationship between nucleus and morphogenesis in ciliates (Dogiel[130]).

Espejoia mucicola has a macrostomatous feeding form which reorganizes as a migratory, non-feeding phase which then reorganizes back to the trophic

form. The differences are traceable to large or small oral primordia in cells of the same size, but this is not a regular cycle and depends on the medium (Fauré-Fremiet and Mugard[171]).

In certain amebae, pseudopodial form can alternate from a lobate *proteus*-type to the monopodial slug-like *limax*-type and then, by the addition of a little KOH, transform into the star-shaped, floating *radiosa*-type (Verworn[490]).

The shelled sarcodinian *Arcella* usually reproduces by extruding a division product which forms a new shell while fission is being completed and one would suppose the same for the very similar *Centropyxis*. Yet in these forms Cavallini[61, 62] described a naked ameboid phase in which the living body leaves the shell as a *proteus*-type, then toughens the outer membrane to make possible *radiosa*-type pseudopods, and with increasing thickness of the outer layer withdraws the pods and forms a shell. In the species of *Centropyxis* studied the shell was at first smooth and later developed spines on the surface opposite the opening.

D. Ameba–Flagellate Transformations

Changes of form in certain protozoa are so striking as to bridge the distinctions between Sarcodina and Mastigophora. In the ameboid phase the animal creeps on the bottom and is of indefinite shape; the swimming flagellate has a definite form and polarity. I am told that the transformations were discovered when a drop of distilled water was placed on certain amebae growing on a semisolid medium, whereupon the "amebae" swam away; for the speed of these transformations is sometimes extraordinary. These remarkable changes, in which there can be no doubt that we are dealing with one and the same cell in different phases, certainly indicate that the cell need not be irrevocably determined in one direction or the other; and perhaps they offer a parallel to the transformation of iris into lens cells in Wolffian regeneration of the vertebrate eye. Similarly, in dissociated sponges it has been observed that the flagellated collar cells can transform into ameboid cells and back again into choanocytes.

The soil-living *Naegleria gruberi* persists as a cyst, emerges as an ameba and can transform into a flagellate. The flagellate neither feeds nor multiplies nor encysts but can only revert to the ameba form. Dispersal could be its major role and exhaustion of its intracellular food reserves may be a stimulus to its reversion (Chang[63, 64]). In this reversion it is noteworthy that the flagella may be resorbed in several different ways, by resorption, shedding or pinching off.[379, 64] In the transformation from ameba to flagellate the origin of the centriolar progenitor of the flagella is problematical. Both Rafalko[379] and Chang[64] report this generative body as already present in the cytoplasm of the ameba; but Wilson[527] said it is intranuclear in origin, which would explain why Schuster[408] could not find it by electron micro-

scopy in ameboid stages. Pittam[369] observed that multinucleate amebae in old or crowded cultures produce flagellates with a larger number of flagella. The centriole probably arises by replication of a pre-existing centriole, outside or within the nucleus. Then from the centriole or its homologous derivatives flagella form (see Section V) as the cell assumes a definite, euglenoid shape. On reversion, the cell gradually becomes more rounded and ameboid as the flagella disappear.

Transformation into the flagellate phase is brought about by flooding an agar culture of bacteria-feeding amebae with distilled water. Hollande[230] related the change to salt concentration of the medium, and Chang concluded that the conversion of ameba into flagellate is brought about by hydration of the cytoplasm; but Willmer[524, 525, 526] found that reduced osmotic pressure of the medium alone is not the stimulus. Rather it is a matter of ionic balance, and Willmer suggested that the flagellate form, with a tougher and possibly less permeable cell membrane, is assumed not so much for dispersal as for conserving essential cations (notably sodium and magnesium) when the medium is diluted. Chang reported that "age" or long culture in broth medium, as well as high temperature, decreases the ameba → flagellate transformation, while growth on agar and pre-freezing of ameba in the cyst stage promotes the reverse transformation. Because this organism may in some circumstance be in balance between ameboid and flagellate phases, it is not surprising that the flagellar formations may be incomplete or abnormal, as described by Chang; and this should be investigated further as potentially of importance with regard to how flagella are formed. The significance of *Naegleria* transformations as a parallel to metazoan cell differentiation was emphasized by Willmer. Lithium, which "vegetalizes" embryos, favors the ameboid form, while iodine ("animalizing") favors the flagellate. Moreover, transformations may be affected by certain steroid hormones.[362]

Coprophilic *Tetramitus* was studied by Bunting,[49] Hollande[230] and more recently by Balamuth[20] and his students. The ameba transforms by first producing a set of flagella and then elongating and assuming a specific shape as a cytostome is developed. Both forms feed and reproduce as such. But if the amebae are grown in a tiny drop they soon transform synchronously into flagellates. This crowding, with exhaustion of food, suggests an adaptive significance for the change to the migratory form; alternatively, with crowding there may be an accumulation of something the amebae themselves produce which favors their transformation.[356] When flagellates were isolated into fresh culture drops they reverted to amebae, depending on salt and nutrient levels, pH, as well as age, recently transformed flagellates reverting the more easily.[355] Because flagellum formation is the earliest indication of change in the amebae, Outka[353] suggested that some activation of kinetosomes or centrioles which sprout the flagella is the basis of this transformation. Hollande considered that the switch is triggered by

changes in salinity. Axenic culture in a defined medium holds great promise for determining the chemical conditions for transformation in either of these genera if it can be developed to sustain both forms (Balamuth and Kawakami,[22] Balamuth[21]).

Gigantomonas herculea living in African termites has a complex flagellate phase, with three anterior flagella, one trailing flagellum with undulating membrane, an axostyle and cresta. Yet preceding each division all these structures are resorbed, leaving a simple, shapeless ameba with pseudopodia and nucleus and a pair of centrioles lying against the nuclear membrane

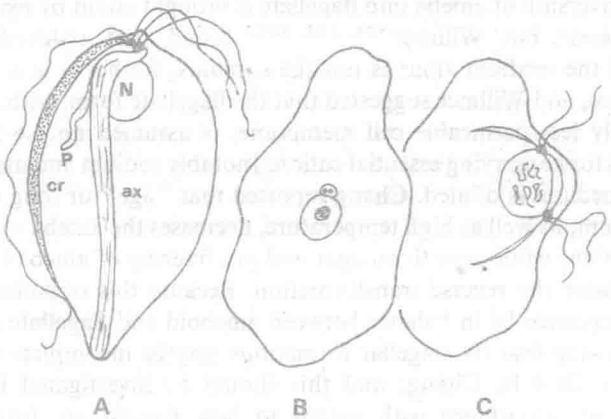

FIG. 2. Cell replication in *Gigantomonas herculea*. **A:** Formed organism with mastigont structures: 3 free flagella and 1 trailing flagellum underlain by the cresta (*cr*), axostyle (*ax*) and parabasal body (*p*) associated with doubled centriole. **B:** Amorphous pre-fissional stage with all extranuclear organelles resorbed except the doubled centriole resting on the nucleus. **C:** Separation of centrioles to divide the nucleus mitotically, each centriole producing a new set of mastigont structures (after Kirby[273]).

(Fig. 2). Flagellates change to amebae at once when placed in 0·67 per cent salt solutions. In the reverse transformation the centrioles officiate in mitosis as well as produce the granules which develop all the fibrous organelles of the flagellate (Kirby[273]). The inherited centriole therefore seems as important in the morphogenesis as the inherited nucleus is presumed to be, but one would have to destroy or remove the extra-nuclear body to prove the point.

Bovee's[39] description of the conversion of *Trimastigamoeba* to the flagellate phase stresses the presence of an axopod-like short-rod stage in flagella formation if not in their resorption, and that after the flagella form near the posterior surface of the ameba they sink into the cell to bring their basal granules to the nucleus (to serve in later nuclear mitosis?). In the reverse transformation, the granules are first resorbed and then the flagella, from

base to tip. The ameba may also convert to a cyst. As with *Naegleria*, distilled water favors the conversion of amebae to flagellates.

Transformations in a new ameba, *Heteramoeba clara*, were studied by Droop.[134] This form seems to have a sexual phase with flagellates of different mating types. If so, a genetic analysis may demonstrate whether nuclear factors are involved in the ameba–flagellate conversions.

Myxamoeba of non-cellular slime molds emerge from spores. In the form studied by Kerr[262, 264] the amebae can encyst or change to flagellates. Eventually amebae fuse to produce the extensive plasmodium. Conversion to flagellates occurs in aqueous media only after the logarithmic growth of a culture attains the stationary phase, as in microstome → macrostome transformations in *Tetrahymena*.

Bovee[40] has reported some remarkable observations on a new species of the helioflagellate *Dimorpha*. The heliozoan form feeds and reproduces; the flagellate form reproduces without feeding in a series of rapid "hunger divisions". In producing the flagellate, the heliozoan form folds up its axopodal spicules like the stays of an umbrella and resorbs them, while two flagella are sprouted from centrioles or their derivatives. The cell then assumes a pyriform shape stiffened by a sort of axostyle, and the nucleus and centriolar progenitors of the flagella resume the close association typical of flagellates. Adding distilled water to the lake water medium can lead to this transformation in 5–30 seconds, the two flagella forming within one second according to Bovee. The reverse change to the heliozoan phase can be brought about by increasing the osmotic pressure of the medium; flagella are resorbed and the body becomes spherical and in one to two seconds the axopods burst forth. If Neff's saline is added under the coverslip the change occurs so fast—in a fraction of a second!—that the nucleus may be impaled by the axopods. These astonishing speeds limit the range of hypotheses of how organelles are formed. This helioflagellate has still another form: when food flagellates are abundant it becomes a lobose ameba. Hence this species has the greatest transforming ability of any so far described.

E. Form Changes in Life Histories and in Sexual Reproduction

Alternate forms in a species of protozoa may be essential stages in its life history or sexual cycle. Consider the ciliate *Ichthyophthirius*, ectoparasitic on fishes (Mugard[344]). After gorging itself, the animal encysts and the meridional rows of cilia or kineties (about 2000) are fragmented into patches. Each patch becomes the cortex of one of the small cells resulting from cleavage-like divisions of the original large cell. Only during the final division does stomatogenesis occur. Even then, in these migratory, infective forms each with only 43 kineties, oral development is not completed until the infector finds a host and begins feeding (MacLennan[319]). Hence there are three forms "young, adolescent, and mature", called tomite, theront and trophont. Processes involved in these transformations are approximated in abnormal

morphogenesis in *Stentor*: self-fragmentation of its kineties is one of the responses to certain salts; division without stomatogenesis can occur, as well as incompleted stomatogenesis under a variety of conditions (Uhlig;[486] Tartar[458])—but of course none of this is part of a normal life cycle.

The *Apostomes* have a complex life cycle correlated with their association with, usually, crustacea. When an infested crab molts, the ciliates enter their only growth period, gorging themselves on the soft linings of the cast-off shell. After engorgement, there is a series of rapid cell divisions, producing tiny swimming forms. With development of new organelles these forms are enabled to fasten themselves to a new host and become feeding forms at its ecdysis. The complex transformations involved, in many genera, were revealed in the monographic contribution of Chatton and Lwoff,[68] summarized later by Lwoff[312] in his exploration of the morphogenetic import of these form changes with presumably constant genome in a line of cells and even within one cell.

These transformations are describable in terms of the cortical structure. There is a fixed number of kineties, and they are not only polarized with respect to the anterior and posterior poles of the cell but also show a left–right difference by the location of the kinetosomes always to the left of the connecting kinetodesma (rule of desmodexy). Kineties increase in length, adding new kinetosomes, in anticipation of the gorging, with the result that the extended kineties undergo torsion and become "stacked" toward the posterior pole like a coil of rope. The vast increase in size from the one big meal takes up the slack in the kineties which again becomes straight and in simple meridional alignment, making possible their equational division. Besides this general behavior, each kinety or sub-set of kineties undergoes distinctive changes: twisting, shortening, forming paired kinetosomes at the anterior end, acquiring trichocysts, etc. Kinety number 1 not only grows in length but produces three new kineties alongside by *elineation* i.e., by lateral addition of kinetosomes which become aligned in rows, *a*, *b*, and *c*. Next to them lie three other rows of kinetosomes, *x*, *y*, and *z*, which undergo extensive waxing and waning. All 6 rows lie close together between kineties 1 and *n*. Before division they also extend from pole to pole so that in the repeated transverse fissions all of the progeny will receive a section of each numbered kinety and each letter-designated row. When divisions are completed rows *a* to *z* shorten and lose their connection with the poles. From *a*, *b*, and *c* are developed the thigmotactic fields which the migrator uses in holding to a host. Later, on molting of the host, the mouthparts are formed, always in association with row *x*. As feeding begins, the kineties elongate and spiral and in time new rows *a*, *b* and *c* arise in association with kinety 1, while *x*, *y* and *z*, reduced almost to nothing, re-extend in preparation for the next spate of fissions. If the pre-divider gets little food, these divisions may not occur or their number may be greatly reduced, but neither condition interferes with the morphogenetic changes. In the first case the same cell simply