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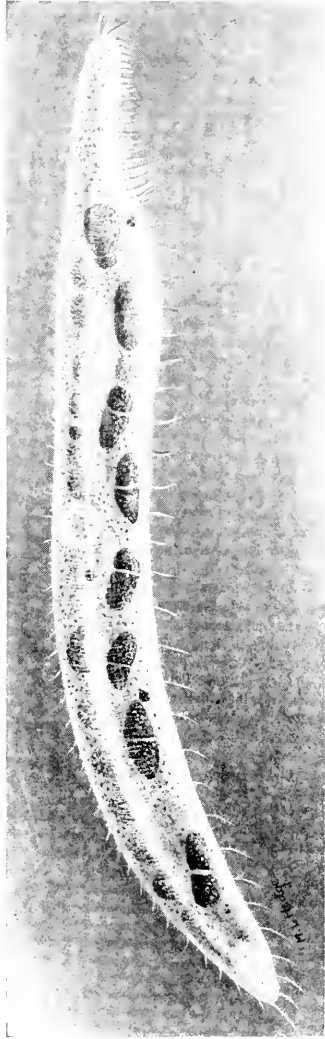


FIG. 1.—*Uroleptus mobilis*. (Drawn by Mabel L. Hedge.)

THE
BIOLOGY OF THE PROTOZOA

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SECOND EDITION, THOROUGHLY REVISED

ILLUSTRATED WITH 223 ENGRAVINGS AND 2 COLORED PLATES



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TO
MY WIFE

WHOSE UNSELFISH DEVOTION HAS MADE THIS BOOK POSSIBLE



PREFACE TO SECOND EDITION.

IN writing this volume the author has made no effort to give a complete account of the Protozoa. As indicated by the title, it is rather a study in biology illustrated by the unicellular animals. The concept of a changing organization brought about by continued metabolism was developed in the first edition. This conception has been amplified in some respects, strengthened and condensed in others, and furnishes the basis for an interpretation not only of life histories but of the significant biological phenomena of cell division, maturity, sex differentiation, fertilization and senescence as well. To strengthen this conception a considerable change in the order of presentation has been introduced. After the first introductory chapter we plunge at once in Chapter II into the substances and structures of the fundamental organization. This is followed in Chapters III and IV by the development of these substances and structures into cytological derivatives (Chapter III) and taxonomic structures (Chapter IV) of the derived organization. In Chapter V the general physiological activities are considered in anticipation of Chapter VI on reproduction. The problem of general vitality and its significance in fertilization and the accompanying phenomena of sex differentiation, maturation, reorganization, adaptation and variations are treated in Chapters VII, VIII and IX. The special chapters on taxonomy, together with more elaborate keys to genera, are transferred from the middle of the book to the end in Chapters XI, XII, XIII and XIV.

Parasitism and disease should be considered in any work on general biology. These topics were omitted in the first edition but are introduced here in Chapter X. Another innovation is the elimination of all references to chlorophyll-forming flagellates, the protozoan flagellates being limited to the Zoömastigophora.

Reorganization or de-differentiation of the derived taxonomic structures at periods of division, endomixis and fertilization whereby

the protoplasm is restored to the condition of the fundamental organization with a renewed potential of vitality, is treated as a special attribute of Protozoa and as an important distinction between Protozoa and Metazoa. Through such reorganizations either by division alone as in the Zoömastigophora and in occasional forms here and there throughout the Protozoa, or by the more drastic means of endomixis and fertilization, the protoplasm is able to continue at an optimum of vitality. With this conclusion and with the recognition of an internal self-regulating mechanism for reorganization, resulting in the continuation of vitality, we are in accord with the essence of Weismann's conclusion that protoplasm of Protozoa is potentially immortal. On the other hand, we cannot agree with Weismann in his further conclusions that natural death is unknown in Protozoa, and that every individual is a potential germ cell.

G. N. C.

NEW YORK CITY.

CONTENTS.

CHAPTER I.

INTRODUCTION	17
Size, Form and Appearance of Protozoa	26
A. Form-relations of Protozoa	30
B. Protoplasmic Structure	39

CHAPTER II

THE FUNDAMENTAL ORGANIZATION.

I. Nuclear Substances and Structures of the Fundamental Organization	49
1. Chromatin	51
2. Other Substances of the Nucleus	57
Intranuclear Kinetic Elements	60
(a) Endobasal Bodies	60
1. Large Homogeneous Endobasal Bodies	61
2. Endobasal Bodies With Centrioles	63
3. Nuclei With Pole Plates and Without Endobasal Bodies	65
II. Cytoplasmic Elements of the Fundamental Organization	68
1. Chromidia	69
2. Volutin Grains	72
3. Mitochondria	73
4. Golgi Apparatus	77
5. Silver Line System	80

CHAPTER III.

DERIVED ORGANIZATION.

I. Cytological	83
A. Derived Nuclei and Derived Nuclear Structures	84
1. The Formation of a Nucleus	84
2. Multiple and Dimorphic Nuclei	84
3. Nuclear Derivatives During Division	88
(a) Origin of Chromosomes and of Intranuclear Spindles at Division	88
(b) Origin of Fertilization (Meiotic) Chromosomes	100
B. Derived Organization; Cytoplasmic Changes	104
1. Cytoplasmic Chromatin	104
2. Cytoplasmic Kinetic Elements	104

CHAPTER IV.

DERIVED ORGANIZATION. TAXONOMIC STRUCTURES.

I. Derived Structures of the Endoplasm. Metaplastids	133
II. Differentiations of the Cortex	135
(<i>a</i>). Cortical Differentiations for Support and Protection	136
(<i>b</i>). Motile Organoids	139
1. Flagella	141
2. Pseudopodia	145
Rhizopodia	148
Filopodia	150
3. Cilia	152
4. Composite Motile Organs	155
Membranulae	155
Membranelles	155
Undulating Membranes	157
Cirri	157
(<i>c</i>). Other Organoids Adapted for Food-getting	162
(<i>d</i>). Oral and Anal Cortical Modifications	164
(<i>e</i>). Contractile Vacuoles	170

CHAPTER V.

GENERAL PHYSIOLOGY.

A. Respiration	174
B. Excretion of Metabolic Waste	176
C. Irritability	179
D. Nutrition	183
1. Food-getting	183
Secretions and Digestive Fluids	193
Digestion of Carbohydrates and Fats	198
Saprophytic Nutrition	199
2. Products of Assimilation	203

CHAPTER VI.

REPRODUCTION.

I. Equal Division and Evidence of Reorganization	209
A. Division in Mastigophora	210
B. Division in the Sarcodina	213
C. Division in Infusoria	215
(<i>a</i>). Evidence of Nuclear Reorganization	217
(<i>b</i>). Evidence of Cytoplasmic Reorganization	218
II. Unequal Division (Budding or Gemination)	225
A. Exogenous Budding	226
B. Endogenous Budding	228
III. Multiple Division (Spore-formation)	233
IV. Development	241

CHAPTER VII.

VITALITY.

I. Isolation Cultures	248
II. Organization and Differentiation	260
1. Inter-divisional Differentiations	260
2. Cyclical Differentiations	266
(<i>a</i>). Cyclical Differentiations Peculiar to Youth	266
(<i>b</i>). Cyclical Differentiations Peculiar to Old Age	269
(<i>c</i>). Cyclical Differentiations Peculiar to Maturity	271

CHAPTER VIII.

PHENOMENA ACCOMPANYING FERTILIZATION.

I. The Environmental Conditions of Fertilization	285
(<i>a</i>) Ancestry	285
(<i>b</i>) Environment	286
II. Internal Conditions at the Period of Fertilization	290
III. The Process of Fertilization	292
A. Meiotic Phenomena	294
(<i>a</i>) Conjugant Meiosis	294
(<i>b</i>) Gametic Meiosis	307
(<i>c</i>) Zygotic Meiosis	309
B. Disorganization and Reorganization	311
(<i>a</i>) Phenomena of Disorganization	311
(<i>b</i>) Metagamic Activities and Reorganization	312
IV. Parthenogenesis	316
A. Endomixis	347
B. Autogamy	322

CHAPTER IX.

EFFECTS OF REORGANIZATION AND THE ORIGIN OF VARIATIONS IN THE PROTOZOA.

I. Effects of Reorganization on Vitality	328
1. Renewal of Vitality as a Result of Conjugation	331
2. Intensity of Vitality and Extent of Renewal	335
3. Relative Vitality of Different Series and Effect of Parents' Age on Vitality of Offspring	339
4. Rejuvenescence After Parthenogenesis (Endomixis)	340
II. Heredity and Variations in Protozoa	342
A. Uniparental Inheritance	343
B. Biparental Inheritance	350

CHAPTER X.

GENERAL ECOLOGY, COMMENSALISM AND PARASITISM.

1. Water-dwelling Protozoa	352
2. Semi-terrestrial Protozoa	353
3. Soil-dwelling Protozoa	353
4. The Sapropelic Flagellates	356
5. The Coprozoic Protozoa	357
Parasitic Protozoa	358
Ectoparasitic Protozoa	359
Endoparasitic Protozoa	359
Effects of Protozoan Parasites on the Host	362
Parasitic Flagellates	364
Trypanosoma in Mammals	372
Trypanosomes of Birds	371
Trypanosomes of Lizards	377
Trypanosomes in Snakes	377
Trypanosomes in Crocodiles	378
Trypanosomes in Turtles	378
Trypanosomes in Frogs, Toads and Salamanders	378
Trypanosomes in Fish	379
Parasitic Rhizopods. Dysentery	385
Early Taxonomic Observations	388
Early Etiological Observations	389
Period of Taxonomic Chaos	392
Other Amebae of the Human Intestine	396
Parasitic Ciliata	397
The More Important Sporozoan Parasites of Man	402
Hemosporidia	406

CHAPTER XI.

SPECIAL MORPHOLOGY AND TAXONOMY OF THE MASTIGOPHORA.

Organization	412
Adaptations and Mode of Life	419
Specific Classification	421
The Water-dwelling Flagellates	421
Classification of the Animal Flagellates	421
Class I. Protomastigota	422
Order Protomonadida	422
Class II. Metamastigota	427
Order 1. Hypermastigida Grassi	427
Order 2. Polymastigida	430
Sub-order 1. Monokaryomastigina	430
Sub-order 2. Dikaryomastigina	431
Sub-order 3. Polykaryomastigina	432

CHAPTER XII.

SPECIAL MORPHOLOGY AND TAXONOMY OF THE SARCODINA.

Class I. Actinopoda Calkins	436
Sub-class I. Heliozoa Haeckel	437
Sub-class II. Radiolaria Haeckel	438
Class II. Rhizopoda von Siebold	442
Sub-class I. Proteomyxa Lankester	443
Sub-class II. Mycetozoa de Bary	445
Order I. Aerasida van Tieghem	447
Order II. Phytomyxida Schroter	449
Order III. Euplasmodida Lister	449
Sub-class III. Foraminifera d'Orbigny	450
Sub-class IV. Amoebae	453
Order 1. Amoebida (Gymnamoebida) Ehrenberg	455
Order 2. Testacea	456
Key to Actinopoda	459
Sub-class 1. Heliozoa Haeckel	460
Order I. Aphrothoraca Hertwig	460
Order II. Clamydophora	460
Order III. Chalarothoraca	460
Order IV. Desmothoraca	461
Sub-class 2. Radiolaria Joh. Müller	461
Class II. Rhizopoda von Sieb.	461
Sub-class I. Proteomyxa	461
Sub-class II. Mycetozoa de Bary	462
Order I. Aerasida van Tieghem	462
Order II. Phytomyxida	462
Order III. Euplasmodida Lister	463
Sub-order 1. Exosporca Rostaf	463
Sub-order 2. Myxogastres Fries	463
Sub-class III. Foraminifera D'Orb.	466
Sub-class IV. Amoebae Bütschli	466
Order I. Amoebida Aut.	466
Order II. Testacea M. Schultze	467

CHAPTER XIII.

SPECIAL MORPHOLOGY AND TAXONOMY OF THE INFUSORIA.

Classification of the Infusoria	486
Infusoria	488
Class I. Ciliata Perty 1852; Bütschli 1889	488

Infusoria—Class I.— <i>Continued.</i>	
Sub-class I. Holotricha Stein 1850	488
Order 1. Astomida	489
Order 2. Gymnostomida	490
Sub-order 1. Prostomina (Prostomata Schewiakoff)	490
Sub-order 2. Pleurostomina Schew. 1886; Em. Kahl	491
Sub-order 3. Hypostomina (Hypostomata Schewiakoff)	491
Key to Genera	491
Order 2. Gymnostomida	491
Sub-order 1. Prostomina	491
Sub-order 2. Pleurostomina (Tribe Pleurostomata Schewiakoff; Kahl)	497
Sub-order 3. Hypostomina Schewiakoff 1896; Em. Kahl	498
Order 3. Trichostomida Bütschli 1889	499
Order 4. Hymenostomida	503
Sub-class II. Spirotricha Bütschli 1889; Em. Kahl 1931	508
Order 1. Heterotrichida Stein	508
Order 2. Oligotrichida Bütschli 1889	512
Order 3. Ctenostomida (Lauterborn) Kahl 1931	516
Order 4. Hypotrichida Stein s. str.	516
Sub-class III. Peritricha Stein	521
Sub-class IV. Chonotricha Wallengren	522
Class II. Suctoria Bütschli	523

CHAPTER XIV.

SPECIAL MORPHOLOGY AND TAXONOMY OF THE SPOROZOA.

Class I. Telosporidia Schaudinn	533
Sub-class I. Gregarinina	534
Order 1. Eugregarinida Doflein Emend	540
Order 2. Schizogregarinida Léger (1892)	541
Sub-class II. Coccidiomorpha Doflein	541
Order 1. Coccidiida Leuckart, Em.	541
Sub-order 1. Eimeriina	541
Sub-order 2. Hemosporidia Danilewsky, em. Doflein	542
Sub-order 3. Babesiina	543
Order 2. Adeleida	544
Class II. Cnidosporidia Doflein	545
Order 1. Myxosporidia Bütschli	548
Order 2. Actinomyxida Stölz	551
Order 3. Microsporidia Ballbani	552
Class III. Aenidosporidia Ceppe	555
Key to Subdivisions and Genera of Sporozoa	558
Bibliography	571

BIOLOGY OF THE PROTOZOA.

CHAPTER I.

INTRODUCTION.

A PROTOZOÖN is a minute animal organism, usually consisting of a single cell, which reproduces its like by division, by budding, or by spore formation and whose protoplasm has passed, or will pass, through various phases of vitality collectively known as the life cycle.

The maze of microscopic life to which the scientific world was first introduced by Anton von Leeuwenhoek in 1675 included a heterogeneous collection of animals and plants. Crustacea, rotifers, minute worms, diatoms and desmids, as well as the more minute Protozoa, were all grouped together during the eighteenth and nineteenth centuries, first under the nondescript term *animalcula* and later under the more ecological term *Infusionsthierc* of Ledermüller (1763). The correct zoölogical position of the higher types was recognized before the middle of the nineteenth century and the group of strictly unicellular forms was first definitely outlined by von Siebold in 1848 under the name Protozoa, a term substituted by Goldfuss (1820) for Oken's suggestive *Urthiere* (1805), while the old name Infusoria has been retained for one of the subdivisions of the group.

The haziness in classification of the older zoölogists has not entirely disappeared in the light of modern knowledge and we are confronted today by the difficulties of distinguishing between Bacteria, unicellular Algae and unicellular animals or Protozoa. It is no reflection on modern science that we are unable clearly to differentiate between these three groups. To accept the problem as insoluble at the present time is merely to admit and apply our conviction that evolution is now, and has been in the past, the primary biological principle underlying the diversities of forms and functions of living things. Few biologists today will refuse to accept the view that higher types of animals—Metazoa—have been derived from forms in the past which were more or less similar to present-day Protozoa; or the view that higher plants have been evolved from unicellular plants. The variations and adaptations

which have been the stepping stones in this evolution have been and are still in progress among all types of unicellular things, so that no artificial definition of Bacteria, of Protozoa, or of Algae will distinguish with strict accuracy either of these groups from the others. Haeckel (1866) undertook to avoid the difficulty by combining all unicellular forms under the common name Protista, but this is, obviously, only another name for the aggregate and an artifice for concealing the real difficulties which we should like to overcome. Minchin (1912), on the ground of structural characters, would distinguish Protozoa from Bacteria by the assumption that the latter are not of "cellular grade" because of the absence in many Bacteria of a typical cell nucleus. Here again, however, the old difficulty shows its head, for in this sense, many well-recognized Protozoa are not, while many Bacteria are, of cellular grade (see Dobell, 1911). The problem after all has mainly an academic interest, and the chief practical value to be gained by its solution would be to set the limits of a text-book or monograph. We may reasonably expect to find therefore, in treatises on Protozoa, some types which with equal right should be included in works on lower plants and on Bacteria. In this connection the greatest difficulty lies in the separation of one group of the flagellated Protozoa from the unicellular algae. We are still tied firmly to the old tradition that animals move and plants are quiescent, and a chlorophyll-bearing organism, if actively moving, is *ipse facto* an animal. Were I to advocate this as the main distinction between animals and plants, there would be, undoubtedly, a storm of protests from all biologists. And yet, what other characteristics do chlorophyll-forming organisms have to justify us in claiming them as animals? At the present time there is a double taxonomic system, one botanical, the other zoological for these questionable forms, and these systems are widely different. We can avoid the resulting confusion by adopting one or the other system of classification. My own conviction is that zoologists should follow the historical precedent furnished in the last century by the elimination from Protozoa of filamentous algae, desmids and diatoms, and now transfer to the botanists the entire aggregate of so-called Protozoa in which the ability to form chlorophyll is a characteristic. (See also p. 412.)

It is less difficult to distinguish between Metazoa and Protozoa; the occurrence of a gastrula stage in the development of a questionable form is sufficient to place it unmistakably with the higher animals. Protozoa, indeed, are often associated in cell aggregates called colonies, the individual cells being held in place by protoplasmic connections, by stalk attachments, or by fixation in a common gelatinous matrix. In some questionable cases, *e. g.*, *Magnosphaera*, these colonial aggregates resemble tissues of Metazoa in their structural appearance, but tissue cells are dependent upon

other parts of the animal for fulfilment of their vital activities while every cell of a colonial protozoön may be self-sufficient and independent, and differentiation among them is limited, at most, to reproductive and somatic cells (*e. g.*, *Epistylis*, *Zoöthamnium* and other vorticellids).

While the single protozoön is to be compared structurally with a single isolated unit tissue cell of a metazoön as a bit of protoplasm differentiated into cell body, or cytoplasm, and nucleus, it is a very different unit physiologically. In its vital activities it should be compared, not with the unit tissue cell, but with the entire organism of which the tissue cell is a part. All animal organisms perform the same fundamental vital activities of nutrition, excretion, irritability with movement and reproduction, which are fundamental attributes of living animal protoplasm. In the higher types of Metazoa these primary activities are performed by complex organ systems, nutrition for example, involving not only the digestive system but the muscular, nervous, circulatory and respiratory systems as well. Each organ has its particular part to play in the economy of the whole and each cell is differentiated for the purpose of its specialized function. Tissue cells, therefore, are physiologically unbalanced cells since they are preëminently specialized for secretion, or contraction, or irritability, etc. Division of labor in a physiological sense here reaches its highest expression.

In the lower Metazoa the organ systems are less highly specialized; fewer organs are present to perform the same fundamental vital activities and the tissue cells have relatively more kinds of work to do for the organism as a whole. Thus the supporting and covering cells of a coelenterate combine the functions of respiration, irritability, muscular contraction, excretion and circulation with the primary functions of an epithelium. Each of them is more nearly balanced physiologically than a single cell of the higher types, but it still needs the activities of other cells, and the organism is again the sum-total of all its cellular parts.

In the protozoön, finally, we find a cell which is physiologically balanced; it is still a cell and at the same time a complete organism performing all of the fundamental vital activities within the confines of that single cell. Whitman, in his essay on "The Inadequacy of the Cell Theory" (1893), clearly expressed the inconsistencies in the common use of the designation "cell" for this variety of structures, and later writers, notably Gurwitsch (1905) and Dobell (1911), have followed in a similar vein.

As organisms the Protozoa are more significant than as cells. In the same way that organisms of the metazoan grade are more and more highly specialized as we ascend the scale of animal forms, so in the Protozoa we find intracellular specializations which lead to structural complexities difficult to harmonize with the ordinary

conceptions of a cell. In perhaps the majority of the Protozoa the fundamental vital activities are performed, as in the simpler Ameba or simple flagellates, by the protoplasm as a whole and without other visible specializations than nucleus and cell body. In other forms,

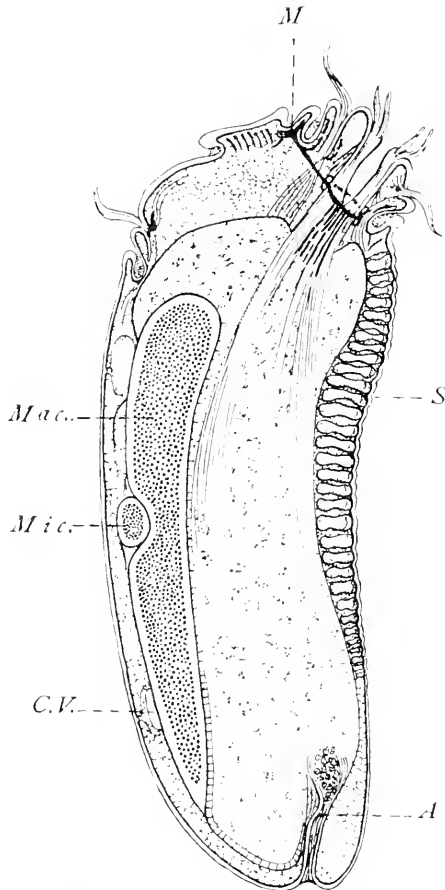


FIG. 2. *Diplodinium caudatum*, a parasitic ciliate in cattle. A, anal canal and defecatory vacuole; C. V., one of the two contractile vacuoles; M, motorium with fiber to circumpharyngeal ring; Mac., macronucleus; Mic., micronucleus; S, skeletal layer. (After Sharp.)

however, intracellular differentiations lead to intracellular division of labor which in some types becomes as complicated as are many of the organisms belonging to the Metazoa. Thus *Diplodinium caudatum*, one of the Infusoria, according to Sharp (1914), has intracellular differentiations of extraordinary complexity (Fig. 2).

Bars of denser chitinous substance form an internal skeleton; special retractile fibers draw in a protrusible proboscis; similar fibers closing a dorsal and a ventral operculum; other fibrils, functioning as do nerves of Metazoa, form a complicated coördinating system; cell mouth, cell anus and a fixed contractile vesicle or excreting organ are also present. All of these are differentiated parts of one cell for the performance of specific functions, and all perform their functions for the good of the one-celled organism which measures less than $\frac{1}{250}$ inch in length. Analogous, if not so complete intracellular differentiations are present in the majority of Infusoria, while many of the flagellates, notably the Hypermastigida, have an almost equally elaborate make-up. In all such cases the single cell is a complicated mechanism and the coöperating parts have the same relation to the organism as a whole as do the organs of a metazoön. Compared with an *Amoeba proteus* or other simple rhizopod such complex organisms are highly specialized and show the extent to which intracellular differentiation may be carried. As Gurwitsch, Hartmann, Dobell and others have pointed out, the application of the term cell which designates a structural unit with specific physiological activity in Metazoa seems to be inappropriate, and, as Whitman argued, inadequate.

A significant difference between Protozoa and Metazoa lies in the phenomenon of reversibility. Differentiations in the protozoan organism are reversible and the derived organization is restored to the fundamental organization (see p. 83) at periods of division, parthenogenesis and fertilization. This does not occur in Metazoa where differentiated cells derived from the fundamental organization of the egg are irreversible and the "somatic" individual dies.

Cell aggregates or colonies are likewise highly variable in their functional specialization. While many of them consist of fortuitous groups of cells with dimensions varying with the number of individuals joined together (*e. g.*, *Ophrydium versatile*, *Poteriodendron petiolatum*, etc.), others are definite in form, number of cells and in arrangement. Here the colony as such has a distinct individuality and in some cases (*e. g.*, *Zoöthamnium alternans*) undergoes a definite developmental cycle. Again some colonies composed of otherwise independent cells do not react as separate individuals but the colony reacts as a coördinated whole. Thus *Zoöthamnium arbuscula*, composed of many hundreds of individual cells in a colony which may attain a diameter of 1 inch, reacts as a unit organism if any one of the component cells is irritated. The entire aggregate contracts into a small ball, so minute that it is scarcely visible. The concerted action is due to the contraction of stalk myonemes which are continuous throughout the entire aggregate, like the coenosarc of some hydroid colonies.

For such colonies of protozoa, as for analogous colonies of hydroids, the expression "individual of a second order" has been applied.

Between the limits of the simplest and the most complex of unicellular organisms are the great majority of the (estimated) 15,000 or more known Protozoa. In each of the main subdivisions simplicity as well as extreme complexity of organization is represented, each subdivision including a series of representative forms ranging from one extreme to the other. Differentiation in the different subdivisions do not follow the same lines of development, however, so that we are able to classify Protozoa according to a fairly natural system. These diverse lines of development make it difficult to treat this branch of the animal kingdom in any general way; the wide range in habitat from the purest waters of lake or sea to the

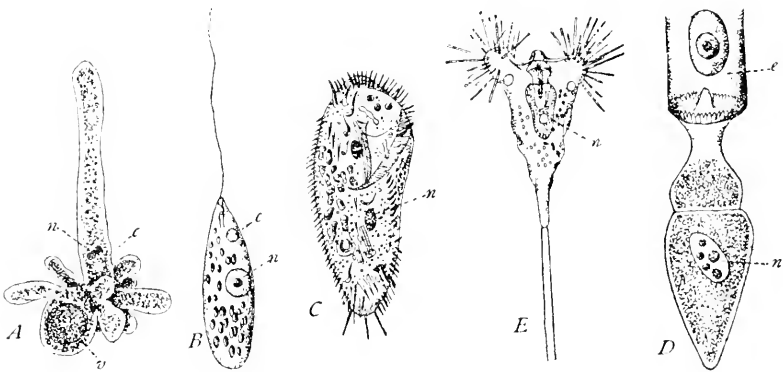


FIG. 3.—Types of Protozoa. *A*, *Amoeba proteus*, a rhizopod; *B*, *Peranema trichophora*, a flagellate; *C*, *Stylonychia mytilis*, a ciliate; *D*, a polycystid gregarine; *E*, *Tokophrya quadripartita*, a suckorian. (*A*, after Calkins, *B*, *C*, *E*, after Bütschli; *D*, after Wasielewsky.)

foulest ditch, and adaptations to environments varying in character from a mountain stream to the semifluid substance of an epithelial, nerve or muscle cell, has brought about manifold varieties of structure. To describe all of these modifications under a few headings, or to attempt to formulate general laws from the different and often highly complicated life histories, is out of the question. The general trends of differentiation, however, permit of grouping the different kinds of Protozoa in four types which were first outlined by the French microscopist Felix Dujardin in 1841. Three of these types—Sarcodina, Mastigophora and Infusoria—are based upon the nature of the locomotor organs—pseudopodia, flagella and cilia respectively—while a fourth type—Sporozoa—includes organisms which are invariably parasitic in mode of life and are essentially without motile organs (Fig. 3).

DISTRIBUTION OF PROTOZOA.

Protoplasm is an aggregate of fluid colloidal substances in which water plays a conspicuous part; exposed to the air it dries and desiccation is fatal to the majority of Protozoa, although it is possible that some forms, like certain rotifers, may reabsorb moisture and again become active. If after losing its water the protoplasm is surrounded by impervious membranes, further evaporation is prevented and within such capsules the protoplasm remains alive. This is the condition of *encystment* and many kinds of Protozoa, protected by their cyst membranes, may live for long periods in a dried state (Fig. 4). Because of their light weight these cysts may be carried in the air and blown by the winds with dust, until surrounded

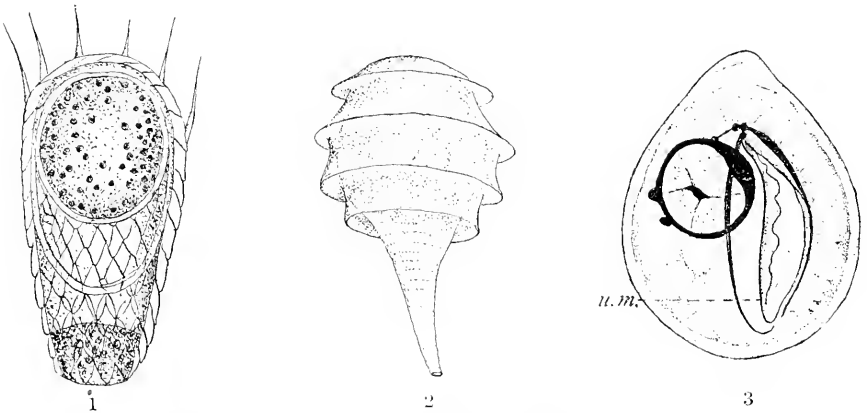


FIG. 4.—Types of cysts. *Euglypha alveolata*, testate rhizopod; *Podophrya fixa*, suctorian; and *Chilomastix mesnili*, a parasitic flagellate. *u.m.*, undulating membrane. (First and second, original; third, after Kofoid and Swezy, University of California Publications in Zoology, 1920.)

again by water the organisms emerge from their cysts and are active once more for a few hours. Such encysted forms account in part for the surprising protozoan fauna in uncovered sterilized water in which food substances come from similarly protected germs of Bacteria and minute plant forms. Similar encysted forms may be present on the blades of dried grass, leaves and other vegetation. In the infusions formed by soaking such dried vegetation in water various species of monads (*Monas*, *Oicomonas*, *Bodo*) and of ciliates (*Colpoda*, *Oxytricha*, *Stylonychia*, *Urostyla*, *Gastrostyla* and *Vorticella*) and the rhizopod *Ameba* make their appearance in the order given (Woodruff, 1912). Puschkarew (1913) concluded that air-borne cysts play only a minor rôle, however, in the spread of Protozoa. It was found that, on the average, there are only $2\frac{1}{2}$ protozoön cysts per cubic millimeter of air and that these are limited

to 13 species and represent the same types for the most part as those listed by Woodruff. Protozoa are very apt to stick to solid substances when they encyst and are carried, in the dried state, with such substances, which accounts in part for the appearance of Protozoa in all kinds of infusions. Similar adhering cysts may be carried from place to place by birds and other flying creatures or by land animals, thus helping to maintain a common type of protozoan fauna in pools and casual waters. The commonest species of Paramecium, viz., *P. aurelia* and *P. caudatum*, are widely distributed over the earth and are almost universally used in general laboratory work as examples of ciliated Protozoa. Their mode of distribution, however, has been a continued puzzle for their supposed inability to form cysts has been generally recognized. Recently, however, Cleveland (1927), upon injecting unknown species of Paramecium in the rectum of a frog, found that a definite cyst membrane is formed by many of the Paramecia. After a few

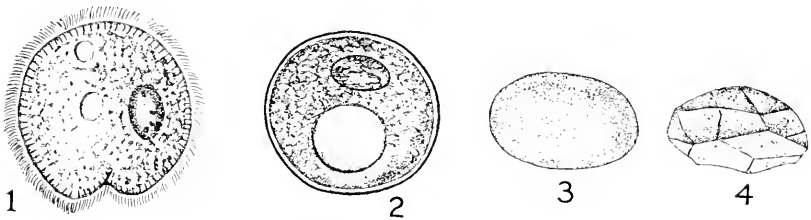


FIG. 5.—*Paramecium caudatum*, stages in encystment. The final product may be easily mistaken for a sand grain. (After Michelson, Arch. f. Protistenkunde, courtesy of G. Fischer.)

days division within the cyst and ex-cystation were observed. Michelson (1928), furthermore, has described encystment of *Paramecium caudatum* under conditions of slow desiccation entailing loss of peristome, vacuoles and cilia. When fully dried the crumpled cyst wall resembles a small sand grain and as such may be overlooked (Fig. 5).

Some forms to which Lauterborn (1901) has applied the term "sapropelic fauna" appear to be able to live without free oxygen. Thus *Frontonia leucas*, *Prorodon ozum*, *Spirostomum ambiguum*, *Pelomyxa palustris*, *P. binucleata*, etc., which usually live in relatively clear waters, may also live in the sulphurous medium of putrefying vegetable and animal matter, while certain species of ciliates of fantastic form seem to require this peculiar habitat for their vital activities (*Dactylochlamys pisciformis*, Lauterb., *Saprodinium dentatum*, Lauterb., *Discomorpha pectinata*, Levand., *Pelodinium reniforme*, Lauterb.). Doflein, following the suggestion made earlier by Bunge, believed that the anaërobic parasitic forms of the

digestive tract may have had their initial start toward parasitism when living as such sapropelic forms.¹

Protozoa are distributed over the entire world. Wherever there is moisture, there will these unicellular animals be found unless conditions of heat or of chemical composition are inimical to life. Oceans and their tributaries, lakes, ponds, pools and ditches, mountain streams and wells contain them, their numerical abundance depending on the available food. They are present, not only in permanent waters, but also in casual puddles of field and road, in droplets caught in the axils of leaves or in hollows of rocks, in rain water of roof or pail and in damp moss. In many cases they are active for only an hour or more until their world dries up, when they may be saved again by encystment, but some forms retain their activity in ordinary garden earth where they are supposed to play an important part in connection with Bacteria of the soil (Cutler and Crump, 1920; Goodey, 1916). The majority of such soil-dwelling forms belong to the Sarcodina and Mastigophora, Gruber's *Amoeba terricola* being a typical case, while other genera and species are discovered from time to time (*Bodo*, *Prowazekia*, *Spirouema*, *Oicomonas*, *Cercomonas*, *Dimastigamoeba punctata* and many others (see Soil-dwelling Protozoa, Chapter X, p. 353).

While excessive heat kills them, excessive cold does little harm beyond retarding vital activities and the melted ice of glaciers may teem with them. They may live, not only in the exposed waters of the earth's surface, but also as parasites in the fluids of other living protoplasm or its products. They may be found in the warm blood of birds and mammals, or in the cold blood of fishes, amphibia and reptiles; in the digestive tract of every type of animal; in the saliva and urine of different types and in the living protoplasm itself of plants, other Protozoa and of tissue cells. No type of animal life is free from the possibility of association with Protozoa either as commensals, or symbionts or parasites (see Chapter X, p. 358).

The common Protozoa of our own ponds and pools are exactly the same in genera and species as those found in similar places in Europe, Asia, Siberia, Africa, South America and Australia; they are cosmopolitan, and the temptation to describe new species because they happen to have been found in some hitherto unexplored locality has no justification from the facts of geographical distribution. This is particularly applicable to the fresh water forms but does

¹ The suggestive experiments and conclusions of Avery and Morgan (1924) give reason for the belief that the inability of some organisms to live in free-oxygen holding media is due to the absence in such forms of a peroxidase capable of breaking down hydrogen peroxide. The latter accumulates under ordinary aerobic conditions and is detrimental to forms which are unable to provide the peroxidase. The limitation of free oxygen may be the explanation of successful artificial cultivation of forms—for example *Spirostomum ambiguum*—which grow best under partly anaerobic conditions (see Bishop, 1923).

not apply equally to the deep sea types. The littoral fauna of salt water, like the fresh water forms, appears to have a cosmopolitan distribution according to the observations of Gourret and Roesser (1886), of Levander and of Hamburger and Buddenbrock in Europe, while in North America the brackish waters are particularly rich in number and variety of Protozoa. The pelagic and deep sea forms appear to be unequally distributed; some types are apparently limited to the Indian Ocean; others to the Atlantic, while many tropical genera and species, especially of Radiolaria and Foraminifera, are not found in the polar seas and *vice versa*. Some strictly pelagic forms, on the other hand, notably *Tintinnidae*, are found on or near the surface of sea water in all parts of the world.

Observations are sufficiently numerous to show that not only is there a certain climatic distribution of salt water forms, but a vertical distribution as well. Certain genera and species of Radiolaria and Foraminifera are present in the surface waters but are rarely found at the depth of from 600 to 3000 feet, while some families, notably the Challengeridae and Tuscaroidae, are present only in the extreme depths of the sea.

Many species are sufficiently adaptable to live either in fresh, brackish or salt water; indeed most of the common forms of rhizopods, flagellates and ciliates seem to be equally at home in either. Many types, however, sometimes entire groups of Protozoa, are not so ubiquitous; the sub-class Radiolaria for example, comprising more species than any other entire class of Protozoa, is exclusively marine, while another large sub-class of the Sarcodina, the Foraminifera, comprises only a few fresh water representative species. Many more types of Choanoflagellates are present in salt than in fresh water. Ciliates are poorly represented in the deep sea, although one family—*Tintinnidae*—is wonderfully rich in salt water forms while fresh water forms are uncommon. Heliozoa, another sub-class of the Sarcodina, on the other hand, are typically fresh water forms with relatively few salt water representatives.

The distribution of parasitic forms belonging to all groups of the Protozoa obviously follows the distribution of their hosts, and we know too little on this subject to generalize; where animals are segregated the opportunities for parasitism are enhanced while some climatic conditions are more advantageous than others for the spreading of germs. Thus the blood-dwelling parasites are more common in the tropics than elsewhere, the biological conditions favorable to the intermediate transmitting hosts being largely responsible for their numbers and variety.

SIZE, FORM AND APPEARANCE OF PROTOZOA.

Although Protozoa belong unquestionably to the microscopic world their sizes vary within wide limits. Some are large enough

to be picked up with forceps (*Parospora gigantea*, a gregarine, up to 16 mm.) and many of the larger ciliates are easily visible to the unaided eye (*Bursaria truncatella*, *Spirostomum ambiguum*) while many smaller types can be seen by the trained eye as mere white specks which, in some cases, may be identified by their characteristic movements (e. g., *Paramecium*, *Frontonia*, *Dileptus*, *Amphileptus*, *Loxophyllum*, etc.). At the other extreme in size are types which are barely visible even with the most powerful lenses of the microscope. From 8 to 16 such forms have ample room for existence in a red blood corpuscle (*Babesia canis*), or 200 to 300 may live simultaneously in a single infected liver or spleen cell of man (*Leishmania*

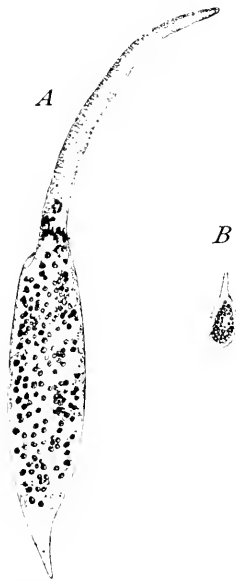


FIG. 6.—*Dileptus gigas*, two sister cells. A, normal individual; B, individual starved for several days. (From Calkins.)

donovani). Between these two extremes of size lie the majority of Protozoa. Their measurements are usually expressed in terms of "microns" or thousandth parts of a millimeter which are represented by the symbol μ , each micron being $\frac{1}{25000}$ of an inch. Thus *Leishmania donovani* measures from $2\ \mu$ to $4\ \mu$, *Paramecium caudatum* upward of $200\ \mu$, *Bursaria truncatella*, $1500\ \mu$, etc.

The same species frequently shows remarkable variations in size due to environmental conditions or to different stages in the life history. Thus normal specimens of *Paramecium caudatum* may measure from $175\ \mu$ to $250\ \mu$ when fully grown and similar variations are characteristic of all species. Environmental factors, especially

food conditions, are frequently responsible for changes in size and character of a species, often rendering them difficult to recognize and affording tempting opportunities for swelling the list of synonyms by new names for the abnormal forms. Thus *Dileptus gigas* when starved has a very different size and character from the normal form (Fig. 6). Again, different normal stages in the life history of a given species are not infrequently mistaken for different species, largely because of difference in size. Thus *Uroleptus mobilis* (see Fig. 1), in its adult vegetative condition, measures about 150 μ , but immediately after conjugation not only is it reduced

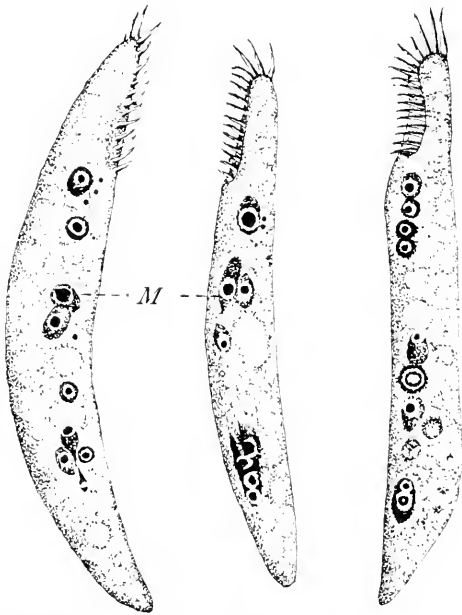


FIG. 7.—*Uroleptus mobilis* Engelm. Old age specimens showing degeneration of macronucleus *M* and loss of micronuclei. See frontispiece. (After Calkins.)

by one-third in size, but its internal structure is entirely different from that of the usual form, while during the period of old age it frequently measures less than 75 μ (Fig. 7), and has a different appearance from the more youthful stages.

Even more striking examples of normal dimorphism are shown by the rhizopod *Dimastigamoeba* and by the ciliate *Glaucoma* (*Dallasia*) *frontata*. Species of the former usually appear as small earth-dwelling ameboid rhizopods, but with the addition of water they develop flagella and become actively moving ellipsoidal flagellates. *Glaucoma frontata* in its usual vegetative state is a more or less quiescent tailed form (Fig. 8), but under certain environmental

conditions not yet fully understood it becomes an active tailless navicular organism which divides repeatedly, giving rise to minute

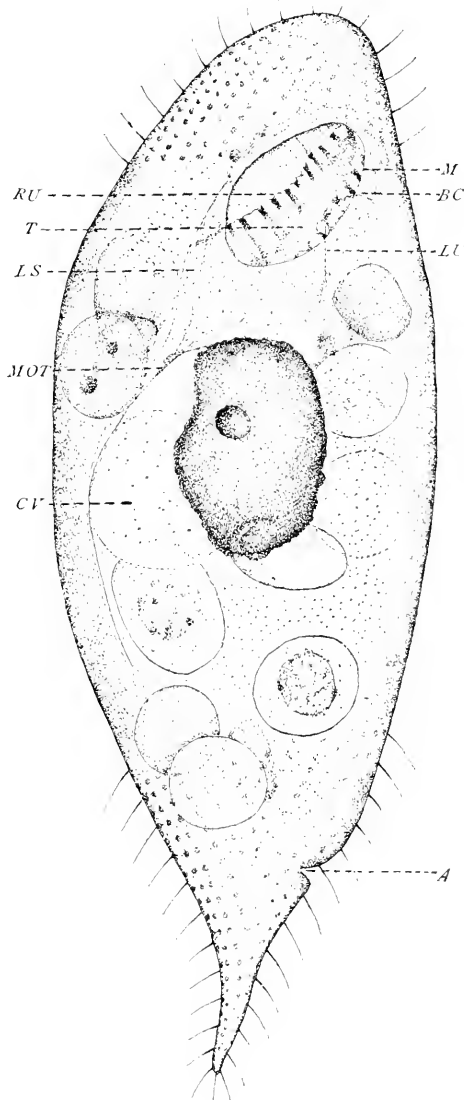


FIG. 8.—*Glaucoma (Dallasia) frontata*. Vegetative individual. A, anus; BC, buccal cavity; CV, contractile vacuole; LS, "ladder" system; LU, left undulating membrane; M, mouth of buccal cavity; MOT, region of motorium; RU, right undulating membrane; T, "tongue" in buccal cavity. (After Calkins and Bowling, Arch. f. Protistenkunde, courtesy of G. Fischer.)

individuals one-sixteenth the original size (Fig. 200, p. 485). To the uninitiated such variations in forms and habits offer great temptation to swell the list of synonyms.

A. Form-relations of Protozoa.—The forms of Protozoa are highly varied and depend to some extent upon the mode of life, to some extent upon the mode of reproduction and to some extent upon their lifeless skeleton elements, but in the last analysis they depend upon the physical consistency of the protoplasm. Fluid types, if not confined by resistant cell membranes, readily change in form according to environmental conditions, or by virtue of forces coming from metabolic activities within. *Amoeba proteus* and other species of *Ameba* are amorphous and are constantly changing in shape, a characteristic phenomenon to which the term *ameboid movement* is applied, and the same protoplasm may be spherical in form, or flattened on the substratum, or extended in various ways. Many forms, under certain pressure conditions in the surrounding medium due to evaporation or reduced volume of water, will suddenly burst and disappear leaving no trace whatsoever of their previous presence. This phenomenon has been repeatedly mentioned by earlier observers in connection with types of Protozoa belonging to all classes, and the term *diffluence* was applied to it by Dujardin. In such cases the fluid protoplasm is usually confined by a resisting membrane or cortex which remains intact during the ordinary phases of activity, but when the pressure from within becomes too great for the resistance of the membrane the latter collapses, the cell disappearing with all the characteristics of a miniature explosion.

Another evidence of the difference in density between different species of Protozoa is the reaction after cutting with a scalpel. Some species, for example *Paramecium caudatum*, are extremely difficult to cut successfully owing to the fluid character of the inner protoplasm which, as soon as the cortex is cut, flows out and disintegrates; in my experience not more than 20 per cent out of more than 1000 operations on *Paramecium caudatum* have been successful, but the percentage is greatly increased by preliminary treatment with neutral red. Other forms of ciliates on the other hand may be cut in any plane, *Urorychia transfuga* and *Uroleptus mobilis* for example, reacting to such operations with all the physical properties of a piece of cheese.

The more fluid Protozoa, when the form is not maintained by resistant cortical differentiations, react to physical properties of the surrounding medium. When forces on all sides are equal, as in suspended water-dwelling types like *Actinophrys sol*, *Actinosphaerium*, many Radiolaria, etc., the form is spherical, or spherical also in parasitic forms enclosed in the protoplasm of the host cell as is the case with the majority of Coccidia. In all types, under certain environmental conditions, or when continuously irritated, there is

a tendency to become globular and this is the form assumed by the great majority of Protozoa when they encyst. The spherical or homaxonic type, furthermore, is characteristic, not only of free floating forms, but also of the most generalized representatives of all classes of Protozoa.

While density or consistency of the protoplasm is thus one of the factors determining form in Protozoa, its effect in the majority of types is offset by the presence of definite membranes, shells, tests and skeletons; by specialized protoplasmic differentiations; or by foreign bodies. Thus the density of the sluggish *Pelomyxa palustris*

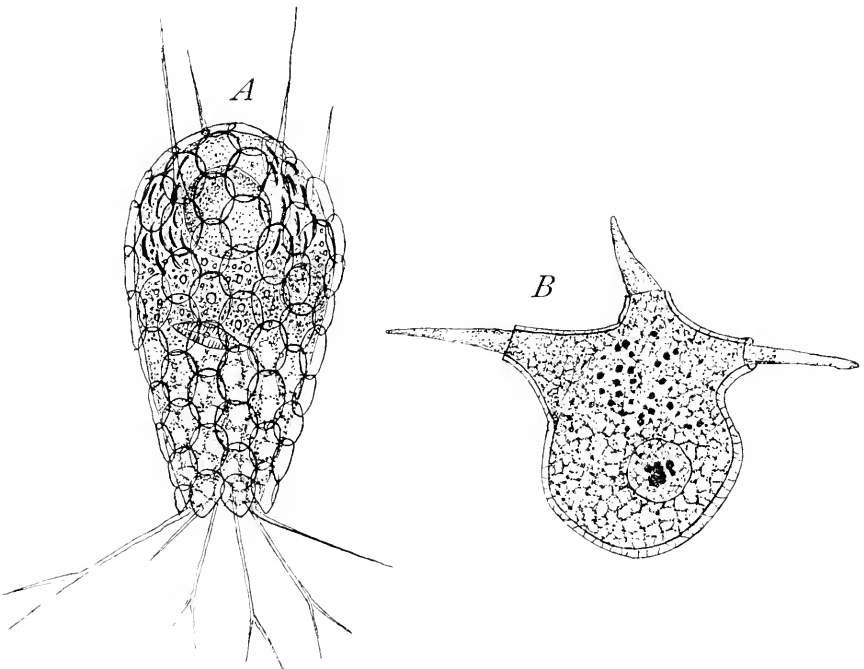


FIG. 9.—*Euglypha alveolata* (A), and *Cochliopodium* sp. (B). (After Calkins.)

is due to the enormous number of crystals of mud and sand, shells of diatoms and peculiar refractile bodies resembling glycogen in make up. Membranes of living substance, as in *Cochliopodium* (Fig. 9) and the majority of flagellates and ciliates, of lifeless chitin as in *Allogromia oviforme* (Fig. 10) or the lifeless materials secreted by the cell and deposited on it are responsible for the forms assumed by many Protozoa. Even delicate types such as *Clathrulina elegans* and the majority of Heliozoa retain their forms by virtue of the protecting shells of lifeless materials deposited on a chitinous membrane. The protoplasmic bodies of many of the fresh water shelled

rhizopods are relatively dense like that of the naked *Amoeba verrucosa* and are more or less globular or pyriform in shape. On such a protoplasmic basis the shells of *Difflugia* species, *Englypha*, *Cypho-*

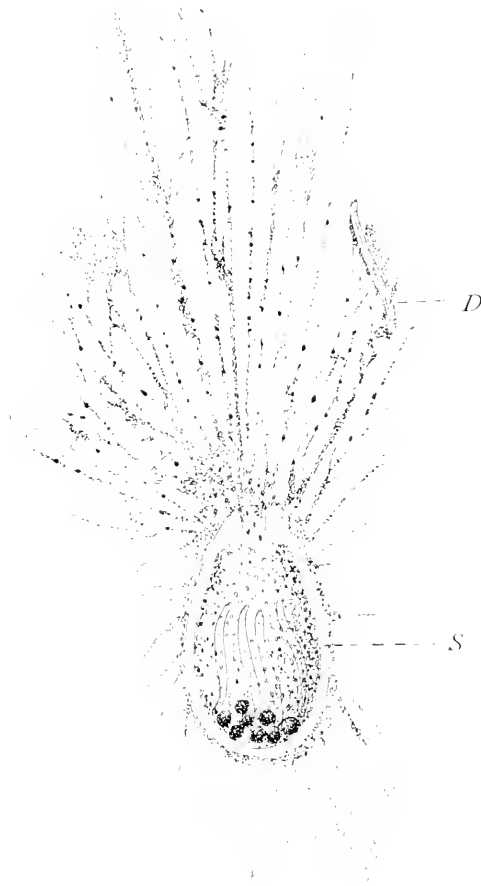


FIG. 10. *Allogromia oviformis*, foraminiferon with chitinous monothalamous shell and reticulose pseudopodia. (D) a recently captured diatom; (S) chitinous shell. (From Calkins after M. Schultze.)

deria, *Centropyxis*, *Arcella*, etc., are deposited and these, once formed, are never changed (Fig. 11). Only rarely are these shelled rhizopods flattened or discoid as in *Hyalodiscus* (see Chapter XII).

The typical form in many shell-bearing or skeleton-forming rhizo-

poles may be due in its last analysis to the finer structure of the protoplasmic body in which the skeleton or shell parts are deposited. Dreyer (1892) has given evidence to show that the form and size

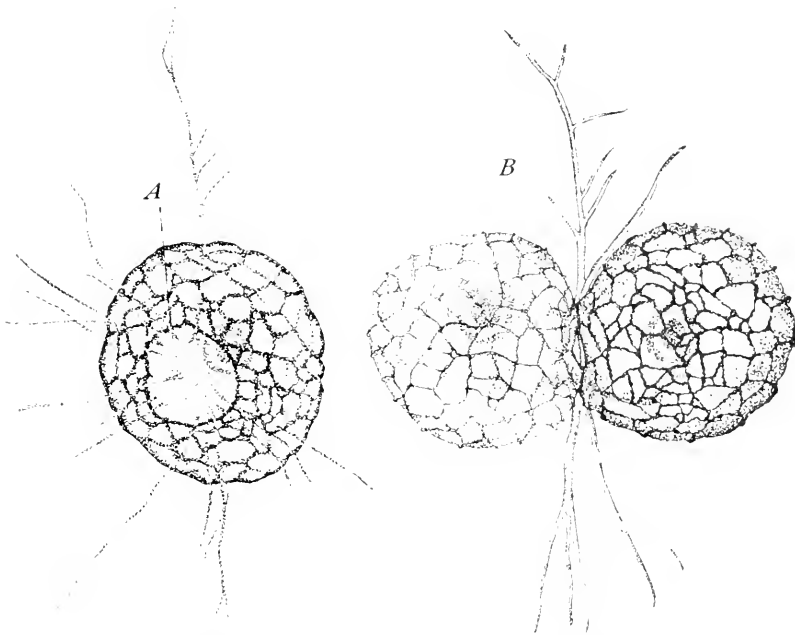


FIG. 11.—*Pseudodiffugia* sp. circular mouth opening and mosaic shell (A). B, division stage. (Original.)

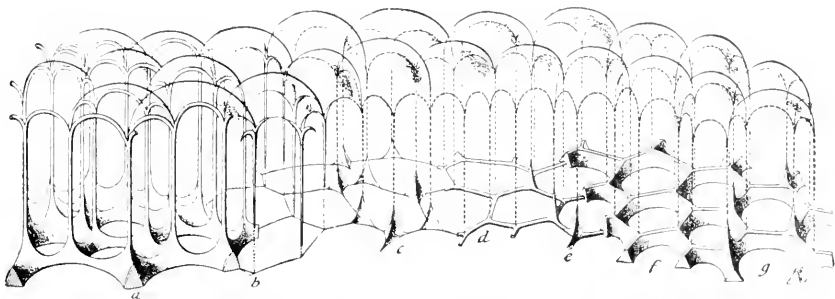


FIG. 12.—Schematic figure illustrating the modifications of skeletons according to mechanical principles of deposition. (After Dreyer.)

of the elements making up the skeletal or shell parts depend upon the alveolar make up of the protoplasm, the interalveolar deposits of silica, etc., taking the form of spicules as in *Heliozoa* and many

Radiolaria, of bars, hexagons, rings, fenestrated capsules, etc. (Fig. 12).

Freely moving types are usually monaxonic. The type form of a freely moving flagellate or holotrichous ciliate is ellipsoidal, the cell being drawn out with its main axis extending in the direction of movement. Attached forms are usually polyaxonic or radially symmetrical, the variations in form depending upon the nature of the

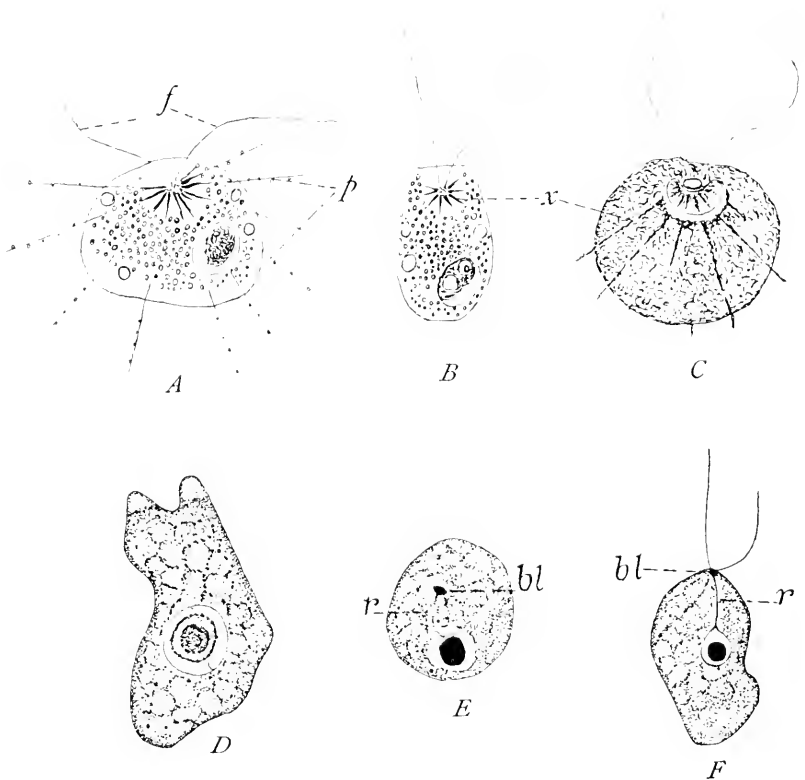


FIG. 13.—Diphasic rhizopods. A, B, C, heliozoa-like and flagellated stages of *Dimorpha mutans*. (After Blochman.) D, E, F, *Dimastigamoeba gruberi*, ameboid and flagellated stages; E, origin of blepharoplast (bl) from endosome; r, rhizoplast. (After C. W. Wilson.)

attaching portion. Some for example are attached by the protoplasm of the posterior end of a cylindrical body (*e. g.*, *Cothurnia*, *Vaginicola*, etc.); others by the more or less stalk-like attenuated end of the body (*e. g.*, *Scyphidia*, *Podophrya*, etc.); and others by chitinous stalks of variable length (*Vorticella* species) which may be more or less branched (*Potriodendron*, *Epistylis*, *Carchesium*, *Zoöthamnium*, etc.). In the same individual the form may change with

change in mode of life, well illustrated by *Dimorpha nutans* (Fig. 13), by *Dimastigamoeba gruberi* or *Trimastigamoeba*. Fantastic types such as *Discomorpha pectinata* or *Tripalmaria dogieli* (Fig. 14) are not uncommon and no evident connection between such bizarre forms and their mode of life is apparent.

Methods of food-getting and the nature of the food are also potent factors in determining form. Many of the diatom- and desmid-eating ciliates, whose food lies on the bottom, are characteristically flattened forms with the mouth on the under, or physiological ventral, surface (holotrichous ciliates belonging to the genera *Chilodon*, *Orthodon*, *Opisthodon*, *Chlamydon*, *Lorophyllum*, etc., and the majority of the hypotrichous ciliates). Special food-getting, or current-directing, organs frequently modify the form as in the collared flagellates (Choanoflagellates) and in types like *Folliculina ampulla* (Fig. 94, p. 169), *Bursaria truncatella* (Fig. 94, p. 169), cephalont gregarines, *Pleuroema* (Fig. 199, p. 482), etc. Shifting of the position of the mouth in response to different food requirements, as Bütschli has shown, has undoubtedly been the cause of some form changes. Thus the proboscis-bearing species and the asymmetrical *Chilodon* types may owe their characteristic forms to such a shifting of the oral region (Fig. 15).

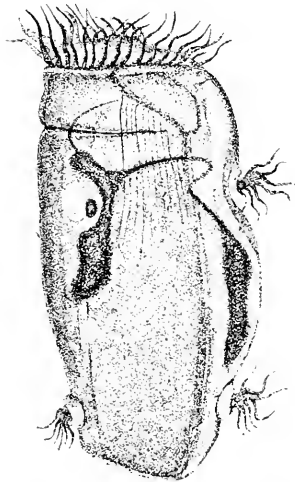


FIG. 14.—*Tripalmaria dogieli* (*minor*). Gut parasite of the horse with three bundles of cilia and internal skeleton. $\times 520$. (After Strelkow, Arch. f. Protistenkunde, courtesy of G. Fischer.)

The monaxonic types, while typically ellipsoidal in form, are frequently characterized by a spiral twisting of the cell body, especially in the rapidly moving forms. In some cases, notably in the flagellates *Streblomastix*, *Spiromonas*, *Holomastigotes*, etc., and in the ciliates *Argyria*, *Paramecium*, *Metopus sigmoides*, etc., the spiral twist is highly characteristic (Fig. 16).

Bilateral symmetry is of rare occurrence among Protozoa; indeed there seem to be few significant cases, that of *Giardia* being the best known (Fig. 17). Here the two nuclei, the motor complex and the eight flagella are arranged in the neatest bilateral manner. One possible mode of origin of such bilaterally symmetrical types is indicated by *Uroleptus mobilis* (Fig. 18). Here two individuals, after conjugation, fused to form a single double and bilaterally symmetrical individual which persisted through 367 generations (see also Fig. 127, p. 245).

Form may be dependent also upon the mode of reproduction.

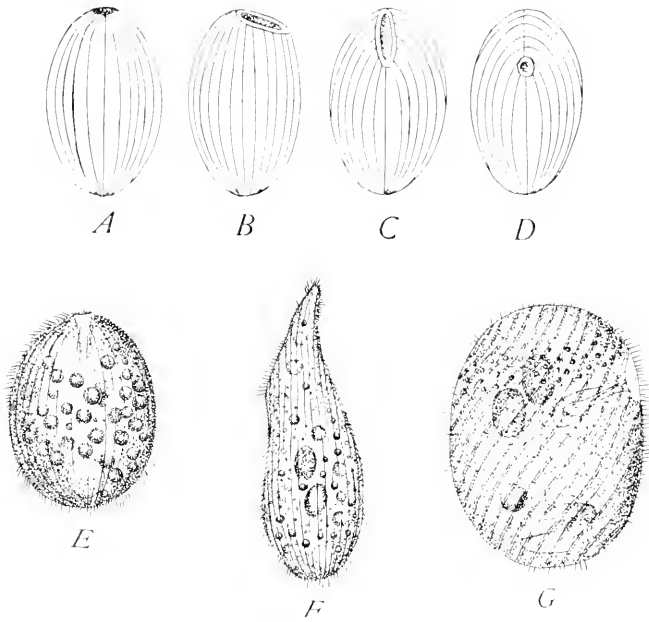


FIG. 15.—Diagrams illustrating shifting of the mouth in ciliates from terminal to lateral or ventral surface (A, B, C, D). E, *Procodon griscus*, corresponds with A; F, *Amphipilus clapedi*, corresponds with B or C; and G, *Nassula microstoma*, corresponds with D. (E and F, after Bütschli; G, after Calkins.)

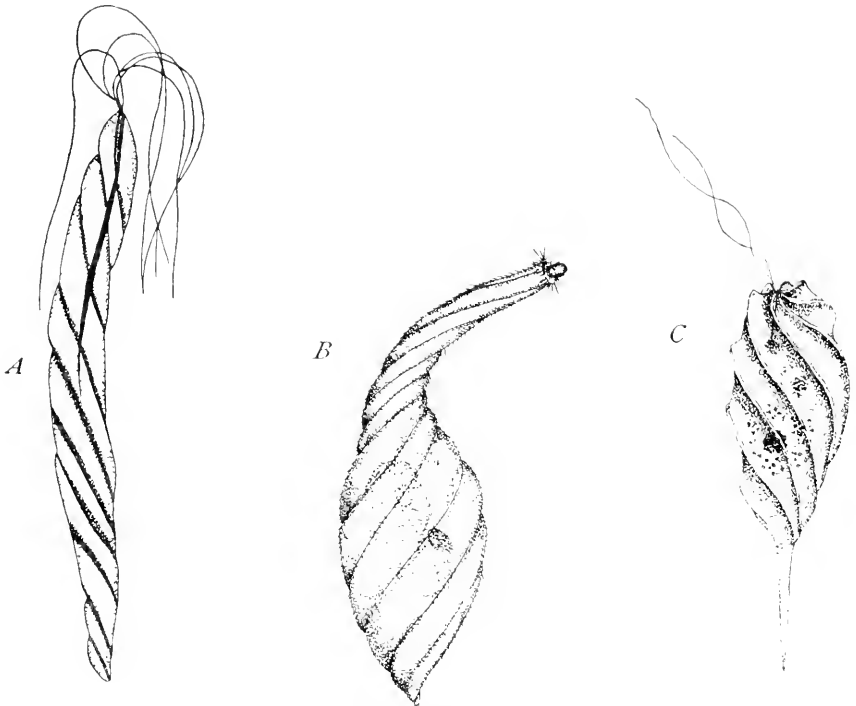


FIG. 16.—Types of spirally wound Protozoa. A, *Streblo mastix strix*. (After Kofoid and Swezy.) B, *Lacrymaria* sp. (Original); C, *Helcrounema* sp. (Original.)

In this connection we have to do only with the multinucleated and with the colonial forms of Protozoa, for in ordinary division the daughter cells separate completely and reproduction has no effect on the form assumed. Thus the foraminiferon *Allogromia oriforme* gives rise by what is termed budding division to a free daughter

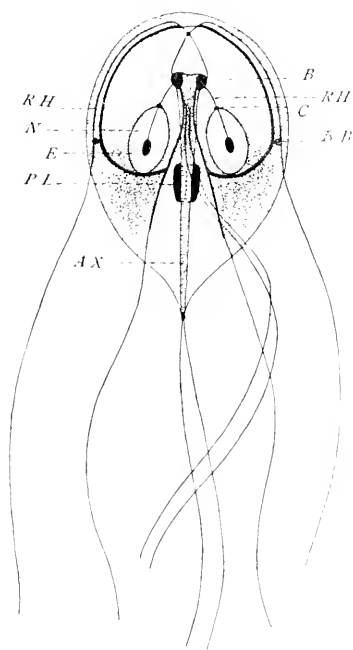


FIG. 17

FIG. 17.—A bilaterally symmetrical flagellate, *Giardia muris* Grassi. AX, axostyle; B, blepharoplast; BB, basal body; C, centriole; E, endosome; N, nucleus; PL, parabasal body; RH, rhizoplast. (After Kofoid and Swezy.)

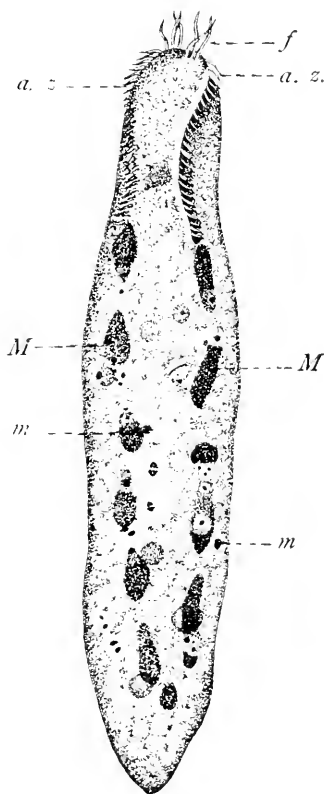


FIG. 18

FIG. 18.—A bilaterally symmetrical ciliate from *Uroleptus mobilis*. A double individual formed by fusion of two individuals after conjugating. With two mouths and adoral zones (a. z.); two sets of cirri (f); and two sets of macronuclei (M) and micronuclei (m). For structure of single individual see Frontispiece. (Original.)

cell which builds an independent test for itself while the other cell remains in the old test. In other forms of Foraminifera, however, the bud of protoplasm does not become separated from the parent bulk of the cell but takes a position in relation to the other portion which possibly depends upon the physical conditions of the proto-

plasm. New shells are deposited about the buds and chambered individuals result (Fig. 19). Repetition of the process gives rise to distinct types of polythalamous or many-chambered Foraminifera, depending upon the position assumed by the bud (Nodosarine, Frondicularian, Rotaline types, etc.).

Dogiel (1929) interprets the duplication (polymerization) of organelles such as contractile vacuoles, macro- and micronuclei, flagella groups, particularly of Polymastigida, somatella formation (see p. 233), multiple nuclei and kinetoplasts of Calonymphidae (see p. 115), etc., as evidence of gradations in cellular differentiations in Protozoa leading to a multicellular condition which is fully established in Metazoa.

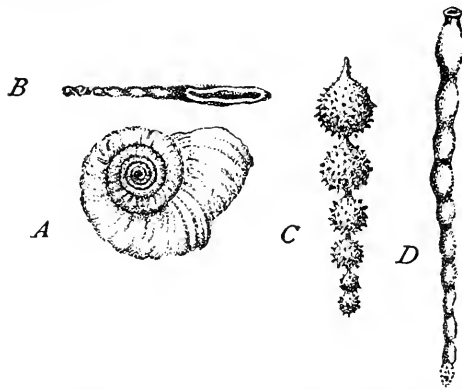


FIG. 19.—Types of shells of Foraminifera. A, B, side and ventral aspects of *Cornuspira* sp.; C, and D, types of *Nodosaria*. (After Carpenter.)

In colonial types the form of the aggregate is determined by the manner in which the individuals are held together after division. The different types are described as spheroid, catenoid, arboroid and gregaloid colonies. In the majority of spheroid colonies, the associated cells are held together by a gelatinous matrix secreted by the individual cells. The typical form of such colonies is spherical as in the genus *Proterospongia*, among the flagellates, or *Ophrydium versatile* among the ciliates. In catenoid colonies the individuals are attached end to end as in some species of ciliates (e. g., *Haptophrya*), or side by side as in the flagellate *Rhipidodendron*. In arboroid colonies the individuals are attached by longer or shorter stalks in a branching, often bush-like colony [*Clathrulina elegans*, *Poteriodendron petiolatum* (Fig. 139, p. 418), *Codosiga cymosa* (Fig. 20), *Epistylis umbellaria* (Fig. 143, p. 280), *Carchesium polypinum*, *Zoöthamnium arbuscula*, etc.] In the majority of these arboroid colonies each individual is borne on its own stem which branches from a common stalk. In some cases, however, especially amongst the flagellates, each stalk bears a cluster of individuals as in *Cladomonas*

fruticulosa, *Anthophysa vegetans* (Fig. 21) or *Phalansterium digitatum* (Fig. 22). In *Rhipidodendron splendidum* the gelatinous branches, colored brown or red by oxide of iron, are arranged in parallel rows, spreading out fan-like as they increase with division of the cells, the aggregate forming an organ-pipe-like arboroid colony. Gregaloid colonies, finally, are fortuitous aggregates of previously independent individuals found mainly amongst the rhizopods and Heliozoa, or in parasitic flagellates under adverse environmental conditions (Spirochetes, Trypanosomes). The origin of gregaloid colonies is not connected in any way with the manner of reproduction.

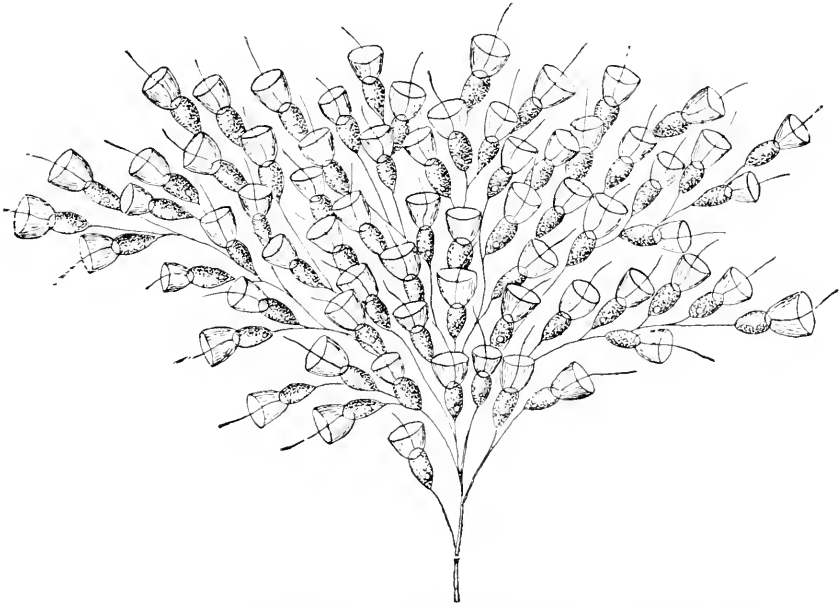


FIG. 20.—Type of flagellate colony. *Codosiga cymosa* Kent, an arboroid colony of collared flagellates.

The combination of all of the above factors effective throughout past ages has resulted in fixed, complex forms which, as in Metazoa, are today associated with the germinal make-up of the protoplasm or genotype, and are transmitted by inheritance.

B. Protoplasmic Structure.—All protoplasm contains the same fundamental chemical elements—C, H, N, O and P—which are necessary for the performance of vital activities. With these are associated mineral elements of one kind or another—Na, K, Ca, Mg, Fe, S, etc., usually as salts of different kinds, and water.

In its last analysis form depends upon the chemical and physical combinations of these elements which indicate specific protoplasmic

organizations and interactions of different protoplasmic substances and which form the physical basis of inheritance. A minute fragment of *Uroleptus mobilis* is difficult to distinguish from a similar fragment of *Dileptus gigas*, yet the former develops into a perfect *Uroleptus*, the latter into *Dileptus*. The encysted forms of many types are impossible to identify until the cysts are opened and vital processes begin again. These facts indicate that the finer or ultimate composition of protoplasm is different in different forms and

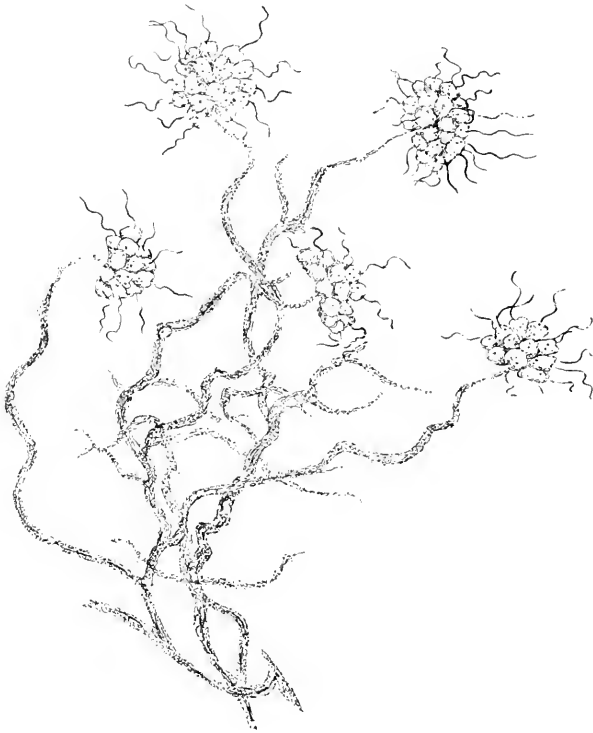


FIG. 21.—*Anthophysa vegetans*. Colony of flagellates with iron encrusted gelatinous stalks. $\times 1000$. (After Doflein, *Lehrbuch der Protozoenkunde*, 1927, courtesy of G. Fischer.)

specific for each species, and justify the view that there are as many kinds of protoplasm as there are species of Protozoa, Metazoa or living things generally. Considerations of this nature inevitably lead us into the lines of thought followed by Whitman, Gurwitsch, Dobell and many others and to question again the adequacy of the cell theory in its application to Protozoa.

The specificity of protoplasm is not at all indicated by its appearance, although obvious differences in many cases may be seen even

with low powers of the microscope. In a living form what we actually see under the microscope in most cases is the external zone of protoplasm which, as the surface of contact between the organism and the outer world, has become modified in various ways. Such outer differentiations are usually transparent so that the nature of the internal protoplasm may be made out in more or less detail. This is particularly true of the so-called "naked" forms such as *Amoeba proteus*, etc., in which the surface protoplasm is

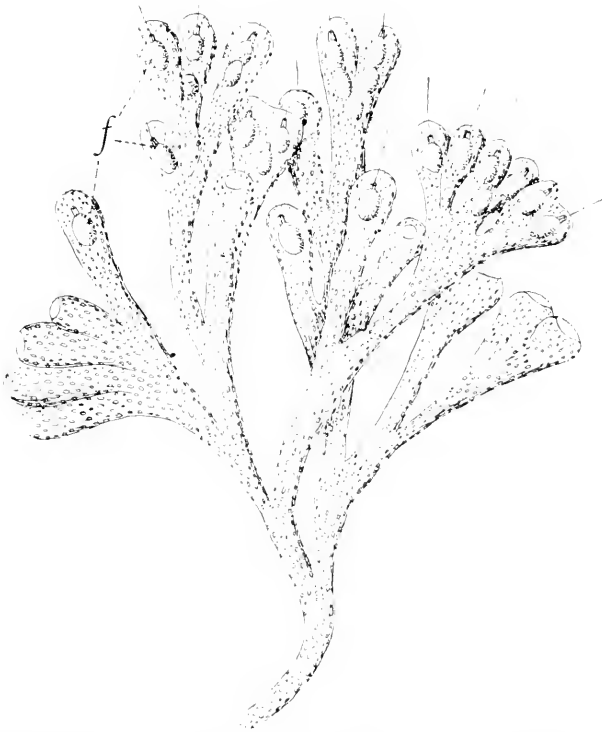


FIG. 22. *Phalansterium digitatum* St. Individuals (*f*) in branched gelatinous colony. (After Stein.)

only slightly different from the internal substance and is made up of living material. Here the entire organism is living protoplasm which appears as a drop of fluid substance, grayish-white in color, viscid in physical character but tenuous and with no tendency to mix with the surrounding water. In such living cells, internal movement of the protoplasm is manifested by the streaming (cyclosis) of distinct granules, some of which are more refractile than others, but which are present in all cells, and invariably character-

istic of the inner plasm. Spherical spaces or vacuoles are also visible in the living forms, sometimes with solid, usually foreign, matter within them (gastric vacuoles, defecatory vacuoles), sometimes filled with clear watery fluid (contractile vacuoles) which is emptied to the outside at regular intervals, or sometimes filled with fluids which are not discharged (stationary vacuoles, or cavulae of Wetzell). The same form, when fixed with a good killing agent, and properly stained, gives a permanent picture of the granules, vacuoles and other cell parts as they were at the instant of fixation. The nucleus now stands out as the most conspicuous part of the cell, while the granules are seen to be of different sizes and to react differently after treatment with different stains.

In most cases the finer physical structure of the protoplasm can be seen both in the living cell and after fixation. It is best described as a foam structure similar to the bubbles of soap suds but with "bubbles" or alveoli of microscopic size. Imagining an optical section through soap suds in which granules of finely-powdered carmine have been distributed by stirring, the picture presented would be a network or meshwork of water, soap and carmine, and with an accumulation of carmine granules where three planes of contiguous bubbles come together, while the spaces within the meshes would be filled with air. The apparent network, however, is merely the optical section of continuous walls of bubbles enclosed on all sides by the water and soap. The physical structure of the protoplasm of a few Protozoa, called spumoid structure by Rhumbler, may be accurately compared with such an emulsion of soap and water. An analogous network, usually of exquisite fineness, represents the more solid substance of protoplasm; the apparent fibers forming the meshwork in some cases at least are the optical sections of continuous walls, which, like the soap bubbles, enclose materials of lesser density. Bütschli who, with Rhumbler, has studied the finer structure of protoplasm of lower plants and animals as well as that of higher forms, was the first to compare such structures with the alveolar structure of emulsions like soap and water, oils and water, etc. The granules of protoplasm, corresponding in position with the carmine of the soap suds, lie in the substance of the denser network of interalveolar material to which Doflein applied the term *stereoplasm*. The alveolar substance, called *rheoplasm* by Doflein, corresponds in position with the air of the soap bubbles.

All who have investigated protoplasm agree that it is not a homogeneous substance but a mixture of colloidal substances in the physical state described by Ostwald as an emulsoid in which the interalveolar materials act in the manner of a dispersing agent while the more fluid intra-alveolar substances are dispersed, but all are subject to reversal of phase.

While the alveolar structure of protoplasm is convincingly demon-

strated by a number of typical forms of living Protozoa, this structure is difficult to make out in other types. Thus in the endoplasm of flagellates like *Chilomonas*, or the endoplasm of *Actinophrys sol*, or *Actinosphaerium eichhornii*, the alveoli are easily discernible, but in *Paramecium caudatum*, in many gregarines, and in many types of flagellates and ciliates, the alveoli, if present, are too fine to be seen with the usual powers of the microscope. Vonwiller (1918) can find no evidence for upholding the alveolar theory of protoplasmic structure in general.

Certainly in many cases the protoplasm appears to be almost homogeneous in structure, the granules alone being evidence of structural configuration. Such forms are illustrations of the granula theory of Altmann, who held that protoplasm is made up of a congeries of such granules or microsomes each of which is termed a bioblast, each bioblast being regarded as a single unit performing all of the functions of living matter including growth and reproduction. Here, however, theoretical considerations have been superimposed on the obvious structure and the physical appearances become clouded in a mist of speculation. Other theories, such as the reticular and fibrillar theories, associated with the names of Heitzmann, Schäfer, Flemming, etc., are based upon the actual pictures of different types of protoplasm.

The larger vacuoles in different types of Protozoa to which the names cavulae, gastric, and contractile vacuoles are given are interpreted according to the alveolar theory as due to the flowing together and fusion of adjacent alveoli. This is certainly the case in the formation of a contractile vacuole of *Amoeba proteus* where the beginnings of a vacuole may be watched under the microscope and the coalescence of minute vesicles noted. In a similar way the relatively huge cavulae or pseudo-alveolae characteristic of *Actinosphaerium eichhornii* and of *Radiolaria* may be accounted for.

Physically, protoplasm is to be compared with an emulsion of colloidal substances which, as Lord Rayleigh and others have pointed out, as a polyphasic system can retain the emulsoid condition only as long as the limiting membranes between dispersed and dispersing media are intact. In the activities of a living, moving cell, there must be a continual disturbance of this physical equilibrium and a constantly changing configuration of the protoplasm due to the manifold chemical actions which are characteristic of living matter.

Chemically, protoplasm is not a substance but a harmoniously working aggregate of different interacting substances which have been identified in general as nucleins, nucleo-albumins, nucleo-proteins, lipoproteins, fats, carbohydrates, salts and the almost endless variety of derivatives from these and from their combinations. But the chemical make up of living substance is, as yet, in an

uncertain and experimental stage. Beyond somewhat glaring generalizations of chemical groups as listed above, we know but little that is definite concerning the chemistry of living matter. It is freely admitted by those who are in the best position to know, that many highly labile substances of active protoplasm are destroyed or changed beyond recognition by the processes of modern chemistry. Some of these are probably quite unaccounted for; another group can be identified as chemically definable substances which, however, we can only assume to be an integral and necessary part of the protoplasmic make-up. Many qualitatively important bodies are overlooked or hidden from observation; others are materials in an absorbed condition or so enmeshed among the colloidal stuffs that their clear demonstration is as yet scarcely possible. The unavoidable destruction, physically and chemically, of protoplasm during analysis must bring about mixtures, or chemical and physical changes amongst the substances originally present, hence the position of different stuffs cannot be definitely ascertained as fundamental or derived until methods are more refined and more exact.

With the exception of the Mycetozoa which have been used extensively for the purpose of protoplasmic analysis, protozoan protoplasm, owing to the minute size of the individuals, has been very little studied in connection with the chemistry of protoplasm, and our present knowledge concerning it is based mainly on morphological considerations together with the results of chemical analysis of protoplasm in higher types of animals and plants.¹

The granules which invariably appear in protoplasm, and which are probably intimately connected with the varied activities going on during life are different in their chemical make-up although, morphologically, they appear much the same. This is shown by their reactions to micro-chemical tests of different kinds and it is not unreasonable to infer that the specificity of protoplasm in different species of Protozoa is due in large part to the chemical and physical composition of these granules and to interactions going on amongst them.

The almost infinite variety of form and structure represented by

¹ An example of one concrete case of chemical analysis may be cited. This is not accepted without question, but it indicates the nature of the substances which enter into the make up of protoplasm—in this case of the plasmodium of the mycetozoön, *Fuligo varians*, as analyzed by Lepeschkin (1923, 1926).

	Per cent.		Per cent.
Monosaccharid	14.2	Globulin	0.5
Albumin	2.2	Lipoproteid	4.8
Amino-acids }	24.3	Neutral fat	6.8
Purin bases }		Phytostearin	3.2
Asparagin }		Phosphatids	1.3
Nucleoproteid	32.3	Other organic stuffs	3.5
Free nucleic acid	2.5	Mineral stuffs	3.4

the Protozoa must be traced back to the chemical nature of the proteins and to their relations and interactions with other substances in protoplasm. Types which have a similar chemical and physical make up, with similar metaplastids and plastids, are practically identical in form and structure and we recognize them as distinct species. Variations in chemical composition, be they ever so little, must result in different chemical reactions and products, and in corresponding variations in form and structure of the organism, and these variations furnish the basis for classification.

Under normal environmental conditions the reactions among the varied substances in protoplasm of the same species, with their products and arrangement of these products, are individual and invariable. Furthermore, the entire organism partakes of this individuality. A fragment of *Stentor* obtained by cutting or by shaking cannot be distinguished from a similar fragment of *Dileptus*, yet the former regenerates into a perfect *Stentor*, the latter into a perfect *Dileptus*. Or an encysted *Uroleptus mobilis* is morphologically identical with an encysted *Didinium nasutum*; both are apparently homogeneous balls of undifferentiated protoplasm; the one emerges from the cyst and develops with the characteristic differentiations of *Uroleptus*, the other of *Didinium*. In short, the homogeneous ball representing *Uroleptus* is as specific and different from the homogeneous ball representing *Didinium*, as the adult *Uroleptus* is different from the adult *Didinium*. We may speak of this undifferentiated chemical and physical make-up as the *fundamental organization* of the species, in a sense similar to the architectonik of Driesch. The adult characteristics result from the interactions of the specific proteins, carbohydrates, salts, water, etc., among themselves and with the environment, and represent what we may call the *derived organization*.

Organization in the above sense is not only specific but is continuous from generation to generation, and has come down through the ages subject, however, to modifications and changes through interaction with the environment or through changes coming from within as in amphimixis.

While organization is continuous the actions and reactions going on within it are discontinuous. More or less prolonged periods of rest are characteristic of all living things, best exemplified in the case of spores, eggs, encysted Protozoa and seeds. At such times the organization is static; the chemical substances making up the specific organization are present but quiescent, or at least, in the absence of water, relatively inactive. A striking illustration is afforded by the phenomenon of desiccation in some types of animals, *e. g.*, rotifers, which has been known for decades. For some years I had on my shelf a bottle of minute amorphous granules which appeared like specks of dust under the microscope. After placing a

few of these granules in water each of them would become an active, living rotifer in an hour or so. Here organization was present but inactive, and activity began with the absorption of water and with oxidation. The rotifer in the active state is the same rotifer that it was in the dried condition, so far as organization is concerned, but it differs in that the organization is now in action. It is a difference of the same nature as that between an automobile standing in the garage, and the same automobile travelling 30 miles an hour. The organization is in action in both moving rotifer and moving automobile; is static in the dried rotifer and in the standing machine.

The automobile simile, however, will not stand analysis. The parts of the machine are little changed by activity and the organization remains the same throughout its period of usefulness. With a living thing, on the other hand, the chemical and physical make up changes with every activity and, as a result of such activities, the protoplasmic organization itself will change. An encysted *Uroleptus* is a motionless and apparently a homogeneous ball of protoplasm; an hour later it is an elongate, cigar-shaped organism with specialized motile organs in the form of membranelles and cirri, and its contractile vacuole pulsates with rhythmical regularity as it moves actively about in the water. The organization has undergone a change in this brief period; the first indication is the swelling and enlargement of the cyst wall, evidently by the absorption of water; oxidation probably occurs and substances already present, or new substances formed as a result of this initial oxidation, are responsible for the newly-developed structures or derived organization not present before. Such structures, however, are the morphological expression of the adult organization and their formation corresponds to the development and differentiation of the metazoön egg.

Continued activity involves other and still more subtle changes in organization; some of these are evident in individual life between division periods; others are evident only in a long series of individuals constituting a life cycle. These will be more fully treated in Chapters VII and VIII.

Other changes in organization may be brought about by environmental conditions; or they may be brought about by changes in one or more of the substances constituting the protoplasm of the species, as when amphimixis introduces a new combination of chromatin into the organization. These are undoubted factors in the phenomena of adaptation and probably play a part in the origination of new species and types.

Consideration of these and of similar activities in living protoplasm lead to questions regarding the nature of life and the nature of vitality. Should we use the two terms life and vitality as synonyms? We are very apt to speak of life as activity, or to say that

life is a series of reactions, integrations and disintegrations. These may be manifestations of life but they are incomplete manifestations and do not tell the whole story. An encysted protozoön, a spore, a seed, a resting egg, or a dried rotifer, shows no more evidence of activity than does a parked car, yet each has life and in a proper environment would manifest activity. An emulsion of oil, salts and water manifests activity strikingly similar to the movements of an *Ameba*, yet such an emulsion has no life. The encysted protozoön or the dried rotifer has protoplasmic organization which the oil emulsion has not, and with absorption of oxygen and water becomes animated. Life thus is incontestably bound up with organization of protoplasm and, for descriptive purposes at least, we find a distinct advantage in a clear discrimination between this concept and the concept vitality. Whatever name we give it, however, brings us no nearer to a conception of what life actually is, for it cannot be measured and endures until the organization is disintegrated. With vitality the case is different; here we have to do with protoplasm in motion and the activities can be measured from beginning to end of a life cycle. While organization has evidently been continuous from the first protoplasm, vitality has been intermittent or discontinuous. Organization may exist without vitality and has always the potential possibility of vitality, but vitality is impossible without organization. I would define vitality, therefore, as *the sum total of actions, reactions and interactions between and amongst the substances making up the organization of protoplasm and between these and the environment*, while life may be defined as *protoplasmic organization manifesting vitality or with a potential of vitality*.

CHAPTER II.

THE FUNDAMENTAL ORGANIZATION.

WEISMANN'S conception of a metazoön as made up of germinal and somatic protoplasm is equally true of a protozoön. Here, however, the two are combined in the make-up of a single cell, and Weismann was not entirely right in considering all Protozoa as equivalent to the germinal protoplasm only of Metazoa. In general the derived organization of a protozoön is a combination of the fundamental organization which retains its fundamental germinal characteristics and the derivatives from it which characterize the adult or fully differentiated individual. Like the metazoan somatic plasm, these derivatives have a limited existence, and again like somatic plasm, new ones are formed from the germinal protoplasm with each successive act of reproduction. An essential difference between the somatic structures of Protozoa and those of Metazoa, is that such structures in Protozoa are reversible while in Metazoa they are irreversible. It is important to make the attempt at least to distinguish between the fundamental or germinal protoplasm and the structures which are derived from it. The latter, as in Metazoa, provide the structural features by which species are differentiated and classified.

Although with our present knowledge it is impossible to analyze protoplasm and to discover the nature of the ultimate fundamental organization which involves the differences between species, it is possible by experiment and upon a morphological basis to determine what protoplasmic parts are necessary for perfect development. Thus, in the experiment with fragments of *Stentor* or *Dileptus* (see p. 45), we find that no development occurs if nuclei are not included in the fragments, and nuclei without cytoplasm are equally impotent. So, too, in all encysted Protozoa, we invariably find a combination of nuclei and cytoplasm. The legitimate inference is that both nucleus and cytoplasm are necessary for continued vitality and that interactions between these two primary components are necessary for the formation of the structures of the derived organization. This is such a fundamental biological truth that it seems hardly necessary to emphasize it here.

It is difficult to distinguish upon a morphological basis between the visible differentiations of the fundamental organization and structures of the cell which should be included more properly in

the derived organization. Some substances are found in all Protozoa and these may be considered the raw materials from which the derived organization is manufactured.

Although they are intimately related, it is convenient to describe the constituents of the nucleus and those of the cytoplasm under separate headings.

I. NUCLEAR SUBSTANCES AND STRUCTURES OF THE FUNDAMENTAL ORGANIZATION.

The term "nucleus" is ordinarily applied in a morphological rather than a physiological sense. If the activities of the component parts of the nucleus are absolutely necessary for the maintenance of life of the cell, then, in some cases such as *Holosticha*, *Trachelocerca*, or *Dileptus*, such activities must be performed by substances which appear to be identical with chromatin but which are distributed throughout the cell. On the other hand, it is highly probable that some functions are possible by virtue of the physical properties of a definite, but permeable, nuclear membrane, as in the tissue cells of Metazoa. It is this type of membrane-bound nucleus that we find in the vast majority of Protozoa.

Certain constantly recurring substances are characteristic of protozoan as of metazoan nuclei, but some types of arrangement and combination of these substances are typical of Protozoa and are rarely found in Metazoa. The most universal of these nuclear constituents are (1) chromatin, which is sometimes called nuclein or identified as such; (2) nuclear sap or nuclear enchylema filling the spaces of the linin reticulum; (3) nuclear membrane which forms a permeable partition between cytoplasm and nucleoplasm; (4) plastin, often so called without being specifically identified as such; also termed paranuclein, or pyrenin. Plastin by itself forms true nucleoli which are comparatively rare in Protozoa. In addition to these, kinetic elements are characteristic of the majority of protozoan nuclei, and these in the present work will be called *endobasal bodies*.

It must be frankly admitted that very little is known in regard to the chemical nature of these various constituents of the nuclei in Protozoa and much confusion exists in the literature owing to the promiscuous use of these terms in relation to structural elements of the nucleus without knowledge of the actual chemical make up.

In their resting stages the nuclei of Protozoa present a bewildering variety of forms and structures, differing in this respect from the much less variable tissue nuclei of the Metazoa. Because of these manifold differences students of the Protozoa have experienced great difficulty in grouping nuclei for purposes of description. They agree, however, in recognizing two primary nuclear types, the

vesicular and the *massive*. Nuclei of the massive type more clearly resemble the nuclei of spermatozoa being filled with small chromatin granules, but they rarely present the homogeneous appearance of a spermatozoön nucleus, the individual granules, although closely packed, being recognizable (Fig. 23). In vesicular nuclei the chromatin granules may be distributed more or less evenly through-

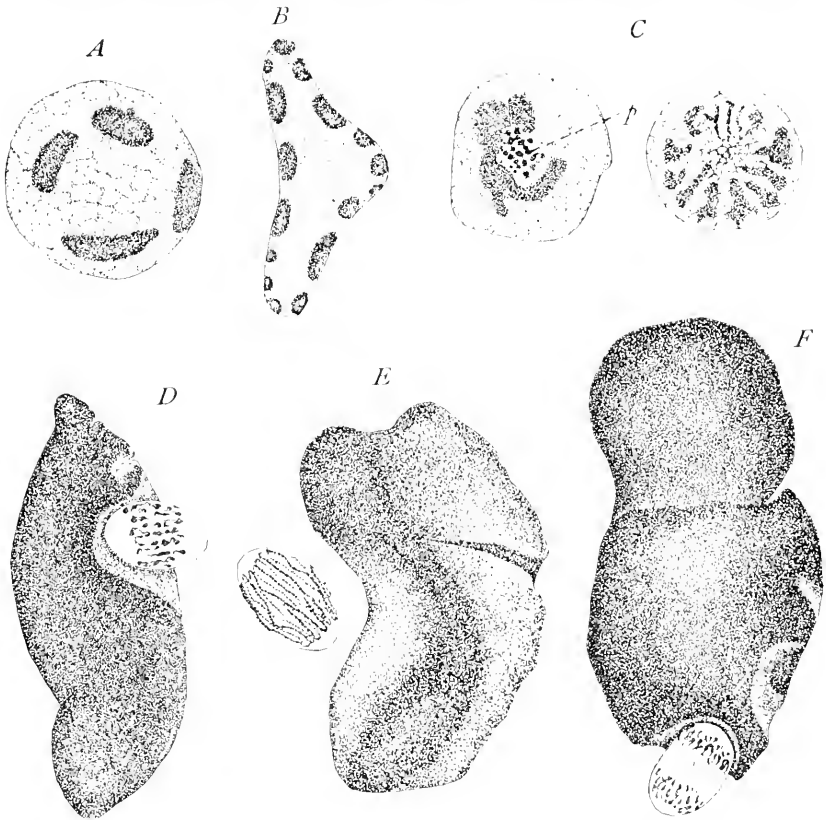


FIG. 23.—Types of vesicular and massive nuclei. A, vesicular type of *Pelmomyxa binucleata*; B, of *Polystomellina crispa*; both with multiple endosomes; C, nucleus of *Actinosphaerium cichhornii* with granular plastin (*p*); D, E, F, macro- and micro-nuclei of *Paramecium caudatum*, the latter in different stages of vegetative mitosis. (A, B, after Doflein; C, after Hertwig; D, E and F, original.)

out the nucleus, or they may be segregated in “net-knots” or either alone or combined with other nuclear substances may be combined in one large central globular mass to which Minchin gives the name *endosome* as an equivalent for the term *Binnenkörper*, or they may be aggregated in several such globular masses or multiple endosomes distributed throughout the nucleus or plastered to the nuclear membrane.

Endosomes may consist entirely of chromatin as appears to be the case in nuclei of some Microsporidia (*Glugea* and *Thelohania*), or some flagellates (*Prowazekia*, Belar, 1920, etc.). Or they may be composed of chromatin and plastin in various combinations. Thus in *Actinosphaerium eichhornii* in some stages of nuclear activity, the chromatin component is in the form of an incomplete ring which partially encloses the plastin portion (Fig. 23, C). In other cases the plastin is entirely surrounded by a cortex of chromatin which may be dense and compact as in the case of many types of rhizopods and Sporozoa or loosely aggregated as in nuclei of *Endamoeba intestinalis* (Fig. 24). The distributed granules of deeply staining material which represent the substitute for a nucleus in *Dileptus gigas* are similarly composed of a plastin core and a chromatin cortex, the former increasing enormously after treatment of the animal with certain kinds of food such as beef broth. Here the term endosome is scarcely applicable since the bodies in question are not inside a nuclear membrane, but they appear to be morphologically equivalent to these intranuclear structures. After treatment with beef broth the body of *Dileptus* is enormously distended due to the swelling of these cytoendosomes (Fig. 25).

The centrally placed intranuclear body is generally described under the name *karyosome*, a term which has been so widely used by students of the Protozoa and for so many obviously different

structures that it is practically synonymous with endosome or Binnenkörper. Thus Minchin describes it as a combination of chromatin and plastin; Dofflein defines a karyosome as a centrally placed, sharply outlined and constant constituent of the nucleus, which may contain no chromatin or may be a combination of other substances with chromatin and which divides during nuclear division, to form two corresponding daughter structures. Hartmann's (1911) definition is more limited, a karyosome in his use of the term being an endosome (Binnenkörper) containing a centriole. Belar (1921) finds a "karyosome" in *Chlamydomorphys minor* which breaks up and disappears, forming neither chromatin nor kinetic elements. If we attempt to combine these different views into a common definition we find that a karyosome may be an intranuclear body which may consist of plastin alone; or kinetic elements alone; or chromatin together with plastin; or a combination of chromatin with kinetic elements; or a combination of chromatin, plastin and kinetic ele-

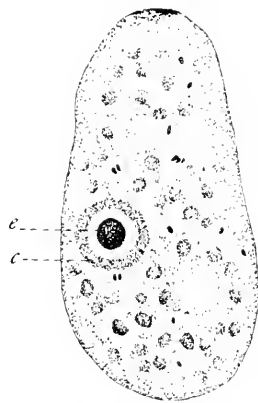


FIG. 24.—*Endamoeba intestinalis*; (e) endosome; (c) cortex of chromatin.

ments. Such a definition obviously would fail to specify any particularly nuclear structure, and so far as its practical value is concerned the term karyosome is no more useful than the non-

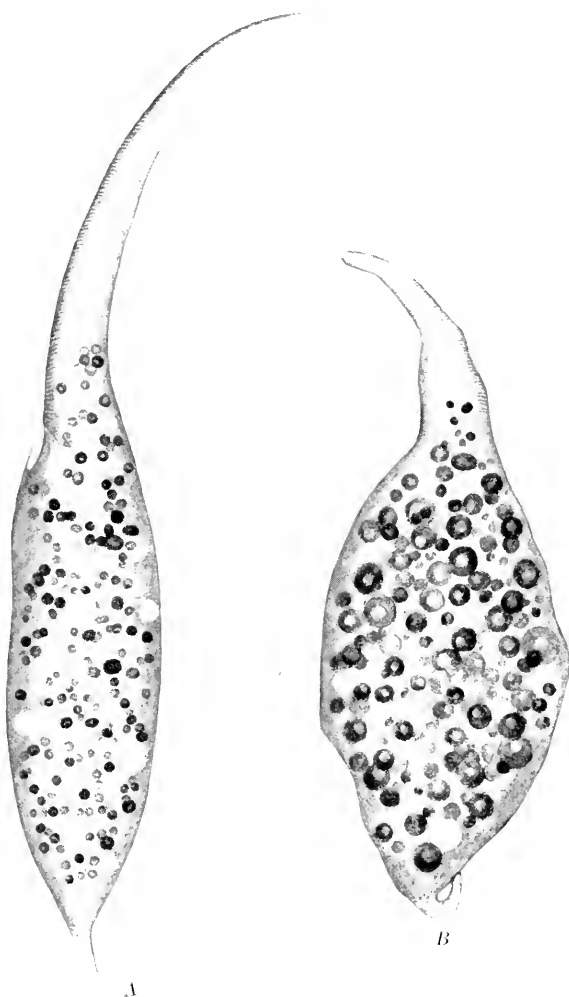


FIG. 25.—*Dileptus gigas*: A, vegetative individual in culture with nucleus in the form of scattered chromatin granules; B, individual showing the effect of treatment with beef extract on the chromatin granules. (Original.)

committal term Binnenkörper or Minchin's equivalent term endosome. I would advocate, therefore, discarding altogether the term karyosome which seemingly bears the earmarks of something definite in the cell, using in its place the general non-committal

expression Binnenkörper, or its equivalent term endosome, the latter as yet, at least, having no specific significance, while for the endosomes having functions characteristic of the kinetic complex a specific term may well be applied. In the present work I shall employ the term endosome in a general way to indicate all central intranuclear structures including those of kinetic function, while for those which are known to be of the nature of kinetic elements I shall use the term *endobasal body*.

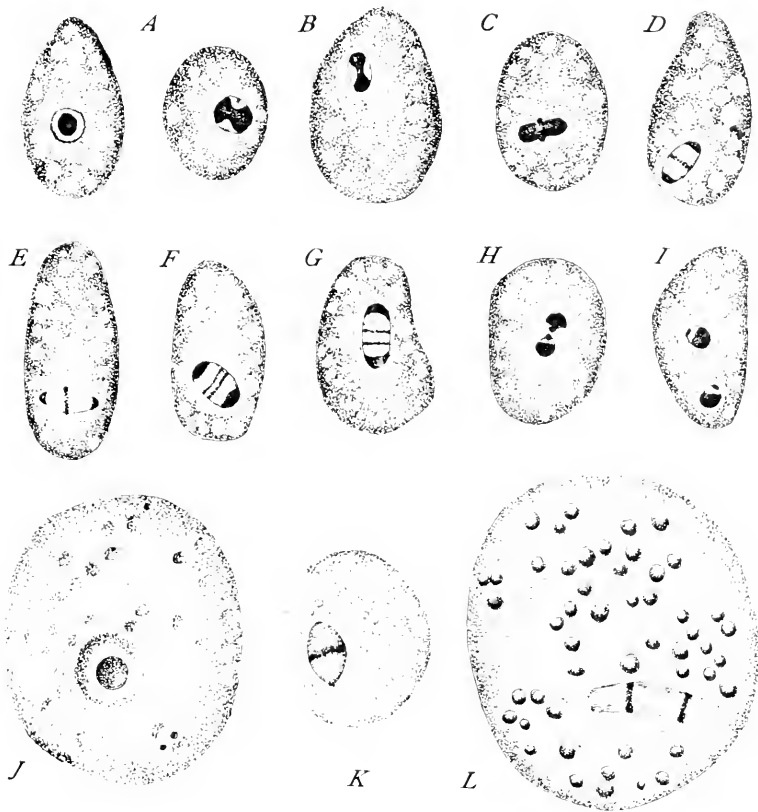


FIG. 26.—Division of amebae. A to I, successive stages in division (promitosis) of *Vahlkampffia limax*; J to L, mitosis in *Endamoeba coli*. (Original.)

The endosome-bearing vesicular nuclei present manifold variations in the arrangement of chromatin. In some the entire chromatin content is confined to the endosome which seems to rest in the center of a colorless enchylema traversed by strands of linin radiating from the endosome to the nuclear membrane (*Aréella vulgaris*, *Cochliopodium bilimbosum* and rhizopods generally, as well as in many

Coccidia and Gregarinida). In other cases the endosome retains only a little of the chromatin, the bulk of which is present as a dense network in the zone between endosome and membrane (*Endamoeba intestinalis*, *A. crystalligera*, etc.). In still other cases the chromomeres are distributed more or less uniformly throughout the nuclear reticulum (*Euglypha alveolata*, etc.).

In vesicular nuclei with endobasal bodies the chromatin may be in the form of more or less regular chromomeres uniformly distributed in the nuclear space (*Euglena* type), or more or less compactly aggregated about the kinetic element (many species of *Endamoeba*, various flagellates, Coccidia and Myxosporidia, etc.). Or, finally, the chromatin may be in the form of relatively large granules collected in a zone just within the nuclear membrane (e. g., *Pelomyxa*), or in fine granular form may make up the chief part of the nuclear membrane (*Vahlkampfia linax*, Fig. 26).

1. **Chromatin.**—Chromatin has been more a conception than a specific thing, the term being used to designate substances which appear under different forms at different phases of cell life. It appears normally in the form of minute granules or chromomeres (chromidiosomes of Minchin) in the resting nucleus, but during division of the nucleus these granules are massed together usually to form characteristic solid and individualized structures, the chromosomes. On *a priori* grounds chromosomes were early regarded as intimately associated with the phenomena of inheritance (Roux, Weismann, Boveri) and the more recent experimental work in genetics has given substantial evidence of the soundness of this early conclusion.

Our conception of chromatin is based largely upon investigations upon the nuclear substances of Metazoa and the higher plants. In ordinary descriptions, however, the term is often used in a vague sense to include any substance or body which stains with the so-called nuclear stains, *i. e.*, the basic anilin dyes, while direct chemical tests to determine the exact chemical composition of chromatin have been made in very few cases. The best of these show it to be composed mainly of nuclein, one of the most complex of protein substances and rich in phosphorus.¹

Vague as is the conception of chromatin in Metazoa it is even more so in connection with the Protozoa, where little has been done in a concrete way to throw light on the subject, although much has been written about it.

Many of the granules found in the cell body of a protozoön as well as those within the nucleus, stain with the usual nuclear dyes and their identification as chromatin is a matter requiring knowledge of their history and fate in the cell. It is only within recent years

¹ For a critical discussion of chromatin, see Wilson, 1925.

that an effort has been made to discriminate between the various granules in the Protozoa which stain intensely with the basic stains, and to distinguish the chromatin granules which enter into the make up of chromosomes from other chromatoid granules which are distributed throughout the cell, particularly the chromidia and the volutin grains. This is the more difficult in Protozoa because chromatin granules are not necessarily confined to the nucleus. Even in Metazoa and plants there are times during division when the chromatin is not confined within a nuclear membrane. In the Protozoa such a condition is permanent in many cases (*e. g.*, in some flagellates; in *Dileptus gigas*, *Holosticha*, etc.). In other cases the nuclear chromatin, by transfusion or by nuclear fragmentation, spreads more or less widely throughout the cell protoplasm (rhizopods, *Actinosphaerium eichhornii*, etc.). Here in different species, the fate of the distributed chromatin varies. In some cases this diffusion of chromatin indicates a degenerative change, the chromatin ultimately losing its characteristic reactions. Thus in *Actinosphaerium eichhornii*, Hertwig has shown that, under adverse conditions such as starvation, or overfeeding, or during periods of depression, such distribution of the nuclear chromatin occurs, the granules ultimately becoming transformed into a characteristic pigment of the cell. In other cases the distributed granules retain their chromatin nature and according to numerous observers are ultimately aggregated into minute secondary nuclei which become the nuclei of conjugating gametes (see p. 69). In these instances, other chromatin which is retained in the "primary nucleus" takes no part in the germinal activities but degenerates and disappears after the gametes are liberated. It must not be inferred that germinal chromatin is thus distributed in the cytoplasm in all cases; on the contrary in the majority of Protozoa the gamete nuclei are derived by division of the morphological nucleus with its contained chromatin, and some authorities, notably Kofoid (1921) deny *in toto* the origin of gamete nuclei from chromidia.

While chromatin thus has a definite germinal function there is equally little doubt of the important participation of the nucleus and presumably of chromatin in the ordinary metabolic activities of the cell. Thus, if an *Amoeba proteus* or the ciliate *Urorychia transfuga* (see Fig. 135, p. 262), be cut into two portions one of which contains the nucleus while the other is enucleate, the former portion only will digest and assimilate food, grow and regenerate the lost part, while the enucleate portion will continue to move and manifest various activities characteristic of destructive metabolism, but it will not take in food, nor digest what food may have been taken in before cutting, and in the course of a week or ten days it dies (Hofer, Verworn, Balbiani and many others).

It is evident that chromatin is directly associated with all of

the important vital activities including reproduction, and the view has been repeatedly advanced that, for these varied activities at least, two different kinds of chromatin are responsible. One kind, the so-called vegetative or trophochromatin, is active in the ordinary metabolic functions of the cell, while the other, the germinal or idiochromatin, has to do solely with perpetuation of the race. While this view of the dual nature of chromatin would seem to be sustained by the phenomena in rhizopods, gregarines, and by the dimorphic nuclei in the ciliates, it is by no means assured that this duality represents a fundamental difference in chromatins. On the contrary it is much more probable, as Hertwig has maintained, that there is only one chromatin and that its functional activity depends upon different factors and conditions which may arise during the life cycle; germinal chromatin in one cell-generation may become vegetative chromatin in the next and *vice versa*. This is particularly clear in the case of the ciliates where the macronucleus, a distinctly vegetative nucleus, and the reproductive micronucleus, arise as subdivisions of a fertilization nucleus after conjugation or its equivalent parthenogenesis.

The importance of chromatin for life of the cell is indirectly indicated by the extreme precision with which it is distributed to daughter cells at the time of division. Like other granules of the cell each chromomere grows and reproduces its exact duplicate by division. Chemically it probably represents the pinnacle of complex structures formed as a result of the activities of constructive metabolism while its derivatives, likewise granular in form and difficult to distinguish as chromatin, give rise to many more or less permanent or temporary structures in the cell body, each of which may perform some cellular activity in its passage through the various stages of chemical breakdown.

Few investigations of a purely chemical nature have been made on protozoan chromatin. The usual procedure is to designate as chromatin all structures of the nucleus which stain with the so-called nuclear dyes, or to interpret chromatin mainly on a morphological basis. Micro-chemical tests of all protoplasmic substances are made primarily on the basis of solubility or insolubility with acids, alkalis, salts, etc., and the conclusion that certain structures are made up of certain substances follows from the microscopic picture presented after such treatment. Such tests do not prove that a given structure is composed of a definite substance and is not a mixture of substances. Kossel, Miescher and others have shown that the chromatin bodies composed mainly of the chemical substance nuclein are not dissolved under the action of artificial gastric juice (pepsin and trypsin in appropriate acid and alkaline media) while other portions of the nucleus such as nucleoli and reticulum are entirely dissolved. Chromatin bodies on the other hand are dis-

solved in strong acids, dilute alkalies, calcium carbonate and sodium phosphate.

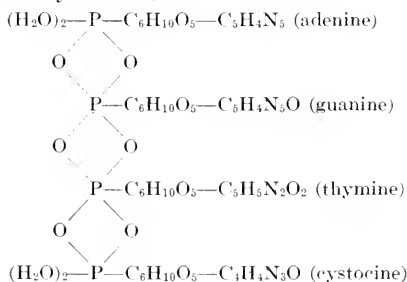
There has been a tendency to regard chromatin as the most important substance of the living cell, and the chromosome as the most important nuclear structure. Important they doubtless are, but in many cases chromatin is known as such only in the form of chromosomes which belong to the derived and not to the fundamental organization (see p. 88). In other words, chromatin is manufactured in the nucleus and the substances or substance from which it is made are still more fundamental. There appears to be little justification for Heidenhain's view of two kinds of chromatin, one—oxychromatin—unstainable with basic dyes, the other—basichromatin—staining readily. A substance in the nucleus is either chromatin or it is something else.

With the growing use of the Feulgen nucleal reaction there is reason to believe that a more precise definition of chromatin will be developed. This reaction finds its explanation in Steudel's (1912) analysis of thymonucleic acid of which the empirical formula is: $C_{43}H_{65}P_4N_{15}O_{34}$.¹ Under moderate hydrolysis with HCl the purin bodies are split off the molecule of thymonucleic acid and reducing groups are freed. These behave like aldehydes and give the characteristic red-violet color with Schiff's test (Magenta in the presence of sulphuric acid).

The nuclei of various groups of Protozoa give positive chromatin reactions with this test, and it is a useful method in tracing the development of chromatin in ex-conjugants or in the chromosomes of the maturation divisions. (See Feulgen and Rossenbeck, 1924; Bresslau and Scremin, 1924; Robertson, 1927; Zuelzer, 1927; Jirovec, 1927; Reichenow, 1928, and *infra* pp. 93 and 315.)

2. Other Substances of the Nucleus.—Bélař (1926) makes this statement concerning nuclei of the Protozoa: "For the most part chromatin of the resting nucleus cannot be distinguished from the ground substance of the nucleus (*loc. cit.*, p. 241)." This refers to the conditions of the living nucleus and not to fixed and stained material. In the latter chromatin in the form of granules can be

¹ This may be written:



distinguished from other substances of the resting nucleus by their color reactions to basic and acidic dyes. Sometimes the chromomeres or chromioles are apparently suspended in a more or less definite "linin" reticulum which is recognized as being a coagulation product of the colloidal ground substance or karyolymph. In other

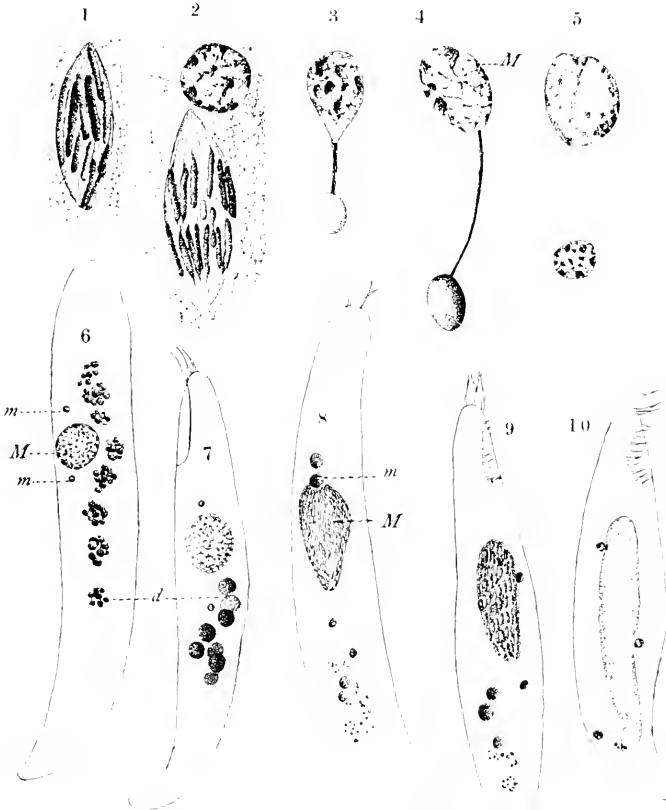


FIG. 27.—Origin of macronucleus after conjugation in *Uroloptus mobilis*. (1) First metagametic mitosis of the amphinucleus; (2) one of the progeny of this division dividing again; (3), (4), (5) telophase stages of second division of the amphinucleus resulting in a new macronucleus (above), and a degenerating nucleus (below); (6 to 10), stages in differentiation of the young macronucleus and disintegration and absorption of the old macronucleus; in (10) two new micronuclei are in mitosis preparatory to the first division of the ex-conjugant. (*M*) new macronucleus; (*m*) new micronuclei; (*d*) degenerating old macronuclei. (After Calkins.)

cases they are combined with the substance "plastin" to form a clearly-defined endosome (karyosome) which, depending apparently on the relative proportions of plastin and chromatin, may or may not be visible in life. Plastin appears to be a well-defined nuclear substance and writers generally speak of it with familiar ease,

despite the fact that very little definite information is at hand concerning it. In pure form it is the nucleolus of tissue cells and stains intensely with acid dyes. Such nucleoli are rare in Protozoa, but the combination of plastin with chromatin in some degree is characteristic of Protozoa, and the staining reaction with basic or acidic dyes varies with the preponderance of one or the other.

The ground-substance of the nucleus or karyolymph (Lundegardh) is difficult to define, a difficulty which Belar (1926) recognizes by the statement: “. . . at best it can be defined as that part of the nuclear space which is neither chromatin nor plastin” (*loc. cit.*, p. 242). From this negative definition and from the fact that it cannot be demonstrated by specific staining reactions or characterized by definite structures, it might seem that karyolymph is a negligible part of the nuclear make-up. Such a conclusion, however, would be a mistake for some of the most important structures of the active nucleus take their origin from this ground substance (see pp. 88, 200).

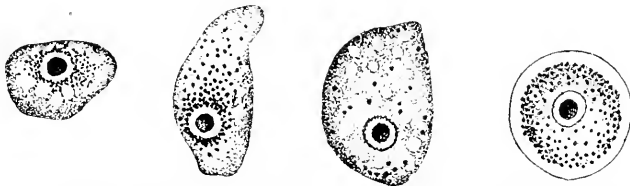


FIG. 28.—*Vahlkampfia limax*; chromatin forming the nuclear membrane and giving rise to chromidia. (After Calkins.)

Membrane.—Like other constituent parts of the protozoön nuclei, the membranes are highly variable, sometimes presenting in optical section only one contour on the outer side (*e. g.*, *Actinosphaerium*); sometimes showing contours both outside and inside (*Amoeba proteus*). In the former case the inner zone adjacent to the membrane shows a decreasing density inwards, until the linin merges insensibly into the intranuclear reticulum. In free-nuclei formation, antecedent to gamete formation described above, the nuclear membranes are probably formed from the cytoplasmic reticulum in which the chromidiosomes are lying. Chromomeres also take part in the formation of nuclear membranes in some cases, *e. g.*, in *Vahlkampfia limax*, where the linin membrane is too delicate to be seen, although the definite limitation of the chromomeres indicates its presence (Fig. 28).

One peculiarity of the nuclear membranes of Protozoa which distinguishes them from nuclear membranes of tissue nuclei, is that in the majority of cases they remain intact during all phases of cellular activity and only rarely disappear, or disappear in part only, during division processes of the cell. (For description of chromatin, membranes, etc., during division, see p. 209.)

Intranuclear Kinetic Elements.—The kinetic elements, some of which are intranuclear and a part of the fundamental organization, are those structures of the cell which are closely connected with the visible expression of the transformation of energy resulting from destructive metabolism. Such expression may be in the form of movement due to the activity of specific motile organs formed as a rule from the substance of kinetic elements, or it may be in the form of intracellular activities as indicated by the transformation and movements of internal attraction centers, center of radiation, of nuclear division, etc. The kinetic elements are justly regarded by many observers as the most elusive and perplexing, but at the same time amongst the most fascinating of all the organoids of Protozoa.

Kinetic elements appear in Protozoa in a multitude of structures, sometimes intranuclear, sometimes cytoplasmic, and often both inside and outside the nucleus. Whether or not they are permanent organoids of the cell is subject to the same arguments pro and con which have been raised for and against the permanency of the centrosome in Metazoa. There is strong evidence, as the following pages will show, that not only are many types of cytoplasmic kinetic elements derived from the nucleus, but also that chromatin and intranuclear endobasal bodies are closely related, while some types that are confined to the cytoplasm are composed in part, or entirely, of a substance which closely resembles chromatin (parabasal bodies). Little is known of the chemical composition of the latter, but both intranuclear and cytoplasmic kinetic elements stain intensely with some of the nuclear dyes and divide by simple constriction at periods of cell division.

In many cases it is impossible to tell from observations on ordinary vegetative individuals, whether a given structure belongs to the kinetic elements or to some other group of the many types of protoplasmic granules. This is particularly true of the intranuclear forms where incomplete extraction of a stain may give the appearance of a granule in some chromatin or plastin mass. In such cases the identity of the structure can be determined only by its history during nuclear division. Cytoplasmic forms can be more easily detected by reason of their relation to motile organs or to more or less complex fibrillar structures.

(a) **Endobasal Bodies.**—Endobasal bodies in nuclei of different Protozoa are highly variable and no general description is possible. In some cases they stain intensely with nuclear dyes, especially with iron hematoxylin; in other cases they stain feebly or not at all with the same dyes that color the chromatin (*e. g.*, *Chilodon*). In some cases they are large and appear homogeneous throughout; in other cases there is a definite, deeply-staining central granule embedded in a more faintly staining plastin (?) matrix, or such a granule may be present without the accompanying matrix; or,

finally, there is no evidence at all of kinetic elements in resting nuclei, but collections of homogeneous substance (karyolymph) are present at the poles of the nucleus during division (pole plates).

1. *Large Homogeneous Endobasal Bodies*.—In this type the endobasal body is conspicuous by its large size and homogeneous structure. It was first described by Keuten (1895) in *Euglena viridis* and was early recognized as a kinetic element connected with nuclear division as attested by the names intranuclear centrosome,

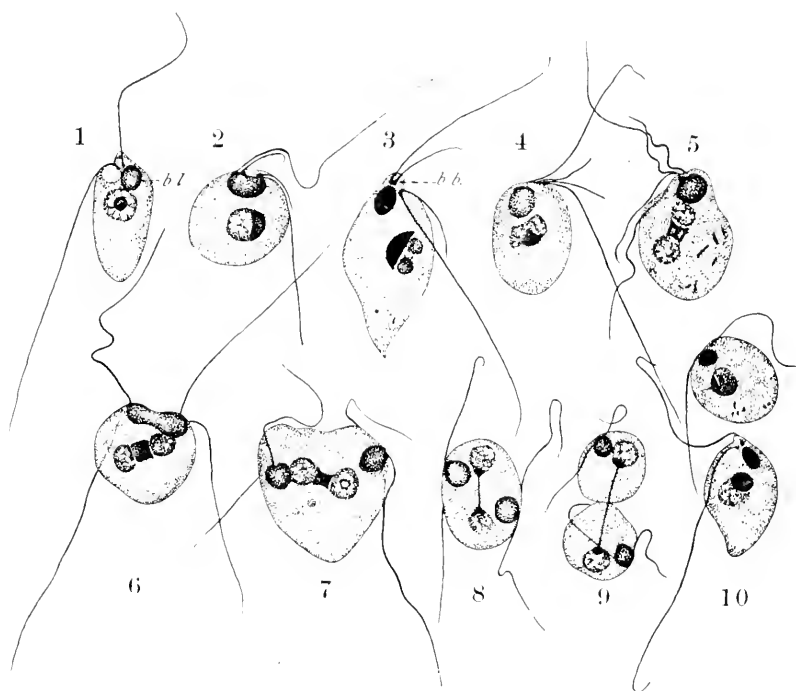


FIG. 29.—*Bodo ovatus* Stein (*dar*, Bělaf). (1) Vegetative individual with two flagella; blepharoplast (*bl*) and nucleus with endosome. (2 to 6) Division of the basal bodies, blepharoplast and nucleus; (7 to 10) completion of nuclear division and division of cell body. (After Bělaf, from Doflein.)

division center, etc., applied to it, while nuclei containing it were included by Boveri in his "centronucleus" type. In *Euglena viridis* and euglenoids generally, this endobasal body according to earlier descriptions of Keuten, Tschenzoff (1916) and others is the most conspicuous structure of the nucleus, where, in the resting nucleus, it appears as a spherical or elongated ellipsoidal body with chromatin granules of limited number suspended between it and the nuclear membrane. It divides prior to division of the chromatin, first elongating with a concentration of its material at the poles. The

elongation continues until a thin fibril, called a centrodesmose, alone connects the two halves. The centrodesmose ultimately

breaks and its substance is absorbed by the two daughter elements. [See also Baker, and Hall (1923).] In the rhizopod *Chlamydrorhrys stercorea*, as well as in the flagellate *Bodo oratus*, the endobasal body which is quite similar to that of *Euglena*, divides subsequently to division of the chromatin (Schaudinn, Bělař, Fig. 29), while in *Amoeba crystalligera* (Schaudinn) there is no centrodesmose formed during division, a condition not uncommon in the rhizopods (e. g., *Arella vulgaris* according to Swarczewsky; *Vahlkampfia limax* [Fig. 28], and many species of *Endamoeba*). Not only is this simple type of endobasal body found in rhizopods and flagellates, but also in some cases in the more complex ciliates, where,

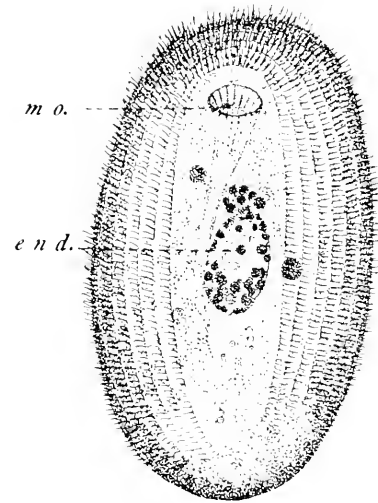


FIG. 30.—*Chilodon* sp. Macronucleus with endosome and endobasal body (*end.*). (*mo*) Mouth surrounded by pharyngeal basket. (Original.)

in *Chilodon encullus*, for example, the macronucleus contains a definite endosome which behaves exactly like that of *Euglena*. It is highly

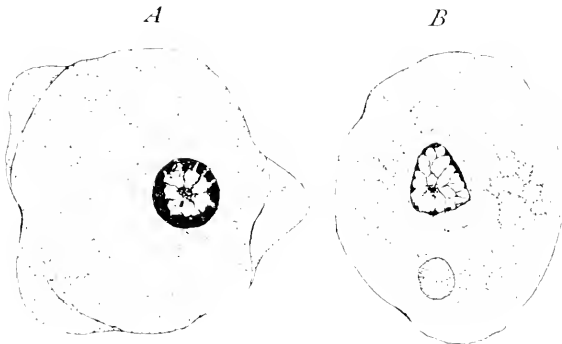


FIG. 31.—*Endamoeba dysenteriac* (Councilman and Laffeur). Two stages in the metamorphosis of endosome and endobasal body. (After Hartmann.)

probable that in all of these cases the endobasal body is embedded in a core of plastin.

2. *Endobasal Bodies With Centrioles*.—Centrioles are kinetic elements in the form of minute granules, which in Metazoa and in some types of Protozoa, form the focal points of the mitotic spindle. In many Protozoa minute granules may be embedded in a matrix of chromatin or plastin, or in a combination of both. These in some cases form the poles of typical spindles, but in the majority of cases, apart from the polar granules and the connecting centrodesmose, there is little evidence of a typical spindle.

In some forms this type of endosome undergoes changes in appearance which Hartmann (1911) and his followers have interpreted as periodic or cyclical in nature. Such variations have to do with the concentration of the chromatin substance about the endobasal body or centriole, being massive and dense in certain phases and distributed in others. In *Eudamoeba dysenteriae* the centriole in the latter phase is distinct and definite but in the former phase it is hidden by the dense chromatin (Fig. 31). From such conditions Hartmann infers that all massive types contain hidden centrioles, a conception applied by Naegler to all of the smaller amebae and endamebae, but, according to Gläser, it is limited to comparatively few types.

Typical endobasal bodies in the form of centrioles are contained in the first maturation nuclei of *Uroleptus mobilis*. Here each massive micronucleus fragments into chromatin granules which remain in a dense reticulum at one pole of the enlarging nucleus until the chromosomes are formed. A centriole, hidden in this mass, divides and one-half traverses the nucleus to form the first pole of the maturation spindle but remains connected by a centrodesmose with the other centriole which, in turn, forms the other pole of the spindle (Fig. 32, *b-g*). Similar centrioles are found in widely separated groups of Protozoa. In *Coccidium schubergi*, according to Schaudinn (1900), the endobasal body divides with a long connecting centrodesmose. Here, however, part of the material of the centrodesmose collects into two granules with a more densely stained connecting thread, thus producing a structure which Doflein interprets as analogous to the mid-body (*Zwischenkörper*) of Metazoa and plant cells. The fate of the centrioles after division differs in different cases. In some, *e. g.*, *Bodo lacertae* (Bělař, 1921, Figs. 33, 34), they come from the nucleus and re-enter the daughter nuclei;¹ in others they arise from basal bodies and become basal bodies of the flagella after division (*e. g.*, *Chilomastix aulostomi*, Bělař, 1921; *Spongomonas*, Hartmann, etc.).

While the embedding matrix in most of the above cases is similar to chromatin in its reaction, and forms an important part of the endobasal body, there are other types (*e. g.*, *Myxobolus Pfeifferi*,

¹ See, however, the earlier contradictory accounts of Prowazek (1904), Alexieff (1914), and Kuczynski (1918).

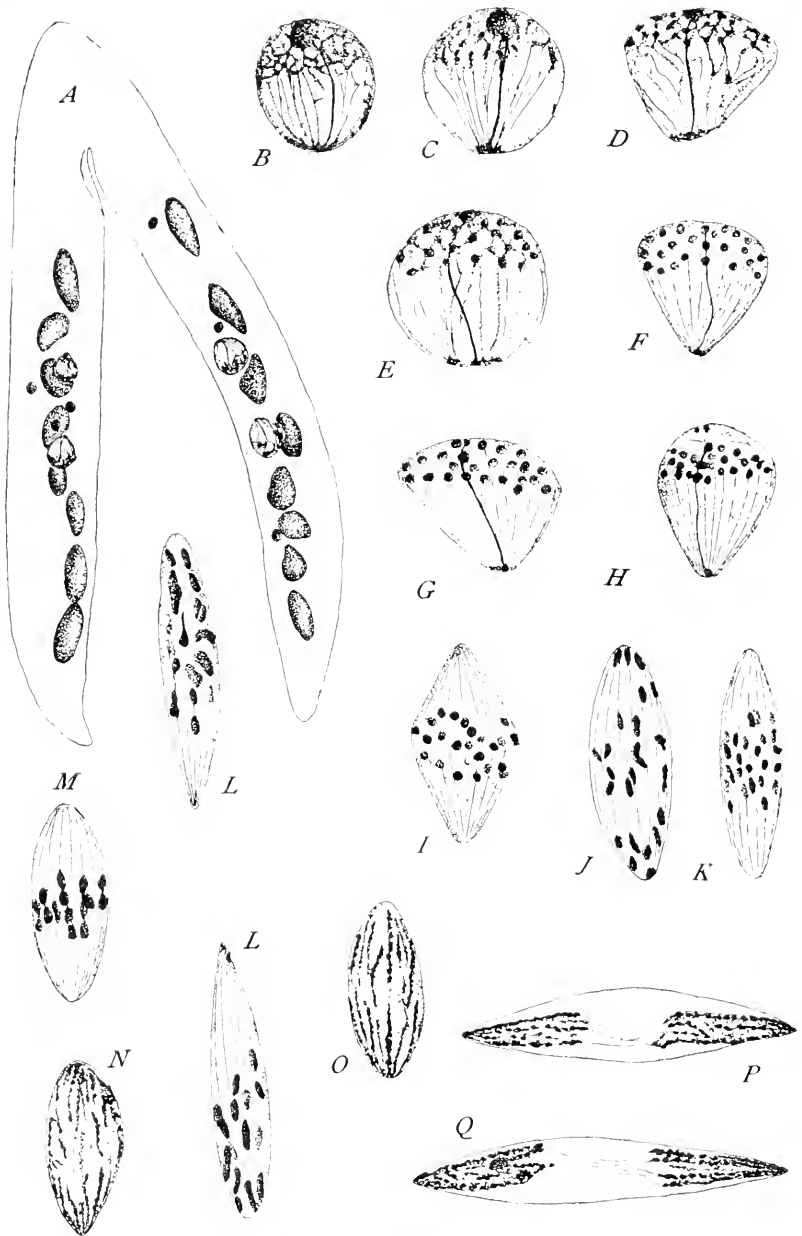


FIG. 32.—*Uroleptus mobilis* Eng. First and second meiotic divisions during conjugation. (A) Two conjugating individuals; (B to G) formation of the first spindle pole by division of the endobasal body (with centrosomes); (H to M) first meiotic nuclear division; (N to Q) second meiotic division. (After Calkins.)

one of the Myxosporidia) in which the centriole emerges from an enveloping plastin-like matrix, which, like a nucleolus, then degenerates and disappears.

3. *Nuclei With Pole Plates and Without Endobasal Bodies.*—This type of nucleus is characterized by the entire absence of endobasal bodies. A hyaline mass, which stains with difficulty, may, however, be present at the spindle poles during nuclear division, but in many cases it cannot be detected in the resting nucleus. During division it occurs in characteristic forms known as pole plates.

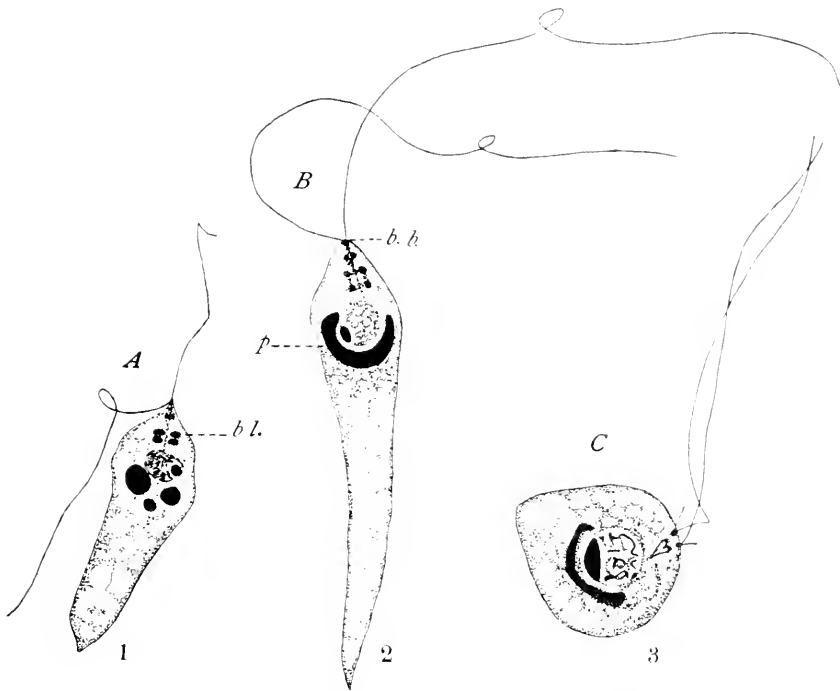


FIG. 33.—*Bodo lacertae* Grassi. Early stages of division of the basal bodies, (*bb*): blepharoplast ring (*bl*); nucleus and parabasal body (*p*). (After Bélař.)

In the micronuclei of *Paramecium caudatum* such a mass forms a hyaline cap at one pole of the otherwise chromatin-filled resting nucleus. Observations are entirely lacking in regard to division of this mass during reproduction, but similar aggregates of non-staining substance are present at the distal ends of the daughter nuclei during stages of division (Fig. 35). Similar pole plates appear as broad, flat and hyaline ends of the spindles of *Actinosphaerium eichhornii* according to Hertwig (1898), in the spindle of *Trichosphaerium sieboldi* according to Schaudinn (1899), or in the macro-

nucleus of *Spirochona gemmipara* (Hertwig). In this group, also, we would include the peculiar hyaline globular bodies at the poles of the nuclear spindles of *Euglypha alveolata* as described by Schewiakoff (1888).

It is quite possible, although direct evidence is lacking, that none of these peculiar pole plate structures belongs to the group of

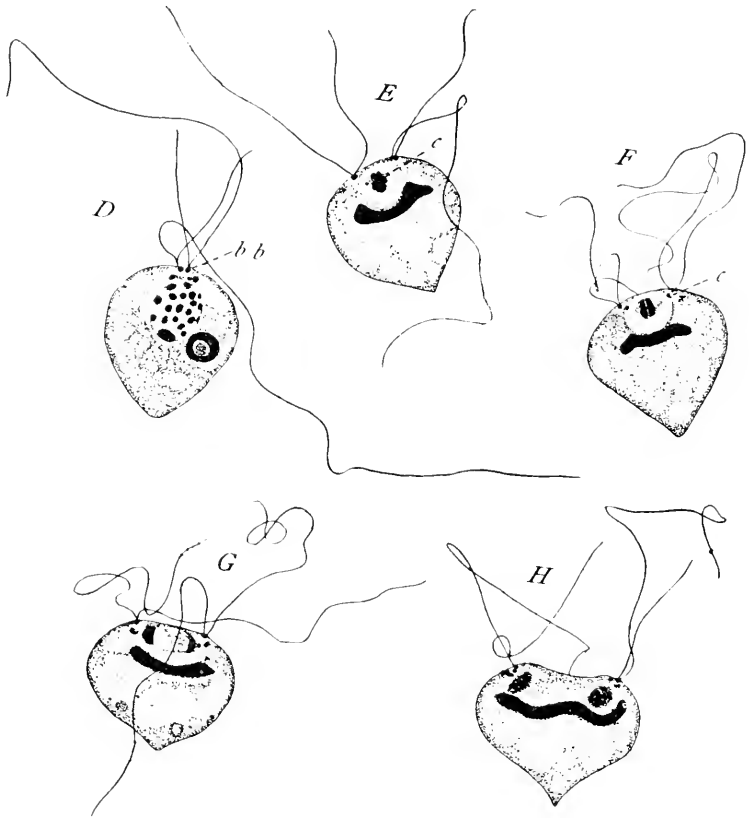


FIG. 34.—*Bodo lacertae* Grassi; division stages continued. (E) Origin of centrioles in the nucleus, and their retention in the daughter nuclei (F to G); (bb) basal bodies, (c) centriole. (After Bélat.)

kinetic elements. Indirect evidence favoring this possibility is furnished by the entire absence of observations on the division of a definite body, the substance of which forms the pole plates. Hertwig (1898) and Doflein (1916) assume that they are formed from the "linin" substance of the nucleus. On this assumption the pole plates might be interpreted as hyaline aggregates of the ground substance of the nucleus, indeed, the hyaline and homogeneous appearance of

the pole plates is suggestive of ameba ectoplasm. With our present knowledge I am inclined to agree with this interpretation of pole plates and to regard *Paramecium caudatum*, with other species of this genus, *Actinosphaerium eichhornii* and the other forms men-

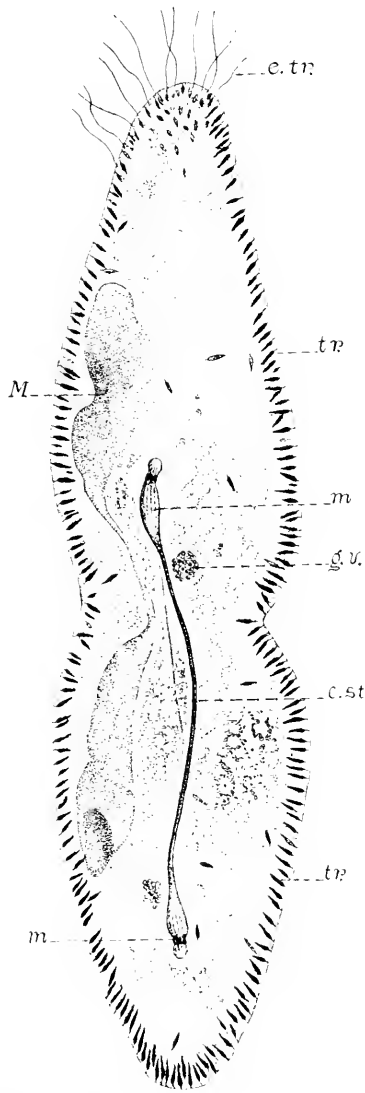


FIG. 35.—*Paramecium caudatum*. Section of a dividing individual; *c. st.*, connecting strand of dividing micronuclei; *c. tr.*, extruded trichoecysts; *g. v.*, gastric vacuole; *M*, dividing macronucleus; *m, m*, divided micronuclei; *tr.*, trichoecysts. (Original.)

tioned above, as containing no permanent intranuclear kinetic elements. To such a group we would also assign forms like *Aulocantha scolymantha* and *Chilomonas paramecium*, in which according to observations of Borgert (1909) and Alexeieff (1911), not only intranuclear kinetic elements but pole plates as well are entirely absent.

On the whole I would interpret the intranuclear kinetic elements of Protozoa as originating by condensation of the ground substance or karyolymph of the nucleus. In *Paramecium caudatum* (Figs. 35, 147) both in vegetative and meiotic divisions, the ground substance forming the pole plates shows but little condensation (Fig. 57), but in the first meiotic division of *Uroleptus halseyi* the karyolymph forms two irregular masses which condense to form the spindle fibers and the two spindle poles which are more like pole plates than like centrioles (Figs. 151, 153). In a similar stage of *Uroleptus mobilis*, however, condensation results in the formation of a definite centriole which divides with a connecting centrodesmose (Fig. 32). In the flagellate type the endobasal body may well be a permanent condition of such condensation. Whether or not such condensations leading to endobasal body formation involve a specific chemical make up, different from that of the karyolymph and from chromatin, is an unsolved problem. The diffuse forms such as may be seen in pole plates do not stain with iron hematoxylin or other nuclear dyes nor do they give a positive Feulgen reaction. The centrioles and permanent endobasal bodies stain with iron hematoxylin but the Feulgen reaction is negative.

II. CYTOPLASMIC ELEMENTS OF THE FUNDAMENTAL ORGANIZATION.

Very little work has been done on the finer structures of encysted Protozoa, and we are relatively ignorant of the make-up of the fundamental organization of the cytoplasm. It is difficult, and often impossible, to distinguish between those elements which are essential parts of the germinal protoplasm and those which are formed as a result of metabolic activities. The latter, obviously, would belong to the structures of the derived organization.

The great majority of the structural elements of the cytoplasm are known only in the adult organism. Many of these are undoubtedly derived structures of the developing individual but some may be essential parts of the germinal protoplasm. Until further knowledge of the origin of such questionable elements is available we may regard them tentatively as parts of the fundamental organization and describe them as such. In most cases they are present in the adult organism in the form of granules which, morphologically, are almost indistinguishable from one another but which react

characteristically with specific staining methods, thereby indicating differences in their chemical composition. Amongst such characteristic granular elements of the cytoplasm are (1) *Chromidia*, found mainly in Sarcodina and Sporozoa; (2) *Volutin* grains, found mainly in flagellates, but also present in Sarcodina and Sporozoa; (3) *Mitochondria*, characteristic of all types; (4) *Golgi* apparatus, probably universal; (5) *Silver Line System* of the Infusoria; (6) *Kinetic* elements (for the latter see pages 88 and 104).

1. **Chromidia.**—The nature and the functions of chromidia have been and still are matters of controversy in which there are wide differences of opinion. Hertwig (1879) early called attention to extra-nuclear chromatin in Radiolaria and later (1899) described the zone of cytoplasmic, deeply staining substance which extends from one nucleus to the other and characterizes the dorsal region of *Arcella vulgaris* and related forms. Hertwig called this the chromidial net and homologized it with the extranuclear chromatin which he had found in Radiolaria. At about the same time (1898, 1902) Hertwig described the breakdown of nuclei and the distribution of chromatin into the cytoplasm of *Actinosphaerium eichhornii*. To such chromatin granules in the cytoplasm he gave the name "Chromidien" and their appearance was regarded as a sure indication of the approaching death of the organism.

These observations mark the commencement of a long controversy over the question of chromidia duality which, so far as the Protozoa are concerned, was first clearly announced by Schaudinn in connection with the life histories of the testate rhizopod *Centropyxis aculeata*, the foraminiferon *Polystomellina crispa*, and some of the endamoebidae.

The chromidial net of *Centropyxis* is similar to that of *Arcella* and according to Schaudinn is the seat of the formation of secondary nuclei by origin *de novo* from the chromatin of the chromidial net. These secondary nuclei become the nuclei of gametes while the primary nucleus degenerates. Similarly in *Polystomellina*, although there is no chromidial net, the cytoplasm of mature individuals of the asexual generation becomes filled with minute chromatin granules—chromidia—which arise by fragmentation of the primary nuclei and ultimately become the nuclei of gametes (Fig. 123, p. 235).

These findings by Schaudinn were subsequently confirmed by Lister (1905) for *Polystomellina crispa*; by Elpatiewsky (1907) and Swarczewsky (1908) for *Arcella vulgaris*; by Goldschmidt (1905) for Mastigina and Mastigella belonging to the flagellate family Rhizomastigidae; by Winter (1907) for *Pencroplis pertusus*, a foraminiferon; by Goette (1917) for *Difflugia lobostoma*. Similar observations were made in connection with Sporozoa of different kinds by Leger and Duboseq for the gregarine *Nina gracilis*; by Swarc-

zewsky (1910) for a species of *Lankesteria* a hemosporidian; by Kuschakewitsch (1907) for *Gregarina cuneata*; by Lebedew (1909) for the ciliate *Trachelocerca phoenicopterus*. The findings and conclusions of these different observers have been criticized by Dofflein (Lehrbuch, Fourth Edition), by Kofoid (1921) and by others, as unconvincing and not, as yet, adequately confirmed, while the suggestion is repeatedly made that the "secondary" nuclei arising thus *de novo* from chromidia may be intracellular parasites.

So far as the dualism of chromidia is concerned Schaudinn (1903) was the first to suggest the idea by the term "somatochromidia" for chromidia which are vegetative in function or the result, as in *Actinosphaerium*, of degeneration, and by the term "gametochromidia" for chromidia which give rise to gamete nuclei. These terms were turned into "trophochromidia" and "idiochromidia" respectively by Mesnil (1905) with a slight difference in interpretation of the former. Goldschmidt (1905) likewise indicated the same interpretation by the terms "chromidia" and "sporetia" respectively.

Before accepting interpretations as above, particularly in connection with chromidia of the testate rhizopods, it is necessary to determine whether or not the granules in question are really chromatin. Khainsky (1910) came to the conclusion that the chromidial net of *Arcella* has an active part to play in nourishment of the organism, and Zuelzer (1904) maintained that the chromidial net of *Difflugia* is the seat of formation of a carbohydrate nutritive substance of the nature of glycogen. If these suggestions prove to be correct it would indicate a different chemical make-up for chromidia and intranuclear chromatin, and a difference which should be detectable by microchemical tests. In this field, however, observations are few and results are discordant. The chromidial net of *Arcella vulgaris* stains black with iron hematoxylin, green with the Borrel mixture and, usually, gives a negative reaction with the usual Feulgen treatment. These results confirm Hartmann's experiment with pepsin under the action of which the chromidial net of *Arcella* is dissolved out while the secondary nuclei are conspicuous after subsequent staining.

Bělař (1926) and others apparently believe that Hartmann's experiment gives a final answer in the negative to the question of the chromatin nature of chromidia. This conclusion, however, is somewhat premature for recent experiments with the Feulgen reaction indicate that nucleic acid is certainly present at some stages. With hydrolysis by strong hydrochloric acid at 60° F. followed by the usual staining method the result is invariably negative, while the primary nuclei show only a faint reaction. If, however, the first part of the operation involving strong hydrolysis is omitted and the *Arcella* material placed directly in the staining solution for

from eight to fourteen hours, a positive reaction is obtained in all forms in which the secondary nuclei are present (Fig. 36). Here the nuclei and the embedding matrix of chromidia are intensely stained. Chromidia at other stages give varying shades of purple depending apparently upon the condition of the organism. Nucleic acid which is formed in the chromidia becomes concentrated in the secondary nuclei; these obviously would resist the pepsin digestion while the residue is dissolved.

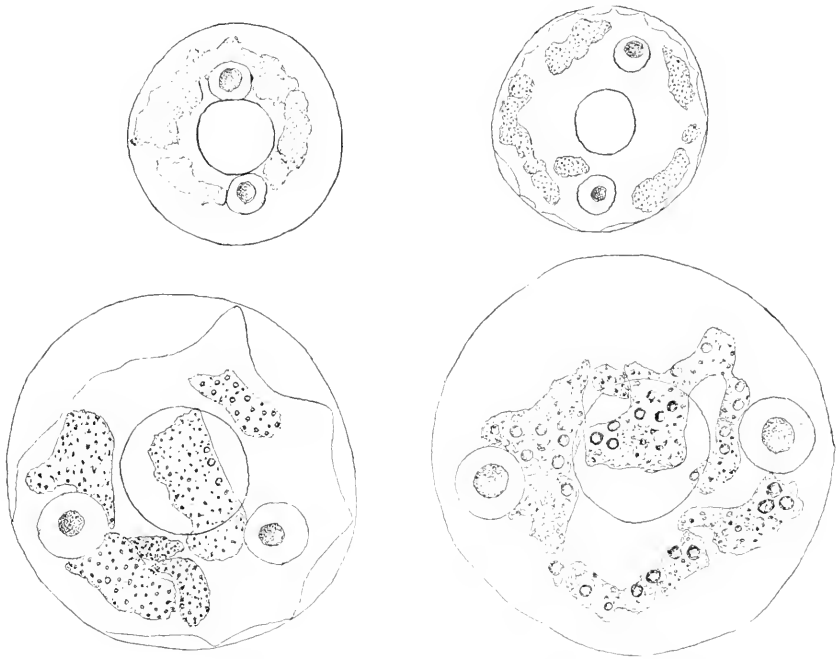


FIG. 36.—*Arcella vulgaris*. Growth of nucleic acid bodies in the chromidial net. (Original, $\times 500$ and $\times 1000$.)

The problem of extranuclear chromatin, or chromidia, assumed a novel theoretical significance with the development of Hartmann's so-called polyenergid theory. Hartmann (1909) suggested a morphological interpretation of Sachs "energid" or nucleus with its sphere of influence, by suggesting an energid as a nucleus consisting of two components, one the chromatin or idiogenerative component, the other a centrosome or homologous structure (kinetic or locomotor component). In 1911 he distinguished three main types of nuclei of Protozoa, *viz.*, monoenergid, meroenergid and polyenergid types. Monoenergid types are in Protozoa having one kind of cell division as in most flagellated Protozoa. Meroenergid types are forms, originally with two nuclei, one of which has lost the idio-

generative component (as in Heliozoa with central granule, or Trypanosomes with "kinetonucleus"). Polyenergid types, finally, involve nuclei containing an aggregate of monoenergid nuclei. Since a monoenergid has but one kind of division Hartmann assumes that this division may take place while in the aggregated condition; or that the monoenergids are freed by rupture of the membrane after which they may divide as monoenergids in the cytoplasm. In all cases the monoenergids become the nuclei of gametes (as in Radiolaria, Foraminifera and gregarines). The conception is interesting, but apart from adding other somewhat unenlightening terms monoenergid and polyenergid it leaves us practically where we were before on the chromidia problem, and separates, without sufficient justification, the chromidial net type from the gamete nuclei type. In all probability the two types are not widely different. The monoenergids which come from a polyenergid nucleus represent chromatin which is formed in the nucleus (see p. 87); the gamete nuclei which arise from the chromidial net represent chromatin which is manufactured by a cytoplasmic substance of the same nature as the karyolymph and a substance which, possibly, may be derived from the nucleus.

2. **Volutin Grains.**—These are widely distributed in Protozoa with the exception of the Infusoria, and are not difficult to distinguish from chromidiosomes. They are usually spherical in form but may be angular and irregular and stain intensely with the basic dyes, retaining the stain even after the chromatin granules are completely extracted. They were discovered by a pupil of A. Meyer in the cells of *Spirillum volutans* from which the peculiar name is derived, and, according to Guilliermond, they are identical with the "metachromatic bodies" of Babes, and with the "red granules" discovered by Bütschli. They take a yellow stain with iodine and a blue stain with methylene blue and 1 per cent solution of sulphuric acid, while their reaction to the usual chromatin stains makes them difficult to distinguish from chromidia. They do not give a reaction with the Feulgen method as usually employed, but Reichenow (1928) found that if the preliminary acid hydrolysis is omitted a typical Feulgen reaction follows upon treatment with the fuchsin-sulphuric acid component alone. He infers from this that volutin substances give a typical Feulgen reaction, which is much more rapid than that of nuclear chromatin, and concludes that volutin consists of free nucleic acid. The same conclusion was reached by Schumacher (1926) on the basis of volutin reactions to his methylene blue phosphin method. Meyer himself regarded them as composed largely of nucleic acid, a conclusion supported by the experiments of Reichenow (1909) on *Hematococcus* in which it was shown that volutin grains disappear in a medium free from phosphorus and that, during the phases of active chromatin increase in the nucleus,

they diminish perceptibly in size and increase in size when the chromatin content becomes stationary. From these results, confirmed by van Herwerden (1917) on yeast cells, Reichenow concluded that volutin grains play a most important part in the vital activities of the cell and he regarded them as a reserve store of nucleo-proteins for the purpose of chromatin growth in the nucleus. They appear to be formed in the cytoplasm and, if these observations are well founded, are entirely different in origin and in function from the other minute granules which they closely resemble. The importance of these conclusions in problems connected with biology of the cell warrants the demand for further and more complete observations and experiments.

3. **Mitochondria.**—The chondriome of a cell consists of the aggregate of cytoplasmic substances of lipoidal nature appearing in the form of minute granules termed mitochondria, as strings of granules termed chondriomites, or as smooth filaments termed chondrioconts according to the terminology of Benda (1903) and Meves (1907).

The lipoidal make-up is shared with the Golgi apparatus, another group of cytoplasmic substances which are equally well distributed and similar in form and in reactions to mitochondria, but which are regarded as distinct from the chondriome and with different functions in the cell.

Some of the lipoidal substances making up the chondriome are evidently autonomous bodies in the cell, while others, more transitory in nature, probably result from metabolic activities. It is quite probable, as Alexieff suggests (1928), that different states or stages of a common type of substance are represented in different organisms and the terms mitochondria chondriomite, chondriocont, etc., have merely a morphological significance. Of these the mitochondria appear to be the original neutral and most widely distributed of the lipoidal substances, and as such they belong to the fundamental organization.

Mitochondria are minute inclusions in the cytoplasm, varying in size from 0.5μ to 1.5μ . They may be spherical granules or rod-shaped, resembling bacteria, or crescentic or sickle form. (Fig. 37.) They have been identified in so many different types of Protozoa that their universal distribution may be assumed with assurance.

Except in a very general way the chemical make-up of mitochondria is unknown. They become reduced in size or disappear after treatment with alcohol or acetic acid, but there are wide differences in the times required to bring this about. They blacken with osmic acid, turn blue green with Janus green B, or red with Janus red (Horning, 1926). Fauré-Fremiet (1910) who was the first to recognize mitochondria in Protozoa regarded them as a combination of albumin and phosphates of fatty acids. Today there is no

great advance beyond this original interpretation, the accepted view being that mitochondria are combinations of a fat-like body (lipoid) and protein, the variations in staining, in solubility, etc., depending upon the relative amounts of protein in the combination, a small proportion making them highly unstable, a large proportion making them more resistant to heat, alcohol and fat solvents in general.

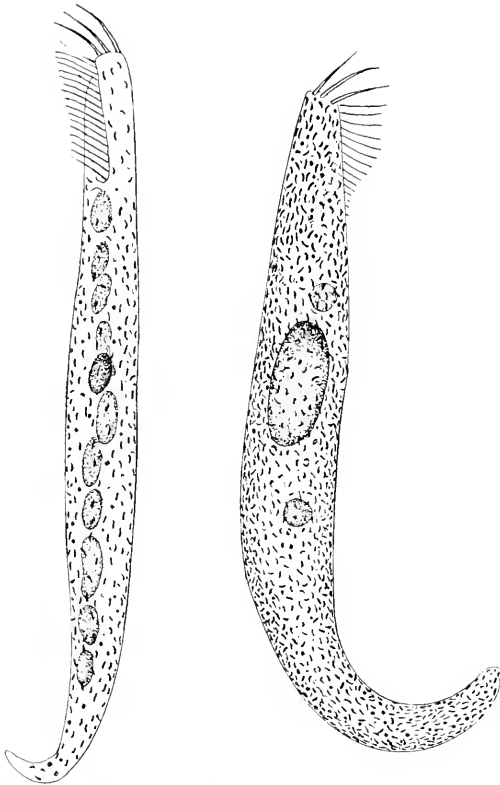


FIG. 37.—*Uroleptus halseyi*. Difference in mitochondrial content of a cultural individual (left) and an ex-conjugant (right). $\times 700$. (After Calkins, Arch. f. Protistenkunde, courtesy of G. Fischer.)

Opinions differ in regard to the autonomy and self-perpetuation of mitochondria. Observations on the living protozoön cell convinced Fauré-Fremiet (1910) that the granules reproduce by spontaneous division and this observation has been confirmed by others upon living and fixed material. Richardson and Horning (1931) in particular, after a slight modification of the pH of the milieu, obtained preparations of *Opalina* showing practically every mitochondrial granule in division (Fig. 38). In other cases, however,

particularly in the early sporozoites of *Monocystis*, Horning was unable to demonstrate the presence of mitochondria and concluded that they are absent in young forms but make their appearance in the process of development. This was interpreted as evidence of their origin *de novo* in the cytoplasm (Horning, 1929).

Suspicious have been aroused from time to time as to the nuclear origin of mitochondria, although little positive evidence has been forthcoming. Some has been obtained recently, however, in connection with observations on the reorganization processes following conjugation of *Uroleptus halseyi* (Calkins, 1930). Here the old macronuclei, eight in number, break up, each into a group of minute spherules. These spherules, at first, have a deeply staining cortex

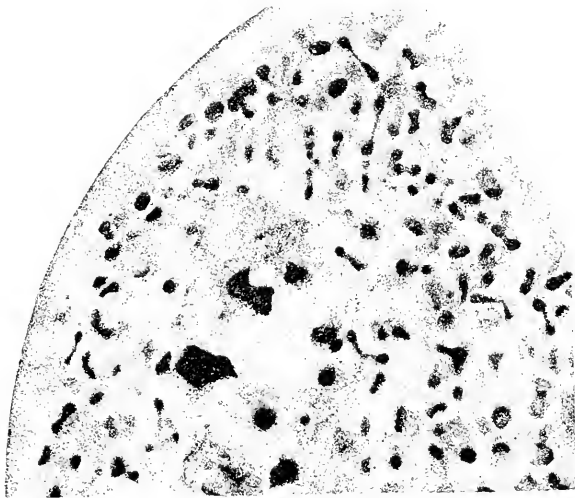


FIG. 38.—Dividing mitochondria in *Opalina*. (After Richardson and Horning, Jour. Morph., courtesy of Wistar Institute.)

(with iron hematoxylin) and a more feebly staining medullary portion, thus giving the appearance of black rings in optical section. At a later stage the apparent rings break up into small crescents and the latter ultimately become rod-like mitochondria filling the cell of the ex-conjugant (Fig. 37).

Opinions are equally divergent regarding the functions of mitochondria in the cell. The earliest suggestion was that of Fauré-Fremiet (1910), who believed that they play some part in connection with the preparation of germ cells, and who was influenced no doubt, by their conspicuous presence in germ cells of Metazoa. Confirmation of this suggestion is furnished in part by observations of Zweibaum (1922), who observed an increase in the fatty acid content of *Paramecium* when ready to conjugate; and confirmed,

in part, by the observations of Joyet-Lavergne (1927) on the differences in number, size, and staining capacity of the mitochondria in the two individuals forming a syzygy in gregarines, thus indicating what he interprets as male and female differentiation.

Numerous observers have maintained that mitochondria are responsible for digestive processes in the cell. The best evidence in support of this suggestion has been furnished by Horning (1928), who, using dark-field illumination, observed mitochondria of heterotrich ciliates adhere to food particles which had been recently ingested; the mitochondria were included in the gastric vacuoles, where they disappeared *pari passu* with the breakdown of the food substances. Horning concludes that, among other possible functions, mitochondria are direct agents in food hydrolysis, playing the part of zymogen granules in the preparation of proteolytic digestive ferments. Causey (1925-1926, etc.) likewise associates mitochondria with food digestion, but he distinguishes between spherical and rod-like forms, the latter being found clustered about the gastric vacuoles (*Endamoeba gingivalis*) while the former are distributed about the cell, where they act as centers of katabolic activity(?). The difficulty of distinguishing between mitochondria and bacteria is obvious, particularly when inside a gastric vacuole, and this has been the main criticism directed against Horning's interpretation, who meets it by describing the stain used which was specific for bacteria and did not stain the mitochondria.

Still other interpretations of the functions of mitochondria have been advocated more or less vigorously by different observers. As active centers they have been associated with the formation of plastids (*e. g.*, leucoplasts, pyrenoids, etc.) filamentous structures of various kinds and with practically all of the cytoplasmic elements of the derived organization. Cowdry (1924) states that more than eighty substances have been claimed to come from mitochondria (see especially Causey, 1926). Not only in cell activities have they been regarded as direct causes, but also as latent or static centers they have been interpreted as cytoplasmic transmitting agents in heredity.

None of the suggested interpretations mentioned above seems to be adequate to explain the purpose of mitochondria. Their universal distribution in Protozoa and in Metazoa indicates some important, possibly fundamental activity which is closely bound up with life of the cell or protoplasm in action. Kingsbury (1912) long since suggested that mitochondria might be associated with cell respiration, a suggestion adopted and enlarged by Joyet-Lavergne (1927) mainly from study of gregarines and coccidia. According to this observer there is a close connection between mitochondria in coccidia and the catalyst glutathion which is a powerful oxidase. (See Needham and Needham, 1926; Tunncliffe, 1926; etc.) He

noted that glutathion is abundant where mitochondria are abundant and *vice versa*. He has also shown that the oxidation-reduction potential (indicated by the expression rH) varies with the distribution of glutathion, low when glutathion is abundant, and high when it is scarce.

While there is considerable evidence to indicate an association between mitochondria and protoplasmic respiration, Joyet-Lavergne himself finds that the association is not always demonstrable and in some cases is highly improbable, and admits that there are probably other functions of the mitochondria.

On the whole we are still in the air as regards the function or functions of mitochondria. The variety of interpretations that have been advanced, and often upon good evidence, suggests that we may have to do here with cellular elements which have a general enzymatic significance and functional both in constructive and in destructive activities. As synthesizing enzymes they may be agents in the selection of different materials from the cytoplasm and in fashioning them into proteins, starch, fats, essential oils, etc. (Cowdry, 1924, 1926; Regaud, 1909, etc.), or by metamorphosis they may give rise directly to plastids of different kinds in the cell (Guilliermond, *et al.*); or by degeneration giving rise to substances like chromidia which Gatenby regards as badly damaged mitochondria. As catalytic enzymes they may act as oxidases in respiration, or as hydrolyzing agents in protein and carbohydrate digestion.

It would seem that we are either demanding too much of one type of protoplasmic substance or that the term mitochondria embraces a large number of substances having different functions, but with a common lipoidal composition in which the protein component is the chief variable. Furthermore, it is not improbable that the Golgi apparatus of the cell represents an extreme variation of this type of substance.

4. **Golgi Apparatus.**—Another cytoplasmic substance which had been identified as a phospholipin (Fauré-Fremiet) or lipoproteid (Bouin, Bowen, Hirschler, King, Horning, Joyet-Lavergne, etc.), and known as the Golgi apparatus, Golgi bodies or (in part) dictyosomes, is also widely distributed in different groups of Protozoa. There are many points of resemblance between this substance and that of mitochondria, particularly in their lipid composition and consequently in their reactions to special stains. In many types the Golgi bodies—dictyosomes—and mitochondria are apparently indistinguishable (*e. g.*, *Gregarina blattarum*, *Spirostomum ambiguum* and *Opalina ranarum* according to Hirschler, 1924), but in cases where, on morphological grounds, they are unmistakable, they differ from mitochondria in their larger size and in their tendency to clump together in masses, or to form a definite reticulum or network (Metazoa) in the vicinity of the nucleus.

In Metazoa the Golgi apparatus appears under two main aspects, one diffused, the other localized. These may be converted one into the other in different stages of cell activity and they should be regarded as variations of the same substance in the cell or of the same structural element. The localized phase was termed by Golgi (1898) the "internal reticular apparatus" from the characteristic net-like structure which it assumes in nerve cells. The granular phase is derived, apparently, from the fragmentation of the fibrils which make up the net structure.

In Protozoa the Golgi apparatus rarely appears in the form of a network, although aggregates of lipoprotein which are found in some cases are regarded as the equivalent of the localized phase typical of metazoan cells. The granular phase, however, is widely distributed in the form of spherules which are larger in size than

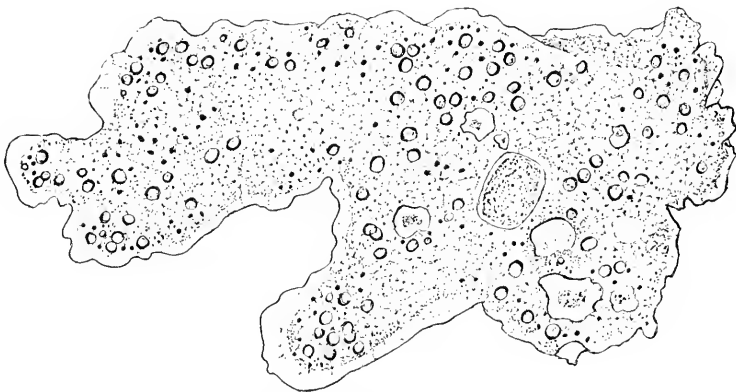


FIG. 39.—Golgi apparatus in *Amoeba proteus*. (After Brown, Biological Bulletin, courtesy of the Marine Biological Laboratory.)

mitochondria and have an osmium blackening lipoidal cortex (osmiophilic portion) and a gray-staining medullary part (osmiophobic portion). This gives them the appearance of black rings or, if imperfectly stained, of crescents or even of rods. In the latter condition they are easily mistaken for mitochondria (Fig. 39).

Golgi bodies as distinct from mitochondria, were first recorded by Hirschler (1914) in *Monocystis ascidiae* a gregarine and similar parasitic forms seem to have been the favorite material for their study. King and Gatenby (1923) and Joyet-Lavergne (1923) described them again in Sporozoa. Since this time, however, descriptions of Golgi bodies from many forms, including representatives from all groups of Protozoa, have been published and various attempts have been made to attach some specific function in the cell to them.

Following the course of development of the subject in Metazoa,

the function of Golgi bodies in Protozoa is generally associated with the secretory activities of the cell. These activities, in turn, fall into different categories but mainly in the group of enzymatic functions. Thus Joyet-Lavergne describes a structure near the tips of young forms (agametes, sporozoites) of coccidia, *i. e.*, that portion which first penetrates an epithelial cell, which he compares with the aerosome of metazoön sperm cells (Fig. 40), the substance of the Golgi body being the source of the cytolyzing agent. There is some evidence also that the so-called parabasal bodies of the Polymastigida and the Hypermastigida are made up of varying proportions of lipid and of proteid substances and have many of the morphological attributes of Golgi bodies (Duboscq and Grassé, 1925). Duboscq and Grassé hold that the parabasals here have a secretory function in connection with the transformation of energy underlying flagellar movements. This, however, has not



FIG. 40.—Golgi apparatus in reproductive cells. 1, 2 and 3, merozoites of *Aggregata cberthi*; 4, sporozoite of same; 5, microgametes of same; 6 and 7, sporozoites of *Gregarina polymorpha*. In all, the Golgi apparatus at anterior end recalls the aerosome of spermatozoa. $\times 1000$ (4 and 5), $\times 2000$ (1, 2, 3, 6 and 7). (After Joyet-Lavergne, Arch. d'anatomie microscopique, courtesy of Masson et Cie.)

been confirmed by later workers and there is high probability that all of the structures which have been called parabasal bodies are not identical in chemical composition (see Hall, 1931; V. E. Brown *et al.*, 1930.). Another type of secretory activity of Golgi bodies in Protozoa is described by Nassonow in connection with the lipoidal membranes, homologized as Golgi apparatus, about the contractile vacuoles and canals of flagellates and ciliates. Nassonow sees in this a special apparatus for the secretion of nitrogenous waste into the vacuole whence it is excreted (see below, p. 170).

There is no satisfactory evidence of the origin of the Golgi bodies in Protozoa. If the parabasals are to be included in this group of substances and there is equal evidence for regarding them as chromatoïd substances, then there is evidence that in some cases they arise from the blepharoplast and the latter from the endobasal body of the nucleus. Causey (1925), upon rather hazy evidence, concludes that the Golgi bodies of *Endamoeba gingivalis* arise as thickenings of the walls of gastric vacuoles.

Further work on these different types of lipoidal elements of the cytoplasm of Protozoa is much needed and a more critical classification of the formed structures of the cell is greatly to be desired, particularly in connection with chromidia, parabasals, mitochondria and Golgi bodies.

5. **Silver Line System.**—Recent technical developments have led to the discovery of a complex system of fibrils in the cortex of ciliates. The way was paved for this by observations of Bresslau (1921) who

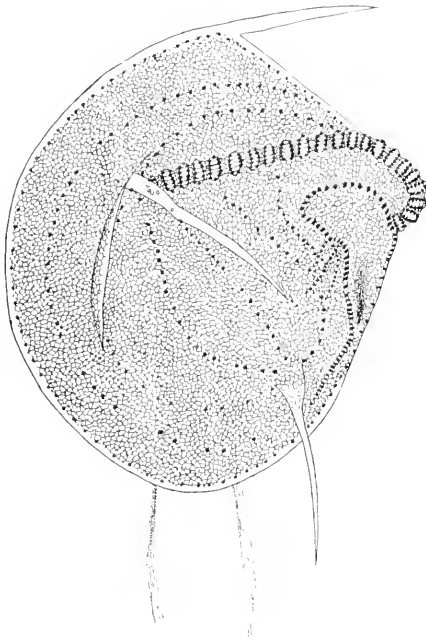


FIG. 41.

FIG. 41.—The silver line system of *Discomorpha pectinata*. Right side. (After Klein, Arch. f. Protistenkunde, courtesy of G. Fischer.)

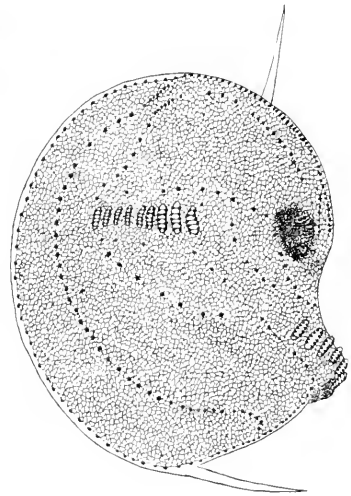


FIG. 42.

FIG. 42.—The silver line system of *Discomorpha pectinata*. Left side. (After Klein, Arch. f. Protistenkunde, courtesy of G. Fischer.)

endeavored to find some chemical (stain) which would cause immediate coagulation of the colloidal structures, especially of the cortex. He used a mixture of equal parts of a 10 per cent opal blue stain and of 6.5 per cent phloxin-rhodamin stain. Ciliates were allowed to dry in this mixture and were then mounted in balsam. Successful preparations made in this way revealed specific types of cortical markings of rectangular or rhomboidal shape. Here areas of coagulation gave evidence of more or less definite boundaries.

B. Klein (1926) also used the method of drying, but drying without coagulation. He argued that small organisms may lose their

water without loss of organization and may re-establish vitality by subsequent hydration (*e. g.*, as in dried rotifers or protozoan cysts). He maintained that normal structures are not disturbed by such desiccation provided the latter process is correctly carried out. Dried forms obtained in this way were treated with a 2 to 3 per cent solution of silver nitrate, which was allowed to act for from eight to ten minutes. The organisms were then submerged in distilled water and exposed to sunlight. Blepharoplasts or basal bodies of the cortex are apparently composed of a substance which

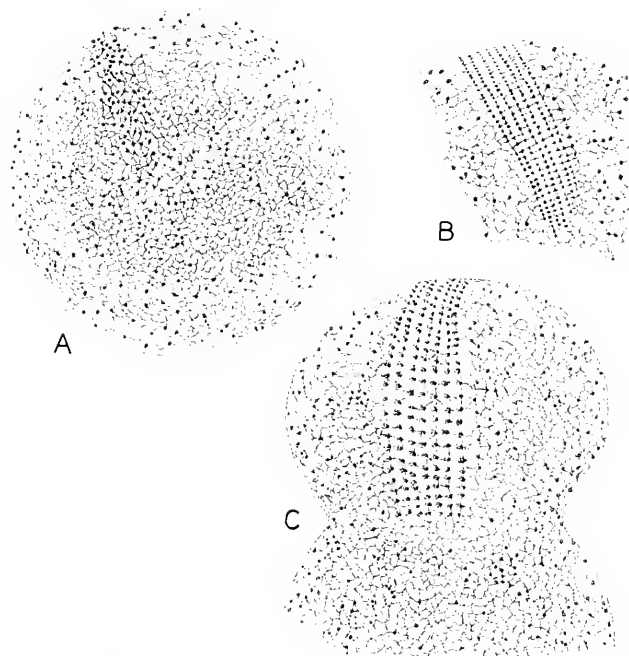


FIG. 43.—*Podophrya fixa*. Silver line system at the time of budding. A, budding region of tentacle-bearing parent organism, aggregation and divisions of primary blepharoplasts; B, later stage with final divisions of blepharoplasts; C, bud in which the blepharoplasts have "satellites" which form the cilia. (After Chatton, Lwoff and Tellier, *Compt. rend. Soc. d. Biol.*, 1929, courtesy of Masson et Cie.)

has an affinity for silver (argentophile substances). The silver is reduced in sunlight and the basal bodies, their connectives and associations are revealed in jet black lines and granules against a yellow background. Klein termed these structures *the silver line system* and has shown that specific systems characterize each species of ciliate (Figs. 41 and 42).

Chatton and Lwoff (1929) have extended the silver nitrate method for fixed material, thus avoiding the somewhat brutal desiccation. Their results in general confirm Klein's.

The silver line systems then are definite aggregates of granules and fibrils which, in some pattern or other, form a part of the cortex of every ciliate. It is present over large stretches of the cell body, even where cilia are absent; for example, throughout the surface of a *Vorticella*. In Suctoria and in some ciliates (*e. g.*, Foettingeriidae) it persists after the embryonic cilia have entirely disappeared, hence to Chatton and Lwoff (1929) the silver line system may have a palingenetic significance, and they term it the *infraciliature*.

The silver line system appears to be, like the nucleus, a definitely organized part of the fundamental organization. It forms a continuum over the cell and persists from generation to generation by division. Cortical structures are formed, apparently under its influence (see Fig. 43) and it may well be a mechanism whereby coördination is effected throughout the organism (see Klein, 1928, 1929, 1930).

CHAPTER III.

DERIVED ORGANIZATION.

I. CYTOLOGICAL.

EVERY protozoön, indeed every organism, has its own particular fundamental organization. This is the specific aggregation of proteins, carbohydrates and fats which, with the imbibition of water containing salts of various kinds and oxygen, will undergo interactions leading to the formation of substances and structures not present before. The changes thus brought about furnish another basic organization in which environmental stimuli, as well as stimuli coming from within, cause interactions which result once more in novel structures or substances. The organization thus is continually changing, each new organization on the basis of that laid down before, until a structural stability results and further changes cease. Such a series of changing organizations is what we usually speak of as *development* or *embryology* or the transition through varying phases from the fundamental to the derived organization. Obviously with a given specific fundamental organization in the same environment the successive changes will always be the same, resulting in the same type of derived organization, and so a species appears to be fixed in type. But different specific types of fundamental organizations have different potentials or possibilities of development which result in different taxonomic types of organisms.

With Protozoa the potential of development is relatively low, but is higher in some groups than in others. Thus the structures of a *Paramecium caudatum* or the endoplasmic structures of a *Giardia* indicate a higher potential in these organisms than in *Amoeba proteus*. But even in the latter there is a vast difference between an encysted amoeba and its actively streaming developed stage, and this difference is brought about by changes in the fundamental organization.

The derived organization then includes the ordinarily invisible structures which result from changes in the fundamental organization, together with the ordinarily visible structures which furnish the basis for classification. The latter for the most part are derived from, or at least are intimately connected with, the former and should not be separated from them. The former are included in the present chapter under the caption *Cytological characters*, while the latter are considered in the following chapter under the heading *Taxonomic characters*.

Changes of the fundamental organization into the derived, occur

in all parts of the cell. The best known are those connected with the nucleus—including its development and differentiation. The changes in the nucleus, like changes in the cell, are brought about through metabolic activity and the results of such changes belong to the derived organization. The formation of nuclei, together with chromatin changes, chromosome formation and spindle formation, belong therefore to the derived and not to the fundamental organization.

A. Derived Nuclei and Derived Nuclear Structures.—1. **The Formation of a Nucleus.**—The formation of the massive type of nucleus during reorganization after conjugation is clearly shown in the case of *Uroleptus mobilis* (Fig. 1, Frontispiece). The young macronucleus is formed by a second division of a fertilization nucleus after conjugation when it appears as a vesicular nucleus with a fine linin reticulum which has no staining capacity. In life it appears like a large, highly refractile vacuole (the so-called “placenta”). It remains in this ghost-like condition for a period of three or four days, enlarging meanwhile and becoming ellipsoidal in form. Chromatin ultimately makes its appearance in the form of minute granules on the nuclear reticulum. These granules increase in number and in size until the characteristic dense nucleus with intense staining capacity results and the nucleus is no longer visible in life¹ (Fig. 27, p. 58). It then divides with the first post-fertilization division of the cell, and each daughter nucleus divides three times (see also p. 315).

2. **Multiple and Dimorphic Nuclei.**—While a single nucleus is characteristic of the vast majority of Protozoa, multiple nuclei are not uncommon and may be found in every group. In some forms, as in many Mycetozoa, the multinucleate condition may be due, not only to repeated nuclear divisions as in *Uroleptus* described above, but to the plastogamic union of originally independent cells, the aggregate being called a plasmodium. In other cases, as in Foraminifera, Radiolaria and Myxosporidia, the multiple nuclei are due to the incomplete division of the cell body after the nuclei have divided; or no attempt at all is made by the cell body to divide. Analogous multinucleate stages are frequently found during certain phases of the life history of many types such as the antecedent stages of sporulation and gamete formation in Rhizopoda and Sporozoa. In still other, and in the typical cases, multiple nuclei are present throughout the entire vegetative life, the number ranging from two to several hundred (*e. g.*, *Actinosphaerium*). Characteristic and familiar examples of binucleate cells amongst rhizopods are *Arcella vulgaris*, *Pelomyxa binucleata*, etc.; amongst flagellates, *Giardia intestinalis* and other species of the same genus.

¹ See also pp. 71 and 315 for development of nucleic acid.

Multiple nuclei are found in *Pelomyxa palustris*, *Actinosphaerium eichhornii*, Calonymphidae and in the majority of Infusoria.

Dimorphic nuclei are examples of multiple nuclei in which a different function in the cell is associated with the different nuclei. Such function may be of a sexual nature as in the Myxosporidia where differences in size and structure indicate a differentiation which may be expressed by the terms male and female nuclei since products of two of them, one from each type, unite to form a fertilization nucleus of the young cell (sporozoite) according to the observations of Schroeder, Keysselitz, Naville and others (see p. 326). Or the function may be of a metabolic nature in one type and reproductive in the other, as in the Infusoria, where the two types show great differences in form and size. Here the nucleus having to do with metabolism makes up a large part of the volume of a cell and is usually of relatively large size, hence is called the *macronucleus*, while nuclei having to do with reproduction and fertilization are always minute and are called *miconuclei* (Fig. 44). Usually the micronucleus is closely attached to the macronucleus and, in some cases, may be partially hidden in a depression or pit in the macronucleus, or it may be entirely independent of the larger nucleus and lie freely in the cytoplasm. A typical example of dimorphic nuclei is shown by *Paramecium caudatum* (Fig. 23, p. 50).

The derived forms assumed by macronuclei and the number in a single cell vary within wide limits. The most generalized condition is a simple, spherical form; but ellipsoidal, rod-like, horse-shoe-shape, beaded and branched macronuclei are not uncommon. The beaded forms frequently appear like several separated nuclei but the segments are usually enclosed in a common membrane contracted at the nodal points, the entire aggregate forming a single nucleus (*Spirostomum*, *Stentor*, *Amphileptus*, *Uronychia*, etc.). The size of the macronucleus bears no constant relation to the size of the organism (Fig. 44).

Miconuclei do not differ much in form but vary in structure from typical vesicular to compact massive types. Their number in the cell likewise varies from 1 to as many as 80 or more (*Stentor*). They are never connected with one another, but are quite independent and distributed at intervals along the sides of the macronuclei.

There is little or no evidence of the phylogenetic origin of these dimorphic nuclei which are distinctive of the Infusoria. In ontogenetic origin the nuclei are invariably derived after conjugation from division products of the fertilization nucleus, the latter being formed by the union of two miconuclear elements. Hence the statement is usually made that macronuclei arise from miconuclei, a statement which is not strictly accurate, since the fertilization nucleus is neither one nor the other, but merely a cell nucleus of a fundamental organization. In some cases macronuclei and micro-

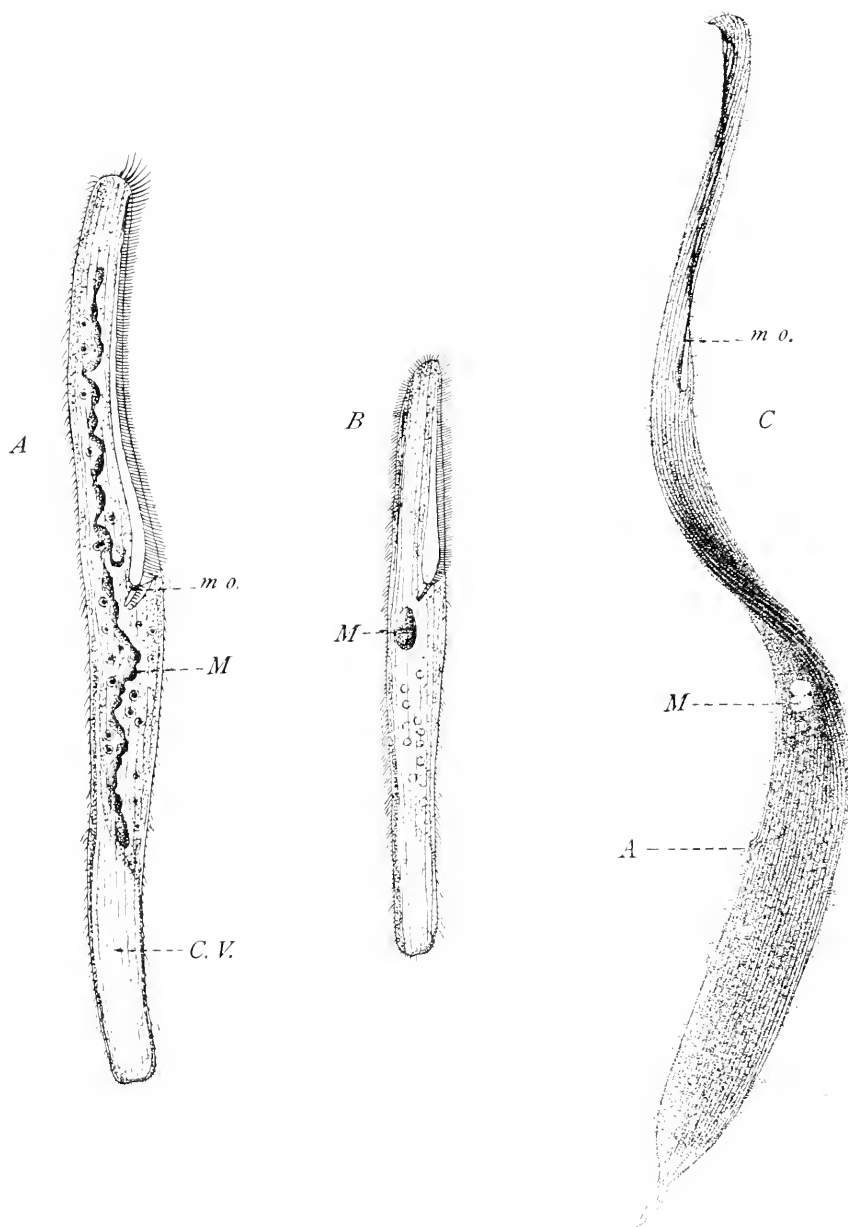


FIG. 44. - Illustrating volume relations of macronuclei and cell body. A, in *Spirostomum ambiguum*; B, in *Spirostomum lutes*; and C, *Lionotus procerus*; (A) anal pore; (C.V.) contractile vacuole; (M) macronucleus; (mo.) mouth. In *Lionotus* the mouth is a long slit, in *Spirostomum* a circular opening at the posterior end of the peristome. (A and B, after Stein; C, original.)

nuclei are not differentiated until the third division of the fertilization nucleus (e. g., in *Cryptochilum nigricans*, *Paramecium caudatum*, *Par. putrinum*, *Bursaria truncatella*, *Carchesium polypinum*, *Opercularia coarctata*, *Ophrydium versatile*, *Vorticella monilata*, *V. nebulifera*, etc.); in other cases differentiation occurs after the second divisions (e. g., in *Anoplophrya branchiarum*, *Colpidium colpoda*, *Dilinium nasutum*, *Glaucoma scintillans*, *Leucophrys patula*, *Lionotus fasciola*, *Paramecium aurelia*, *Par. bursaria*, *Blepharisma undulans*, *Spirostomum teres*, *Euplotes patella* and *charon*, *Onychodromus grandis*, *Stylonychia pustulata*, *Uroleptus mobilis*, etc.); and in still other cases the differentiation takes place after the first division (e. g., *Chilodon uncinatus*). In all cases both macronucleus and micronucleus are formed by metamorphosis of such products of division of the original nucleus after conjugation, the former by a remarkable increase in size and in quantity of chromatin, the latter by reduction in size and concentration of the chromatin; the former becomes a metabolic organoid of the cell, the latter a germinal organoid.

Mention may be made here of the vesicular nuclei which arise by a process of so-called free-nuclei formation from chromidia, the evidence for which is difficult to interpret otherwise. It rests, in the main, on the observation of Hertwig as early as 1876, and again in 1899; of Schaudinn in 1903; of Lister, 1905; of Goldschmidt in 1907; Elpatiewsky in 1907, and Swarczewski in 1908. In all cases the free nuclei arise by the association of chromidia or chromidiosomes which have been derived from the nucleus and distributed in the cytoplasm (see p. 69). Both Elpatiewsky and Swarczewski describe the formation of the minute gametes of *Arcella vulgaris* by the fragmentation of the cytoplasm into minute cells about these free nuclei. These gametes move off as minute amebae leaving the parent with its "primary" nuclei, which ultimately degenerate. Each of these gametes contains at first a few scattered granules derived from the chromidial mass which ultimately unite to form the gamete nucleus. The process is more minutely described by Goldschmidt in connection with the mastigameba *Mastigella vitrea*. Here a chromidial mass forms on the outside of the nuclear membrane by transfusion of chromomeres (Fig. 45). After separation of this mass from the nucleus, the chromatin granules come together in groups and form nuclei about which minute gamete cells are cut out from the cytoplasm while the primary nucleus remains intact. The same thing in principle is illustrated by the origin of the germ nucleus inside the nucleus of *Gregarina cuneata* and other gregarines as well (see Fig. 55, p. 101). A somewhat similar mode of formation of the microgamete nuclei of *Coccidium schubergi* was earlier described by Schaudinn. This type of nucleus formation, according to Minchin, represents the possible origin of Protozoa of "cellular grade" from

bacteria-like organisms of non-cellular grade, in which the chromatin is permanently distributed. Doflein (1916) remains skeptical in regard to this type of free-nuclei formation and Kofoid (1921), apparently without investigation of free-living forms, maintains that such free nuclei are intracellular parasites. It is evident that the burden of proof here rests with the critics. (See also p. 71.)

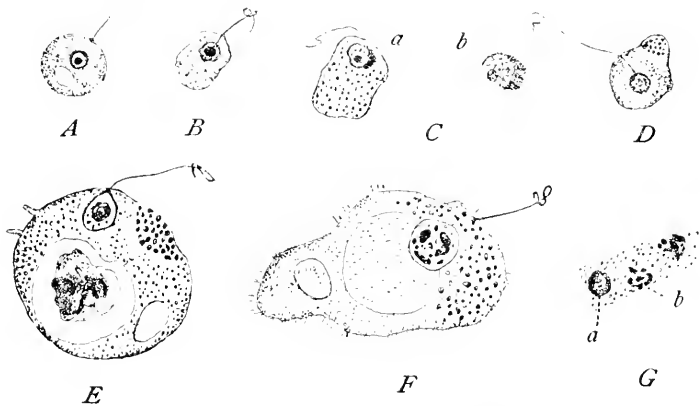


FIG. 45.—Chromidia formation in *Mastigella* and *Mastigina*. A, B, young forms of *Mastigella vitrea* prior to chromidia formation; C, chromidia arising from the nucleus; D, young form of *Mastigina setosa* with accumulation of chromidia; E, F, mature stages of *M. setosa*; G, formation of gametic nuclei (a) from scattered chromidia. (After Goldschmidt.)

3. **Nuclear Derivatives During Division.**—The substances composing nuclei—karyolymph, plastin, chromatin and kinetic elements—are apparently inert during vegetative life, inert at least so far as demonstrable activity is concerned. Metabolic activities which result in cell division, however, are manifested periodically by characteristic changes in these substances, and structures not present before—spindle elements and chromosomes—are formed which, after a brief existence, pass again into the apparently inert condition of the vegetative nucleus, that is, they are reversible. Theoretically such transient phases are the most important of all stages in the life history for they involve the formation and division of chromosomes, which are regarded as the vehicle of hereditary characteristics, and the kinetic elements, which are regarded as instrumental in bringing about such formations and divisions.

(a) *Origin of Chromosomes and of Intrannuclear Spindles at Division.*—The nucleus is the most complex of the formed organoids of the cell, and its reproduction involves growth and division of

its different elements. These may be more or less independent in their division, or they may be united in various simple or complex combinations during the division processes. Or the nuclear elements may be combined with extranuclear cytoplasmic elements to form a characteristic division figure representing a most highly perfected mechanism for the equal distribution of the more important cell elements which are thus perpetuated from generation to generation by equal division. Such a perfected mechanism, termed a karyokinetic or mitotic figure, is characteristic of nuclear division in cells of the Metazoa and of higher plants, the combination of processes whereby the constituent parts are equally distributed to daughter cells being known as indirect division, karyokinesis, or mitosis. In Metazoa such processes involve division of centrioles and centrosomes, formation of a fibrillar spindle figure, dissolution of the nuclear membrane, aggregation of chromomeres into compact chromosomes which are identical in size, shape and number in corresponding cells of all individuals of the same species, and the longitudinal division of each chromosome in all somatic cells, separation of the daughter chromosomes and reconstruction of the daughter nuclei. In all Metazoa the processes of mitosis differ only in minor details and mitosis is the characteristic type of nuclear division, although direct division, whereby the nucleus divides without the formality of centrosomes and spindle or chromosome formation is known in a few cases.

In Protozoa, on the other hand, there is no one type of nuclear division common to all forms. Here we find gradation, in the association of constituent nuclear and cytoplasmic kinetic elements during division resulting in an enormous variety of division types. These vary in complexity from a simple dividing granule to mitotic figures as elaborate as in the tissue cells of higher animals and plants. Some observers see in these diverse types a possible evolution of the mitotic figure of Metazoa and use them as one would use the separate pieces of a picture puzzle to reconstruct its past history in development. Terms like "promitosis" (Naegler), "mesomitosis" (Chatton) and "metamitosis" (Chatton) may serve a useful purpose to indicate general types of the association of nuclear and cytoplasmic elements during division, but when an effort is made to give a specific name to each step in an increasingly complex series the result is a confusion of terms which defeats the useful purpose intended. Thus Alexieff proposes a large number of specific names, not all his own, it is true, for protozoön division types which he regards as sufficiently definite to permit of recognition.¹

Because of the multitude of diverse types of division figures in the

¹ These terms include Promitosis, Proteromitosis, Haplomitosis, Cryptohaplomitosis, Eurypaimitosis, Cyclomitosis or Polymitosis, Polyrheomitosis, Metamitosis, etc.

Protozoa the difficulty of treating them in any general way has been admitted by all students of cytology as well as by protozoölogists. I shall endeavor here to convey an idea of this diversity and at the same time to describe some of the more frequent types of division figure without confusing the issue still more by my own views as to their possible relations to one another or to any process of evolution. The apparent object of the complex mechanism of a mitotic figure is to ensure the exact bipartition of the hereditary complex represented by the chromosomes. These elements, and the chromatin of which they are composed, are the most important, while the kinetic elements with which they are associated in division, as agents in the process, are of secondary importance.

The conception of chromosomes, as they appear in Metazoa, is definite and consistent throughout. They are formed at certain periods of cell activity (prophase of division) by the aggregation of chromomeres into nuclear bodies of definite form and size, and the number is constant for all somatic and germ cells in the same species. Each chromosome is specific and retains its individuality from generation to generation by cell division. At the end of division it resolves itself into an aggregate of chromomeres which, in some cases, are found to be confined to a definite part of the nucleus (chromosomal vesicle), at the prophase of the following division these same chromomeres re-collect to form the chromosome which divides into equal parts by longitudinal division. The chromosomes, furthermore, are qualitatively different, no two of them being identical. During meiosis, finally, the number of chromosomes is reduced to one-half by the separation of half of them from the other half, thus resulting in two types of nuclei which are quite different in chromosomal make-up.

An analysis of the literature dealing with the so-called chromosomes of Protozoa shows that there has been little or no consistent use of the term. To many observers the word is used to describe any chromatin which happens to be in the center of a division figure and without regard to other conditions which limit and define the chromosome as a definite thing, viz.: A definite number in the cell, longitudinal division, qualitative differences, reduction in number at maturation, etc. It is true that in only a few cases among the Metazoa has it been demonstrated that chromosomes have a specific individuality combined with qualitative differences, but the striking similarity in dividing chromosomes of all Metazoa and the same complicated mechanism in all cases for their equal distribution to daughter cells, give a basis upon which the generalization rests. We have no basis, however, for extending the generalization to Protozoa, for here we have absolutely no evidence of qualitative differences and but little evidence of individuality. In some cases we have evidence that structures in the center of a division figure

are formed by the fusion of chromomeres, and some evidence that such structures divide longitudinally. These two conditions, which are relatively rare, are the only conditions whereby many of the so-called chromosomes of Protozoa resemble those of Metazoa, and if we use the term chromosome at all it should be in a definite, limited, morphological sense and only for those nuclear structures of Protozoa which conform in origin and in fate to chromosomes of Metazoa. I shall use the term chromosome, therefore, only for those compact intranuclear aggregates of chromomeres which divide as unit structures and which are resolved into chromomeres after such division.

A brief review of some of the frequently recurring types of chromatin structure at the time of nuclear division will show how difficult it is to speak with assurance of chromosomes in Protozoa. The series is not to be construed as an effort to establish a phylogenetic chain of stages culminating in well-defined chromosomes, nor as a means of pointing out that one is a "higher" type than another. Certain vital functions are undoubtedly associated with the nucleus and with the chromatin of the nucleus, and the fact that some types of organisms with peculiar nuclei continue to live and reproduce is evidence enough that such nuclei are adequate for their needs. The variations in type arise through the association of chromatin with other nuclear or cytoplasmic constituents, and this involves more or less formality in preparation for its perpetuation by exact bipartition to daughter cells. All traces of chromosome formality, however, as well as reduction processes, appear to be absent in gamete nuclei formed by rhizopod chromidia.

One group of types is represented by massive nuclei as found in the macronuclei of the Infusoria. Here the resting nuclei are made up of closely packed granules or chromomeres and there is little formality or mechanism associated with their division during reproduction. Each granule elongates and divides into two parts, thus doubling the number of chromomeres. The mass thus formed is passively distributed to the daughter cells by division of the nucleus through the center. It is a quantitative distribution, for the daughter nuclei do not contain representative halves of the individual chromomeres and the inference is that all of the chromomeres are qualitatively identical. To this type also I would assign the peculiar chromatin granules of *Dileptus gigas* which are distributed throughout the protoplasm unconfined by a nuclear membrane. Each granule divides where it happens to be and with the majority of granules both halves remain in one daughter cell after division (Fig. 46).

These macronuclei, however, particularly the band-form types of the hypotrichous and peritrichous ciliates and the multinucleate chain-form types of hypotrichs, may undergo characteristic pre-

divisional changes which for lack of a better term may be called "purification" processes. These are associated with the so-called "Kernspalt" or nuclear cleft which for decades has been an enigma. A simple case is that of *Uroleptus halseyi*, an hypotrichous ciliate



FIG. 46.—Division of *Dileptus gigas*. The longated chromatin granules (C) divide where they happen to lie. (Original.)

with, normally, eight macronuclei which are separate and arise by three consecutive divisions of the division nucleus (Fig. 47).

When first formed these eight nuclei are composed of homogeneous chromatin granules similar in size and in staining capacity. After a period of normal growth and activity, and particularly at the approach of a division period, a different type of granules appears in each of the nuclei. These, which I have called the "X granules" (Calkins, 1930), stain intensely with iron hematoxylin but disappear entirely, by hydrolysis of the Feulgen technique; furthermore, they stain green with the acid component of the Borrel stain. One of these X granules, usually more prominent than the others, lies in the anterior third of each nucleus. Its substance spreads out in a zone or flat plate extending transversely through the nucleus (Fig. 47, *b, c*). This plate reacts to stains exactly like the X bodies and disappears by hydrolysis in the same way. The nuclear cleft forms just posterior to this plate and the anterior third of each nucleus, viz.: that portion anterior to the cleft is thrown off and disappears in the cytoplasm. Other X granules which may be present are similarly discarded, leaving the bulk of each nucleus with only one type of granule. The process occurs in all eight nuclei at the same time, and after it is completed, the residual "purified" nuclei all fuse to form a single macronucleus which, after condensation, becomes the division macronucleus (Fig. 128, p. 246). The substance of the X granules thus appears to have a cytolyzing effect on the nucleus and is the agent in formation of the nuclear cleft.

Ivanic (1929) describes two deeply-staining (iron hematoxylin) granules which appear at the ends of the curved macronucleus of *Euplotes patella*. These he interprets as centrosomes, and argues for a promitotic division of the macronucleus. It is more probable that these are X granules marking the beginnings of two nuclear clefts which pass from the extremities of the nucleus to the center where they disappear, as shown by Kidder (1932) in the case of *Conchophthirius mytili*. Turner (1930)

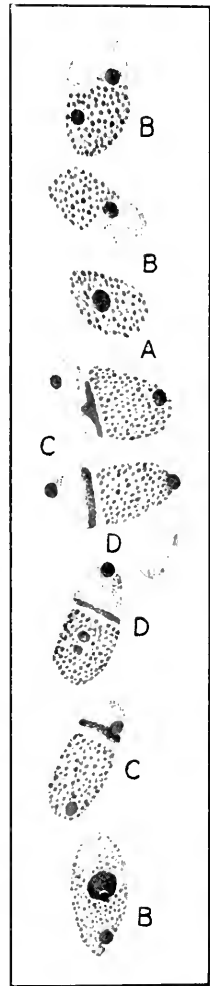


FIG. 47.—*Uroleptus halseyi*. X bodies. Chromatin elimination and nuclear cleft in preparation for division of the macronucleus. (Original.)

describes these as "reorganization bands," each band consisting of a "reconstruction plane" (unstained) and a solution plane (Fig. 48). Turner suggests that "The reorganization bands cause a phase reversal of a colloidal system in which the chromatin changes from a continuous (reticulum) to the dispersed (granular) phase." Certainly his descriptions and figures indicate a marked change in the chromatin after the "absorption bands" have passed by. A similar difference is apparent in the chromatin granules anterior and posterior to the nuclear cleft in *Uroleptus halseyi*, but here the portion with the finer granules (reticulum?) is cast out. With this change in the chromatin granules the macronucleus of *Euplotes* is ready for division.

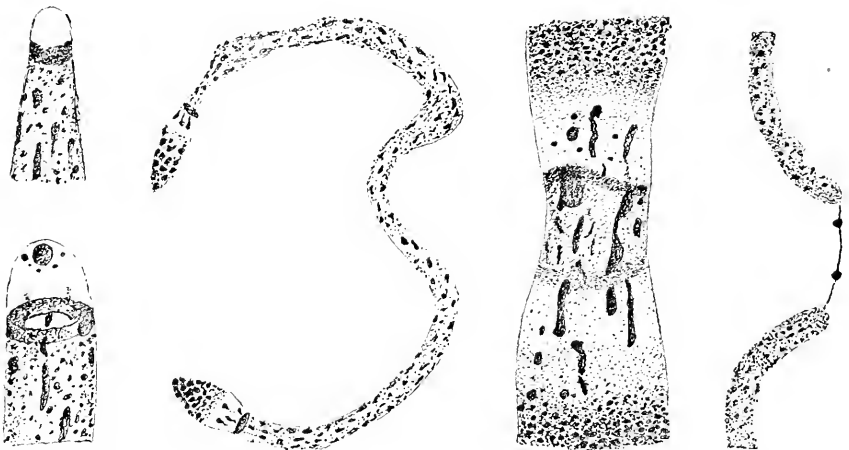


FIG. 48.—*Euplotes patella*, macronucleus with "absorption bands" which start at the two ends and progress to the middle where they meet. At division, two small granules are discarded in the cytoplasm. (After Turner, from University of California Publications in Zoology, 1930.)

In another group of types we have to do with vesicular, endosome-containing nuclei. The endosome may or may not contain an endobasal body. It is well represented by the nucleus of *Spongomonas splendida* according to the observations of Hartmann and Chagas (Fig. 49). Here, according to the description, the mass of chromatin of the resting nucleus divides into two equal masses without fragmentation at any stage. Similar conditions are shown by the gregarine *Gonospora varia* according to Brasil (1905), by *Sappinia diploidea* according to Hartmann and Naegler (1908), by the simpler amoebae and, in a striking way, by *Haplosporidium ctenodrilae* according to Granata (1915).

In another group of types the chromatin of the resting vesicular nucleus is contained also in a definite endosome, but, in preparation

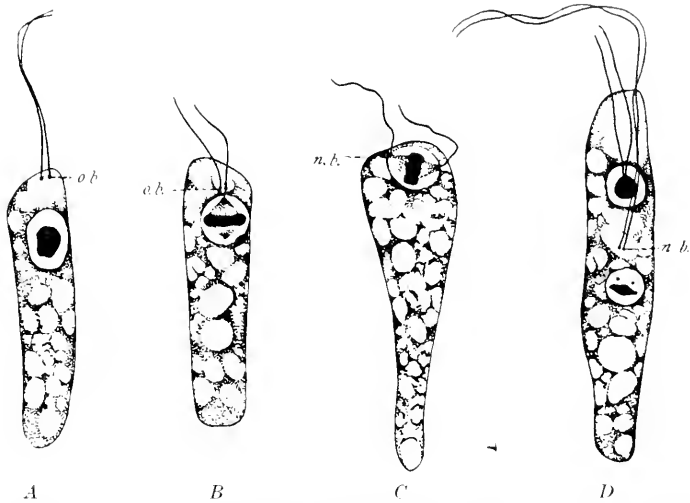


FIG. 49.—Division of *Spongomonas splendida* Hart. and Ch. The old flagella are discarded and new ones form from the centrioles (C and D). (o.b.) old blepharoplasts; (n.b.) new blepharoplasts. (After Hartmann and Chagas.)

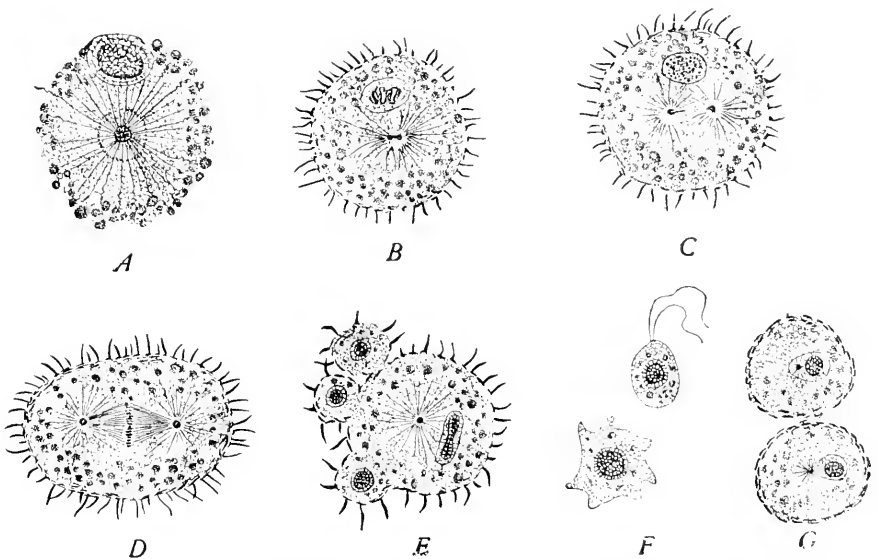


FIG. 50.—Nuclear division and budding in Heliozoa. A, Vegetative cell of *Sphaerastrium* with axial filaments focussed in a central granule (centroblepharoplast); B, C, D, division of central granule and spindle formation in *Acanthocystis aculeata*; E, F, formation of buds of same; G, exit of central granule from the nucleus of young cells. (After Schaudinn.)

for division, the endosome fragments into minute chromomeres, which may be strung out in lines through the nucleus, these strings being divided transversely at division. Or the chromomeres may be aggregated in a fairly homogeneous transverse plate in the center of the dividing nucleus (Fig. 51). The former condition is illustrated by the nucleus during vegetative division of *Actinosphaerium eichhornii* according to Hertwig, the latter condition by *Sphaerastrum* and *Acanthocystis* (Fig. 50), *Collodietyum* (Fig. 51), *Paramoeba chaetognathi*, or the myxomycete *Comatricha obtusata* according to Lister.

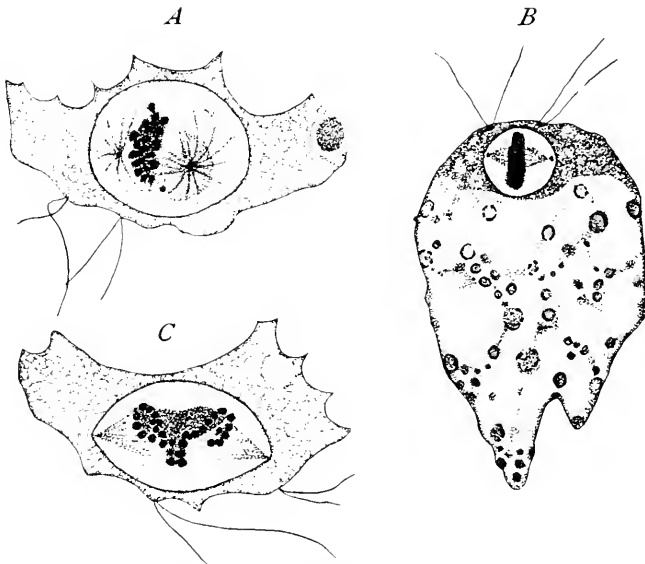


FIG. 51.—Nuclear division in *Collodietyum tricitiatum*. (After Bělař.)

A slight modification of this type is shown by nuclei containing multiple endosomes as in *Pelomyxa binucleata* which fragment at periods of division, giving rise to a granular nuclear plate (?) which presumably divides to form the daughter plates as shown in Schaudinn's well-known figure or to division figures like that of *Centropyxis aculeata*.

Another widely distributed type of division figure is derived from vesicular nuclei in which the chromatin is not contained in one or more endosomes but is distributed peripherally about the nucleus where it usually forms a distinct chromatin reticulum. Such nuclei usually contain an endosome which may be the most conspicuous structure of the nucleus. In *Amorba crystalligera* the peripheral chromatin appears to be passively divided without any appreciable change in its make up. In *Amorba respertilio* the peripheral chro-

matin is similarly divided and distributed but the endosome apparently contains some chromatin in addition for a complete division figure is formed from its substances, chromatin-like granules forming a nuclear plate (Fig. 52). In other cases, as for example *Endamoeba intestinalis* and *E. cobayae*, the peripheral chromatin is broken up into chromomeres, which collect in the center of a spindle

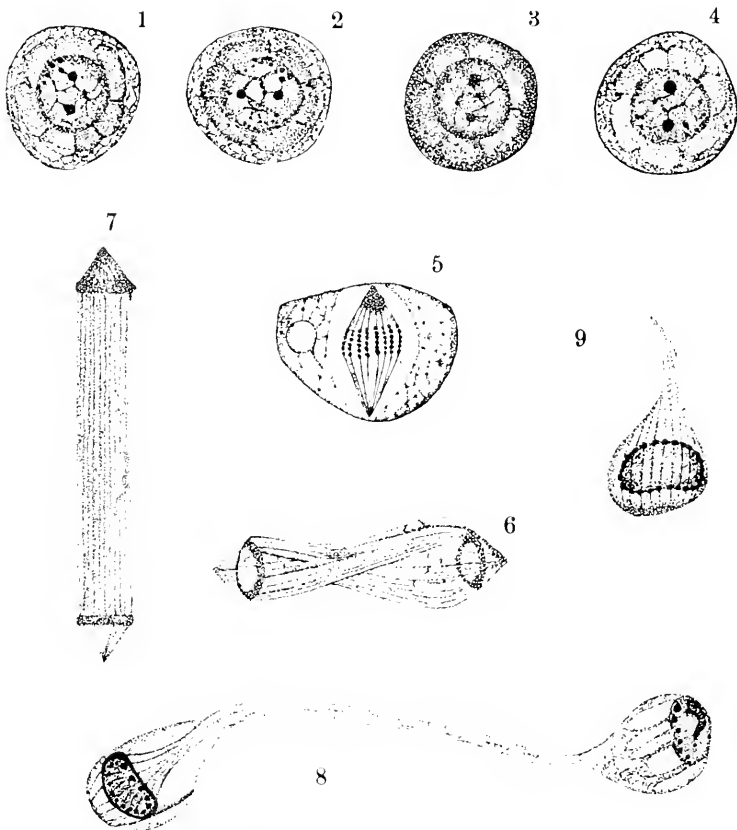


FIG. 52. — *Amoeba respertilio* Dof. Origin of the spindle within the nucleus (1, 2), nuclear division (5, 6, 7), and reconstruction of nuclei after division (3, 4, 8, 9). (After Doflein.)

from the lining of the nucleus and with centrioles at the poles. In *Chlamydomorphys* the endosome apparently divides before it disappears, the chromosomes being formed from the peripheral chromatin.

In still another general type, derived also from vesicular nuclei, the chromatin in the form of chromomeres is suspended in a loose reticulum. In *Opalina* chromatin appears to be aggregated in a few larger granules, which divide where they happen to be without

further formality, the nucleus meantime assuming an indefinite division figure. More frequently, however, the chromomeres are suspended between an endosome and the nuclear membrane, as in *Eimeria schubergi*, or various species of *Trypanosoma*. In some of these, at division the chromomeres appear to form a nuclear plate, and are distributed in equal groups to the daughter nuclei (Fig. 51).

In a final group of types of nuclear division figures either from massive or vesicular nuclei, the chromomeres are derived from the fragmentation of endosomes or from a chromatin reticulum. The common feature in this large group is the fact that these chromomeres unite secondarily to form definite chromatin bodies which satisfy, in part at least, the definition of chromosomes as given above. These chromosomes are divided equally, one-half going to each pole of the division figure. In some cases it is obvious that their

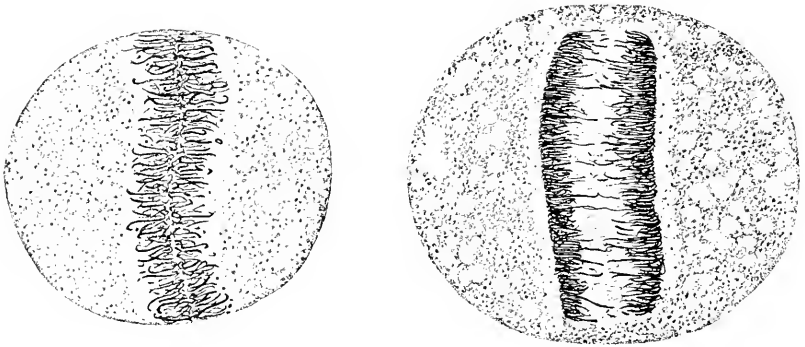


FIG. 53.—Metaphase and anaphase of nuclear division in the radiolarian *Aulacantha scolymantha*. $\times 300$. (After Borgert, Zoolog. Jahrbücher, courtesy of G. Fischer.)

division is longitudinal, but in the majority of cases it cannot be ascertained with assurance whether their division is longitudinal or transverse. Nuclear figures of this general type may be divided into two groups, in one of which the chromosomes are too numerous to permit of decision as to their constant number, and the second comprising forms in which the chromosomes are constant in number and in some of which this number is reduced to one-half at meiosis. In the first of these groups we would include types like *Euglypha alveolata*, the various species of *Paramecium* and some Radiolaria (Fig. 53). In the second group we would place such forms as *Actinophrys sol*, *Aggregata eberthi*, *Trichomonas* and allied flagellates, *Trichonympha* and related forms, and the majority of ciliates in which the maturation processes are known.

In *Euglypha alveolata* the chromatin of the vesicular nucleus is distributed throughout the resting nucleus. During the early divi-

sion stages the chromomeres are rearranged in rods or fibrils which form a more or less definite skein within the nucleus; this skein fragments into a large number of chromosomes which, according to Schewiakoff, are longitudinally divided. A more aberrant history is followed by the chromatin of the nuclei of various species of *Paramecium*. In *Paramecium caudatum* the micronucleus belongs to the massive type, and there is no satisfactory account of the origin of chromosomes in vegetative division (Fig. 35, p. 67), but the number is much smaller than in the meiotic divisions (see Fig. 147, p. 297).

A more definite metazoan type of chromosome formation is shown by the organisms with a definite number of chromosomes which is reduced to one-half at meiosis. Here the number of chromosomes is usually smaller and their individual history during nuclear division is less difficult to make out. A good example, typical of the more complex flagellates, is *Trichonympha campanula*, as described by Kofoid and Swezy. Here the resting nucleus contains a large granular endosome. In the prophase of division the granules of this endosome give off chromatin along the walls of the linin reticulum until a definite skein stage results (Fig. 54). Double chromosomes, 26 in number, and formed by the splitting of the spireme segments, make up a definite nuclear plate. They are attached by intranuclear fibers to the daughter blepharoplasts and are divided longitudinally with the division of the nucleus. The original connecting fibrils between the separating halves of the blepharoplast ("centroblepharoplast") remain at all times outside the nuclear membrane, hence it is called a paradesmose by Kofoid and Swezy. One of the chromosomes appears to be different from the others, both in resting and division stages, and is called the heterochromosome, although its function or significance is quite unknown. Similar odd chromosomes are known in some Gregariniidae and Coccidiida where the vegetative stages are haploid, as well as in other polymastigote flagellates. Except for the complications brought in by the extensive neuromotor apparatus of *Trichonympha campanula*, the division figures of other related flagellates are quite similar, although the number of chromosomes is usually smaller. Thus Kofoid and his collaborators found about 24 in *Leidyopsis sphaerica*, 12 in *Trichomitus termitidis* and 4 in *Giardia muris* (Fig. 54, p. 100).

A smaller number of chromosomes is likewise found in a number of the Gregariniida, and their history in division approaches that of metazoan chromosomes. Thus in the case of *Monocystis rostrata* Mulsow describes 8 definite chromosomes formed from a portion of the nuclear chromatin, the number being reduced to 4 in the gamete-forming divisions (Fig. 55). Shellack and Léger, also, have described similar chromosomes in *Monocystis ovata* and in *Stylorhynchus longi-*

collis. In the latter case, also, there is a peculiar lagging heterochromosome ("axial chromosome") of unknown significance.

(b) *Origin of Fertilization (Meiotic) Chromosomes*.—In practically all Protozoa the sequence of stages leading to formation of chromosomes which enter into pronuclei is quite different from that of the division nuclei. This phenomenon is one of the final acts of development and in Protozoa represents a last stage of differentiation of

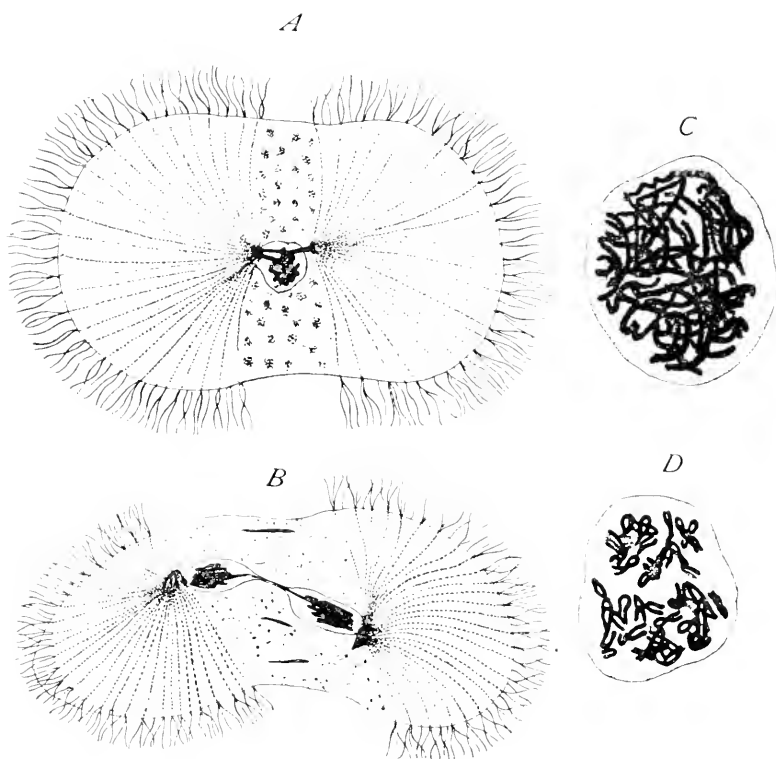


FIG. 51. *Trichonympha campanula* in division. A, and B, prophase and anaphase of nuclear division; the divided centrophlepharoplast forms the poles of the spindle and are connected by a paradesmose. C and D, breaking up of chromosome spireme into chromosomes which show a tendency to unite in pairs. (After Kofoid and Swezy.)

the derived organization of the nucleus. Here, as in Metazoa, there are at least two maturation divisions, while in ciliates the number is increased to three. As in Metazoa, one or the other of the maturation divisions is a reducing division or reduction may be parcelled out in both divisions, the end-result being that the number of chromosomes is reduced by one-half, *i. e.*, from the diploid to the haploid number. As in Metazoa, the first of the meiotic divisions

is usually preceded by activity in the nucleus resulting in a skein-like arrangement of the chromatin (spireme) from which definite chromosomes emerge. This spireme, in Metazoa, is the stage of pairing of homologous chromosomes, *i. e.*, chromosomes representing the same characteristics in the two parents. By such association

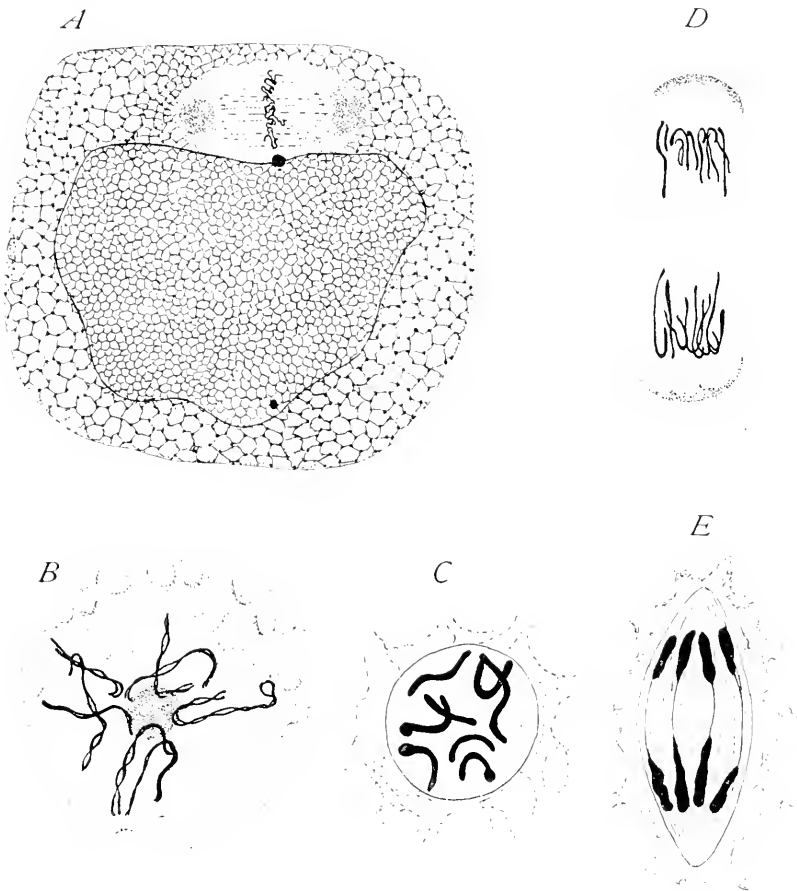


FIG. 55.—*Monocystis rostrata*; chromosome reduction. A, Formation of spindle in pseudo-conjugant; B, C, nuclear plates of progamous divisions, 8 chromosomes; D, anaphase of same; E, anaphase of last progamous division, the number of chromosomes is here reduced from 8 to 4. (After Mulsow.)

the chromosomes when fully formed are apparently reduced to the haploid number, but each is double, and the actual reduction occurs in the ensuing divisions.

In Protozoa the antecedent or prophase stages of the first meiotic division rarely conform to the metazoan scheme, but in most cases

there are stages which have some resemblance, at least, to spireme formation of the metazoan type. For *Actinophrys sol*, Bělař (1922) has described in great detail the transformations of the chromatin of the vesicular nucleus in the first maturation division. A spireme,



FIG. 56. Chromosomes of *Aggregata berthi*. Letters *a* to *f*, or *a'* to *f'*, designate the haploid groups. *A*, prophase of the first division (male); *B*, nuclear plate of same; *C*, anaphase groups at first division; *E*, chromosomes in macrogamete nucleus before fertilization; *F*, chromosomes in zygote nucleus (diploid); *G*, paired chromosomes in nuclear plate of first zygote division; *H*, early anaphase groups of first zygote division, and separation of homologous haploid groups. (After Dobell and Jameson.)

passing through bouquet, pachytene, strepsineme and synapsis stages, into double chromosomes of the metaphase nuclear plate, are strikingly similar to analogous stages in metazoan meiosis (Fig. 157, p. 309). Here there is very little to suggest individuality

of the chromosomes, but in the coccidian *Aggregata cherthi* where reduction is zygotic (the vegetative stages being haploid) the twelve chromosomes unite in six pairs of homologous chromosomes (Dobell) (Fig. 56) and a modified spireme occurs in the progamous divisions. Similar but less definite conditions are shown in the gregarine *Diplocystis schneideri* as described by Jameson (1920) (Fig. 158, p. 310). A somewhat simplified history of the chromatin was given by Mulso (1911) for the progamete nucleus of the gregarine *Monocystis rostrata* (Fig. 55). Here, differing from *Diplocystis*, reduction is gametic and the vegetative stages are diploid. The resting nucleus is vesicular and the chromatin granules join chain-wise to form eight chromosomes. These split lengthwise in the metaphase stage, a preliminary spireme stage, apparently, being absent.

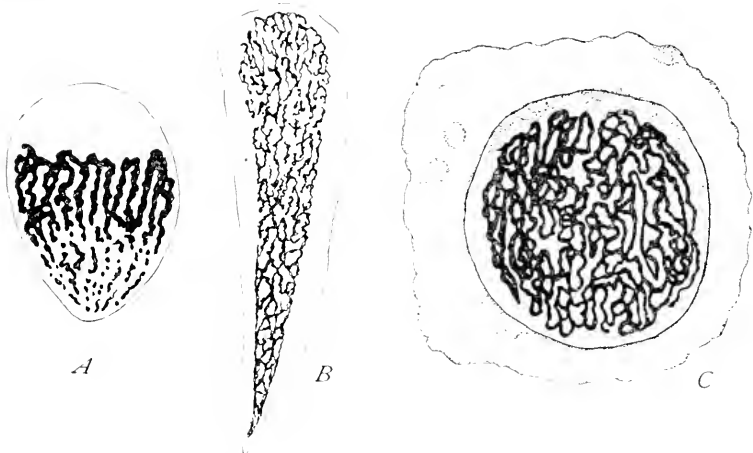


FIG. 57.—Micronucleus of *Paramecium caudatum* in the prophases of the first meiotic division. *A*, Early stage in the formation of chromosomes; *B*, elongation of the nucleus prior to crescent formation; *C*, metaphase of the first division. Dehorne describes the entire chromatin aggregate as forming one highly convoluted chromosome. (After Dehorne.)

In the hypermastigida (*Trichonympha*, *Dincunympha*, *Staurojoenina*, etc.) flagellates, fertilization is unknown, but ordinary nuclear division is preceded by formation of long chromosomes which give the appearance of a spireme.

Quite a divergent type of spireme formation is found in the ciliates where the chromatin is massed in homogeneous micronuclei. In *Paramecium caudatum* the micronucleus elongates to form a bar nearly equal in length to the macronucleus (Fig. 57). The massed chromatin becomes granular, and the granules stretch out in an elongate network which, in the following crescent phase, breaks up into a multitude of double chromosomes.

In other ciliates the massive micronucleus gives rise to a group of chromatin granules which form an umbrella shape mass at one pole of the nucleus (*Didinium*, *Oxytricha*, *Euplotes*, *Uroleptus*, etc.). This has been described as the "candelabra" stage by Collin (*Auoplophrya*) or the "parachute" stage by Calkins (*Uroleptus*). The number of granules is much larger than the number of chromosomes of the later reducing division, but this large number is halved at the first meiotic division (Fig. 32, p. 64). With the second division the remaining granules usually fuse to form the diploid number of chromosomes and this number of chromosomes is finally reduced to one-half. At the third division these resulting haploid chromosomes become granular and are divided transversely.

B. Derived Organization; Cytoplasmic Changes.—1. **Cytoplasmic Chromatin.**—During the metabolic activities of the cell, substances which are undoubtedly derived from the nucleus are cast off into the cytoplasm. The majority of these are not represented by demonstrable structures of the cytological organization. Thus in *Uroleptus (mobilis* and *halseyi*) fully one-third of the macronuclear chromatin is shed into the cytoplasm at each division and disappears as chromatin, while in ciliates generally the entire substance of the macronuclei and a variable proportion of micronuclear substance (fifteen-sixteenths in *Uroleptus mobilis*) is absorbed in the cytoplasm at periods of conjugation. In the latter case, again, this nuclear substance cannot be definitely traced into cytoplasmic structures (see, however, the described origin of mitochondria in *Uroleptus halseyi*, p. 75).

Secondary nuclei which are formed in the cytoplasm of Foraminifera, Radiolaria and some ameboid forms are traced directly back to nuclear chromatin. Thus in *Polystomellina crispa*, *Pencroplis* and other foraminifera the nuclei fragment distributing quantities of chromatin granules (chromidia) in the cytoplasm. These granules in groups of two or three form minute secondary nuclei, one such nucleus in each swarm spore (amebula) which then develops into a megalospheric generation with hundreds of small nuclei formed by division (see p. 69). When mature the protoplasm breaks up into swarms of flagellated gametes, each with one of these minute nuclei (Schaudinn, Lister, Winter *et al.*).

The testate rhizopods secondary nuclei develop from chromidia which form the nuclei of ameboid swimmers (*Centropyxis* Schaudinn, *Arcella*). Similarly in pseudopodia-forming flagellates (Rhizomastigidae) Goldschmidt (1905) describes the formation of secondary nuclei in *Mastigella* and *Mastigina* (Fig. 45, p. 88) from the cytoplasmic chromidia.

2. **Cytoplasmic Kinetic Elements.**—It is in the cytoplasm that kinetic elements are most highly differentiated, and the often perplexing structures which appear in different types of Protozoa

have led to much confusion in terminology as well as in interpretation. Indeed the type of development of the kinetic elements in flagellates is entirely different from that in ciliates and at the present time, at least, they cannot be homologized. Any attempt, therefore, to present a clear picture of the diverse elements and to distinguish one type from another inevitably leads to contradictions in interpretation. The facts may be marshalled, however, into fairly logical series indicating increasing complexity in the organization of the cell. Such series are presented in the following pages with the understanding that they involve no claim of finality, nor do they indicate phylogenetic relationships.

The kinetic structures most frequently found in the cytoplasm of Protozoa are relatively simple, the more complex types which have been revealed being found in comparatively few cases. In considering Protozoa as a group, therefore, too much weight should not be attributed to these more complicated forms. For purely descriptive purposes they may be considered in the following order: (1) Kinetic elements, which are morphologically and functionally equivalent to intranuclear centrioles forming parts of endobasal bodies and usually derived from them; (2) blepharoplasts equivalent to basal bodies, or independent of basal bodies, which lie at or near the bases of motile organoids and give rise to the kinetic structures in them; (3) basal bodies derived from and independent of blepharoplasts; (4) parabasal bodies which are closely connected with the blepharoplasts and probably derived from them; (5) centrodesmoses and paradesmoses, or connecting fibrils between kinetic elements at the spindle poles; (6) rhizoplasts, or fibrils originating as outgrowths from the substance of specific kinetic elements and connecting two such elements or ending blindly in the vicinity of the nucleus; (7) astrospheres and centrosomes, similar to analogous structures in the cells of Metazoa; (8) miscellaneous kinetic elements such as centroblepharoplasts, axostyles, parastyles and the neuromotor apparatus of flagellates. An entirely different series involves the motorium, conductile fibrils, and myonemes of Infusoria together with the silver line systems of the ciliates which we have included in the structures of the fundamental organization (see p. 80).

Since many of these are characterized by their functional activities as well as by their specific structures, it is not illogical to find that the same organoid performs generalized functions. Thus a blepharoplast may be the same as a centriole, or as a basal body; rhizoplasts may arise as a broken centrodesmose or paradesmose; a myoneme as a conductile element, etc. The complexities of organization arise from the simultaneous presence of many of these different kinetic elements in the cell where they may form a coördinating system of organoids which Sharp and Kofoid have aptly designated the *neuromotor system*.

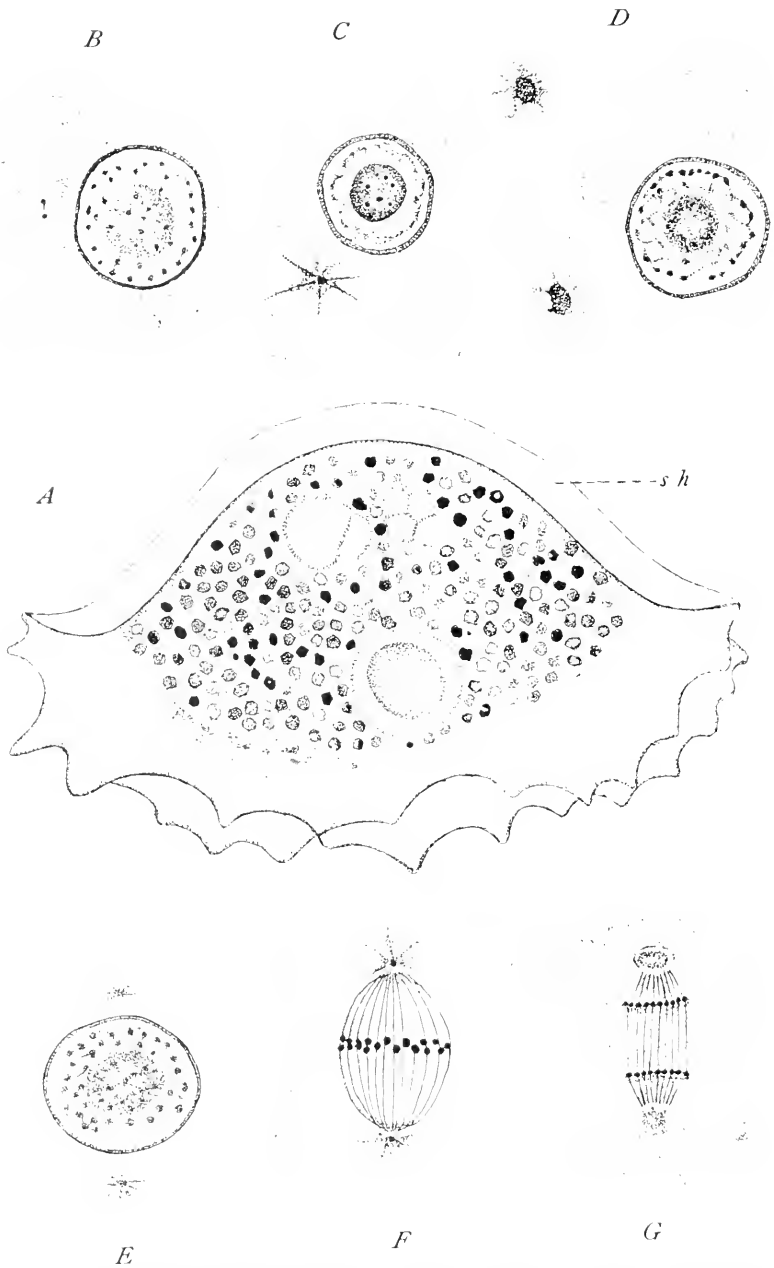


FIG. 58.—*Hartmannella klitzkei* Arndt. Centrosome and centriole in a testate rhizopod. A, Animal with watch-glass-like shell; B to F, origin of the centrosome in the cytoplasm, its division, and position on the spindle; G, anaphase stage of nuclear division. (After Arndt.)

1. *Blepharoplast, Basal Body and Centriole.*—In many of the comparatively simple Protozoa which have no specialized motile organoids, the cytoplasm apparently lacks all traces of specific kinetic elements. Thus in the entire group of Sporozoa, in the simpler Gymnamebida and in testate forms of rhizopods, kinetic elements, if present at all, are in the form of endobasal bodies within the nucleus or as centrosomes close to it. Arndt (1924), however, described a centrosome, with centriole, which divides and forms the poles of the mitotic figure in *Hartmannella klitzkei*, a testate rhizopod (Fig. 58). In some of the relatively simple rhizopods, however, especially those belonging to the family which Doflein has called the Bistadiidae, from the fact that two distinct phases—an ameboid and a flagellate phase—are interchangeable, we find organisms which throw light on the origin of cytoplasmic kinetic elements. Such dimorphic types of rhizopods have been repeatedly observed since Dujardin first called attention to them, but details concerning the origin of kinetic elements and the flagellum have been made out only through use of modern cytological methods.

In some Protozoa, e. g., *Codosiga botrytis*, the kinetic elements of the flagellum grow directly out of an endobasal body of the nucleus, indicating their origin from an intranuclear kinetic element (Fig. 59, A), in other simple forms the flagellum arises from a kinetic element situated in the cytoplasm but connected with the intranuclear kinetic element by a rhizoplast at some stage (Fig. 59, B). In the phytoflagellate *Polytoma urella*, according to Geza Entz (1918), the relation between intranuclear and cytoplasmic kinetic elements varies with the age of the cell. The usual condition in adult cells is two basal bodies, one at the base of each flagellum, and neither of them is connected by a rhizoplast with the nucleus. In young individuals, however, the original single blepharoplast (= basal body) is connected by a rhizoplast with an intranuclear endobasal body, or a larger rhizoplast from the blepharoplast may break up into a calyx of fibrils which enter the nucleus at different points. The inference might be drawn in all such cases that the cytoplasmic body represents one of the daughter halves formed by division of the nuclear endobasal body, while the connecting fibril represents the rhizoplast formed during such division. Such stages are well illustrated by the dimorphic forms of rhizopods during the transition from the ameboid to the flagellated phase. Thus Whitmore described a cytoplasmic kinetic element functioning as a basal body which is connected by a fibril with the nucleus and which lies at the base of the flagella in *Trimastigamoeba philippineusis*, and Puschkarow described a similar condition in *Dimastigamoeba bistadialis* (Fig. 59, C). The most complete observations, however, were made by Charlie Wilson in connection with the transition from ameboid to flagellated stage in a closely-related form, *Dimastigamoeba*

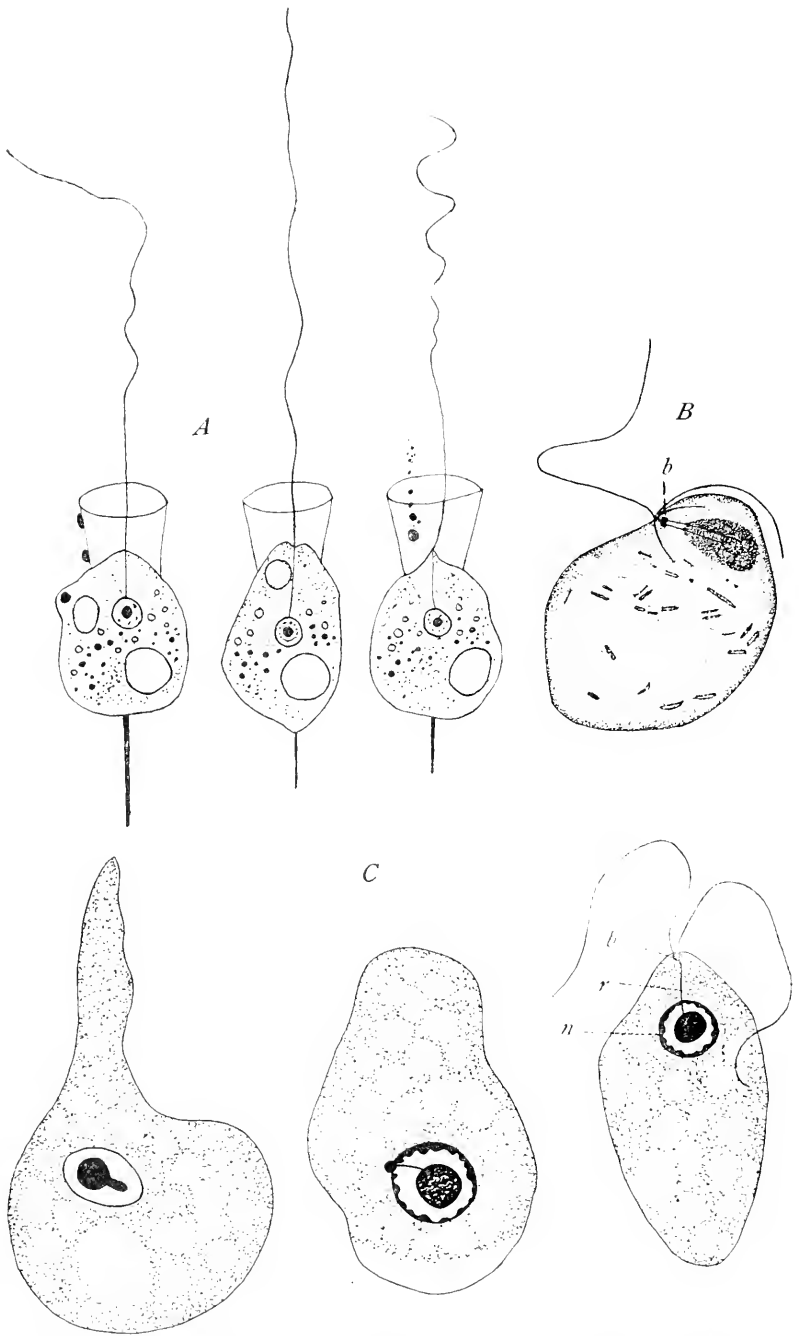


FIG. 59.—Flagellum insertion. *A*, *Codosiga botrytis*, with flagellum arising from the nucleus. *B*, *Dimastigamoeba bistadialis* Puseh, with blepharoplast connected by rhizoplasts with the nucleus, and with independent basal bodies. *C*, *Dimastigamoeba gruberi* and origin of the blepharoplast from the endosome in the nucleus: (*b*) blepharoplast; (*n*) nucleus; (*r*) rhizoplast. (*A* and *B* from Doflein, *C* from Wilson.)

gruberi, one of the soil amoebae. She describes the nucleus of this organism as containing a typical endosome within which an endobasal body is embedded. At the period of flagellation this endobasal body divides and one daughter element migrates through the substance of the endosome and through the nucleus to the cytoplasm, retaining its connection throughout with the intranuclear kinetic element (Fig. 59, C). In the cytoplasm it becomes a basal body which gives rise to the kinetic elements of the flagella. In these cases the extruded kinetic element combines the functional characteristics of a blepharoplast and a basal body or group of basal bodies. In this dual capacity it may be regarded as a blepharoplast—basal body. In *Diuvastigamoeba bistadialis* according to Puschkarew it divides, one part remaining as a blepharoplast, the other becoming a basal body; the two parts, however, are connected by a rhizoplast and rhizoplasts connect the blepharoplast with the endobasal body (Fig. 59, B).

In *Bodo lacertae* according to Bělař the centrioles after division are taken into the daughter nuclei. Here the kinetic elements, although originating from an endobasal body, are different in function from those described in the preceding paragraph. Forming the poles of the mitotic spindle they are correctly described as centrioles, but apparently they again become endobasal bodies (Figs. 33, 34, p. 65).

While the flagella appear to emerge directly from the nucleus in some cases, *e. g.*, in *Mastigamoeba invertens* according to Prowazek, or *Codosiga botrytis* according to Doflein, in many cases they take their origin actually from kinetic elements in the form of centrioles which lie on the outside of the nuclear membranes, as in *Mastigina setosa*, *Phialoecma cyclostoma*, *Cercomonas longicauda*, *Oicomonas termo*, or *Chilomastix gallinarum* (Fig. 60). In such cases, illustrated by *Chilomastix aulostomi* according to Bělař (1921), centrioles become the basal bodies, and the latter become centrioles. In such cases the basal bodies are unquestionably blepharoplasts.

In other cases the blepharoplast does not remain connected with the nucleus by any fibrillar process, but as an entirely separated and independent kinetic element gives rise to the flagella at or near the anterior end of the cell (*Leptomonas jaculum*) or *Herpetomonas gerriidis* (Fig. 169, p. 366). In *Chilomastix mesnili* Kofoid and Swezy (1920) describe three blepharoplasts, one of which gives rise to two flagella, another gives rise to one flagellum and the parastyle, the third to the parabasal, peristomial fibril and the cytostomal flagellum (Fig. 60, B). Boeck (1921) has confirmed these findings. Or, the blepharoplast may migrate toward the posterior end of the cell where with or without division to form blepharoplast and basal body it gives rise to a flagellum, which becomes the vibratile margin of an undulating membrane as in the majority of trypanosomes (Fig.

61, E). In still other cases the blepharoplast also gives rise to one endoplasmic fibril or rhizoplast, which extends deeply into the cell as in *Rhizomastix* (Mackinnon), or a number of such rhizoplasts may be formed as in *Mastigella vitrea*. In these cases the blepharoplast divides independently of the nucleus at periods of cell division.

2. *Parabasal Body and Blepharoplast*.—As a centriole may be contained in an endobasal body which consists largely of chromatoid substance, so may a basal body be enclosed in chromatoid substance

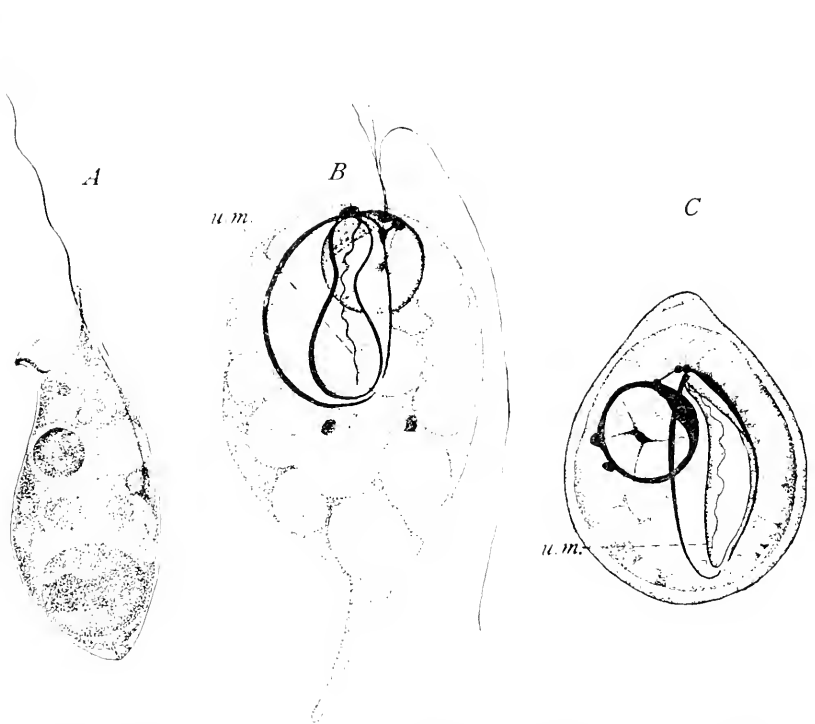


FIG. 60.—Flagellum insertion. A, *Phialomema cyclostomum*; B, *Chilomastix nassuli*; C, the same, encysted. (u.m.) Margin of undulating membrane in cystostome. (A, Original; B, C, after Kofoid and Swezy.)

of a blepharoplast, as shown by Goodey (1916) in the flagellate *Prowaczekia (Bodo) saltans*, or by Kofoid and Swezy (1915) in *Trichomonas augusta*. Again, just as a centriole may be freed from its enclosing chromatoid substance in an endosome, so may the basal body be freed from the blepharoplast. In a similar way the blepharoplast may be contained in an embedding chromatoid mass of a cytoplasmic kinetic element, or it may be free from such a mass. We may then have in the same cell a kinetic complex consisting of one or more basal bodies, one or more blepharoplasts,

and a residual kinetic element in the form of a chromatoid mass. To this residual chromatoid mass the name *parabasal body* is applied, the term originating with Janicki (1915). Kofoid (1916) interprets its function as a storage or feeding reservoir for the kinetic elements, its substance in turn being derived from the nucleus.

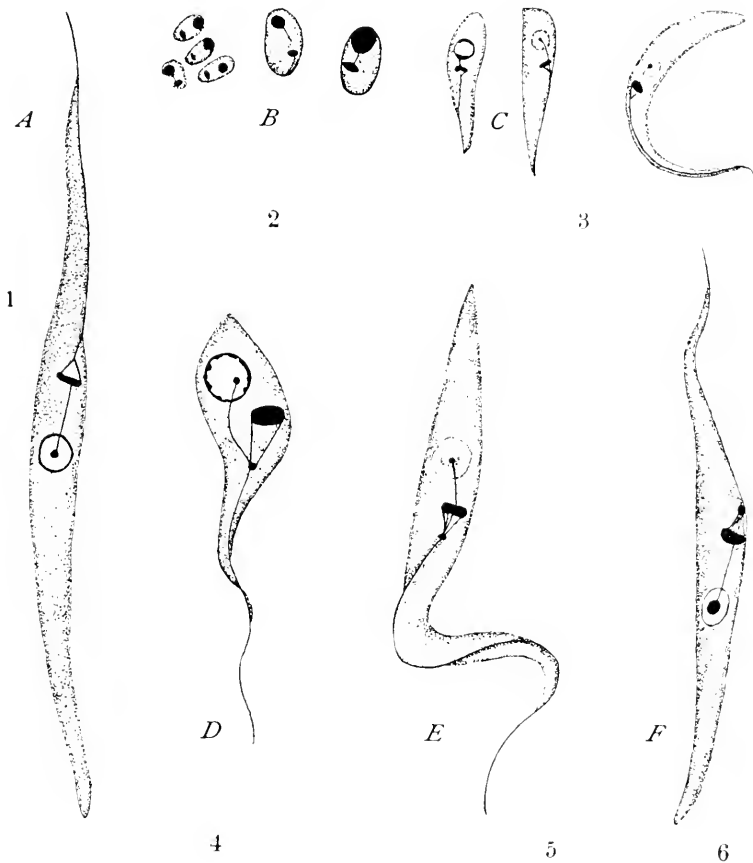


FIG. 61.—Relation of parabasal to nucleus. *A*, *Crithidia caryophthalmi* endosome of nucleus and parabasal connected by rhizoplast; *B*, origin of parabasal from endosome of nucleus; *C* and *D*, differentiation of parabasal and rhizoplasts; *E*, *Trypanosoma cruzi*, and *F*, *Crithidia leptocoridis*, for comparison. (After McCulloch.)

It is in connection with the parabasal body that most of the difficulties have arisen concerning the interpretation of cytoplasmic kinetic elements. It is still in the stage of polemics and controversies continue over the chemical nature of its substance. The difficulties began with Schaudinn's work (1904) on the trypanosome

of the little owl (*Glaucidium [Athene] noctuae*). Schaudinn's description and figures of the history of the kinetic elements at the base of the flagellum have been cited and copied in practically every text-book dealing with the Protozoa and have had a wide influence in theoretical protozoölogy. Other keen observers, however, have sought in vain for evidence corroborating this history. In the absence of such confirmation and in view of the multitude of different observers who find a simpler explanation in many different types of trypanosomes, including that of the little owl (see Minchin, Robertson, Sergeant, *et al.*), Schaudinn's interpretation and conclusions can be accepted only with many reservations.

The essential point in Schaudinn's description was the origin by heteropolar mitotic division of the nucleus of a recently "fertilized cell," of a larger nucleus which becomes the nucleus of the cell, and a smaller nucleus which forms the kinetic complex. This smaller nucleus divides again by mitosis, also heteropolar, the smaller portion becoming the basal granule which forms the flagellum and the "myonemes" of the undulating membrane, while the larger portion remains intact as a homogeneous deeply-staining granule. The contested points in regard to this phase of Schaudinn's work are, first, the "fertilized cell" of the trypanosome, which is now generally regarded as a stage in the life history of an entirely different parasite of the little owl (Minchin enumerates no less than five different types of protozoön parasites which may live simultaneously in the blood of this owl). A second contested point is the origin of the kinetic elements of the cytoplasm by mitosis. Other contested points and untenable conclusions drawn from them have to do with sex differentiation and parthenogenesis which need not be considered here.

It is not at all impossible that Schaudinn may have seen the emergence of a kinetic element from the endosome of the nucleus as described above in the case of *Dimastigamoeba gruberi*, and the similar emergence of a basal granule or blepharoplast from a chromatoid mass in the cytoplasm. The interpretation of such possible stages as mitotic nuclear division, and the smaller products of such division as nuclei, has led to numerous theoretical developments which have only a narrow basis of fact. Two years after Schaudinn's paper appeared, Woodcock translated it into English and conferred the name "kinetonucleus" on the smaller body resulting from the heteropolar mitotic division and the name "trophonucleus" on the nucleus of the cell. Schaudinn himself was the first to announce this binucleate character of the trypanosome body and the hypothesis was taken up by his followers, Prowazek, and notably Hartmann (1907). The latter developed the conception into an elaborate view of original nuclear dualism upon the basis of which he created a special group of the Protozoa including trypanosome-like flagel-

lates and hemosporidia, which he called the "Binucleata."¹ As Doflein points out, not only do the hemosporidia have no blepharoplasts as do the trypanosomes, but blepharoplasts in the latter are not to be considered nuclei. In this use of the term blepharoplast Doflein includes the structure to which Woodcock gave the name kinetonucleus, but he employs the term in a special sense as a kinetic element, while German writers generally use it for structures of widely different significance. Thus Schaudinn, although convinced of its nuclear character, nevertheless called it a blepharoplast. French writers, as a rule, speak of it as a centrosome (*c. g.*, Mesnil, Laveran, etc.) as do some English observers (*c. g.*, Moore and Breinl); many of the latter, however, follow the original nuclear interpretation, Bradford and Plimmer following Stassano, regarding it as a "micronucleus" and comparing it with the smaller nucleus of the ciliates, while Woodcock and Minchin considered it a "true nucleus."

The essence of the problem indicated by the various usages of these familiar terms comes down to a decision as to whether the so-called kinetonucleus, by which is meant the relatively large chromatoid body in the cytoplasm and closely connected with the basal granule, is a nucleus, or a kinetic center of the cell, or neither. Woodcock's term connotes a happy combination of both nuclear and kinetic possibilities; the kinetic function evident from its relation to basal granules or blepharoplasts, while its nuclear characteristic is seen mainly in the deeply-staining chromatin-like substance of which it is composed as well as by its frequent connection with the nucleus. Some writers, notably Rosenbusch (1909), giving free play to the imagination, and under the conviction that it is a nucleus, describe it as such, with centriole, "karyosome," nuclear space which may contain chromatin granules, and a nuclear membrane. The extremely minute size of this organoid and the pranks which the Romanowsky stain or any of its modifications may play with it, as they do with structures of the actual nucleus, together with a fertile imagination, are sufficient to account for the perfect nuclear type which Rosenbusch, for example, described. Other observers, while maintaining its nuclear character, do not accept this extreme interpretation; Minchin, for example, describes it as a "mass of plastin impregnated with chromatin staining very deeply, rounded, oval, or even rod-like in shape" (Prot., p. 288).

If we bear in mind the many types of granules in the cell which stain like chromatin with certain dyes, it seems unnecessary, to say the least, to make the term nucleus, which stands for a well-known and easily recognized organoid of the cell, elastic enough to embrace cytoplasmic bodies in regard to which there is so little evidence of nuclear structure or nuclear function. In well fixed and stained

¹ For critiques of the Binucleata, see particularly Minchin (1912), Dobell (1911).

material the so-called kinetonucleus affords little evidence of nuclear make-up; it appears as a homogeneous mass of chromatoid material which divides into equal parts prior to division of the nucleus. Such features do not make it a nucleus any more than similar features make nuclei of pyrenoids, or of other plastids of the cell. Functionally, and unlike the nucleus, it is not necessary for the vital activities of the organism, as shown by the experiments of Werbitski (1910), confirmed by others, in which by the use of certain chemicals (*e. g.*, pyronine) the "kinetonucleus" of *Trypanosoma brucei* disappears without any effect upon the movements and reproduction of the trypanosome, a race being formed in which this organoid is absent. Nor can the "kinetonucleus" be regarded as a centrosome, for although closely connected with basal granules, it never behaves like an attraction center. With the exception of Schaudinn's account and the overdrawn account by Rosenbusch there is no evidence that it divides by mitosis; it never develops chromatin structures which by any stretch of the imagination can be called chromosomes.

If the "kinetonucleus" is not a nucleus nor an active kinetic center of the cell, then any misleading appellation such as kinetonucleus, centrosome, or blepharoplast, which indicates co-partnership with the actual cell nucleus or other easily recognizable organoid, should be discarded together with the supplementary term trophonucleus. Among names suggested to replace the term kinetonucleus is "kinetoplast" used by Wenyon, Dobell, and Alexieff, and "parabasal body" (Janicki) as used by Kofoid.

The non-committal term parabasal body was first employed by Janicki (1915) to designate an accessory structure in the kinetic complex of *Lophomonas* (Fig. 105, p. 211). Analogous structures have since been found in practically all of the parasitic flagellates thus far described, although not found in free-living types generally. It is present as a globular mass of deeply-staining substance close to the blepharoplasts of types like *Trypanosoma brucei*, *Bodo edax* or *Bodo lacertae* (Fig. 33, p. 65); as an elongate mass in most of the *Cryptobia* species (Fig. 61, C); as a long basal filament in *Trichomonas augusta* (Fig. 77, p. 145); or *Chilomastix mesnili*; as a spirally coiled mass in *Devescovina striata* (Fig. 62, F), etc. It apparently differs in size and form in different phases of the same organism as in *Bodo lacertae* where, in addition to the globular form, it may be rod-like or partly coiled or absent altogether. In *Chilomastix mesnili* an homologous rod-like body, termed the *parastyle*, arises from a second blepharoplast (Kofoid and Swezy, 1920) (Fig. 60).

The most extensive work on the parabasal body has been carried out by Kofoid and his followers who regard this structure not as a nucleus nor as a kinetic center, but as a "kinetic reservoir" or a reservoir of substances which are used by the animal in its kinetic activities under the conditions of its dense environmental medium.

This substance, according to Kofoid, appears to form at the expense of the nuclear chromatin and increases or decreases—that is, the parabasal body becomes larger or smaller apparently in relation to metabolic demands. When the parabasal body is poor in chromatin the blepharoplast and nucleus may be rich and *vice versa*. “Our data are too incomplete to give a clear picture of the process, but as far as they go they suggest the origin of the parabasal at the expense of the chromatin of the nucleus, the movement of stainable substance on the rhizoplast, either to or from the blepharoplast at the base of the flagella, and the wax and wane of the parabasal” (Kofoid, 1916, p. 5). This interpretation is strengthened by the positive reaction of the parabasal of some species to the Feulgen nuclear test (see p. 57).

Kofoid's interesting and suggestive interpretation of the nature of the parabasal is very well sustained by the morphological relations of blepharoplast, nucleus and parabasal body in widely divergent types of flagellates. Morphologically, a series representing a gradually increasing complexity is illustrated by: (1) *Dinastigamoeba gruberi*, in which the blepharoplast arises by division of the intranuclear kinetic center and remains connected with it by a centrosome or, in this case, a cytoplasmic rhizoplast; (2) *Seytmouas subtilis* in which the blepharoplast is not connected with the nucleus and gives rise only to the flagella; (3) *Bodo edax*, or species of *Cryptobia* in which a large chromatoid mass, the parabasal body, is connected by rhizoplasts with the blepharoplast, or may be independent of it; (4) *Bodo lacertae* in which basal bodies (arising from the blepharoplast), blepharoplast and parabasal body are all independent; (5) *Giardia augusta*, in which the independent blepharoplast, basal bodies and parabasal body are all double and arranged in perfect bilateral symmetry; (6) *Calonympha grassii* (Fig. 63), in which nuclei, parabasal bodies, blepharoplasts and basal bodies are multiple and in which axial threads (rhizoplasts) unite to form a central axial supporting rod; (7) *Trichonympha campanula*, in which the blepharoplast (centroblepharoplast) acts as a centrosome in mitosis while long rhizoplasts connecting distal basal bodies with the blepharoplast form a complex radial system of astral rays (Figs. 61 to 65).

In many cases the blepharoplast, which is the central element of the kinetic complex, remains connected with the nucleus by a rhizoplast as a permanent record of the intranuclear origin of the entire complex (Fig. 62). In many cases the blepharoplast is double, as in most biflagellated forms; in others it is triple as in *Trimastigamoeba philippinensis* or *Chilomastix mesnili* (Fig. 60, B); in some it is quadruple, or contains four basal bodies as in *Trichomonas*; in others it is multiple, forming a ring of blepharoplasts about a bundle of flagella as in *Lophomonas blattarum* (Fig. 105, p. 211).

Finally in flagellates with multiple nuclei (family *Calonymphidae*), in addition to a number of free blepharoplasts and parabasal bodies, each nucleus is accompanied by a blepharoplast which gives rise

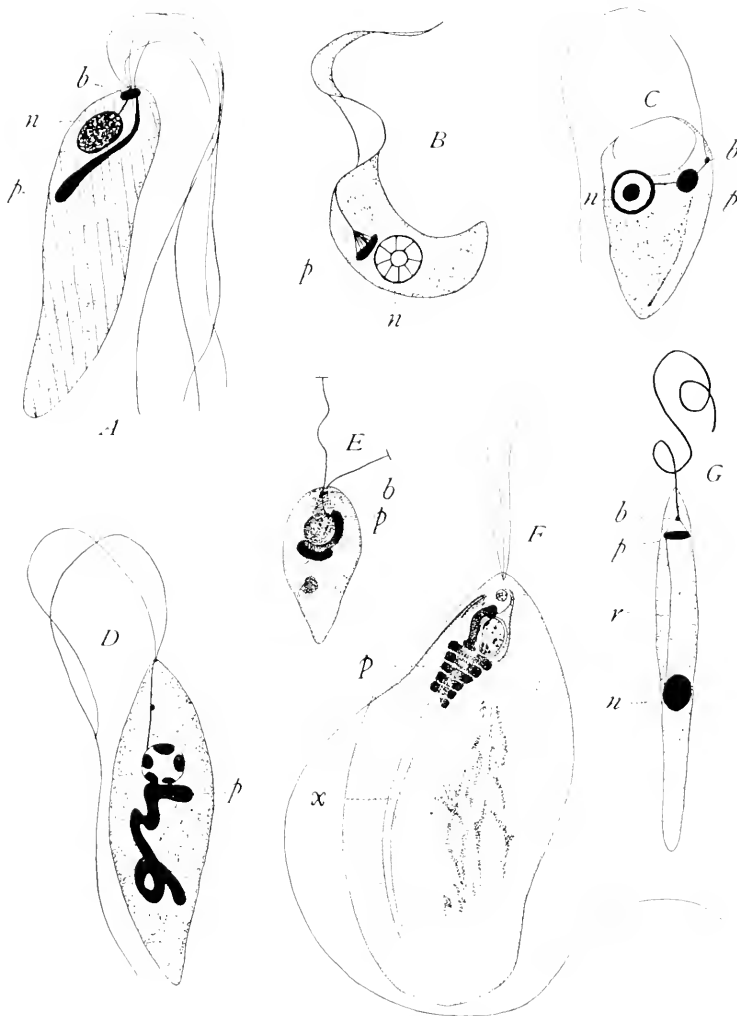


FIG. 62.—Types of parabasal body. A, *Polymastix*; B, *Trypanosoma cruzi*; C, *Cryptobia* sp.; D, *Bodo lacertae*; E, *Prowazekia* sp.; F, *Derescoquina striata*; G, *Herpetomonas musca-domesticae*. (b) Blepharoplast; (p) parabasal body; (n) nucleus; (x) axostyle. (A, C, D, G, after Swezy; B, after Chagas; E and F, after Doflein.)

to three uniform flagella and one longer, band-formed flagellum, by a parabasal body, and by a rhizoplast (axial strand, Fig. 63).

Many of these aggregations of kinetic elements are sufficiently

complex to justify the term neuromotor system of Sharp and Kofoid and appear to form a coördinated whole, as shown by the reaction after maceration when they retain their connections and remain together for some time after the supporting protoplasm has disappeared (*Trichomouas*, Kofoid). The term is certainly justified in connection with the remarkable kinetic structures of flagellates belonging to the family Trichonymphidae. In *Trichonympha campanula*, Kofoid and Swezy (1919) describe the system as composed of an external coating of cilia-like motile organs, three zones of

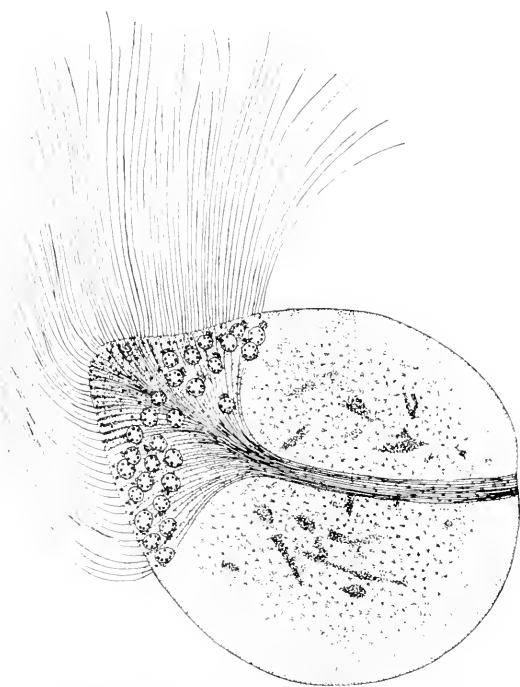


FIG. 63.—*Calonympha grassii* Foa. (From Doflein.)

flagella with their basal bodies, rhizoplasts connecting basal bodies with a great anteriorly placed blepharoplast, and more deeply-lying myonemes which apparently are not connected with the blepharoplast (Fig. 64). Kofoid and Swezy regard the central organoid as a kind of superblepharoplast, calling it the "centroblepharoplast" since it has the attributes of a centrosome. When it divides the entire aggregate of kinetic elements of the cortical zone divides with it, forming a mitotic figure with centrosomes, central spindle and astral rays (Fig. 54). The connecting fibrils of the centrosomes, unlike

the centrosomes in Metazoa, remain outside of the nucleus (as it does in many other flagellates) and is called the *paradesmose* by Kofoid to distinguish it from the centrosome or central spindle.

From this review of the cytoplasmic kinetic elements in the flagellates it is apparent that in endobasal bodies, basal bodies, and parabasal bodies we have to do with structures closely connected with the kinetic activities of the organism and closely related to each other. The chromatoid substance of which they are composed may or may not be chromatin, although the evidence adduced indicates that it arises from the nucleus and in some cases is similar to chromatin in its staining reactions. It does not behave like chro-

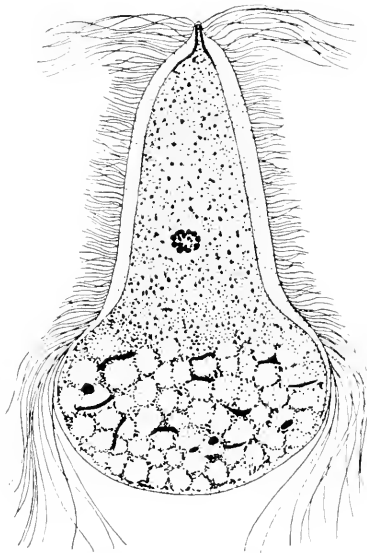


FIG. 64.—*Trichonympha campanula* Kof. and Swez. (After Kofoid and Swezy.)

matin during division of the cell, but like pyrenoids, or chromatophores, where each granule reproduces its like by division; nor does it afford any evidence of constructive metabolic activities in the cell. For these reasons I believe, with Kofoid, that the term "parabasal body" expresses the relationships and functional activities of the so-called "kinetonucleus" much better than does the latter term and should take its place in literature dealing with the Protozoa. The interpretation of kinetonucleus and parabasals, however, is still incomplete. In *Trypanosoma*, as stated above, the kinetic element known as the "kinetonucleus" (*auct.*) or the "parabasal" (Kofoid, Swezy, *et al.*) gives a positive Feulgen nucleal reaction, indicating the presence of thymonucleic acid (Bresslau and Scremin, 1924; Robertson, 1928; Jirovec, 1927; DaCunha and

Muniz, 1928). Lwoff (1931) finds that this nuclear reaction is confined to a cortical zone of the body in question, and holds that probably in all cases the so-called kinetonucleus is composed of two quite different substances, one of which, the medullary substance according to the observations of Grassé (1926), is apparently of lipoid nature. Lwoff (1931) gives a new interpretation of parabasals and kinetonuclei in the simpler parasitic flagellates such as *Leptomonas etnocephali* (Fig. 65). Here the so-called "parabasal filament" (p.f.) does not originate from the blepharoplast ("mastigosome" of Lwoff = m) but from a flagellar ring (r) quite removed from and not connected with the blepharoplast. The latter, however, gives rise to and is connected with what he terms the "kinetonucleus," which he shows has a chromatin cortex (k). The latter gives rise to still another element which he calls the "paranuclear" body (c.Bm). In this case the "parabasal" is not derived from the blepharoplast, but is of entirely different origin from parabasals of other forms. What Lwoff calls the "kinetonucleus" has the same relation to the blepharoplast as do the majority of parabasals (e. g., *Crithidia*, *Trypanosoma cruzi*, etc., Fig. 61). Further study of these perplexing fibrils in flagellates and particularly in the hypermastigida, must be made before the puzzle of exact homologies can be solved.

3. *Other Cytoplasmic Kinetic Elements.*— A unique cytoplasmic kinetic element, apparently homologous with the centrolepharoplast of certain flagellates, is found in some types of Heliozoa. The non-committal name central granule (Centralkorn) was given to this structure by Grenacher (1869), who was the first to observe it. In some types it lies in the geometrical center of the cell (*Acanthocystis aculeata*, *Sphaerastrium fockei*, *Raphidiophrys pallida*, etc.); in other types it is excentric (*Dimorpha nutans*, *Wagnerella borealis*) or absent altogether (*Actinophrys sol*, *Actinosphaerium eichhornii*, *Camptonema nutans*, etc.). In the ordinary vegetative activities of the cell, radiating fibers starting from the central grain extend through the protoplasm to the periphery, where they form the axial filaments of the pseudopodia (Fig. 66). In division stages of the cell, the central grain first divides forming an amph-

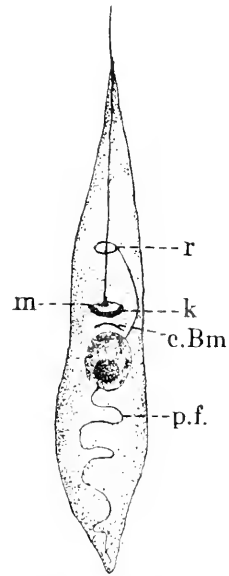


FIG. 65.—*Leptomonas etnocephali*. Parabasal apparatus consisting of periflagellar ring and posteriorly directed filament; "kinetonucleus" and "mastigosome" (basal body). (After A. and M. Lwoff, Bull. biologique de la France et de la Belgique, courtesy of Prof. N. Caullery and Les presses Universitaires de France.)

aster consisting of centrosomes, centrodesmose and astral rays made up of the radiating fibrils (Fig. 50, p. 95—see also *Trichonympha campanula*). Stern (1914), however, found that mitotic spindles may arise in *Acanthocystis* without any connection with the central granule (Fig. 67). The central grain, however, takes no part in reproduction by budding, whereby amoeboid or flagellated buds are formed which contain a nucleus derived from the parent cell nucleus, but no central grain. This nucleus, however, contains an endobasal body which divides and one of the daughter granules emerges from the nucleus as it does in *Dimastigamoeba gruberi* (p. 34), but retains its centrodesmose for some time and ultimately forms the central grain of the

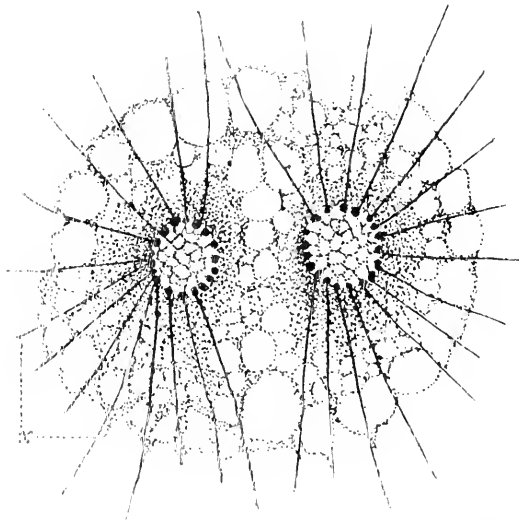


FIG. 66.—Relation of axial filaments to nuclei. Section of *Actinophrys sol* with axial filaments arising from intranuclear granules in recently divided nuclei. (After Schaudinn.)

adult organism (Schaudinn, 1896; Zuelzer, 1909; *Acanthocystis aculeata*, *Wagnerella borealis*, Fig. 50). Similarity with the centroblepharoplast in flagellates is thus shown (1) by its origin from an intranuclear centriole; (2) by its relation to axial filaments which are homologous with rhizoplasts; (3) by its history during mitosis. The analogy is further strengthened by its relation to the flagella and to the axopodia which are simultaneously present in some of the Helioflagellida (*Actinomonas mirabilis*, Kent, *Ciliophrys marina*, Caullery, and *Dimorpha mutans*, Gruber). In *Dimorpha mutans* (Fig. 13, p. 34), the central grain lies near one pole of the cell where it forms the basal body of the two flagella as well as the focal point for the axial filaments; here flagella and axial filaments appear to be homologous

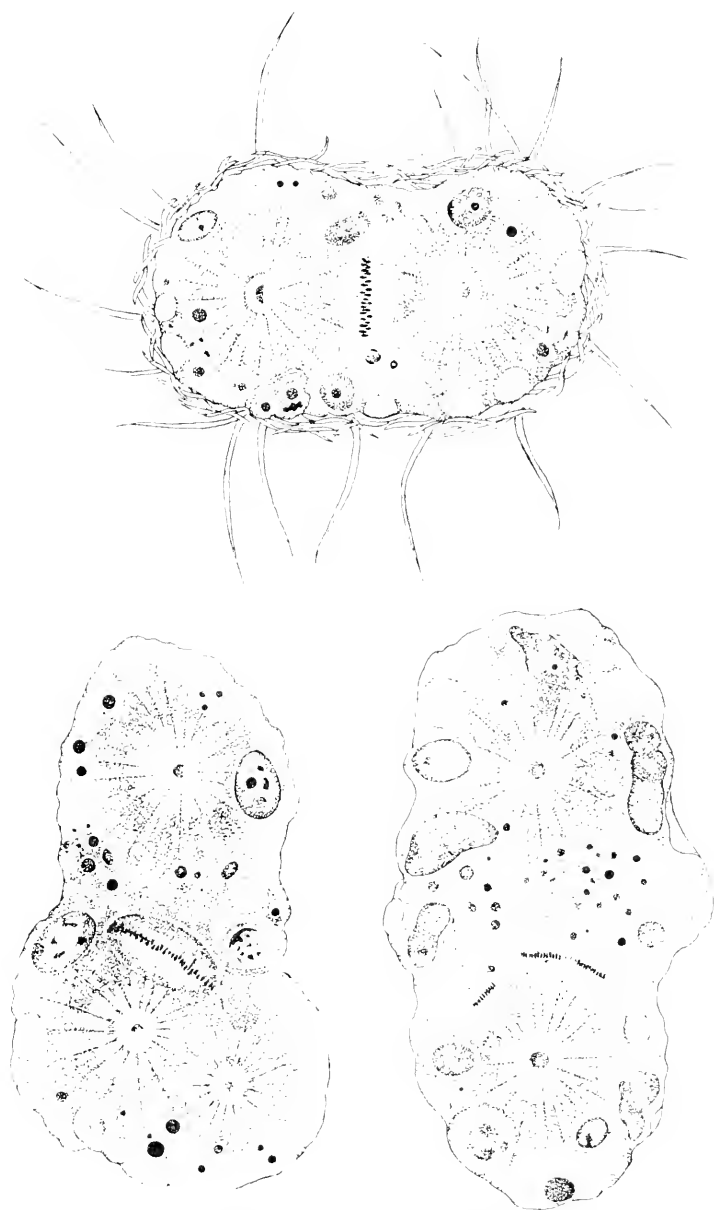


FIG. 67.—*Acanthocystis aculeata*; centropharoplasts disconnected from nuclear spindle. (After Stern.)

structures. According to Zuelzer the pseudopodia of *Wagnerella borcalis* are withdrawn at times, owing to the contraction of the entire complex of radiating fibrils, and basal bodies lying at the bases of the axopodia become grouped in a zone of granules about the central grain. When the pseudopodia are again formed the granules migrate centrifugally to the periphery and, as basal bodies, give rise to the axial filaments.

In Heliozoa without a central granule the axial filaments in some cases center in the nucleus in which there are many distinct and definite granules of uniform size distributed about the outer zone, from each of which an axial filament appears to rise (Fig. 66). In *Camptonema nutans* the nuclei are multiple and, according to Schaudinn, each one gives rise to a single pseudopodial element, but in *Actinosphaerium eichhornii*, which is also multinucleate, the axial filaments apparently have no connection with either nuclei or central kinetic elements.

Apart from kinetic elements like centrobalepharoplasts which, at the same time, are centers of mitotic activity of the nucleus and of kinetic activity of the motile organs, there are comparatively few examples of kinetic elements comparable with centrosomes of Metazoa. These are best represented in non-motile organisms such as Sporozoa, whereas in freely-moving types there is always some peculiar feature which makes the homology with centrosomes doubtful.

A frequently cited example of a centrosome in Protozoa was first described by Hertwig in the case of *Actinosphaerium eichhornii* (Fig. 68). Here, during the formation of the first maturation spindle minute granules of chromatoid substance are cast out of the nucleus and condensed into one or two minute centrioles from which fibrillar structures radiate into the cytoplasm and throughout the nucleus. This structure, however, has no permanent relation to the cytoplasm or nucleus, but disappears after the first maturation spindle is formed while subsequent maturation spindles and spindles of division stages are characterized by pole plate formation (see p. 65). Much more typical centrosomes are found by Arndt (1924) in *Hartmannella klitzkei* (Fig. 58, p. 106) and in the Gregarinida, especially in the *Monocystis* types, where they have been described by Léger, Brasil, Mulsow, Doflein, and others. In *Monocystis rostrata*, for example, a single centrosome with marked astral radiations lies outside the nuclear membrane (Fig. 55, p. 101). An amphiaster is formed as in egg cells of Metazoa, and a complete mitotic figure results. Similar centrosomes occur in *Urospora lagidis*, St., *Gonospora varia*, Léger, and *Stylorhynchus longicollis*, St.

In general we do not find the same types of kinetic elements in Infusoria that are found in other forms of Protozoa. Balepharoplasts, parabasal bodies and centrosomes are still unknown in ciliates, although certain peculiar kinetic elements are present here

which may turn out to be homologous with one or more of these structures. Endobasal bodies, however, are known in micronuclei of a few types (*e. g.*, *Uroleptus mobilis*, *Oxytricha fallax*) and in some macronuclei (*e. g.*, *Chilodon cucullus*, Fig. 30, p. 62). On the other hand, certain special types of cytoplasmic kinetic elements such as myonemes, motorium, and conductile fibers, are characteristic of the ciliates, some of which become highly complicated coördinated neuromotor elements.

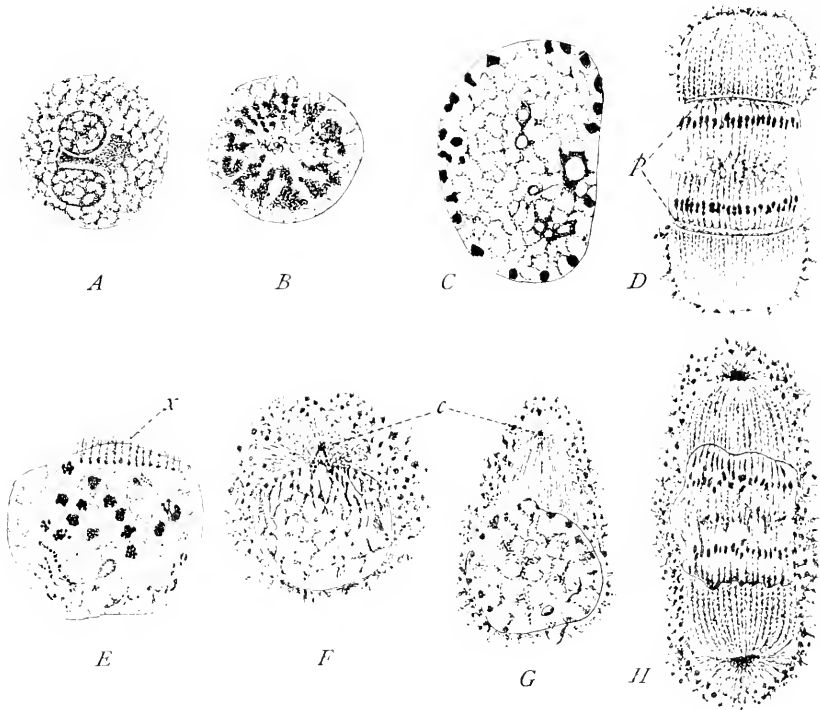


FIG. 68.—*Actinosphaerium richhornii*; origin of centrosome from nucleus.
(After Hertwig.)

The most widely distributed of the kinetic elements are the basal granules of the cilia, which are situated in the contractile zone of the cortex and form a part of the silver line system (see p. 80). The exact nature of these extremely minute bodies is unknown and their origin or renewal is purely hypothetical. Collin (1909) and Entz (1909) record some observations which suggest their derivation from nuclei (Entz) or at least some connection with them (Collin). A single basal body gives rise to a single cilium (Fig. 69) but groups of them are found at the bases of the more complicated membranes,

membranelles and cirri, the number varying with the species. Thus Maier describes 2 in the membranelles of *Nyctotherus cordiformis* and many of them arranged in a row in the membranelle of *Stentor uiger*; in undulating membranes of the vorticellids Maier and Schröder describe 3 rows of basal granules while in the "paroral" and "endoral" membranes of *Glaucoma scintillans* there are 5 and 10 rows of basal granules respectively (Maier). In the cirri of *Stylonychia histrio* which are circular in cross-section, according to Maier, there is a discoidal plate of basal bodies. Alverdes (1922) found that an isolated cilium will beat if the basal body is attached, not otherwise.

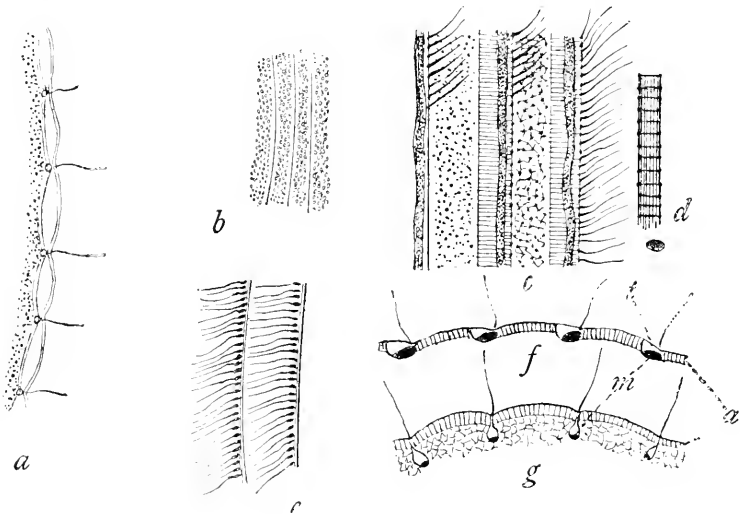


FIG. 69.—Cilia and myonemes of Infusoria. *a*, Membrane and periplast of *Stentor coeruleus*; *b*, *c*, and *e*, rows of cilia of same; *d*, myoneme of same; *f*, optical section of membrane and myonemes of same, and *g*, optical section of cortex of *Holophrya discolor*; *m*, myoneme; *t*, myoneme canal. (*a*, *b*, *c*, after Johnson; *c*, *d*, *f*, and *g*, after Bütschli.)

A perplexing series of structures consisting of granules and connecting fibrils is found in some holotrichous ciliates. In *Chlamydomon mucosynve*, for example, a double row of granules with connectives running around the body near the margin and visible in life as a hyaline band, and a similar but more ladder-like structure is present in the oral vestibule of *Glaucoma frontata* (Fig. 8, p. 29). It is possible, but not demonstrated, that these structures belong to the same category as the girdle around the posterior end of *Vorticella* and represent the infraciliature (Chatton) or special tracts of the silver line system.

Myonemes.—One of the most striking characteristics of certain types of ciliates is their power of contraction. A fully-expanded

Spirostomum ambiguum may be 2 mm. in length but, on irritation, it suddenly contracts to one-quarter that size, or a *Trachelocerca phoenicopterus* contracts to one-twelfth its original length (Læbedev); a *Folliculina ampulla* with its great peristomial lobes widely outspread quickly folds itself completely into its comparatively narrow tube (Figs. 94, 206), or an entire colony of widely distended individuals of *Zoöthamnium arbuscula* contracts instantly into a minute ball. These varied movements which are quite independent of movements of translation or rotation, are due to the contraction of specialized muscle-like fibrils, the myonemes. These are long, delicate, contractile threads, circular or band-like in cross-section situated in the cortical zone and running throughout the entire length of the body, either straight (*Stentor*) or spirally (*Spirostomum*). In some cases a second set of myonemes run transversely about the body as in the peristomial regions of *Campanella umbellaria* or various species of *Stentor*. The myonemes of *Stentor coeruleus* or *Prorodon teres* lie in characteristic canals, which appear hyaline in contrast with the granular adjacent "ribs" of the ectoplasm. Their finer structure has been made out in only a few types, in *Stentor coeruleus* perhaps better than in any other. Here Schröder describes a typical cross-stripping due to alternate rows of light and dark substance (Fig. 69, *d*).

In the majority of cases the contractile effect of the activity of myonemes is possible only by their intimate connection with the firm membranous cortex which encloses the entire animal, a connection which makes it possible for a coördinated contraction of the whole animal at once. A retraction of special regions of the organism involves the attachment of one end of the contractile element to some relatively fixed structure, as muscles in vertebrates are attached to the endoskeleton (Fig. 70). In many cases the general cortex serves this purpose as in the sphincter-like myonemes of the Vorticellidae (Schröder), or the retractile elements of the "seizing organ" or "tongue" of *Dilinium nasutum* (Fig. 98, p. 187), or the closing apparatus of the operculum-bearing types of ciliates. In some cases, however, especially in parasitic ciliates like *Ophryoscolex* or *Diplodinium caudatum*, there is a specialized differentiation of the "cuticle" discovered by Gunther and well described by Sharp. These peculiar differentiations function according to the latter observer, as endoskeletal structures for the attachment of conspicuous band-form myonemes, which serve as retractor strands for drawing into the body a characteristic gullet and adjacent organoids. These skeletal elements are formed from the ectoplasm and are hardened, according to Eberlein, by a deposit of silicic acid which, as Sharp implies, may be the explanation of their rigid but brittle nature.

Myonemes or analogous organoids are not confined to the ciliates

but may be found in some types of Gregarinida (see p. 535) and in one group of the Radiolaria. The so-called myonemes of the Trypanosomidae, however, are very doubtful kinetic elements but, more probably, are analogous to the cuticular markings which are

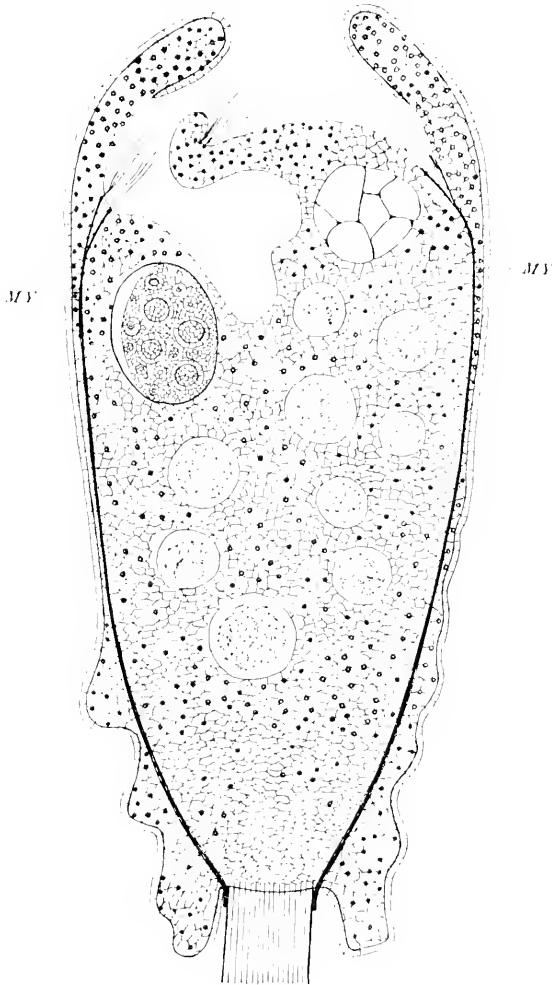


FIG. 70.—*Epistylis plicatilis*; longitudinal section showing myonemes (MY) from membranelles to base of cell. (After Schröder.)

frequently found on the periplast of flagellates. In some of the gregarines, myonemes form a thick layer of extremely fine fibrils in the cortex, running longitudinally and circularly, or possibly spirally, about the cell, their contractions giving rise to the peristaltic movement so characteristic of these forms (see p. 535.)

Myophrisks of the Radiolaria are contractile strands which are fastened by their distal ends to the extremities of the axial bars of the Acantharia. The proximal ends fray out into fibrils which are lost in the reticulum of the gelatinous mantle or calymma, of the ectoplasm. By their contractions the calymma is drawn up to the ends of the axial bars whereby the diameter of the organisms is increased and its specific gravity decreased, the reverse occurring with their relaxation. The myonemes thus seem to play a part in the hydrostatic activities of these Radiolaria, although this function is difficult to understand, since the change in specific gravity is usually interpreted as a means by which these motionless forms escape from adverse conditions on the surface. We should expect, however, that rough water or other surface conditions detrimental to the organisms, would be sources of stimulation which should cause the contractile elements to contract and thus to defeat their apparent purpose by decreasing the specific gravity.

Coördinating Fibers.—If a single cilium of a resting *Pleuronema* be touched the entire organism responds. Here and in similar cases there appears to be a definite tactile function. In flagellates also it is not improbable that certain flagella, as the anterior flagella of *Caduceia theobromae* described by França (1918), or indeed possibly all flagella have a more or less well-developed sensory function. In ciliates, such as *Paramecium caudatum*, with a uniform coating of cilia, the motile elements do not all beat simultaneously, but a wave of contraction, beginning at the anterior end, passes down the cell to the posterior end. Cilia in the same transverse row beat synchronously, but each cilium in a longitudinal row begins its beat shortly after the cilium anterior to it has started and before it has ended its beat (Verworn). The cilia of transverse rows are thus synchronous, those of longitudinal rows metachronous in their contractions, a phenomenon which accounts for the wave-like movement of undulating membranes which are formed of fused cilia of longitudinal rows (well shown in the undulating membranes of the Pleuronemidae). According to Alverdes (1922) isolated cilia with basal body may act independently of a coördinating system but they do not react to stimuli.

This regularity of cilia movement which may be easily seen in the uniform ciliary coating of *Nyctotherus ovalis* from the cockroach, indicates the transmission of impulses and the activity of some coördinating mechanism in the cell which today we attribute to the silver line system. Entz, Maier, Schuberg and many other observers have found distinct fibers connecting the basal bodies of protozoön cilia and have generally interpreted them as myonemes. Since forms like *Nyctotherus*, *Frontonia*, *Paramedium*, etc., which do not contract, show the same rhythmical action of the cilia, it is probable that the threads connecting their basal bodies are not myo-

nemes but coördinating fibrils. It is conceivable, moreover, that myonemes in a generalized condition may be both coördinating and contractile in function. In some cases, however, two distinct sets of fibrils have been observed, one of which is interpreted as contractile, the other as conductile. Thus Neresheimer described "myophanes" and "neurophanes" in *Stentor coerulesus*, and *Climacostomum virens*, the former extending the entire length of the body, the latter only from the base to the center (Fig. 71). On *a priori* grounds it would seem that, as Yocom points out, Neres-

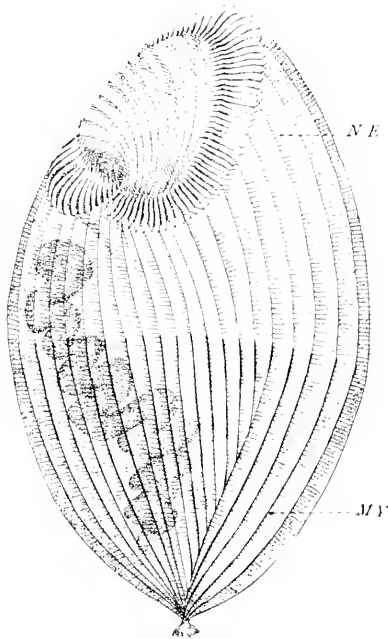


FIG. 71.—*Climacostomum* sp. To show neurophanes (NE) and myophanes (MY). (Original.)

heimer made an unfortunate application of his two terms, his neurophane fibers, for example, to which he ascribes a transmitting function, being situated in the least advantageous position for the functions of irritability or conductivity, Jennings having shown that the first and most strongly marked reactions to certain stimuli in ciliates appear in the anterior region, a result confirmed by Alverdes (1922).

The more recent observations of Sharp, Yocom, and Taylor, all from Kofoid's laboratory, afford more striking evidence of specific conducting or coördinating fibrils in ciliates, although not connected

with the silver line system. In connection with *Diplodinium ecaudatum*, Sharp described, for the first time in the literature, a system of connected fibrils emanating from a common mass of differentiated protoplasm, which he called a "motorium," the whole system being termed the "neuromotor apparatus." The motorium is situated in the ectoplasm of the anterior end of the organism between the two zones (adoral and dorsal) of membranelles (Fig. 2, *M*, p. 20.) From it as a center a number of fibers pass to different regions of contractile activity. These fibers are named and interpreted by Sharp as: (1) A circumesophageal ring strand running to a definite ring of substance similar to that of the motorium encircling the gullet (esophageal ring), from which other fibers apparently take their origin and run posteriorly along the retractile gullet; (2) a dorsal motor strand running to the bases of the adoral membranelles; (3) opercular fibers or a group of fibers running to the operculum (see Fig. 2).

The delicacy of structure and the position of this amazingly complex aggregate are sufficient evidence to disprove any hypothesis of a supporting function. Self-perpetuation of the elements by division indicates no relationship to supporting structures such as trichites (oral basket) in the mouth regions of forms belonging to the family Chlamyodontidae. Their position in the cell and the attachments of the several fibrils are arguments against their interpretation as myonemes.

McDonald (1922) has recently described a somewhat similar neuromotor system in *Balantidium coli* and *B. suis*. Here an anterior motorium gives rise to (1) a ring-form fibril which passes around the adoral cilia region and (2) a similar ring fibril passing around the gullet. Other elements of the system consist of basal granules of the cilia, from which rhizoplasts pass inward to the central region of the cell. At the point where each rhizoplast enters the endoplasm is a granular thickening from which a radial fibril passes toward the periphery where it ends blindly.

Many other ciliates have been added to this list of motorium-bearing forms, but we are still ignorant as to the origin and history of the motorium during division. Amongst these forms are: *Paramecium* (Rees), *Glaucoma frontata* (Calkins and Bowling, 1929), *Uroleptus halseyi* (Calkins, 1930), *Conchophthirius mytili* (Kidder, 1933), and others. Until we have some positive evidence of its origin and perpetuation by division, the interpretation of the motorium as a definite organoid of the cell must be held in reserve (cf. Rees, 1931; Turner, 1933).

Evidence in favor of a conductile function of such a neuromotor system is furnished by the observations of Yocom (1918) and the micro-dissection experiments of Taylor (1920) on *Euplotes patella*. In Euplotidae, apart from the motile organs, contractility is un-

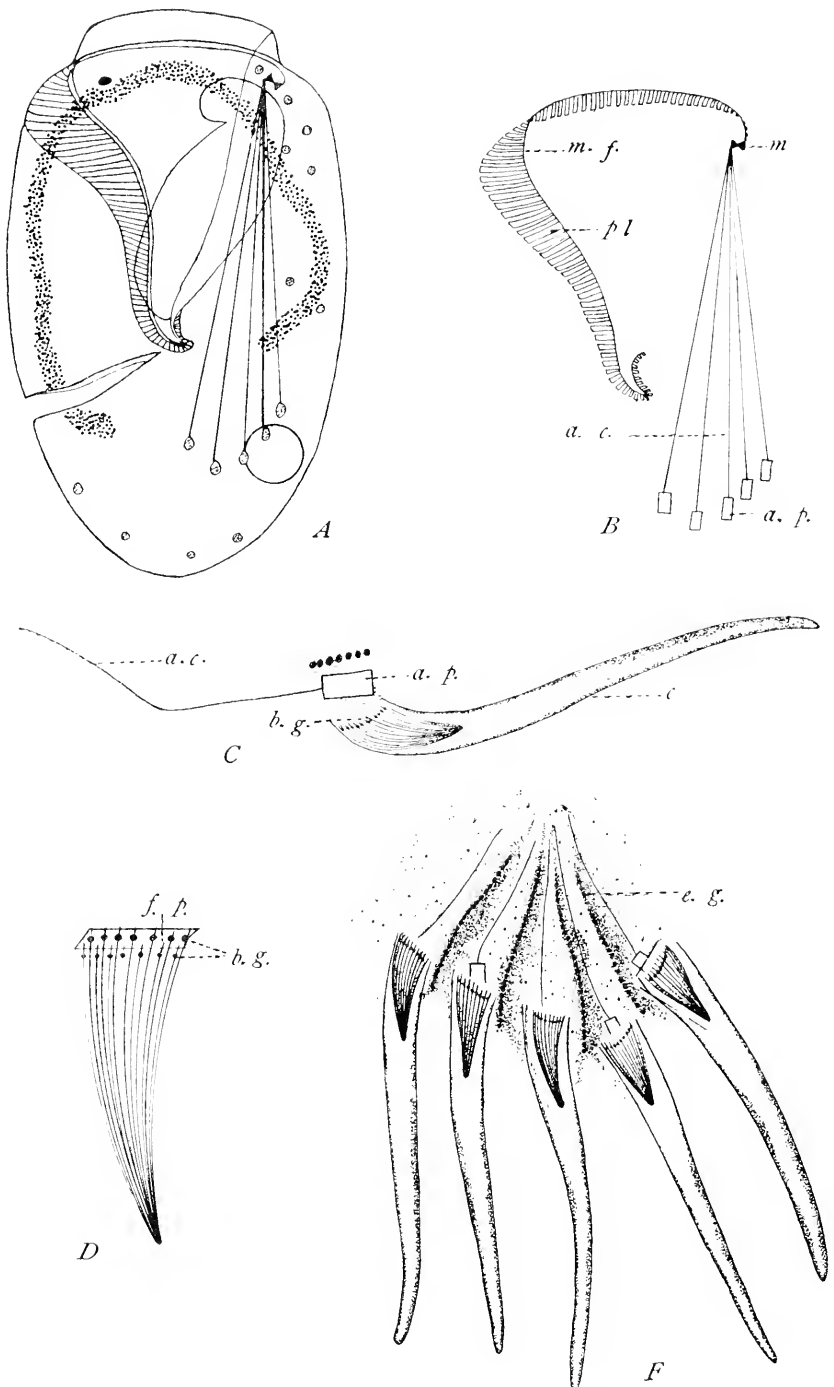


FIG. 72.—Micro-dissection of *Euplotes patella*. A, individual with lateral cut; showing distribution of the cellular structures; B, neuromotor apparatus isolated; C, an anal cirrus with accompanying structures; D, an isolated membranelle; F, the five anal cirri; (a.c.) anal cirri fibers; (a.p.) basal plates of the anal cirri; (b.g.) basal granules; (c) cirrus; (e.g.) ectoplasmic granules; (f.p.) fiber plate; (m.f.) membranelle fiber; (m) motorium; (p.l.) membranelle plates. (After Taylor.)

known, nevertheless the literature contains many references to myonemes in the several species. Distinct fibrils in these hypotrichs which Engelmann regarded as nerve-like in function, have been interpreted in the main as supporting or contracting elements (Maupas, Bütschli, Schuberg, Maier, etc.). Prowazek worked them out in some detail in the case of *Euplotes harpa* and Griffin (1910) in the case of *E. worcesteri*, both observers regarding them as contractile in function. Yocom has studied them more recently in *Euplotes patella* and a complex system, comparable with that of *Diplodinium caudatum* is described. A definitely staining bilobed mass of differentiated protoplasm which Yocom identifies as a motorium is situated in the ectoplasm near the right anterior angle of the triangular peristome (Fig. 72, *m*).

From one lobe of this mass a set of five prominent longitudinal fibrils which seem to emerge as a single strand, run to the bases of the five anal cirri near the posterior end (*a. c.*); from the other lobe a single fibril passes along the inner margin of the anterior lip and down the left side of the peristome closely following the bases of the frontal and peristomial membranelles. In the anterior lip it gives rise to a simple network of branching fibrils (Yocom). The other cirri of the ventral surface are not thus connected with the motorium, and each appears to have an entirely independent set of fibers which run into the endoplasm and disappear in different directions.

Yocom attempted, rather unsuccessfully, to homologize the motorium with the blepharoplast of flagellates; until further observations are forthcoming in regard to the activities of this structure at different periods of cell life it seems more expedient to regard the motorium as a structure peculiar to the ciliates than to add it to the already over-burdened conception of the blepharoplast.

The only direct evidence of the physiological nature of the neuro-motor complex is furnished by Taylor's micro-dissection experiments with the same organism, *Euplotes patella* (Fig. 72). Cutting the fibers connecting the anal cirri with the motorium had a noticeable effect on the normal reactions of creeping, swimming and turning, while severing the membranelle fiber led to characteristic irregularities in the usually coördinated activities of the membranelles and to abnormal spiral revolutions while swimming. Destruction of the motorium, finally, resulted in uncoördinated movements of the membranelles and of the anal cirri. This evidence, excellent as it is, rests upon an exceedingly delicate technique and upon the personal interpretation or estimation of minute differences between normal and induced reactions. It is a line of work, however, which invites further research and promises fruitful results.

CHAPTER IV.

DERIVED ORGANIZATION. TAXONOMIC STRUCTURES.

ALTHOUGH fundamentally important in vital functions, the various granules and structures which have been described can hardly be regarded as obvious or visible characteristics of Protozoa. Careful study, involving elaborate technical methods, is necessary to reveal the parts they play, and for some, at least, even this has not yet yielded positive results.

The visible characteristics, those we see upon casual examination with a microscope—form, color, movement, shells, tests, stalks, etc.—are secondary in importance in respect to the ultimate vital activities. It is in connection with these, however, that the Protozoa are best known and the peculiar fascination which they have for the microscopist is mainly due to these obvious features. The outer structures which please the eye, or the motile organoids which cause the fascinating endless variety of movements, represent the outcome or product of the activities going on between the various constituent elements of the protoplasm. Some of them are necessary for the continued life of the organism, some are useful in one way or another, but not absolutely necessary, and some, *e. g.*, the scalloped cuirass of *Entodinium* or the fantastic forms of many sapropelic types, have no obvious reason for being. These structures represent the completed derived organization and furnish the obvious characteristics upon the basis of which the Protozoa are classified.

In some types of Protozoa, even on superficial examination, it is evident that the aggregate of substances making up the protoplasm is differentiated into an external zone and an internal, medullary part. The external portion is usually called ectoplasm, the inner part endoplasm. The ectoplasm is that part of the protoplasm which comes in direct contact with the environment. It is the part through which food substances must pass into the organism and through which the waste matters of destructive metabolism, as well as undigested food, must be voided to the outside; it is the part which first receives external stimuli of various kinds, and it is the part which gives rise to the more easily visible portions of the locomotor structures, and to the specializations for support and protection.

Acting thus as a medium of exchange between the living protoplasm and the external world, the ectoplasm has become modified

in ways that would be impossible for the endoplasm. In simple cases as, for example, in *Amoeba proteus*, it is not strikingly different from the endoplasm, but in other cases it becomes a complex of special adaptations and the seat of many important organoids of the cell. The external zone of protoplasm thus becomes practically an organ system with structures and functions quite different from the inner protoplasm. In view of these distinctive features it is frequently called the cortex.

I. DERIVED STRUCTURES OF THE ENDOPLASM. METAPLASTIDS.

In the protoplasm of all Protozoa, in addition to the permanent granules of one kind or another described in the preceding chapter, there are many types of transitory or fixed products of cell activity collectively known as metaplastic granules or metaplastids. All of these are formed during the vital activities of metabolism some of them as reserve stores of food substance formed as products of the building up or anabolic processes of metabolism, others by the destructive or catabolic processes. In the former group are included fats, glycogen, paraglycogen, oils, albumin spheres, etc. In the latter group, as products of destructive metabolism, are included a great variety of crystals, pigment granules, chitin and pseudo-chitin, and other more or less widely distributed products. These products of destructive metabolic activities are frequently so abundant as to give the protoplasm a densely granular appearance.

The form and appearance of these various products of protoplasmic activities vary within wide limits and will be discussed more fully in connection with the different classes of Protozoa. Many of them serve a useful purpose as reserves in nutrition and other physiological processes, while a number of them are used for purposes of support, protection, or shell and skeleton building. Glycogen-like bodies are found in a few types of flagellates; true glycogen occurring in the protoplasm of *Pelomyxa palustris* according to Stolç (1900), and in the ciliates *Paramecium*, *Opalina*, *Glaucoma* and *Vorticella* according to Barfurth. Paraglycogen, also called zoöamyllum, which differs from glycogen in its solubility and in its color reactions when subjected to sulphuric acid and iodine, is present in many ciliates and flagellates as well as in some gregarines.

Oils and fats are widely distributed. Great oil globules are particularly characteristics of the Radiolaria where, in addition to serving a useful purpose as reserves of nutriment, they also serve a hydrostatic function in the activities of different organisms. Globules of smaller size but conspicuous by their frequently brilliant coloring are found in many types of flagellates and ciliates.

Protein derivatives in the form of chitin and pseudochitin are

more widely distributed through the entire group of Protozoa, forming the substratum upon which, or between layers of which, shell materials are deposited, while cups, tests or "houses," cyst membranes, stalks, etc., are formed directly from its substance. Shell and skeleton materials such as calcium carbonate, silica, strontium sulphate, etc., are likewise formed as results of metabolic activity, sometimes continuously, as in the lime-stone shells of the Foraminifera, and sometimes periodically at intervals of saturation (dictyotic or lorication moment) as in the formation of the characteristic silicious skeletons of the Radiolaria.

Pigments of various hues are also frequently found in Protozoa. In some cases, as in *Actinosphaerium eichhornii*, they are formed as a final product of degeneration of chromatin granules (chromidia); in other cases they are products of metabolic activities following the digestion of specific kinds of food, as melanin pigment, brown or black in color, which follows the digestion of hemoglobin by malaria-causing hemosporidia (*Plasmodium* species). Specific coloring matters are found here and there, especially amongst the ciliates, which have nothing to do with chlorophyll and which are named according to the organism in which they are found. Thus the blue coloring matter sometimes called stentorin, is characteristic of *Stentor coerulens* and some species of *Folliculina*; a red pigment of *Mesodinium rubrum*; violet of *Blepharisma undulans*, etc.; the colors being due, probably, to the kind of food that is eaten, since the pigmentation of the same species is not constant, some forms in the same culture of *Blepharisma undulans*, for example, may be colorless while others are more or less bright pink, or violet, or even purple in color. The suggestion has been made that specific products of hydrolysis of certain kinds of food act as intravital stains on the protoplasm, thus producing the characteristic colors. In many cases the pigment is accumulated in masses of varying size representing excretory matters of one kind or other. Thus we find the black pigment granules of *Metopus sigmoides* and of *Tillina magna*, or the brown pigmental masses (phaecodium), characteristic of the triplarian Radiolaria.

Other metaplastids that are useful for purposes of protection or support, are the peculiar trichocysts and trichites found in the ciliates and about which there is very little definite information (Fig. 35, p. 67). They are usually embedded in the cortex when fully formed but the trichocysts at least appear to be formed in the vicinity of the nucleus as Mitrophanow has shown for *Paramecium*, and as I have also observed in the case of *Actinobolina radians*. The trichocysts at rest are capsules filled with a densely staining (with iron hematoxylin) substance which is thrown out in the form of long threads when the organisms are violently irritated as with poisons of one kind or another. They appear to be connected with

the silver line system and, according to Bresslau, Kahl and others, are here represented by granules when the trichocysts are undeveloped. In such granular form they are sometimes called "protrichocysts" and Bresslau regards them as the source of the "tektin" which forms artificially produced tests and houses (see p. 137). The trichites are stiff, usually rod-like supporting structures and are rarely discharged.

II. DIFFERENTIATIONS OF THE CORTEX.

It is quite probable that there is no such thing as an entirely naked cell among the Protozoa. Even in *Amoeba proteus*, the classical example of a naked cell, the ectoplasm is covered by a delicate, viscous hyaline zone of modified protoplasm. Hofer, Verworm, and others, have noted it in connection with food taking; Schaeffer (1917), in connection with movement claiming that it is a third kind of protoplasm in addition to ectoplasm and endoplasm and Chambers (1915) came across it in connection with micro-dissection experiments. Among Sporozoa and Infusoria it has been described in many species, and in flagellates and ciliates it is not infrequently characterized by definite markings or sculpturing. It is the most external portion of the cell and is distinguished from the remainder of the cortex by the special name *periplast* or *pellicle*.

The periplast always fits the body closely, dividing when the body divides. In *Paramecium caudatum* during plasmolysis it is extremely delicate, but may be seen when it becomes separated from the rest of the cortex and distended by the accumulation of fluids. In other cases it is much more definite and membrane-like as in *Cochliopodium bilimbosum* (Fig. 9, p. 31), or in the loricate ciliates such as *Euplotes harpa*, *Uronychia setigera* and their allies. Periplasts are frequently delicate enough to give way to forces generated within the body, but elastic enough not to break, a phenomenon resulting in peristaltic movement which is not infrequent in Gregarinida (e. g., *Monocystis agilis*) and in some flagellates. Such organisms are said to be "metabolic" and the peculiar motion is sometimes called "euglenoid movement."

In many cases the periplast is ornamented by striations which usually run obliquely down the cell; in some cases by ridges; by furrows or by nodules as in the ciliate *Vorticella monilata*. In *Coleps hirtus* the periplast is differentiated into definite plates of characteristic form arranged in four girdles which compose an armature for the organism (Fig. 73, A, C). The skeletal structures of endoparasitic ciliates, e. g., *Diplodinium caudatum* are likewise differentiations of the periplast (p. 21).

Not only the periplast, but the entire cortex has become differentiated in a great variety of ways in response, apparently, to the

many demands made upon it as a result of contact with the environment. These may be grouped as cortical differentiations for (a) support and protection; (b) locomotion and irritability; and (c) food-getting and defecation.

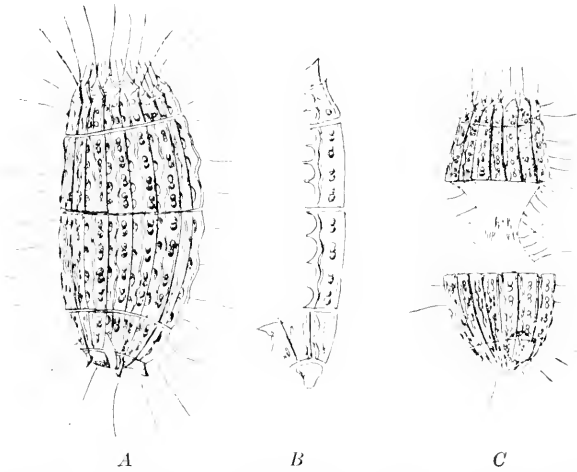


FIG. 73.—A, B, C, Form, structure of plates, and division of *Coleps hirtus*. (After Maupas.)

(a) **Cortical Differentiations for Support and Protection.**—Apart from the thickening and hardening of the periplast which furnishes sufficient protection and support for the great majority of flagellates and ciliates, the cortex is the seat of precipitation of different mineral substances; of secretion of gelatinous substances; or of protoplasmic modifications into lifeless organic substances of various kinds. These various products of cortical activity are moulded into close-fitting, lifeless membranes of chitin, pseudochitin, and cellulose, or into loosely-fitting shells, tests, skeletons, cups, tubes and the like. These are not divided when the cell divides but are either left as empty shells and tests, or one of the daughter individuals after reproduction remains in the old shell while the other individual makes a new shell for itself.

Gelatinous mantles are common in flagellates and are occasionally found in the ciliates (e. g., *Ophrydium versatile*), but gelatinous materials are secreted by all types of Protozoa. Usually, when the secretion is abundant, daughter cells remain embedded in it as a matrix after division, and the so-called spheroidal types of colony result (see p. 38). The ability to secrete gelatinous mantles as a reaction to unusual stimuli appears to be very widely distributed, if not universal amongst Protozoa. Bresslau (1921), using a variety

of chemical stimuli, was able to demonstrate a voluminous gelatinous envelope secreted by *Colpidium campyllum*. Similar secretions were also demonstrated in other ciliates and in certain rhizopods and flagellates as well. The secreted material, which he called "tektin," appears to be a combination of an albumin complex and a carbohydrate complex and, according to Bresslau (see also Schneider, 1930) it is instrumental in forming shells and tests of Protozoa, as well as trichocysts of many types.

The most characteristic shell-forming material manufactured by Protozoa is chitin and pseudochitin. Chemically chitin is a modified protein ($C_{30}H_{50}O_{10}N_4$ or multiple) and is undoubtedly polymorphic in composition. Its mode of formation is still unproved, but conditions in Protozoa support the view of Chatin that it arises by transformation or differentiation of the peripheral cellular protoplasm. Not only are cups, tests, "houses" of various kinds formed of these substances, but cyst membranes, spore capsules of the Sporozoa and "central capsules" of the Radiolaria as well, while impregnated with calcium carbonate, silica, strontium sulphate, etc., or covered by foreign bodies of different kinds, the chitinoid membranes furnish the framework for the up-building of the most complex shells and skeletons. In encysting ciliates the animal becomes spherical, much condensed by loss of water and is surrounded by an envelope of fluid-like material which condenses more and more with exposure until the definite membrane, impervious to moisture and resistant to all unfavorable conditions of the environment, results. In Radiolaria the central capsule is a spherical wall of chitin, separating the endoplasm from the external protoplasm and perforated in various ways to permit of communication between the different regions of the cell (see p. 439).

In flagellates and ciliates the chitinous houses, tests, cups, etc., are usually colorless and very transparent, but in the rhizopods this is unusual, the chitin shells being colored by oxides of iron usually red or brown (*Arcella* sp., etc.). In the majority of fresh water rhizopods the outer surface of the chitinoid shell is covered by foreign particles of various kinds, such as sand crystals, diatom shells, or even living algae, which are glued to the membranes by a chitinous cement. Similar shells, which are generally known as arenaceous shells, are found amongst the Foraminifera. In other cases, plates of silica are deposited in the inner protoplasm and passed out during reproduction to be cemented on the chitinous membrane in regular patterns (*Euglypha alveolata*, Fig. 9, p. 31). Foreign bodies caught up in the wrinkles of withdrawing pseudopodia are similarly stored in the protoplasm to be used for shell-building purposes, Verworm, for example, compelling *Difflugia* to build its shell of differently colored powdered glass.

The lime shells of Foraminifera are formed in quite a different manner. Here, calcium carbonate is precipitated between two lamellae of chitin very much as a cement wall is made between board surfaces. Except for a single mouth opening such limestone shells may form an unbroken wall about the organism (imperforata) or they may be perforated by myriads of minute pores (foramina) through which the pseudopodia pass to the outside, a condition which gave rise to the name Foraminifera. In the more complicated types of these lime-stone shells, which may reach a diameter of 2 or 3 inches, the calcium carbonate may be deposited at successive intervals of growth, thus giving rise to chambered structure of the cells. Such polythalamous shells are complicated by the presence of an intricate system of canals which, in life, are filled by moving protoplasm (Fig. 74).

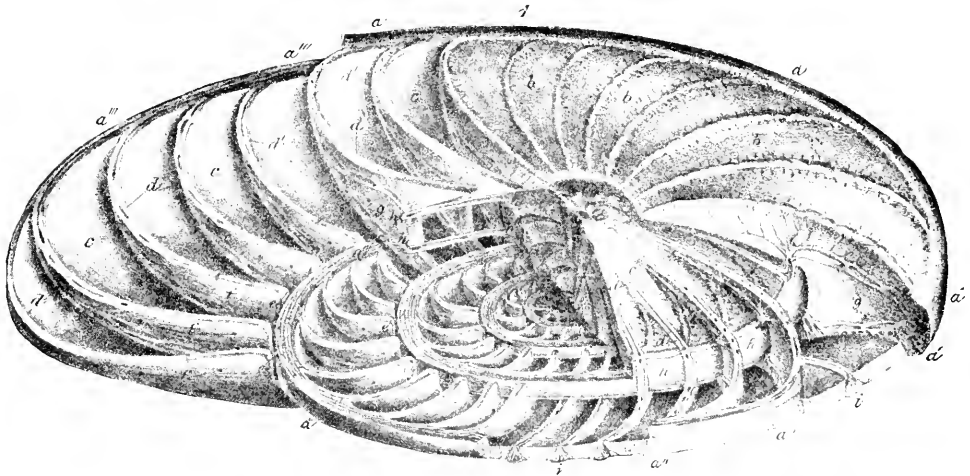


FIG. 74.—A complex polythalamous shell of *Operculina* (schematic). The shell is represented as cut in different planes to show the distribution of the canals and the arrangement of septa and chambers. (After Carpenter.)

Skeletons of Heliozoa and Radiolaria, unlike the more clumsy shells of the Foraminifera, are usually delicate in structure and graceful in design. They are formed for the most part by a deposit of silica upon a chitinous base. Dreyer has given evidence to indicate that such skeletons have their beginnings in spicules which conform in shape and size with the nodal points in the alveolar walls of the cytoplasmic reticulum (Fig. 12, p. 33). Isolated spicules are characteristic of several Heliozoa and Radiolaria where they form a loose or felted covering in the outer protoplasm. Such spicules invariably grow by accretion, that is, by the addition of new substance to the outside of that already formed. If such added material is formed in a limited region of the protoplasm, the result is a con-

tinued accretion of silica to the end of a spicule which is pushed farther out with each increment, thus giving rise to long bars and spines which are radially arranged in forms like *Acanthocystis aculeata*, etc. (Fig. 75). The silicious deposit, again, may be made throughout a zone completely surrounding the center, resulting in clathrate or latticed skeletons of varying grades of complexity (*Clathrulina elegans*, *Nassellaria*).

While cellulose mantles and shells are more usually found in



FIG. 75.—Types of spicules in Heliozoa. A, *Raphidiophrys pallida* with curved silicious spicules; B, *Pinaciophora rubiconda* with tangential plates and forked spines; C, *Acanthocystis tuffacea*, with separated plates and forked spines; D, *Pinaciophora fluviatilis*. (From Calkins after Penard.)

chlorophyll-forming organisms, there are some types in which internal skeleton elements are composed of this or a closely related substance. In the parasitic Ophryoselecidae skeletal structures are present which are made up of a substance resembling cellulose to which Dogiel gave the name Ophryoselecin.

(b) **Motile Organoids.**—The organoids by which Protozoa move are to be considered as modifications of the cortex, although some types, as shown in the preceding chapter, are derived in part from internal kinetic elements (flagella and some pseudopodia). Three main types are distinguishable—flagella, pseudopodia and cilia, each of which is sufficiently distinct from the others to furnish a natural basis for classification of the Protozoa, a basis of classi-

fication which Dujardin first employed to create the three great groups *les flagellés*, *les rhizopodes*, and *les ciliés*. Each type is subject to many variations, due to inherent differences in the motile organoids themselves, or to fusion in various ways leading to structures of considerable complexity.

It is extremely difficult to decide whether flagella or pseudopodia are the more primitive in type. From most general text-books on Zoölogy we learn that the matter admits of no question, and are taught that the pseudopodium is the most primitive form of motile organ in the animal kingdom. This certainly has been the most widely accepted view. Many a generalization referring to Protozoa, however, which has found its way into general works on Biology, appears to have been drawn from the conditions in some one organism which is conspicuous by reason of its abundance and ease of study. It would sometimes appear, indeed, that the common species of *Paramecium* and *Amoeba proteus*, to many general writers constitute the Protozoa. This seems to be the case with the problem of pseudopodia and flagella, the argument being that a pseudopodium of *Amoeba proteus* is certainly a less complex motile organ than the flagellum of *Eugleua viridis*, and therefore more primitive. Had the comparison been made between the pseudopodia of *Actinophrys sol* or *Acauthocystis aculeata* and a typical flagellum, the conclusion would not have been so obvious. There is a good deal of evidence against the generalization as it is usually expressed. In the first place, a pseudopodium of *Amoeba proteus* cannot be interpreted as a motile organ. It is not a definite structure in the cell, nor does it cause the body of *Amoeba proteus* to move. On the contrary, it exists because of the movement of the body protoplasm and the pseudopodium is merely the visible, physical expression of this movement which, in turn, is due to the transformation of energy in destructive metabolism. This energy finds its vent in that portion of the ectoplasm which, for the time being offers the least resistance; the ectoplasm gives way at this point, the endoplasm gushes through and a pseudopodium results (see Chapter XII, p. 435). Such pseudopodia are not the source of movements of the cell, they are results, not causes, of movement. The pseudopodia of some Heliozoa, on the other hand, are motile organs, and the axial filaments which they contain are regarded as equivalent in structure and in mode of origin to the kinetic elements of flagella. The pseudopodia of Foraminifera are intermediate between those of Heliozoa and those of testate rhizopods. The problem, then, comes down to a theoretical question of probabilities. Is it more probable that pseudopodia of the type found in *Amoeba proteus* become progressively differentiated into motile organs through stages like the finger-formed pseudopodia of the testate rhizopods, the reticulate pseudopodia of Foraminifera and axopodia of Heliozoa and Radio-

laría, to the typical motile organ of the flagellate type? Or is it more probable that a motile organ originating from a definite kinetic center (basal body or blepharoplast) has become progressively indefinite with loss of the kinetic elements through the same series of forms, but in the opposite direction, and ending in types like *Amoeba proteus*? To my mind, the pseudopodia of *Amoeba proteus* and its immediate relations, have no place at all in such a series; they are merely expressions of the physical conditions of the protoplasm and of the forces operating within, and they may appear in any cell having an appropriate physical make up. Thus we find them in certain types of cell (leukocytes and phagocytes) widely distributed throughout the animal kingdom, and we find them here and there, in every group of the Protozoa.

An illuminating illustration in support of this conclusion is afforded by the transitory flagellated stages of one group of ameboid organisms, the Bistadiidae (see p. 108). Here, in *Dimastigamoeba gruberi*, for example, the organism loses its pseudopodia under certain conditions, and develops flagella, not by metamorphosis of the pseudopodia, but from blepharoplasts which, as centrioles, emerge from the nucleus (Fig. 59, p. 108).

Although only a matter of academic interest, I believe that the flagellum type of motile organs is the most primitive type we know while axopodia and myxopodia, the former with kinetic elements of weakened function, the latter with denser axial protoplasm which Doflein also interprets as equivalent to axial filaments, represent stages in the deterioration of the kinetic function coincident with the absence of definite kinetic centers (see also p. 120). For these reasons also, together with others which will be given later, we hold with Doflein (1916), Klebs and many others, that the group of flagellates furnishes more evidence of original ancestry than do the rhizopods (see p. 411).

1. **Flagella.**—Flagella are widely distributed throughout the animal and plant kingdoms, forming the motile elements of animal spermatozoa and of plant zoöspores, or current-producing organs of many types of Metazoa. They are sometimes combined with pseudopodia (*Dimorpha mutans*, Fig. 13, p. 34, *Mastigamoeba invertens*, *Ciliophrys infusionum*, etc.), sometimes with cilia (*Myriaphrys paradoxa*, Fig. 197, p. 478).

Flagella are usually excessively fine and delicate fibers extremely difficult to see and to study in the living organism. In the great majority of cases the finer structure has not been made out, but in a few favorable types some progress has been made. In these cases it is known that the flagellum is made up of two definite elements, an axial, highly vibratile filament, which is formed as an outgrowth from the basal body or blepharoplast, and an enveloping elastic sheath which is formed from the proplastic substance of the cor-

tex. In some cases the sheath is circular in cross-section (see Plenge), in others ellipsoidal, while the contractile thread which is usually attached firmly to the sheath may run in a straight line the entire length of the sheath, or may follow a spiral course. In the majority of flagellates the sheath undulates and vibrates in unison with the contractile axial thread, but in a few types, such as *Peranema trichophora* or certain species of *Bodo*, the sheath remains passive while the axial thread extends freely beyond the limits of the sheath, where its activity in the surrounding medium results in a steady progressive movement of the cell. Under the influence of somewhat violent stimuli, however, the sheath itself may undergo fibrations in such forms.

Owing to the nature of flagella and to their delicacy of structure, there are not many possibilities of variation in type. In addition to those which are circular or ellipsoidal in cross-section, there are some which are band form. Such band-form flagella suggest the possibility that vibratile membranes, which are not uncommon in parasitic types of flagellates, may, morphologically, be regarded as flagellum sheaths which remain attached throughout their length to the cortex while the axial thread forms the contractile margin (Fig. 169, p. 360). Such vibratile membranes are characteristic of the genera *Trypanosoma*, *Cryptobia*, *Trichomonas*, *Trichomastix*, etc., all of which are parasites in the blood or digestive tract of different animals.

There are, however, abundant variations in size, number and position of flagella in the cell. When there is but one it usually emerges from a pit or funnel-shaped opening at the anterior end of the cell (flagellum fissure). When two are present they may be equal in size and length (*e. g.*, *Spongomonas splendida*, Fig. 49, p. 95), or one may be considerably thicker and longer than the other (heteromastigote types). Both may be directed forward as in *Amphimonadidae* or one may be directed forward, the other backward, as in *Bodo*, *Anisonema*, etc. In such cases the posteriorly directed flagellum (trailing flagellum or *Schleppgeissel*) appears to act as a runner upon which the cell body glides, and has little to do with the actual locomotion of the animal (Fig. 76).

Delage and Hérouard have attempted to explain the dynamics of flagellum action whereby the comparatively heavy body is moved forward by reason of the vibrations of the exceedingly delicate thread. In the usual type the extremity of the flagellum describes a rather wide circle so that it is in a certain focus of the microscope for only an instant of time. With this circular movement, which varies in different species, constant undulations pass from the base to the tip. A forward pull results from the combination of such movements and the cell either glides smoothly after its active propeller or rotates more or less rapidly on its long axis while freely

swimming. When two flagella are present a curious shaking movement may accompany rotation and translation.

With such energetic motile organs exerting a constant strain on the body there would seem to be some danger of their being pulled out, especially in those types with soft fluid bodies without firm

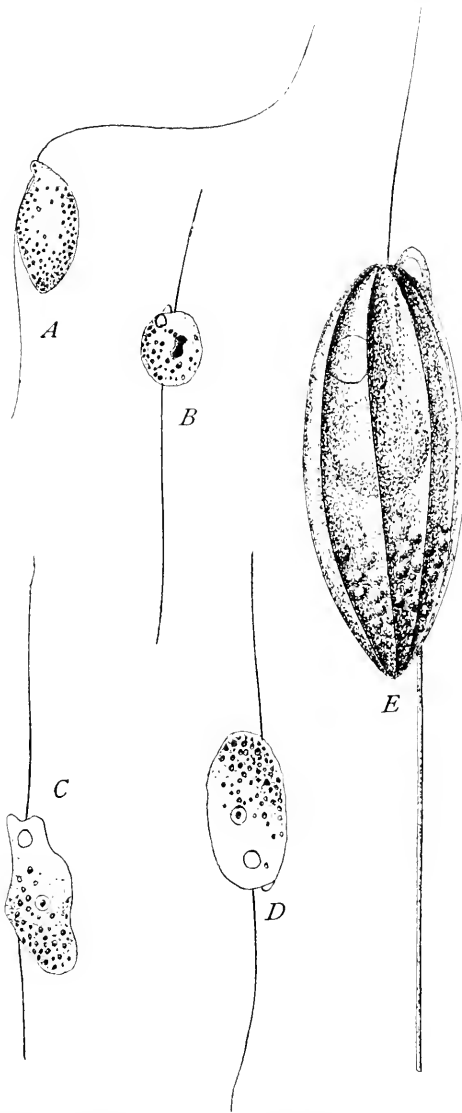


FIG. 76.—Free-living flagellates with trailing flagella. A, C, D, *Bodo caudatus* St.; B, *Bodo globosus* St.; E, *Plocotia vitrea* Duj. (After Calkins.)

periplasts. This phenomenon has indeed been recorded by some observers, the flagellum, freed from the body, moving off like a spirochete (Klebs, Bütschli, Fischer, etc.). Such observations may or may not be well founded, at any rate accidents of this character are guarded against by the manner of flagellum anchorage in the cell. As described in Chapter III a flagellum is derived from a blepharoplast which may be just below the periplast or deeper in the protoplasm, or it may arise from the nucleus (Fig. 59, p. 108). Its anchorage is further assured by rhizoplasts which sometimes run to the posterior end of the cell as in *Herpetomonas* or species of *Rhizomastix* (Fig. 62, p. 116), or which form a branching complex deep in the body substance as in *Dimastigamoeba* (Fig. 59, p. 108). In the various species of *Giardia* the basal bodies of the eight flagella are connected by a complete system of rhizoplasts (Fig. 17, p. 37).

Another type of structure which is regarded by some (*e. g.*, Kofoid) as a modified flagellum is represented by the axostyles or internal motile organoids of the parasitic flagellates. In *Trichomonas* this appears like a glassy, hyaline curved bar of considerable diameter, extending from the nucleus to the posterior end of the cell where, like a spine, it projects from the periphery (Fig. 77). It is usually interpreted as a supporting axial rod to give rigidity of form to an otherwise soft and variable body (Doflein). Dobell regards it as a remnant of the centrodesmose left in the cell after division of the blepharoplast, a view supported by Hartmann and Chagas (1910) who interpret it as a centrodesmose formed during division of the intranuclear centriole. Martin and Robertson (1909), on the other hand, found that axostyles arise after division quite independently of the nucleus or of centrodesmose, and regarded them as independent organoids of the cell. Kofoid and his associates discard the assumption that axostyles are supporting or skeletal structures and place them in the category of kinetic elements. They are interpreted as intracellular organoids with a contractile function characteristic of flagella and serve as organs of locomotion in the dense media in which the parasites live and in which the flagella would be ineffective. They are closely connected with the blepharoplasts in all species of *Giardia* (Fig. 17, p. 37), and are regarded as independent, self-perpetuating organoids which may be the first to divide in the processes of reproduction (*Giardia*) or the last to divide (*Trichomonas*). In all cases, according to these observers, but denied by others, the axostyle divides longitudinally throughout its entire length, beginning with divisions of the anterior end in which the blepharoplast may be embedded (Fig. 77).

In regard to the two opposing points of view as to the function of axostyles the evidence rather supports the interpretation of Kofoid and Swezy (1915). The necessity of a supporting struc-

ture, or a form-rectifying organ, in these parasitic types is difficult to conceive. On the other hand, their intimate relation to the blepharoplasts and their activity in reproduction indicate a common function with the kinetic elements. The observations of Kofoid and Swezy on the energetic movements of the axostyle while the organism works its way through the mucus afford a more plausible interpretation of the function of this organoid than the *a priori* view of those who see in such movements only the efforts of an elastic supporting structure to restore the form of a plastic cell.

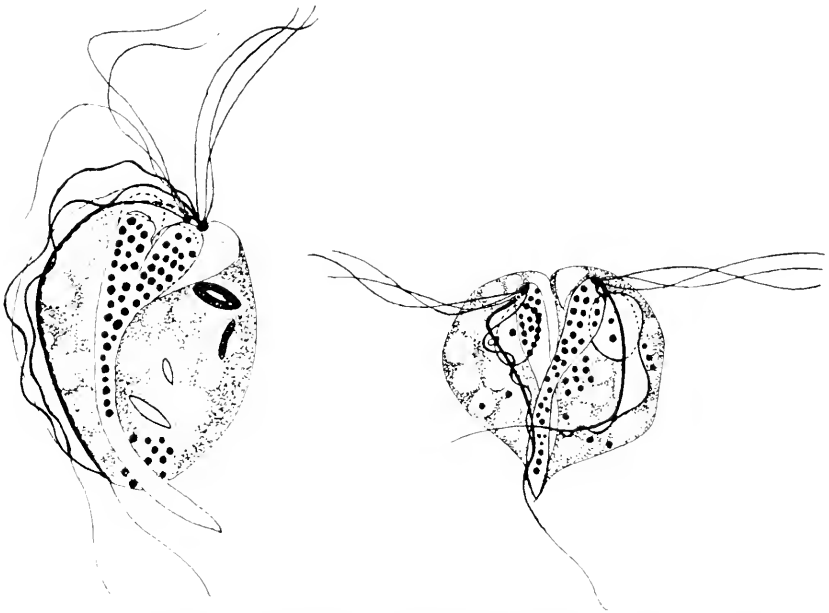


FIG. 77.—*Trichomonas augusta* Alex. Two successive stages in division of the axostyle. (After Kofoid and Swezy.)

2. **Pseudopodia.**—Pseudopodia are more or less temporary projections of the cortex which may serve for purposes of locomotion or, more often, as food-trapping or food-catching organoids. Four types are recognized, axopodia, rhizopodia (myxopodia), filopodia and lobopodia, which differ widely in their structural make up. Of these only the first type can be regarded in a strict sense as motile organs (see p. 140), the others functioning as food-catching organoids, or mere protrusions of the semifluid body.

Axopodia.—Axopodia are different from other types of pseudopodia in possessing, like flagella, central axial fibers of specialized protoplasm derived from endoplasmic kinetic elements. They are found only in organisms belonging to the groups Heliozoa and

Radiolaria, in which they radiate out in all directions from a usually spherical body (Fig. 78).

Unlike flagella, the outer coating of an axopodium is not a smooth periplast-like sheath, but consists of fluid protoplasm in which the movements of granules out on one side and back on the other are

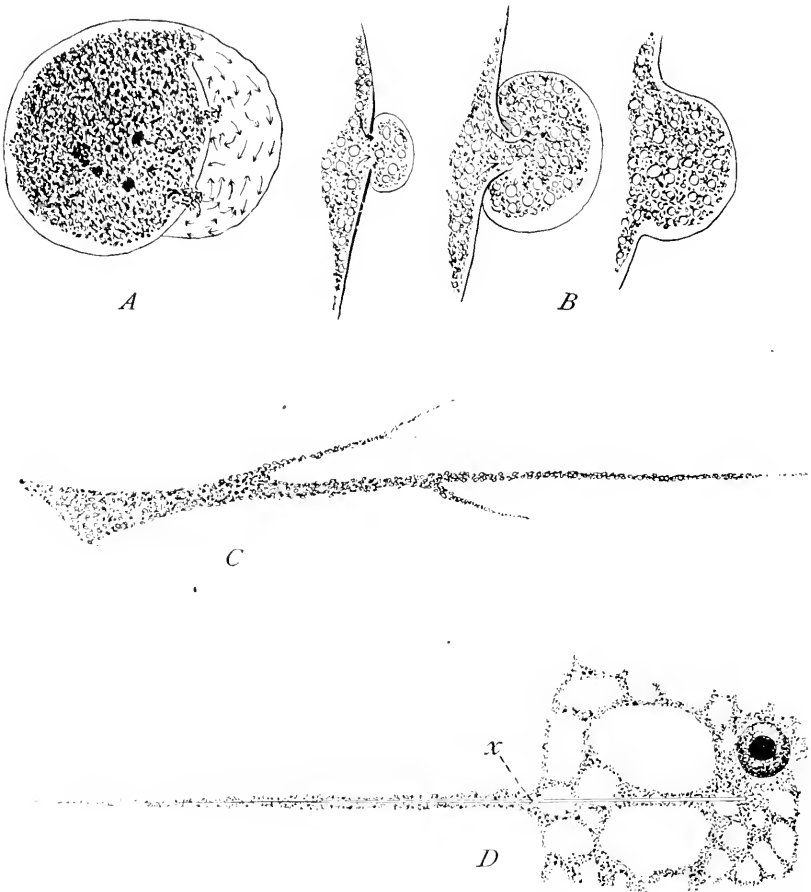


FIG. 78.—Types of pseudopodia. *A, B*, Eruptive type of lobopodium; *C*, myxopodia type of Foraminifera; *D*, axopodia type of Heliozoa. (After Calkins.)

clearly discernible. In this manner the outer protoplasm is continually changing about the central axial filament, which alone is constant or fixed. Upon prolonged irritation, or in preparation for division or encystment, the axial filaments themselves, together with the enveloping protoplasm, are withdrawn.

Like flagella the axial filaments are formed as outgrowths from

endoplasmic kinetic elements. *Gymnosphaera*, *Raphidiophrys*, *Sphaerastrum*, *Acanthocystis*, *Dimorpha*, etc., possess characteristic "central granules" which, from their activities in cell division, are unmistakably centroblespharoplasts (see p. 117) from the substance of which the axial filaments are formed (Fig. 50, p. 95). *Wagnerella borealis*, in addition to the central granule, possesses a zone of basal bodies which give rise to the axial filaments and which at times of retraction of the pseudopodia are drawn into the central granule. In still other cases, as in *Actinosphaerium eichhornii*, the axial filaments do not arise, apparently, either from central granules or from nuclei, but appear to start indefinitely in the cytoplasmic reticulum (Fig. 78, D).

While the more common forms of Heliozoa are quiescent, floating types, some of the Heliozoa are freely motile. *Acanthocystis aculeata*, as well as other species of the same genus, turns slowly over and over in a rolling movement; *Camptonema nutans*, according to Schaudinn, bends and straightens its axopodia in food-getting and in other activities. *Actinosphaerium eichhornii* and *Actinophrys sol* are practically motionless. The active movements are due to the axopodia and the structure of axopodia is strikingly like that of flagella. That the contractile axial filament is the seat of this movement, and not the enveloping protoplasm, is not open to reasonable doubt. Structure, function and mode of origin thus justify the inclusion of axopodia with the kinetic elements of the cell.

On the other hand, in types with axopodia which are practically motionless, the axial filaments have apparently lost the vibratile function and now serve as supporting elements for the long radiating pseudopodia. There is little reason to doubt that such elements are homologous with the axopodia of motile types and that the latter are homologous with flagella. This is well illustrated by the case of *Dimorpha nutans* where two flagella and many axial filaments of axopodia originate from the same blepharoplast (Fig. 79.)

Speculations as to phylogeny on purely morphological grounds are not profitable, but in this group of Heliozoa we have pretty good evidence of a close relationship between flagellates and Sarcodina, and equally good evidence of the transition from an active kinetic element to an inactive, supporting axial rod, as seen in the pseudopodia of *Actinosphaerium eichhornii*. This change in type is probably associated with the loss of specific kinetic centers for neither in the cytoplasm nor in the nuclei are such elements to be found. In some forms, finally, notably in *Clathralina elegans*, the ends of the axopodia are frequently branched, a condition which points the way to pseudopodia of the rhizopodia type in which the supporting element is not in the form of an axial rod, but in the form of stiff stereoplasm (Fig. 78, C). The pseudopodia of *Clathralina*, which

have no axial filaments, appear to be transitional to those of the testate rhizopods to which group Valkanov (1928) assigns them. In this stalked form (Fig. 82), however, the stalk takes its origin from the nucleus, as Valkanov clearly shows, and at some stages, at least, has a fibrillar structure. This suggests the possibility that the stalk of *Clathrulina* (and of *Hedriocystis*) may represent the concrescence of ancestral axial filaments.

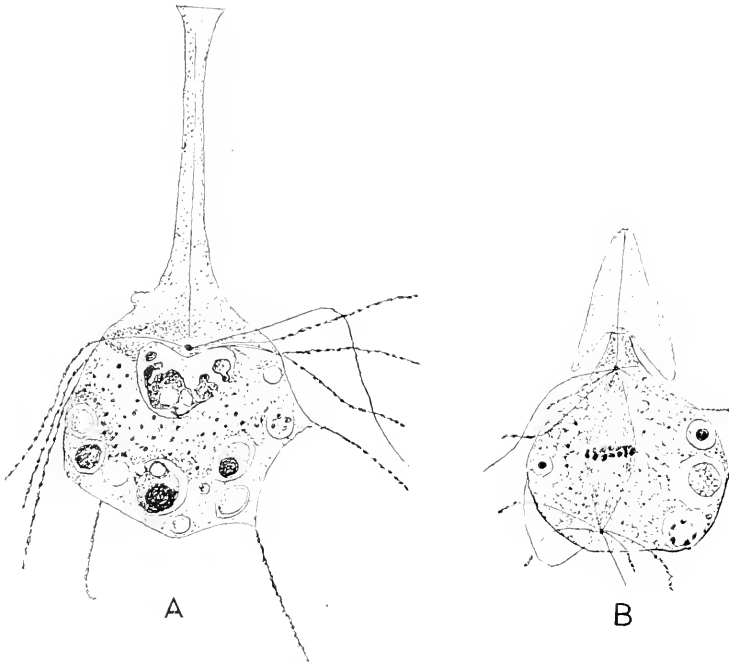


FIG. 79. — *Dimorpha mutans*. Vegetative individual with two flagella and axopodia. Axial filaments of axopodia and flagella meet in a common central granule. At division the central granule divides and forms the poles of the mitotic figure, while the axial filaments form astral rays. $\times 1950$. (After Bělař, Allgemeine Biologie, 1927; B. Ergeb. u. Fortschritte d. Zoologie, courtesy of G. Fischer.)

Rhizopodia. This type of pseudopodia differs from others, first, in the tendency to branch and, second, in the tendency to fuse or anastomose when such branches meet. From these characteristics they are sometimes called reticulose pseudopodia and myxopodia. So far as number of species is concerned, this type is the most characteristic form of Sarcodina pseudopodia. They occur in all forms of Foraminifera, Radiolaria and Mycetozoa which include the great majority of Protozoa. As a result of their unlimited power to branch and to anastomose, great meshworks of reticulated protoplasm are created which make ideal traps for the capture of food.

In many types, especially in Radiolaria, they may be long and ray-like, with relatively little tendency to fuse; in other cases a main trunk gives rise to so many branches that it is lost in the reticulum, great accumulations of protoplasm collecting at the branching points (Fig. 10, p. 32).

Doflein includes axopodia and these branching anastomosing pseudopodia in the one type (rhizopodia), and sees in the axial filament of the former and the inner protoplasm of the latter only

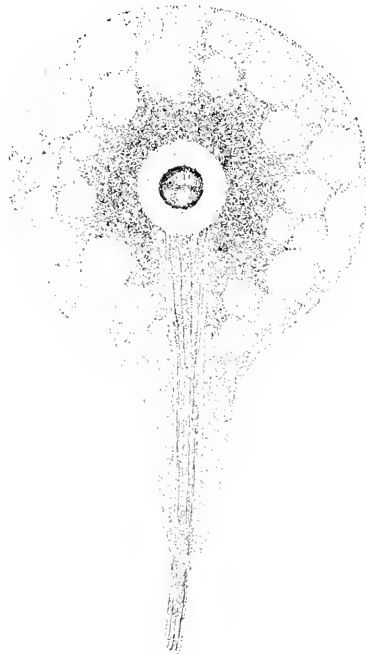


FIG. 80.—*Clathrinula elegans*, stalk formation. (After Valkanow, Archiv f. Protistenkunde, 1928, courtesy of G. Fischer.)

different states of the same fundamental stereoplasm. Axial filaments, however, derived from the substance of kinetic centers, are quite different from structureless axial stereoplasm which has no relation to kinetic elements. The enveloping protoplasm is apparently the same in both types and granule streaming is a common property, but the physical consistency is quite different. In rhizopodia the outer protoplasm is soft and miscible, leading to fusion on contact with one another, while axopodia never anastomose. The denser core of rhizopodia, while not condensed to a single fiber, serves the same function of support as the axial filament of *Actinosphaerium* and gives stiffness and rigidity to long ray-like pseudo-

podia of many Foraminifera and Radiolaria which stand out in all directions from the cell.

Filopodia.—Structurally filopodia are entirely different from the types described above, being formed of clear hyaline ectoplasm in typical cases, or they contain a few granules indicative of endoplasm (Fig. 11, p. 33). They are usually long and slender and with rounded ends giving the impression of slender glass rods. In some forms there is a tendency to branch at the ends as in *Euglypha alveolata* (Fig. 9, p. 31), but there is never anastomosis. Sometimes they sway back and forth like a filament of *Oscillaria*, but usually they creep along the substratum where they serve mainly for food capture.

Filopodia are characteristic of the fresh water testate rhizopods, but are sometimes present in naked types like *Amoeba radiosa*.

Lobopodia.—Lobopodia are made up of granular endoplasm and hyaline ectoplasm, and are temporarily projected portions of the body protoplasm not to be compared with definite locomotor organs of other Protozoa. The inner protoplasm of nearly all kinds of Protozoa with granules of various kinds, food substances more or less digested, and waste materials, is in constant movement called cyclosis. In more highly differentiated forms, and in organisms with a firm cell membrane, this movement is confined to the internal protoplasm and the form of the cell is not affected by it. In the shell-less rhizopods, however, there is no such outer covering, and the peripheral protoplasm gives way at the weakest points, and an outward flow of protoplasm with corresponding change in the form of the body results (see Chapter V). If such a weak point is constant in position, a constant flow in its direction is the outcome, and the *Amoeba*, consisting of practically one pseudopodium, as in the *limax* types, moves in one direction. In *Amoeba verrucosa* a delicate periplast surrounds a somewhat dense protoplasm which, accumulating on one side (according to Rhumbler, 1898), causes the cell to roll over.

Withdrawal of pseudopodia is accomplished by their absorption into the body substance, and is accompanied by a wrinkling of the denser ectoplasm preparatory to its transformation into endoplasm (see Schaeffer).

In pseudopodia generally it is evident that we have to do with different types of structure which, in only a few instances, can be regarded as motile organs. Axopodia, with their axial filaments derived from kinetic elements, are closely related to flagella and may be regarded as organs of locomotion, but the other types, which may represent highly modified axopodia, have lost the kinetic elements, if they ever had them, and are useful only as food-catching organs. In most rhizopods the entire organism is the motile element, rhizopodia, filopodia and lobopodia being expressions of energy trans-

formations comparable with the rotation of protoplasm in *Nitella* or circulation in *Tradescantia*. Axopodia of the motile Heliozoa, axial filaments of the inactive species and stereoplasmic cores of the rhizopodia may be regarded as successive phases in the modification of vibratile flagella. These types of pseudopodia have in

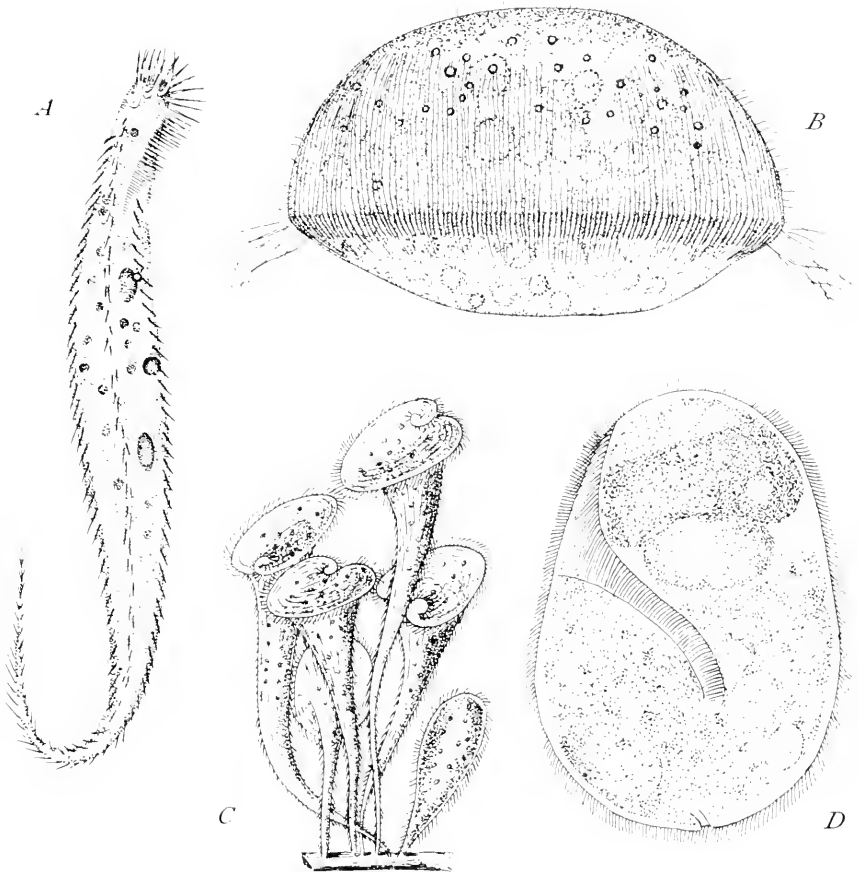


FIG. 81.—Types of Ciliata. *A*, *Uroleptus pisces* (after Stein); *B*, *Cyclotrichium gigas* (after Fauré-Fremiet); *C*, *Stentor polymorpha* (after Bütschli); *D*, *Nyctotherus ovalis* (original).

common an enveloping layer of granular protoplasm, but filopodia and lobopodia represent a different type, being made up in large part, or entirely, of ectoplasm and without any evidence whatsoever of kinetic elements. So-called "contractile elements" of this type of pseudopodia are largely figments of the imagination.

3. **Cilia.**—Cilia are the motile organs of Infusoria and accompany the most highly differentiated types of cortex to be found in the Protozoa. Individually they are shorter, more delicate and less powerful than flagella and owe their importance as motile organs to their large numbers and synchronous beating. Their action may be compared with that of oars in rowing, while flagellum action might be compared with sculling, and the results of cilia and flagella activities bear a relation similar to that between a racing shell and a gondola (Fig. 81).

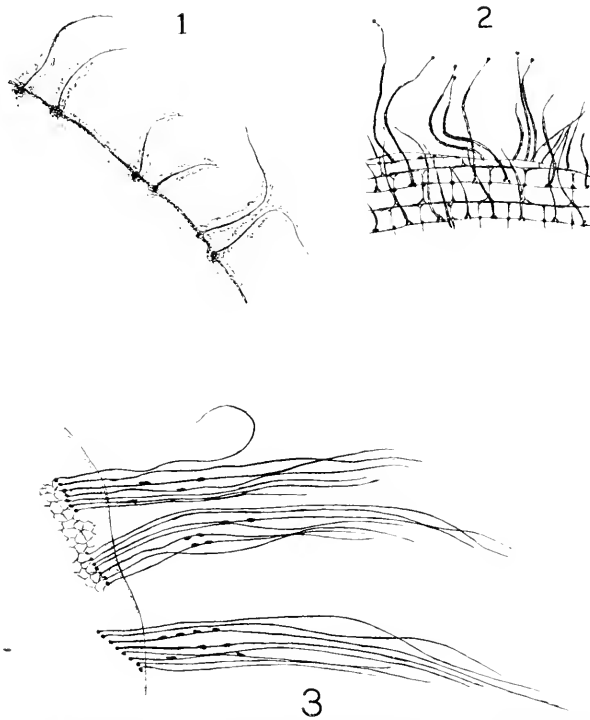


FIG. 82.—Cilia structure. Axial filaments protruding from protoplasmic sheaths in cilia of (1) *Coleps hirtus*, (2) *Paramecium*; (3) cilia make up of three lateral cirri of *Stylonychia*. Silver line technique. (After Klein, *Archiv f. Protistenkunde*, 1929, courtesy of G. Fischer.)

According to the interpretation of several observers, mainly Schuberg, Maier, Schubotz, Schröder, etc., the cortex of ciliates is a composite of zones of differentiated protoplasm. In the majority of cases such zones cannot be made out, for one shades into the other, and the whole into the alveolar endoplasm. In favorable cases, however, we can distinguish: (1) A superficial periplast perforated for the exit of cilia and trichocysts when present; (2) an alveolar

layer containing trichocysts if the latter are present; (3) a contractile zone containing the basal bodies of cilia, myonemes and coördinating fibers; (4) a denser zone which shades off into the endoplasm and supplies an anchorage for nuclei and contractile vacuoles.

A single cilium is constructed on much the same plan as a flagellum, consisting of a central axial filament or fiber, and an elastic sheath of protoplasm. Movement is due to the active contraction



FIG. 83.—*Cyclopidium glaucoma*. Cilia with axial filaments protruding from plasmic sheaths. Silver line technique. (After Klein, *Archiv f. Protistenkunde*, 1929, courtesy of G. Fischer.)

in one plane of the axial fiber and recovery to the elasticity of the enveloping sheath. The contractile element originates from a basal body in the contractile zone. In many organisms local thickenings occur at intervals along the axial filaments. These are similar to basal bodies and are clearly demonstrated by silver nitrate impregnations for bringing out the silver line system (Figs. 82 and 83).

The arrangement of cilia on the surface of the body varies in

different species; sometimes they form a complete coating for the organism as in the majority of Holotrichida (Fig. 84); sometimes they are limited to certain zones as in *Urocentrum turbo*, *Didinium*

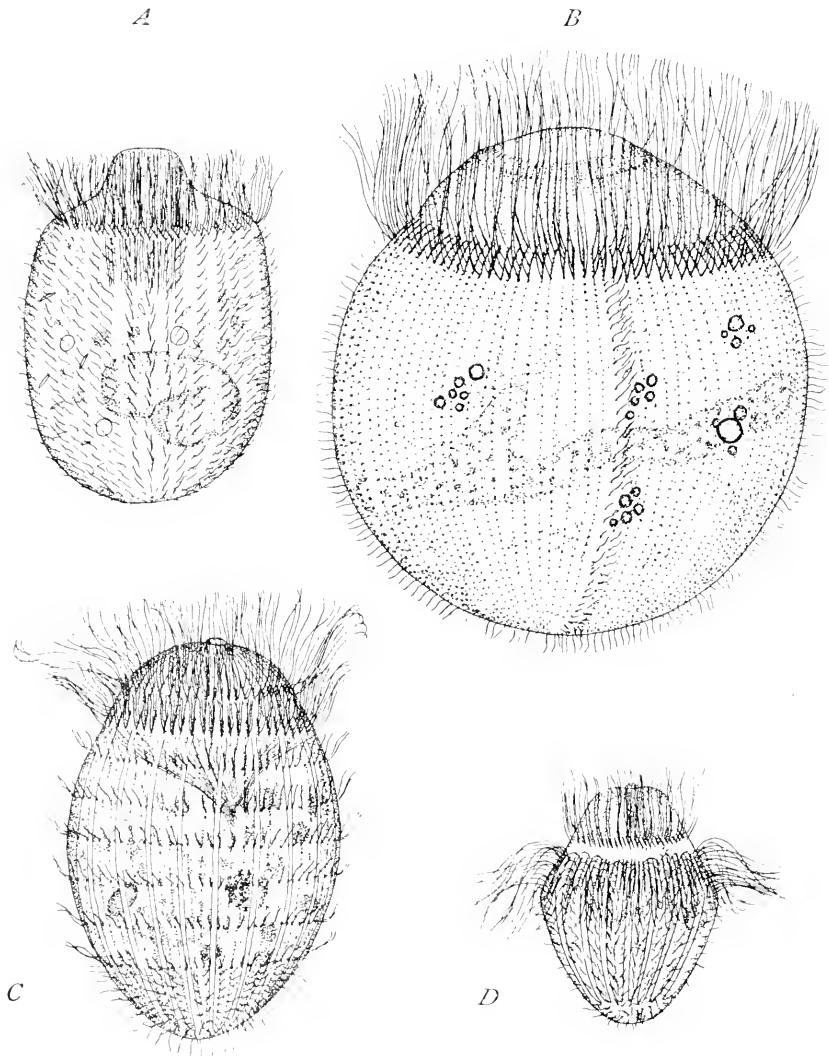


FIG. 84.—Types of Ciliata. A, *Monodinium balbianii*; B, *Cyclotrichium sphaericum*. C, *Dinophrya lieberkühni*; D, *Askcnasia elegans*. (After Fauré-Fremiet.)

nasutum, etc. (Fig. 205, p. 504); or sometimes to the ventral surface, as in generalized Hypotrichida (Fig. 88, p. 159). In all cases they are arranged in longer or shorter rows running straight or spirally, and

giving the striped appearance characteristic of the ciliates. Waves of contraction pass from the anterior end posteriorly, cilia of the same transverse rows beating synchronously, those of the same longitudinal rows metachronously.

The periplast is variously sculptured in different species, giving the appearance superficially of a different mode of origin of the cilia. In some cases they appear to come from the centers of minute cups or dimples as in *Paramecium aurelia*; in other cases from longitudinal grooves or furrows between ridges of periplast (Fig. 69, p. 124), and in some they appear to come from the ridges themselves.

Rhizoplasts or endoplasmic prolongations from the basal bodies are comparatively rare but occur in some cases as in *Didinium nasutum* (Fig. 98, p. 187). Coördinating fibrils apart from the silver line system have been described in a few types (*Euplotes*, *Diplodinium*, see p. 129), and center in a specialized neuromotor body, the so-called motorium (Yocom, Taylor, Sharp).

In some cases cilia are uniform in length over the entire body (*Opalina*); in other cases they are longer in the region of the mouth or around the posterior end, but no sharp dividing point separates short from long ones (Fig. 84). In some cases they are uniformly long and vibrate like flagella (*Actinobolus radians*, Fig. 91, p. 163).

4. **Composite Motile Organs.**—A well-marked characteristic of cilia is the ability of two or more to fuse into motile organs of variable complexity. Such combinations give rise to membranulae, membranelles, undulating membranes and cirri, each of which, although composed of fused cilia, originates or grows as an independent and complete organoid. In each case also the component cilia may be demonstrated by use of dilute alkalies such as potassium or sodium hydrate. It is often difficult to distinguish lines of closely set cilia from fused cilia, and loosely bound cilia are sometimes present, the aggregates being spoken of as "pseudomembranes."

Membranulae.—Membranulae are very long, delicate, finely-pointed aggregates of cilia which differ from the somewhat similar cirri in movement and in composition, while their basal granules, in *Didinium nasutum* at least, are connected with the vicinity of the nucleus by definite rhizoplasts (Fig. 98, p. 187). Similar membranulae form the basal ring in Vorticellidae (Schröder, Schuberg, etc.).

Membranelles.—Membranelles are formed by the fusion of cilia in the region of the mouth. In many of the Holotrichida the cilia are longer just posterior to the mouth than in other regions of the body, frequently forming circlelets about the mouth as in *Lacrymaria olor* or *L. lagenula* (Fig. 85). In the other Orders of Ciliata oral cilia are fused to form membranelles. In the oral regions the body

is usually differentiated into a specialized food-collecting, frequently funnel-like structure called the *peristome*. Cilia on the floor of the peristome are usually longer than in other parts of the body, and in

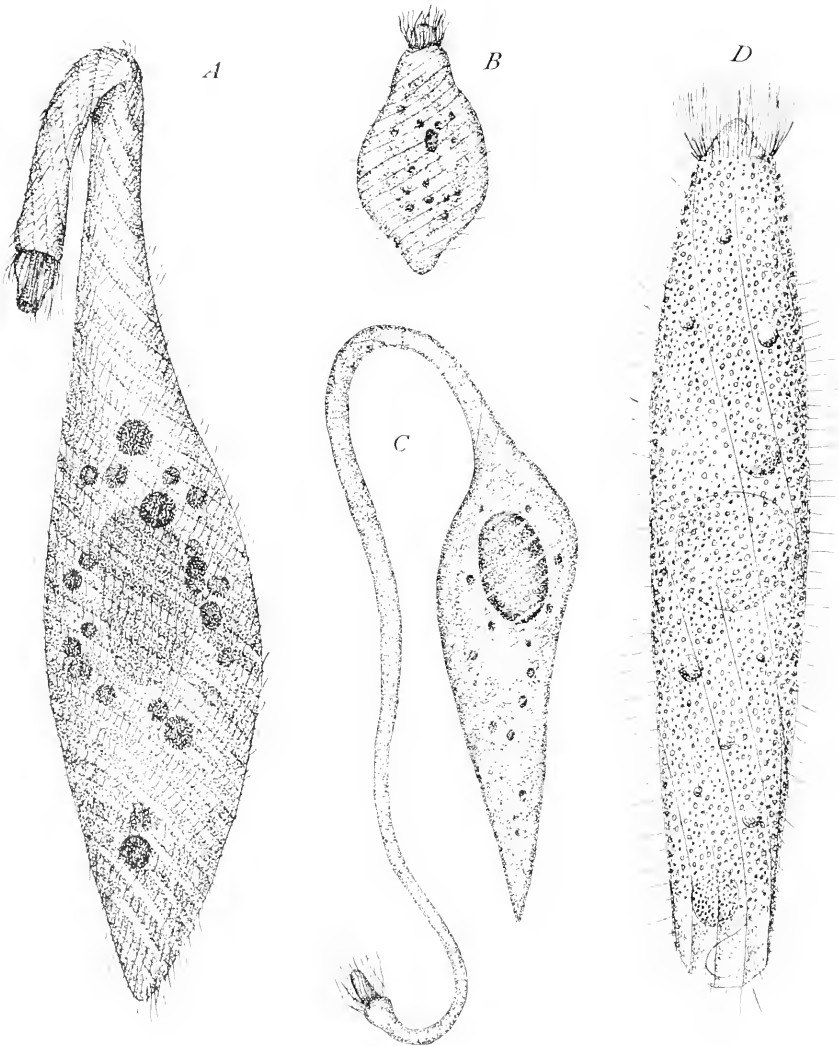


FIG. 85.—Types of *Lacrymaria*. A, *Lacrymaria* sp.; B, and C, retracted and expanded phases of *Lacrymaria olor*; D, *Lacrymaria lagenula*. (After Calkins.)

four of the five orders of ciliates some of these are invariably aggregated in triangular, quadrilateral or ribbon-like membranelles and membranes for producing food-bringing currents of water toward

the mouth. In every order except the Holotrichida a fringe of such specialized motile organs, known as the *adoral zone*, lies on a margin of the peristome (Fig. 88).

Membranelles are usually made up by the fusion of two rows of cilia as shown by the double row of basal bodies (Maier) and their flat or curved faces make powerful sweeps in the water. According to Schuberg, Gruber, Maier and others, the anchorage of these organoids is quite complex. The basal granules form a double row immediately below the periplast; fibrils from these, analogous to rhizoplasts, form a broad triangular basal plate and are then brought together to form an end thread which connects the membranelle with coordinating fibers (Fig. 72, p. 130).

While in most cases the membranelles represent the fusion of comparatively few cilia in transverse rows of the peristome, making them relatively narrow at the base, in other cases, notably in the Tintinnidae, such fusion includes practically all of the cilia of the transverse rows, making membranelles as broad as the peristome. In the Vorticellidae there are two rows of membranelles, the double adoral zone winding about the peristome usually in a direction opposite to that of the Heterotrichida and Hypotrichida (Fig. 86.)

Undulating Membranes.—Undulating membranes are found in all orders of the ciliates and range in size from delicate aggregates no broader from base to tip than ordinary cilia to relatively enormous balloon-like structures equal in width to more than half the diameter of the body and in some cases, as *Lembadion conchoides*, almost equal to length of the body (Fig. 87). In the simplest cases these membranes are composed of a single row of longitudinally placed cilia, the basal bodies of which form a single basal strand. Since cilia of the longitudinal rows beat metachronously the result of their contraction when fused in these undulating membranes is a series of waves passing from the anterior to the posterior end. In more complex forms undulating membranes may be composed of 3 to 10 rows of cilia, fused in longitudinal rows, the length varying from a few microns to great waving sheets of protoplasm almost as long as the entire cell (Fig. 87). They are usually found in the peristomial area inside the adoral zone and are named preoral, endoral, paroral, etc., according to their positions in relation to the mouth.

Pseudomembranes are present in numerous types. Here the component cilia are not firmly united and the membrane is easily disrupted. Such a membrane, which is rather easily disintegrated, is characteristic of *Blepharisma undulans*. Chambers and Dawson (1925) were able to hold down a portion of the pseudomembrane with a needle whereupon the distal portion broke into fibrils which later reunited after the obstruction was removed.

Cirri.—Cirri are the most highly specialized of all the motile organs of ciliates, the most characteristic forms occurring in the

Hypotrichida. They are placed more or less definitely on the ventral surface, a group, variable in number, at the anterior end being known as the *frontal cirri*, a similar group, also variable in number, near the posterior end being known as the *anal cirri*, while other groups may form *caudal cirri*, *ventral cirri*, *marginal cirri*, etc. (Fig. 88).

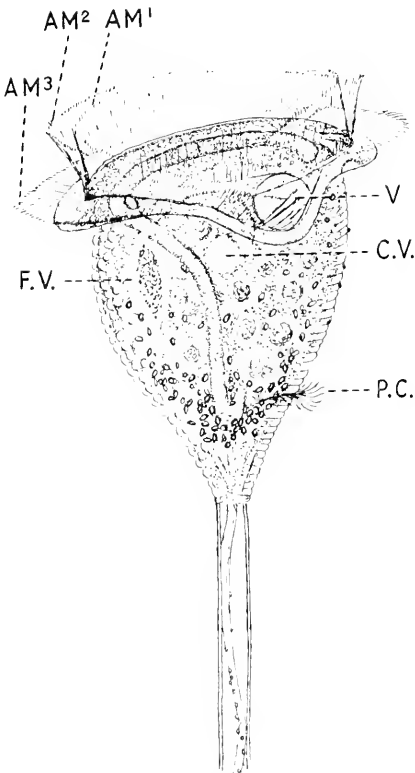


FIG. 86.

FIG. 86. Structure of typical Vorticella showing the adoral membranes, AM^1 - AM^3 ; vestibule, *V*.; contractile vacuole, *C.V.*; food vacuole, *F.V.*, and posterior cirret of cilia. (After Noland and Finley, from *Trans. Am. Microscopical Soc.*, 1931.)

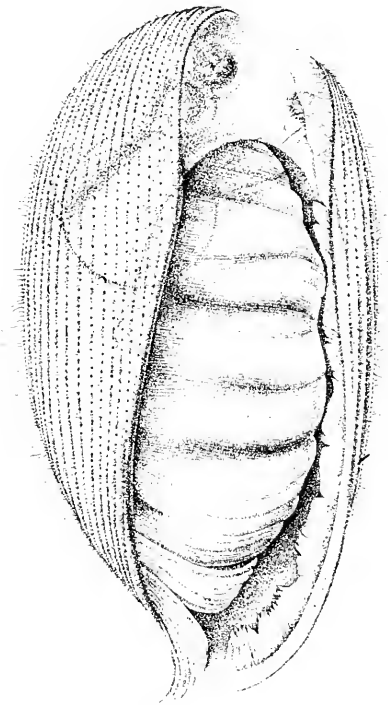


FIG. 87.

FIG. 87.—*Lumbadion conchooides* F. F. (After Fauré-Fremiet.)

Cirri are always broader at the base and taper gracefully to a fine point. In cross-section near the base they are either circular, ellipsoidal, quadrilateral or irregular, and always have a basal plate made up of the basal granules of the fused cilia. Under unfavorable conditions of the medium in which the organisms live, and usually after imperfect fixation, the constituent cilia become separated par-

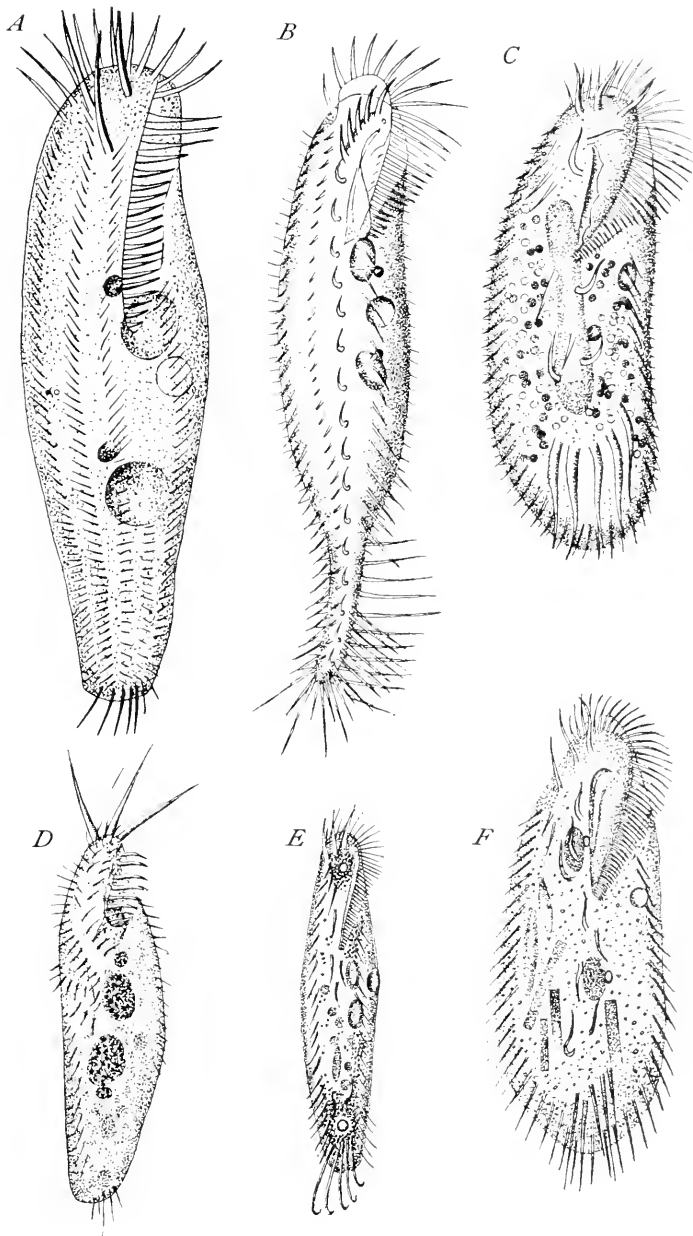


FIG. 88.—Types of Ciliata. A, *Amphisia kessleri*; B, *Uroleptus piscus*; C, *Histrio pellionella*; D, *Strongylidium* sp.; E, *Oxytricha pellionella*; F, *Oxytricha fallax*. (A, after Calkins; B, C, D, E, after Bütschli; F, after Stein.)

ticularly near the tip, and the cirri then present a most frayed-out or ragged appearance. They vary in size from extremely minute cilia-like marginal and ventral cirri to great ventral brushes in forms like *Aspidisca* (Fig. 90) or huge hooked structures as in *Uronychia*, *Diophrys* and other Euplotidae (Fig. 89) (see also p. 221).

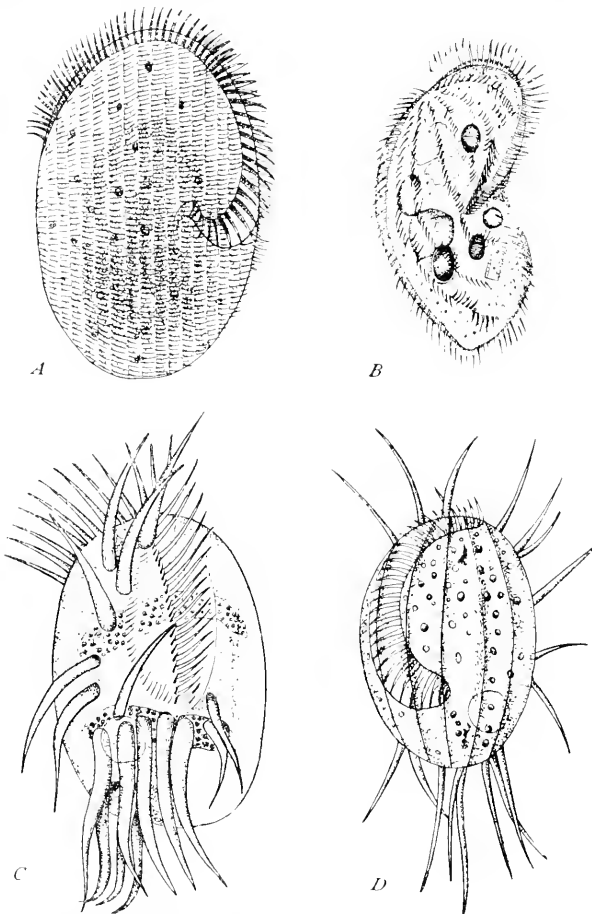


FIG. 89. Types of ciliates. A, *Peritromus cinnam*; B, *Kerona pediculus*; C, *Diophrys appendiculatus*; D, *Euplotes charon*. (A, C, D, after Calkins; B, after Stein.)

Cirri are preëminently organs of locomotion, but, unlike other motile organs of the ciliates, their stroke is not confined to one plane but may be in any direction. This gives to the Hypotrichida an extreme variety of movements unparalleled by any other group of Protozoa. Many of them walk or run on the tips of their frontal and ventral cirri (*Stylonychia*); others swim with a peculiar jerky

movement (*Aspidisca*); others combine swimming due to the adoral zone with sudden jumps or springs due to the anal or caudal cirri (*Uronychia*, *Euplotes*, etc.). Such saltations are not limited to the Hypotrichida, however, but are characteristic of organisms in all groups where cirri are developed as in *Halteria grandinella* among Oligotrichida, *Mesodinium cinctum* among Holotrichida, etc.

In some cases cirri are differentiated as tactile organs, especially the more dorsal ones of certain Hypotrichida. It is probable that such cirri are no different from other motile organs of the ciliates in this respect, extreme irritability being a common characteristic. Few observers can have failed to note the instantaneous effect of a slight local irritation on a quietly resting *Pleuronema chrysalis*, for example, with its long cilia radiating out in all directions, yet there are no cirri here.

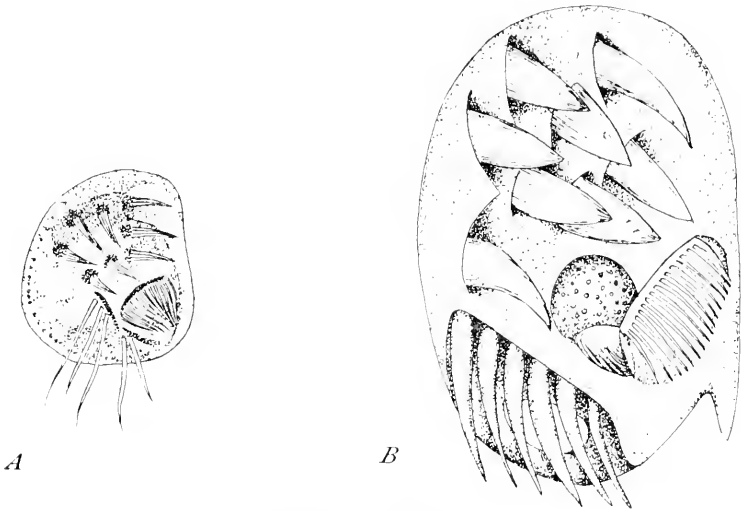


FIG. 90.—Two species of *Aspidisca*. (Original.)

The synchronous and metachronous vibrations of cilia and cilia aggregates are probably regulated by coördinating fibers with highly developed irritability. This is the interpretation given by Schuberg to the basal fibrils in the contractile zone of *Paramecium caudatum*; by Neresheimer (1903) to certain fibers distinct from the myonemes in *Stentor corruleus*, and by Sharp, Yocom, Taylor and others, to conspicuous fibers in *Diplodinium caudatum* and *Euplotes patella* (see p. 127); others, however (*e. g.*, Jollos, and Bělař), interpret them as supporting structures. In the latter organism Yocom (1918) and Taylor (1920) found fibers running from the posterior anal cirri and from the adoral zone of membranelles to a common anteriorly placed structure termed the motorium, which

they regard, with Sharp (1914), as a center of the neuromotor system (see p. 129). The ventral and frontal cirri, however, are not connected by similar fibrils with this motorium, but possess bundles of fibrils, described earlier by Prowazek in *Euplotes harpa*, and by Griffin in *E. worcesteri*, which may run in any direction until lost in the endoplasm. The inference is that these cirri are independent of the coördinated system of fibrils which regulate the adoral zone and the anal cirri, and that their movements, which are always irregular, are not affected by cutting the coördinating fibrils of the motor system (Fig. 72, p. 130, also see p. 131).

(c) **Other Organoids Adapted for Food-getting.**—Mention may be made here of a few special types of cortical differentiation apart from the cell mouths, which Infusoria use for purposes of food-getting. The most striking of these are the tentacles of *Actinobolina radians*, the "tongue" or "seizing organ" of *Didinium nasutum* and the tentacles of the Suctoria.

Contractility due to myonemes is a widely-distributed phenomenon in ciliated Protozoa and in most cases involves the activity of the entire organism (see p. 124). When it is limited to restricted portions of the body, such as the peristomial complex of *Diplodinium caudatum*, or the "vestibule" of Vorticellidae, it acquires a special interest. Even more remarkable than these, however, is the power, possessed by *Lacrymaria olor*, of projecting its mouth-bearing extremity any distance up to three times the length of the flask-shaped body, or until the rubber-like neck is reduced to a mere fibril. The "head" thus projected dashes here and there with amazing rapidity, the body meantime remaining quiet and unmoved, until finally the head and neck are withdrawn and the cell swims off with no visible trace of contractile structures (Fig. 85, p. 156). No special myonemes have been described in this form and the projection and retraction of the "head" must be due to the elasticity of the cortex of the "neck" region, combined with activity of the oral circle of cilia while the body cilia are at rest or relatively quiet.

Another remarkable and special phenomenon, seen apparently by few observers, is the method of food-getting by *Actinobolina radians*. This organism, when at rest, protrudes a forest of radiating tentacles which stand out like axopodia, sometimes stretching a distance equal to two or more times the body diameter. The ends of these tentacles carry trichocysts (Entz, Calkins, Moody) which upon penetrating an individual *Halteria grandinella* completely paralyze it. The tentacle, then, with prey attached, is withdrawn entirely into the body, the *Halteria* is worked around to the mouth and swallowed (Fig. 91). *Actinobolina vorax* (Wenrich) has a similar food-getting mechanism but is not as fastidious about its food as is *A. radians*.

In *Didinium nasutum* the proboscis bears a peculiar protrusible plug or tongue of protoplasm termed the "seizing organ" by Thon (1905) and Prandtl (1907) (Fig. 98, p. 187). A zone of trichocyst-like fibrils lies near the extremity of this plug and when certain types of ciliates, preferably *Paramecium*, are struck by *Didinium* the plug, with trichocysts penetrates the cortex of the prey, paralyzing it. While this process takes place too rapidly to be seen, the results show that it must have taken place for, after striking and anchoring in the *Paramecium*, the seizing organ with prey attached is retracted and the prey, often larger than the captor, is swallowed whole (Fig. 98, p. 187). No satisfactory explanation of this phenomenon has yet been given.

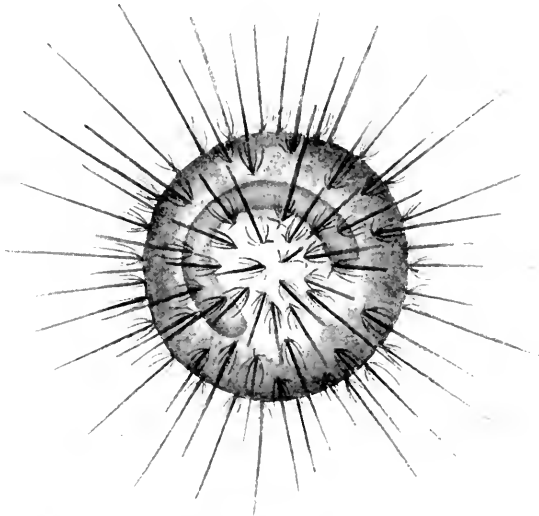


FIG. 91.—*Actinobolina radians* St. (After Moody.)

Still another type of cortical organs is illustrated by the various kinds of tentacles of the Suctoria. Some of these are constructed for piercing, while others are hollow, forming sucking tubes through which food is taken into the body. They are evidently provided with some type of poison, for active ciliates, coming in contact with these tentacles, become suddenly quiet and remain so while the suctorial tentacles penetrate the cortex and suck out the endoplasm of the prey which can be followed through the feeding tubes to the endoplasm of the captor (Maupas, 1883). Like the tentacles of *Actinobolina radians*, these suctorial tentacles are retractile, but again there is no satisfactory explanation of their activity and no description or mention of specialized motile apparatus.

Like the majority of formed organoids of the cell, the more complicated of the motile organs described above are formed anew at each division of the cell. This does not apply to the majority of pseudopodia nor has it been observed in the case of cilia, but is well-established for flagella and for the aggregates of cilia, such as membranelles, undulating membranes and cirri. In a few cases the flagella themselves are said to divide, but this is questionable, the flagella probably arising in all cases from the substance of blepharoplasts or basal bodies which have divided. Young (1922) has shown that a cirrus of *Uronychia transfuga* if cut does not regenerate, but if the protoplasm is partly included in the operation a new cirrus is regenerated. Demboska (1925) has shown that if a single cirrus of *Stylonychia* is cut out all of the cirri are renewed.

(d) **Oral and Anal Cortical Modifications.**—In all naked forms of Protozoa and in corticate forms which, like *Opalina*, take in food substances by osmosis through the general body surface, there are no portions of the ectoplasm differentiated as cytostomes or cell mouths. In such forms, furthermore, where there is no undigestible matter, there is no modification as cytopyge (cytoproct, or cell anus). In testate forms, obviously, there is only a limited region of the body substance which is open for the reception of food. In testate rhizopods the shell openings are due to the physical conditions under which the lifeless shell materials are deposited and no definite mouth parts as protoplasmic differentiations are present.

In all Protozoa, on the other hand, which take solid food and which are covered by more or less highly differentiated cortical plasm, there are permanent openings in the cortex serving for the intake of solid bodies and for defecation of undigested remains. In many cases such openings in the cortex merely expose a limited region of soft receptive protoplasm as in *Oikomonas termo* (Fig. 97, B, p. 186), but in other cases complicated cortical differentiations with supporting and food-procuring adaptations give rise to complex and permanent cytostomes and cytoprocts.

In flagellates such an area of softer protoplasm is situated at or near the base of the flagellum, or two such areas may be present, each at the base of a flagellum or group of flagella, as in *Trepomonas* and *Hexamitus*. In one group, the Choanoflagellidae, a collar-like membrane arises as a protoplasmic fold around the base of the flagellum and forms a cuff or funnel surrounding the flagellum for a distance equal to one-third or one-half its length (Fig. 92). These are extremely delicate, the margins alone in many cases indicating their presence and dimensions. According to Françé, they are somewhat spirally rolled like a cornucopia, the free margin arising from the softer food receptive area and by its movements directing food particles toward this area. This, according to de Saedeleer (1929), is an erroneous interpretation, the appar-

ent spiral roll of the collar being due to the presence of two prehensile tentacles. In some cases two such collars, one within the other, are present as in *Salpingoeca entzii* or *S. marinus* (Fig. 92). The second, outer, collar in some types is regarded by Doflein as a periplastic rigid structure which forms a part of the cup or

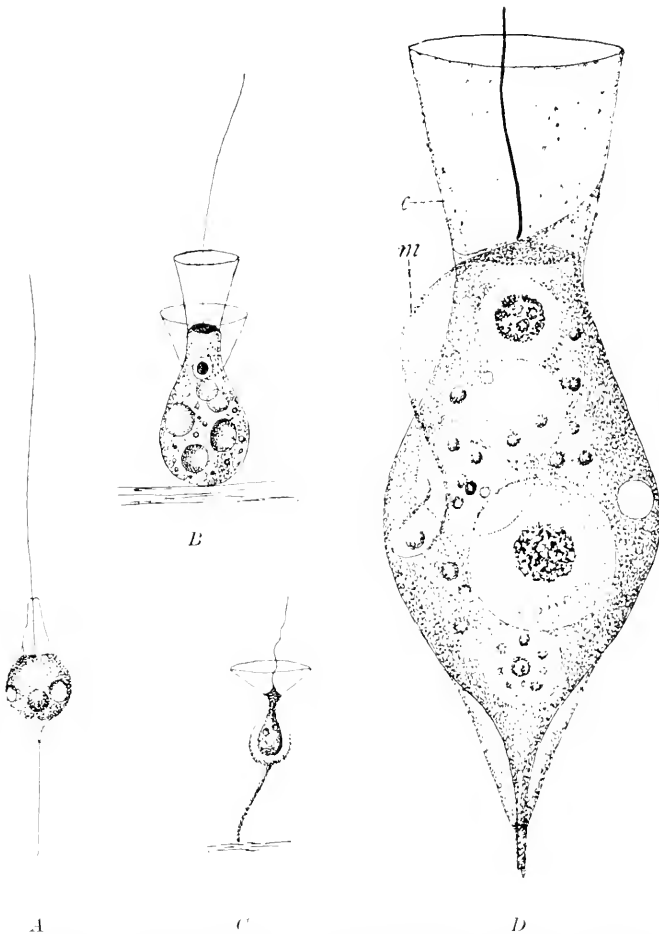


FIG. 92.—Types of choanoflagellates. A, *Colosiga pulcherrimus*; B, *Diplosiga socialis*, C, *Salpingoeca marinus*; D, collar type according to Francé. (After Calkins.)

house and is not morphologically equivalent to the inner collar, which, like a pseudopodium, may be shortened or lengthened, or drawn in and formed anew by the living cell. According to the older interpretation these protoplasmic collars assist in food-taking by forming a sticky directive course for particles down the inside

to the receptive area at the base of the flagellum (Kent), but according to Francé granules on the inside of the collar are moving away from the cell as defecatory material while the food particles move down the outside to a receptive area not included by the collar base (Fig. 92, D).

In the majority of corticate flagellates the food-taking receptive area is continued as a pit or groove known as the flagellum fissure, or as the cytopharynx. The flagellum arises usually at or near the base of such a pit and in many cases the contractile vacuole empties into it.

It is in the ciliate group, however, that we find the most characteristic and most complicated types of cytostome. Here they may be mere pores in the cortex which remain closed except during the process of ingestion and without accessory current-producing motile organs, or they may be permanently open and provided with undulating membranes or other vibratile elements. The former type, known as the Gymnostomina, eats only occasionally and then by a definite swallowing process, the soft mouth region widening into a huge opening to receive the prey. Thus *Didinium nasutum* ordinarily swims about with little evidence of a mouth at the extremity of the conical proboscis (Fig. 98, p. 187), but when swallowing a *Paramecium* which may be larger than itself, the entire anterior end appears to be nothing but mouth, the body wall of the *Didinium* being reduced to a thin enveloping sheath about the *Paramecium* (Figs. 98, 5). Similar, but not so spectacular cytostomes are present in other types of Gymnostomina. *Spathidium spathula* may swallow smaller ciliates like *Colpidium* (Fig. 99, p. 188); *Nassula aurea*, *Chilodon cucullus*, etc., still smaller forms. In all such forms the protoplasmic region around the mouth is strengthened by simple or complex metaplastic structures—the trichites (Fig. 195, p. 475). The Trichostomina are always provided with food-getting motile organs and a constant stream of water with suspended bacteria and other minute living things passes through the permanently open mouths making these creatures, according to Maupas, gluttons *par excellence* of the animal kingdom (see, however, p. 190).

The complications in regard to structure in these two types of cytostome have to do with the support of the walls of the mouth and of the gullet into which the mouth opens, and for the perfection of the current-producing apparatus. Such support is obviously important in preventing rupture of the soft protoplasmic bodies of forms like *Didinium nasutum*, *Euchelys farrimen*, *Prorodon teres* or *Spathidium spathula* (Fig. 99, p. 188). In all of these cases there is an armature of elongated rods, trichites, formed of stereoplasmic substances, embedded in the walls of the mouth and gullet, and these, like spiles in a ferry slip, take up the strain when the mouth is opened. In many cases, however, the perfection and

strength of these cytostomial supports seem to be entirely out of proportion to such hypothetical needs of the organism. Thus in all of the Chlamyodontidae the trichites form a tubular armature, the ends making a circumoral ring which may project beyond the ventral surface (*Chilodon cucullus*). Such an aggregate, known as an oral or pharyngeal basket, or pharyngeal armature, forms a more or less definite cytopharynx. In some cases the trichites are replaced by a compact corneous tube which extends deep into the endoplasm as in *Nassula aurca*, *Orthodon hamatus*, *Trachelius ovum*, etc. (Fig. 93).

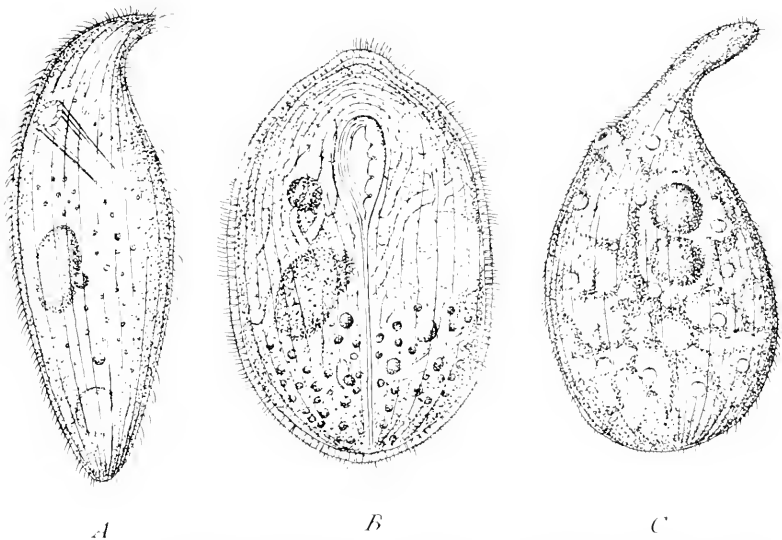


FIG. 93.—A, *Orthodon hamatus* with oral tube; B, *Frontonia leucas*, with undulating membrane on left margin of mouth; C, *Trachelius ovum*. (A and C, after Bütschli; B, after Calkins.)

In the Trichostomina the permanently open mouth always leads into a more or less highly-developed gullet or cytopharynx, while peristomial cortical differentiations of various kinds lead to it. The cytopharynx is usually provided with one or more undulating membranes, while membranelles, undulating membranes and cirri may also be present in the peristome. These are well illustrated by the complex oral apparatus of *Glaucoma (Dallasia) frontata* (Fig. 8, p. 29).

The mouth region of the ciliates appears to be the focal point of the longitudinal rows of cilia. In the generalized forms, such as *Actinobolina radians*, *Prorodon teres*, *Holophrya discolor*, etc., the mouth is exactly terminal and the rows of cilia run symmetrically

to the posterior end (Fig. 84, p. 154). In the majority of cases, however, the mouth is not terminal but may be found at various points on the side or upon the ventral surface. Thus it may be on the side in forms like *Nassula aurea*, or *Glaucoma (Dallasia) frontata* (Fig. 8, p. 29), on the ventral anterior surface in *Frontonia leucas* (Fig. 93, B), or various species of *Chilodon*, or at the extreme posterior end as in *Opisthodon mnemiensis* (Fig. 191, p. 472). Wherever the mouth is found the rows of cilia are correspondingly altered from symmetrically placed lines as in the generalized forms, to all kinds of asymmetrical arrangements. This has led to the view, first elaborated by Bütschli, that the ancestral position of the mouth in ciliates was terminal at the anterior end, and that by adaptation to different modes of life, and to various types of food, the mouth has shifted from the anterior end to the various positions as now found in different types. With this shifting the focal points of the ciliary rows have similarly shifted, and the positions of the lines of cilia in some forms are used as evidence to indicate the path of this shifting and the mode of evolution of the present-day cytostomes. A familiar illustration of such shifting is the series of forms represented by the genera *Holophrya*, with terminal mouth, *Spathidium*, with oblique mouth, *Colpidium*, *Glaucoma (Dallasia)* and many others, with subterminal mouths, *Amphileptus* and *Lionotus* with elongated slit-like mouths extending from the anterior end far down the ventral surface, such types leading to the various proboscis-bearing genera like *Dileptus* in which the mouth is limited to the posterior end of such an ancestral slit-like aperture, now represented for the most part by a row of trichocysts (Figs. 6, 13, 203).

In *Chilodon* there is an oblique line of cilia running from the anterior left-hand margin of the ventral surface to the circular mouth which in some species may be shifted well over on the right side. The lines of ventral cilia begin at this line and not at the mouth, while an oblique row of specialized cilia suggests the beginnings of adoral zone formations characteristic of the majority of Trichostomina, while the line itself may well represent the positions held by the mouth in ancestral forms.

In many types of ciliates, a special region of the body, not found in the more generalized forms, is developed as a feeding surface. Such regions, known as *frontal fields*, are characteristic of ciliates which live permanently or temporarily as attached forms. There is some evidence to indicate that such frontal fields as occur in *Stentor*, and the Peritrichida, are derived from the anterior ventral surface of more actively moving forms. In *Peritromus*, for example, the line of the peristome cuts out a definitely limited frontal region of the ventral surface, which is provided with special motile organs, the frontal cilia. Bütschli (1888) suggested that such a peristome, if continued around the right side of the organism, would completely

separate an anterior frontal field from the remainder of the body, as seems to be the case in *Climacostomum virens* (Fig. 71, p. 128). With the development of an attaching portion of the body as in *Stentor*, and in the interest of feeding, such a frontal field becomes directed upward, reaching its most perfect development in types like *Vorticella* and its allies (Fig. 86, p. 158).

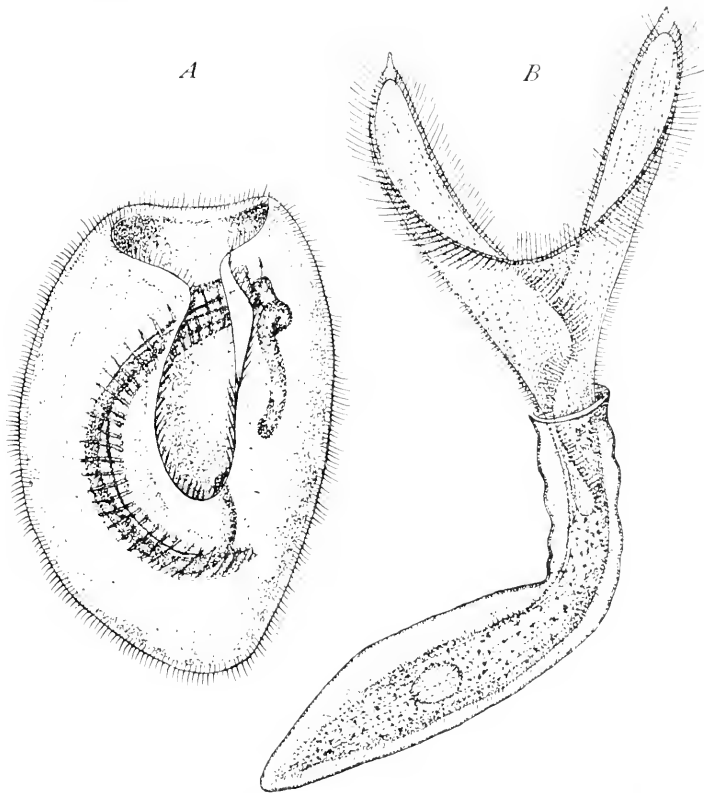


FIG. 94.—A, *Bursaria truncatella*, frontal field deeply insunk; B, *Folliculina ampulla*, with frontal field drawn out into two flexible arms. (A, original; B, after Doflein.)

Such frontal fields are flat in the various species of *Stentor*, or they may be greatly invaginated as in *Bursaria truncatella*, or drawn out into two ciliated food-getting arms as in *Folliculina ampulla* (Fig. 94), or into a tripartite frontal field in *Triloba paradoxa*, or rolled up in spiral folds as in *Spirochona gemmipara* and *Bursalinus symspiralis*.

The cytoproct is rarely differentiated as a definite opening in the cortex. In many cases, especially in the flagellate group, the cytopharynx and anus are the same. In the majority of ciliates, on the other hand, there is a constant opening or pore, usually in the pos-

terior region of the body, which is closed and invisible except during the process of defecation (Fig. 44, C, p. 86). In some forms, notably in *Pycnothrix monocystoides* and *Diplodinium caudatum*, a definite anal apparatus is developed. In the latter case Sharp describes a "rectum" with distinct walls opening to the outside by a permanent cytopyge, while at the inner end there is a "cecum" which acts as a collecting vacuole for the fecal matter (Fig. 2, p. 20).

(e) **Contractile Vacuoles.**—In the rhizopods and most of the soft-bodied flagellates the contractile vacuole can scarcely be called a cortical differentiation. In these cases they are more or less casual organoids, moving freely with the endoplasmic granules. In the corticate flagellates and ciliates, however, there is a permanent spot in the cortex through which the contents of contractile vacuoles, fixed in position, are emptied to the outside. As a rule, the salt water forms of Protozoa do not have contractile vacuoles (see p. 176) and the number in fresh water forms is variable, sometimes in the same organism (testate rhizopods and Heliozoa). In many types, however, the number as well as the position is fixed; one, as a rule, in Hypotrichida and Peritrichida, and variable numbers in the Holo-trichida and Heterotrichida.

In rhizopods the roving vacuole adds to its volume by picking up fluid substances from all parts of the endoplasm until it becomes too heavy to be easily moved with the flowing endoplasm. The vacuole is thus gradually left behind, so to speak, until it finally breaks through the thinning wall of protoplasm and empties its contents to the outside, usually at that part of the body which for the time being is posterior. In the fixed forms of vacuoles the fluids to be excreted are brought to the excretory organoid by more or less definite routes or canals, through the endoplasm. Such canals are highly characteristic of many types of ciliates. A familiar example is afforded by the different species of *Paramecium* where the five to ten radiating canals form a characteristic rosette about each of the two contractile vacuoles (Fig. 95). In the Hypotrichida there are usually two such canals leading to the dorsally placed vacuole, and two in *Stentor*, one following the margin of the body to the "foot," the other following the rim of the peristome in a circular course around the body. In *Ophryoglena flava* there may be as many as thirty fine feeding canals leading from all parts of the body to the centrally placed vacuole, and in *Frontonia leucas* eight to twelve such canals follow a tortuous course throughout the body substance. In *Pycnothrix* the canals form a branching network through the endoplasm. Such canals are replaced by a ring of feeding vacuoles in many of the corticate flagellates.

In corticate Protozoa the contractile vacuole usually opens to the outside in the vicinity of the anus when such a structure is present. In many cases it opens into the cytopharynx as in the majority of flagellates or in the vestibule of forms like *Vorticella*.

In *Campanella umbellata* such a reservoir is replaced by two definitely walled evacuation canals, while in *Pycnothrix* the excretory canal is said to be provided with special cilia.

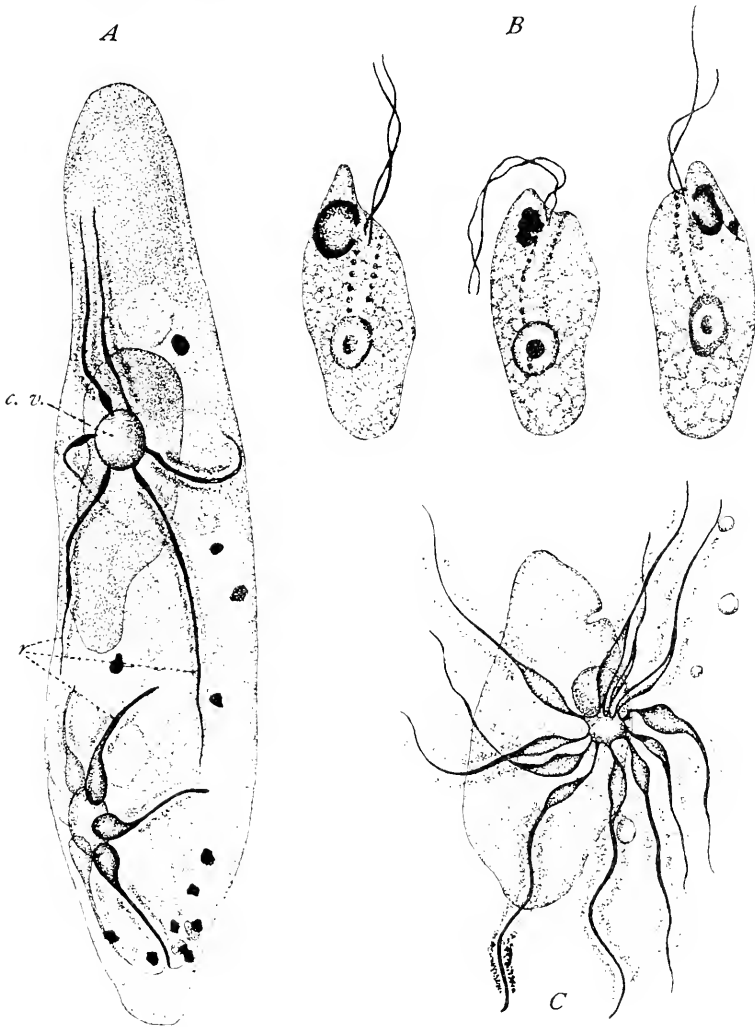


FIG. 95.—Golgi bodies in *Chilomonas paramecium* (B) and *Paramecium caudatum* (A and C). *c. v.*, Contractile vacuole; *r.*, radial canals of *Paramecium*. (After Nassonov.)

In some types of parasitic ciliates (Bütschliidae and Paraisotrichidae) a peculiar type of "concrement vacuole" has been described by Dogiel (1929) which appears to be a normal part of the derived organization. These are interpreted, not as excretory, but as special structures with a statolith function.

CHAPTER V.
GENERAL PHYSIOLOGY.

THERE is no doubt that our knowledge of the structures of Protozoa far outstrips our knowledge of their functions. The minute size of the individuals and the inadequacy of micro-chemical tests make it extremely difficult to follow out any physiological process to its end. Furthermore, it must not be overlooked that physiological problems here for the most part begin where similar problems of the Metazoa leave off, namely in the ultimate processes of the single cell. Here the functional activities have to do with the action and interaction of different substances which enter into the make-up of protoplasm and, for the most part, these are beyond our powers of analysis. A few of these activities may be duplicated individually and apart from correlated functions, in the laboratory. Or specific reactions between specific chemical substances may be obtained as, for example, the digestion of fibrin by fluids extracted from the protozoön protoplasm; or in a physical sense the reversal of the sol and gel states in colloidal mixtures. Such individualized processes, however, give little idea of the infinite play of forces continually operating in living protoplasm, all of which, harmoniously working together, make up the phenomena of vitality and distinguish living from lifeless matter.

As Mathews points out, the essential differences in chemical actions in protoplasm and in physical nature are: (1) The orderliness with which they are carried on, and (2) the speed of the reactions.

A starving *Dileptus gigas* will slowly decrease in size although its form remains about the same (Fig. 6, p. 27). This is due to disintegration through continued oxidation and other catalytic processes which lead to the exhaustion of protoplasmic constituents unless new food is added. If the process is continued the organism will ultimately die in from one to three weeks. If a *Dileptus* is accidentally crushed its protoplasm will completely disintegrate within a few seconds. The process of disintegration in the first case is orderly, in the latter completely disorganized. Other normal vital activities are equally orderly; the orderliness dependent possibly on the regulation of permeability by the colloidal membranes, the alveolar membranes, nuclear membrane and investing membrane of the cell; and regulation of permeability in turn is dependent upon the chemical make up

of the constituent parts, and salts or electrolytes and the continued activity between them (cf. Clowes, Overton, Mathews).

The speed of specific chemical actions is a characteristic vital phenomenon due to the participation of subtle and elusive, but specific, catalytic agents, the enzymes.

This aggregate of colloidal substances forming polyphasic physical systems in protoplasm is the seat of the multitude of activities characteristic of life. Huxley's definition of protoplasm as the physical Basis of Life does not carry us very far in the analysis of living matter. In a moving protozoön there is a constant interaction of the various substances making up its protoplasm—oxidation, enzyme formation and action, amidization and deamidization, disintegration and regeneration, protein break-down and protein reconstruction, all taking place simultaneously or *seriatim*. Substances in this whirlpool of action may be regarded as living so long as they are, or may be, drawn into the vortex of protoplasmic activities. The results of these multitudinous activities contribute to the well-being of one organism. In another moving protozoön a similar bewildering complex of activities likewise results in the well-being, in this case of a distinctly different type of protozoön. The first protozoön, let it be a *Didinium nasutum*, captures and swallows the second, say a *Paramecium caudatum*. It is well known that a fragment of a protozoön will regenerate into a perfect organism of its type and we might well be perplexed by the problem why is it that the *Paramecium* protoplasm in *Didinium* does not manifest itself as *Paramecium* and not as *Didinium*. The answer to this apparently simple problem is a matter of organization or the manner in which the fundamental substances making up the protoplasm in the two organisms are put together and interact. The architectonic of Driesch, or protoplasmic architecture, is specific for each type of organism and the form and structures of the organism are expressions of this architecture. When this organization disintegrates, life and the possibility of controlled reactions are lost and the erstwhile living protoplasm becomes dead matter. This happens when *Paramecium* is paralyzed by the seizing organ of *Didinium* (see Fig. 98, p. 187). The vital activities of *Paramecium* are suddenly stopped, and disintegration of its organization, through hydrolysis, continues with the digestive processes in *Didinium*. The inert proteins, probably as amino-acids, are re-integrated in the *Didinium* protoplasm, and what was living substance in *Paramecium* now enters again, through a form of transmigration, into the vortex of vital activities of quite another type of organism.

The sum total of the various physiological processes of the individual may be grouped for the Protozoa, as they are for the Metazoa, into aggregates of special activities which we call the fundamental vital functions, and distinguish as respiration, nutri-

tion, excretion, irritability and reproduction. In Metazoa these are performed by specialized cells, grouped into tissues, organs and organ systems, the complexity varying with the specialization of the organism. In Protozoa they are all performed by the single cell and all are more or less dependent on the activities of the diverse substances and structures which compose it. All work together in a harmonious cycle of matter and energy.

A. Respiration.—The scientific beginnings of the modern mechanistic conception of vital activities is traced to Lavoisier and his comparison of animal heat with physical heat due to combustion through oxidation. The utilization of chemical energy, or energy of combination liberated by oxidation, is possibly the keynote to the multiple vital harmonies of animal life (see Verworn, 1907). Oxygen necessary for such physiological combustion is obtained by all protozoa without the aid of specialized respiratory organs. It is readily absorbed through permeable membranes from the surrounding water, or obtained by reduction from oxygen-holding substances, as in anaërobic forms. In one way or another it is ever present to initiate the round of vital functions.

Oxygen may be taken into the cell directly from the surrounding medium as in the aërobic forms, or it may be obtained by breaking down oxygen-holding substances in protoplasm, so-called reducing processes of all types but especially of anaërobic forms. Through the use of chemical indicators the degree of oxidizing power of a cell, including both direct oxidation and reduction, may be determined and is expressed by the symbol rH in values from one to forty. This factor, known as the "oxidation-reduction potential," varies from time to time and is used in much the same way as the expression pH, indicating the hydrogen-ion concentration from intense acidity (pH 1 or 2) to strong alkalinity (pH 10). It is highly probable that a definite rH is as important for cell activity as a definite pH, and that this oxidation-reduction potential is maintained by the R-SH compounds (cystine, cysteine and glutathione) of the protoplasm (Krogh, 1916; Hopkins, 1921; Meyerhof, 1924).

The intake of oxygen and the voiding of CO_2 constitute the essential needs of the cell in respiration. The relationship between the oxygen taken in by an organism and the CO_2 produced by its metabolic activities is indicated by the expression R. Q. (respiration quotient). Daniel, 1931, found that the R. Q. of *Balantidium coli* under aërobic (sic) conditions is 0.84, which is very nearly the same as the usual R. Q. for man (0.85). For *Amoeba proteus* and *Blepharisma undulans* Emerson (1929) found the R. Q. to be "about unity."

To a certain extent the oxygen intake and CO_2 output are measurable, but always with a large experimental error. Kalmus (1927).

for example, by an ingenious method, made out that a single *Paramecium* consumes 0.0052 c.mm. of O_2 in one hour at $21^\circ C.$, a figure which Howland (1931), using the same method, slightly modified, changed to 0.00049. Adolph (1929) made out a typical rate of 0.55 cc. of oxygen intake per million individuals per hour at $19.7^\circ C.$

In a similar way R. Emerson (1929) obtained results with *Amoeba proteus* and *Blepharisma undulans*; Peters (1921) with *Colpidium colpoda*; Hulpieu (1930) with *Amoeba proteus* found that the rate of movement is not noticeably affected by changes in the amount of available oxygen from 0.005 to 100 per cent; below or above these limits the animals are slowly killed. He found, furthermore, that amoebae are able to move for some time in the absence of oxygen which indicates that its energy is not derived by direct oxidation. Verworn (1896), on the other hand, found that *Rhizoplasma kaiseri* in an oxygen-free medium ceases its centrifugal pseudopodial movements while centripetal movements continue for some time but ultimately stop. Addition of oxygen restores both types.

It is the function of catalytic enzymes to expedite chemical processes which are under way and catalases of different kinds result from metabolic activities going on in protoplasm. Amongst these are the oxydases which aid in oxidation and reduction in the cell. Indications of such agents as the "reductase" of Becker (1926) and the extraction of glutathion have been obtained, while Joyet-Lavergne (1929) adduces considerable evidence in support of his view that glutathion is intimately associated with the mitochondria of the cell.

Correlated with the intake of oxygen is the output of CO_2 and water. While these are perhaps more properly treated in connection with the functions of excretion there is good evidence of a gaseous exchange, but quantitative results are not altogether satisfactory.

The energy of combination, released by oxidation, is paid for by loss in the chemical compound oxidized. Other compounds may be formed with lessened energy of combination, and end-products, notably CO_2 and urea $((NH_2)_2CO)$, are not only useless to the organism but positively harmful unless voided. Excretion, therefore, must follow oxidation. To make good the loss of substance new food materials must be taken in, digested and assimilated, but this is possible only through movement, and movement in turn is an expression of irritability. Excretion and irritability thus are fundamental vital functions, while a third, nutrition, is closely correlated. Excess of food intake over waste by oxidation leads to growth of the diverse protoplasmic substances and to their reduplication by division, while the aggregate of such divisions, expressed visibly by division of the cell, constitutes reproduction. The fundamental vital functions are intimately bound together; external con-

ditions, such as decrease in temperature of the medium in which a protozoön lives, means decreased oxidation, retarded movements, less food and a lower division rate. Increase in temperature involves a speeding up of all activities and, if food is abundant, a higher division rate. External conditions involving absence of food lead to starvation and death of the cell through uncompensated loss by oxidation. In short, interference with any one of the fundamental functions leads to disturbance of them all, and the various phases of vitality of the protoplasm during a typical life cycle may be due to inadequate functioning of one or another or all of these activities.

B. Excretion of Metabolic Waste.—The waste matters of oxidation and continued metabolism are frequently voided in the same manner that water and oxygen are taken in, namely, by osmosis. In such cases there is no physiological need of specialized excretory organs. It is possible that all Protozoa excrete in this way, although the majority of fresh water Protozoa possess contractile vacuoles which are generally regarded as excretory organs. In marine forms and in parasites they are generally absent. If the latter forms, and these are in the majority of Protozoa, are able to dispose of the products of destructive metabolism without definite organs for the purpose, why are the latter necessary in fresh water forms? Hartog (1888) has long maintained that contractile vacuoles are not obligatory excretory organs, but are primarily hydrostatic organs for the purpose of maintaining a pressure equilibrium between the fluids within the cell and those in the surrounding water. Degen (1905) interprets the vacuole in a similar way, its variations in size and pulse depending upon permeability of the membrane which varies with the environmental salts. Here difference in density of the surrounding medium is largely responsible for loss of the organ characteristic of fresh water forms, but changes in permeability of the cell membrane due to salts in the new medium undoubtedly play an important part. Other experiments by different observers bear out the same principle. Thus dilution of the normal neutral salts in the medium causes enlargement of the contractile vacuoles in ciliates according to Massart (1891), while increased concentration leads to reduction in size, retardation in rate of contraction, or total disappearance of the vacuole.

While there is justification for Hartog's view of the purely physical significance of the vacuole, there is every reason for believing that water in protoplasm picks up any soluble waste matter that may be present, and holds it in solution. Early experiments to prove this, by Brandt (1885), Griffiths (1889) and others using chemical indicators, or the murexid test for uric acid, were not convincing, and the function of the contractile vacuole as a primitive type of excretory organ remained an hypothesis.

Not only water, CO_2 (see Lund, 1918) and urea, but other prod-

ucts of metabolism as well, are found in the protoplasm of different Protozoa. These are usually present in crystalline form or in amorphous heaps, which are rather loosely spoken of as "excretory stuffs" without evidence as to their origin or significance. The crystals often seen in *Paramecium* were identified by Schewiakoff (1893) as calcium phosphate combined with some organic substance. Similar crystals have been described by Schaudinn, Schubotz and others from the protoplasm of different kinds of Protozoa. Schewiakoff found that the crystals of *Paramecium* are not defecated as are undigested food substances, but are first dissolved and then disposed of—presumably with the water of the contractile vacuoles.

The function of the contractile vacuole in Protozoa thus has long been a disputed problem. The views of the older students of the group, with their conceptions of structural complexity of these unicellular organisms, fantastic today, nevertheless have a certain historical interest. The idea that a vacuole is a rudimentary beating heart as interpreted by Lieberkühn (1856), Claparede and Lachmann (1854 and 1859), Siebold (1854) and Pritchard (1861) was no less incongruous than the supposition of Ehrenberg (1838) that the contractile vacuole is an organ connected with the gonadal system.

With development of knowledge of structure and function of the Protozoa, and particularly of the mechanism of vitality, more reasonable hypotheses of the function of the contractile vacuole have been developed. There is, first, some ground for the belief of Spallanzani (1776), Rossbach (1874) and Dujardin (1841) that it is an organoid having to do with respiration of the organism, together with other possible functions, a view supported in modern times by Bütschli (1877, 1888) and Degen (1905). There is, second, ground for the belief held by Stein (1859), Gruber (1889) and the majority of modern students of Protozoa, that it is an organoid for the excretion of katabolic waste, despite the unconvincing experimental evidence by Brandt (1885), and by Griffith (1889). Howland (1924), however, by using a much more delicate test (the Benedict blood-filtrate test) obtained unmistakable evidence of the presence of uric acid in cultures of Protozoa; in *P. caudatum* analyzed by Benedict, a color reaction was obtained equivalent to 4 to 5 mg. of uric acid per liter. There was no proof here, however, that the uric acid came from *Paramecium*. Weatherby (1929) showed that the usual ingredients of a culture medium contain measurable quantities of uric acid. He found, however, that the extracted fluids of contractile vacuoles of *Paramecium* and *Spirostomum* contain urea, whereas the vacuole of *Dilinium nasutum* contains ammonia, but in no case does the nitrogenous waste of the vacuole represent all of the nitrogenous output of the cell, much being voided by exosmosis. There is, third, ground for the belief that the contractile vacuole is an organoid for the regulation of osmotic pressure in the cell, a view

first advanced by Hartog (1888) and supported by Degen (1905), Stempel (1914), Khainisky (1910) and by Nassonov (1924).

These three beliefs are not necessarily exclusive and the possibility of all three functions is still open. The osmotic function is well supported by evidence furnished by Gruber's (1889) experiments in transferring fresh-water, vacuole-holding *Actinophrys sol* and *Amoeba crystalligera* to salt water, and *vice versa*, or by Zuelzer's similar experiment with *Amoeba verrucosa*, the protoplasm becoming more condensed and the vacuole lost in salt water. Hogue (1923) found that *Vahlkampfia calkensi* when transferred from salt water to fresh water media developed 1, 2, 3, or even 4 contractile vacuoles. More extensive experiments by Degen (1905) with salts of different kinds and with varied conditions of the environment show that the contraction of the vacuole is a function of osmotic pressure, and irrespective of the type of salt or neutral solution introduced. With Hartog, he concludes that protoplasm of fresh water forms, with its salts in solution, has a higher osmotic pressure than the surrounding medium, which leads to continued intake of water. Such intake, if not balanced, would lead to inflation and to diffuence, a conclusion strengthened by Botsford's (1926) merotomy experiments with *Amoeba proteus* in which it was shown that the size of the vacuole depends upon the size of the fragment cut off. According to Degen and Hartog it is the function of the contractile vacuole to establish this balance.

This hypothesis, with further evidence supplied by the absence of contractile vacuoles in marine forms where osmotic relations of protoplasm and environment are more evenly balanced, is theoretically correct. There is no reason to doubt, however, the further possibility that the water expelled by the contraction of the vacuole contains water-soluble, katabolic excretory substances such as CO₂ and nitrogenous waste, positive evidence for which is supplied by several observers. This indeed was admitted by Degen although he obtained no evidence of the nature of the substances excreted. He saw in the membrane of the vacuole the possibility of an excretory mechanism. The actual existence of such a membrane, however, is still in dispute, indeed the majority of investigators deny its existence (Bütschli, Rhumbler, Schewiakoff, Taylor). Others, however, give evidence to show that a true membrane, although very delicate, is actually present. Howland (1924, 1) for example, by micro-dissection methods has been able to remove the contractile vacuoles of *Amoeba verrucosa* and of *Paramecium caudatum* after which they retain their integrity for considerable periods as free vacuoles in the surrounding water. She also has punctured the vacuole with needles while in the endoplasm, causing the expulsion of its contents into the surrounding endoplasm and resulting in the wrinkling of the vacuole membrane. Nassonov (1924) also not

only demonstrates the presence of a membrane in various types (*Paramecium caudatum*, *Lionotus folium*, *Nassula lateritia*, *Campanella umbellaria* and other *Vorticellidae*) but, by use of fixation methods employed for demonstrating the Golgi apparatus in metazoan cells, comes to the conclusion that the membrane of the contractile vacuole is a part of the Golgi apparatus. This, in Metazoa, he had earlier (Nassonov, 1923) identified as an organoid intimately bound up with secretory activities of the cell (see also Bowen). In different Protozoa the contractile vacuole, which he unhesitatingly calls an excretory apparatus with a definite lipid membrane, is variously complicated, from a simple vesicle with osmiophilic membrane in forms like *Chilomonas paramecium* (Fig. 95, B, p. 171), to complex aggregations of vesicle and canals as in *Paramecium* (Fig. 95, A, C). In the latter case the canals appear to contain the material by activity of which substances are chemically differentiated for secretion and these are passed on to the vesicle by which they are excreted. According to Nassonov the lipid-containing membrane (confirmed by Chatton, 1925, and by Gelei, 1928) must be semi-permeable and its contents must have a higher osmotic pressure than the surrounding plasm. Hence fluids would flow into the vacuole completely distending it until the pressure would burst the retaining membrane and the fluid would be ejected. The highly viscous membrane would mend but for a new flow into the vacuole a new supply of osmotically active stuff would be necessary. This, Nassonov assumes, is formed by secretion from the osmiophilic membrane into the canals and vacuole. This secreting activity is compared with the secretory activity of the Golgi apparatus in Metazoa. Gelei holds, however, that the function here is to condense and to conduct concentrates from the plasm into the canals, not a secretory function, but excretory. (See also Lynch, 1930.) With this work of recent investigators we have a very definite argument for the excretory functions of the contractile vacuole and for the presence and function of the lipid membrane. In quite a modern way it brings us dangerously near to an Ehrenbergian conception of a kidney and bladder in Protozoa.

C. Irritability.— In the absence of all knowledge as to the manner in which protoplasmic particles respond to stimuli of different kinds, we are constrained in speaking of irritability of Protozoa, to limit descriptions to aggregates of such responses as manifested through movement, as energy transformed by oxidation from the potential or stored chemical energy to the active or kinetic condition, or as manifested by adaptations to changes in environment. But the manner in which such kinetic energy is utilized in pseudopodia formation or by the elements of flagellum, cilium or myoneme, is a matter of pure speculation. The reactions which characterize the resulting movements, however, can be analyzed and measured

and these form the chief basis of our knowledge of protozoan irritability.

Attempts to explain pseudopodia formation and ameboid movement have varied with the changes in our conceptions of the physical make up of protoplasm. The protoplasm of *Ameba* regarded as a fluid substance was supposed to follow the laws of surface tension characteristic of all fluids. Pseudopodia formation, according to the views of Berthold (1886), is the attempt of one fluid (protoplasm) to spread out between water and the substratum as Quincke's well-known experiments demonstrated for fluids. As physical conditions on all sides of the *Ameba* are not equal, variations in tension result in local diminution, and the tendency to spread is focussed in a local area and the pseudopodium results. Bütschli's (1894) observations and experiments with emulsions of oil, salts and water, and Rhumbler's (1898) analysis of the causes of movement in lobose rhizopods led these observers also to interpret pseudopodia formation as a result of surface tension phenomena. With the more modern conception of protoplasm as a colloidal aggregate in the physical state of an emulsoid in which the external and internal protoplasm of *Ameba* are in the relation of gel and sol, the difficulty of applying the laws of fluids became apparent and the hypothesis based upon surface tension has been generally abandoned. Rhumbler himself (1910 and 1914) recognized this difficulty and materially changed his conception of ameboid movement, while Hyman (1917) greatly enlarged and perfected his later point of view. According to Hyman the ectoplasm of *Ameba*, by virtue of its relatively solid state, becomes tenuous but elastic, as demonstrated by the experiments and observations of Jennings (1904), Kite (1913), Schultz (1915) and Chambers (1915, 1917), and exerts an elastic tension on the inner fluid protoplasm. Bancroft (1913) and Clowes (1916) demonstrated the reversibility of phase in diphasic physical systems through the agency of electrolytes, and the conclusion followed that the ectoplasm represents a reversal phase of the more fluid inner protoplasm. Hyman argues that, owing to the tension of the enveloping ectoplasm, if any local region of the more solid ectoplasm becomes liquefied, the resistance gives way at such a point and the fluid endoplasm is pressed out, thus forming a pseudopodium. The immediate cause of such liquefaction she traces to a local increase of, or change in, metabolic activity resulting in the production of hydrogen-ions which, with the surrounding medium, form an acid appropriate for dissolution of the more solid ectoplasm. By the use of Child's potassium cyanide test for metabolic gradients, she was able to demonstrate that such local regions of greater metabolic activity actually occur on the periphery of *Amoeba proteus* before a pseudopodium breaks out, also that the extreme tip of the advancing pseudopodium is the most actively metabolic part.

Whether changes in the nature of protoplasmic response or changes in direction of movement after repeated shocks should be interpreted on the basis of "memory" and "learning" or in some other way is largely a matter of personal idiosyncrasy on the part of the observer. Numerous writers have described processes of food "selection" by *Amoeba* (e. g., Gibbs and Dellinger, 1908; Schaeffer, 1917 and elsewhere; Metalnikoff *et al.*, 1910). Mast and Pusch (1924) interpret an observed change in the protrusion of pseudopodia of *Amoeba proteus* in respect to a beam of light as evidence of something analogous to "learning" in higher animals, etc. "Learning" involves "memory," and such terms connote processes of an entirely different nature which we associate with the highest types of animals. It is conceivable that fatigue, to use the term in its broad sense implying total or partial exhaustion of protoplasmic constituents necessary for a reaction, and therefore a purely physical matter, is adequate for explanation without calling upon any obscure pan-psychic interpretation. Similarly with Kepner and Taliaferro's (1913) evidence of "purpose" in methods of food-getting by *Amoeba proteus*.

Many of the reactions of Protozoa are bound up with the coördinating mechanism of the cell through which the organism acts as a unit. The specific response of an organism to a stimulus is the result of its particular protoplasmic architecture expressed through its coördinating mechanism and motile organs. This has been elaborately worked out by Jennings (1904 to 1909) in connection with the "motor response" of many different kinds of Protozoa.

The discussions and controversies over the matter of directive stimuli or tropisms in Protozoa have evidently been due in large part to a lack of common understanding of the definition. If by "tropism" is meant the orientation of an organism in respect to the path of a stimulus, then tropisms, as Jennings was the first to point out, play little part in the activities of the Protozoa. If, however, by "tropism" is meant "the direct motor response of an animal to an external stimulus" (Washburn, 1908), then tropisms play a most important part in such activities. The two definitions are not compatible; the former conveys the idea of a directive stimulation upon local motor organs or controlling elements; the latter implies the complex reaction of a definite mechanism characteristic of any specific protoplasm, and the same reaction follows upon stimulation by any type of stimulus (Pütter, 1903, Jennings, 1909). It follows further that the reaction is called forth regardless of the particular elements first to receive the stimulus.

We owe Jennings the credit for first clearly distinguishing between these two conceptions, as well as for careful analyses of the movements of lower organisms (1904 *et seq.*), and for demonstrating the particular motor response distinctive of specific types of Protozoa.

He also showed that the nature of the motor response in some organisms, *e. g.*, in *Stentor*, is correlated with the physiological state of the organism, and adduced evidence which indicates that phenomena of fatigue are involved. The classical example of a

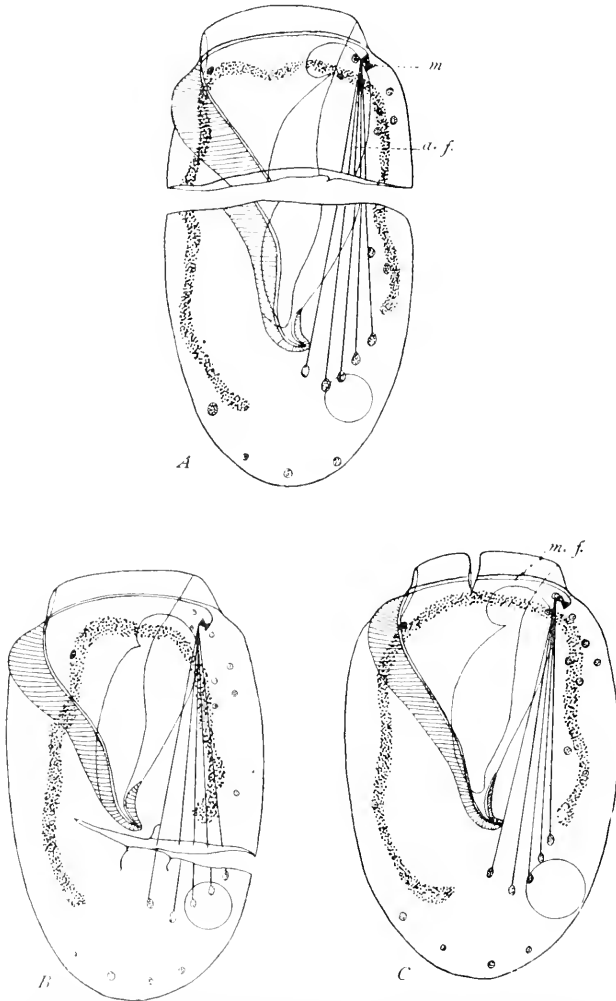


FIG. 96.—Merotomy in *Euplotes patella*. (After Taylor.) *a. f.*, Anal cirri fibers; *m.*, motorium; *m. f.*, membranelle fiber. (See also Fig. 72.)

motor response, formerly interpreted as chemiotaxis, is the case of *Paramecium caudatum* or *aurelia* in a drop of dilute acid. Casual swimming brings the individual to the outer limit of the drop; the transition from water to drop does not provide a stimulus strong

enough to bring about the motor response and the individual continues through the drop until it strikes the farther limit. Here the stimulus is sufficiently strong to cause the motor response which is manifested as a backward swimming, due to reversal of cilia, turning on the long axis and recovery of normal forward swimming movement. Repetition of this procedure keeps the individual in the acid drop. Others enter in a similar way and are similarly trapped until many are confined in the acid drop where they are ultimately killed. Such motor responses unquestionably play an important rôle in food-getting and in vital activities generally.

The stereotyped nature of the motor response in any specific organism may be due in the main to the characteristic silver line and neuromotor systems which the higher types of flagellates and ciliates possess. The observations of Sharp (1914), Yocom (1916) and McDonald (1922) on ciliates, of Kofoed on flagellates, and the experiments of Taylor (1920) in cutting different regions of the neuromotor complex of *Euplotes*, indicate that the motor response of Protozoa is bound up with coördinating systems possessing some of the attributes of coördinating systems in Metazoa (Fig. 96). Knowledge of these complex systems and their reactions is quite sufficient to dispel any lingering belief in tropisms as due to stimulation of special motile elements acting independently in such a way as to orient the organism in respect to the path of the stimulus. Through coördinating fibrils all parts work together; cutting the system at any point leads to inharmonious or uncoördinated movements of the motile organs as Taylor has demonstrated. All reactions depend upon the organism as a whole; enucleated fragments are unable to react as do nucleated fragments (Hofer, 1890, Willis, 1916). Jennings' careful observations, which led him to the conclusion that the protozoön organism always acts as a whole is fully confirmed by these later observations and experiments.¹

D. Nutrition.—Under the heading nutrition are included all physiological processes involved in the replacing of substances exhausted by destructive metabolism. Groups of activities including: (1) food-getting; (2) secretion and digestion; (3) assimilation; (4) defecation, find their place here. Certain specialized structures adapted for these various activities have been described for the most part in the preceding chapters, and the following is supplementary in nature dealing with the functions which these structures perform.

1. Food-getting.—The varied methods by which Protozoa acquire the needed materials for replenishing protoplasmic substances reduced by oxidation are all correlated with the phenomena of

¹ For discussion of different types of stimuli and the resulting reactions by Protozoa see Minchin (1912), Khainsky (1910), Mast (1910-1918), Pütter (1900, 1903), Jennings (1904, 1909).

irritability. The particular method employed by any one type of organism is probably the result of many factors of organization and adaptation combined with mode of life, all of which are traceable to adaptations resulting from the effects of external stimuli and response through irritability. It would indeed be remarkable, considering the endless variety of endoplasmic and cortical differentiations, were we to find a common method of food-getting amongst the Protozoa. On the contrary, it is probable that no two types of organism follow an identical method. Nevertheless it is possible, and it is certainly convenient, to group these manifold activities under a comparatively few main types which are designated: (1) Holozoic nutrition; (2) saprozoic nutrition; (3) autotrophic or holophytic nutrition; (4) heterotrophic nutrition. Many authorities introduce a fifth type under the caption parasitic nutrition, but as this does not differ in principle from saprozoic nutrition, it is included with the latter type.

While these terms apparently indicate different modes of nutrition they are more applicable to methods of food-getting, and the differences have to do in the main with the nature of the raw materials taken in and the subsequent processes necessary for their elaboration. Thus holozoic nutrition in Protozoa as in Metazoa involves the ingestion of raw materials in the form of proteins, carbohydrates and fats which are usually combined in the protoplasm of some other living organism eaten as food. It is an expensive method of acquiring raw materials for it necessitates capture and killing of living prey, preparation and secretion of digestive fluids and ferments necessary to make the proteins and carbohydrates soluble, and disposal of the undigestible residue. On the other hand, it assures the supply of capital in the form of chemical energy without the labor of storing it up. Saprozoic nutrition is, so to speak, a more economical method, for the organism does away with the elaborate processes of secretion and digestion and relies upon the activities of other organisms for the preparation of its raw materials and the "storage of energy." Dissolved proteins and carbohydrates made soluble through the agency of bacteria and other organisms in infusions, or prepared by the digestive processes of the host in the case of parasites and some commensals, are absorbed directly through the body wall or through special receptive regions, by endosmosis. This type of food-getting may be regarded as a degeneration or adaptation of the holozoic method, the specialized absorptive areas being reminiscent of former mouths, while the pathogenic effects of some types of parasites are interpreted as due to the secretion by the parasite of digestive fluids which cause cytotoxicity of the host cells. Holophytic or autotrophic nutrition, characteristic of plants, is quite different in principle from the other two. Digestive processes typical of the majority

of animals, as well as the intake of solid or dissolved food, are absent. A highly labile substance, chlorophyll, is manufactured in the presence of light and usually by specialized plastids—chromoplastids—of the cell. Chlorophyll is very sensitive to light and in some way not yet understood is instrumental in utilizing the radiant energy of the sun to form complex, energy-holding compounds. Plants thus become the great banking house for animals and their capital is the apparently inexhaustible energy of the sun. Heterotrophic nutrition, finally, is characteristic of those Protozoa which combine any two of the above methods of acquiring raw materials.

The great majority of Protozoa are holozoic in their methods of food-getting, and we may distinguish two main groups, the continuous feeders, and the occasional feeders. Continuous feeders are those forms with permanently open mouths through which a constant current of water is maintained by action of the peristomial motile apparatus (see p. 164). Minute forms of life, especially Bacteria, are carried by these currents into the endoplasm where they undergo digestion in improvised stomachs or gastric vacuoles (see p. 193). Chejfec (1929) estimates that *Paramecium caudatum* may thus ingest and digest from two to five million *Bacterium coli* in twenty-four hours. The majority of ciliates, including many of the holotrichous, hypotrichous, heterotrichous and peritrichous ciliates, belong in this group.

The occasional feeders, like carnivorous types of Metazoa, feed whenever chance brings prey within the radius of their activity, and many of them, like cannibals, are guilty of feeding at times upon their close relatives (Maupas, 1883, Joukowsky, 1898, Dawson, 1919, Lapage, 1922). In some cases balloon-like membranes are unfolded and spread out like sails for the direction of food currents to the mouth as in *Pleuronema chrysalis* (Fig. 199, p. 482). Such forms are intermediate between the constant and occasional feeding types. In other cases great net-like traps are spread for the capture of unwary diatoms, desmids or smaller Protozoa, as in the Foraminifera (Fig. 10, p. 32). In other cases the microscopic hunters, like men in shooting boxes, lie in wait for their prey. Here long tentacles usually radiate out from the body in the surrounding water as in *Actinobolina radians* or in Suctoria, until a victim comes in contact with one or more of the outstretched processes (Fig. 91, p. 163); in the same way axopodia of the Heliozoa capture chance organisms which serve as food (Fig. 97).

The most interesting of these holozoic types are the predatory forms which hunt their prey and capture them, while in full motion. The small but powerful ciliate, *Didinium nasutum*, belongs in this group. It darts here and there with an erratic movement while rotating at the same time on its long axis. In its sudden darts,

it strikes a *Paramecium* or other ciliate purely at random; the proboscis with seizing organ is buried in the victim which is then swallowed whole (Fig. 98, 1-6). *Lionotus fasciola*, *Spathidium spathula* and other gymnostomatous ciliates capture living organisms in a similar way (Fig. 99) while less spectacular methods are employed by *Frontonia leucas*, *Ophryoglena flava*, *Prorodon nireus*, etc., in swallowing diatoms, desmids and other relatively stationary organisms.

A special type of food-getting, illustrated by the Rhizopods, may be interpreted in some cases as the result of physical properties of semifluid bodies. Rhumbler has made the most exhaustive studies

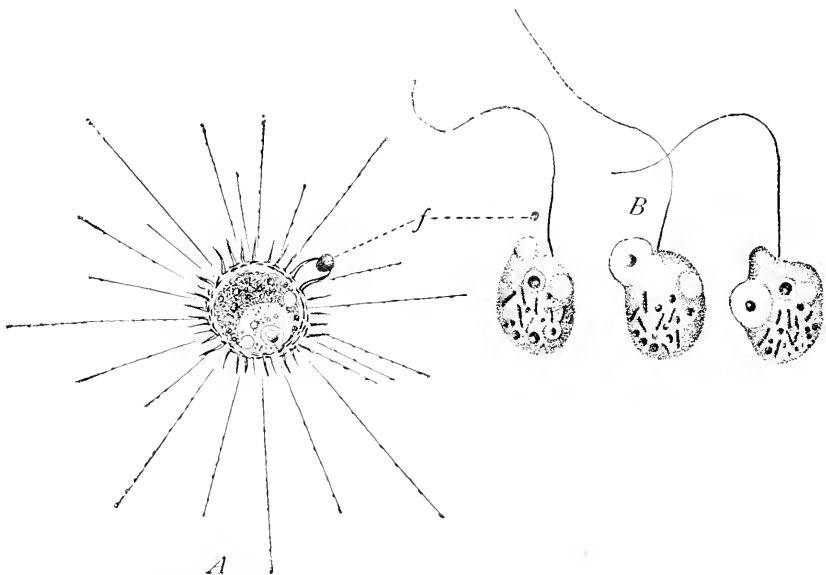


FIG. 97.—Types of food getting. A, *Acanthocystis* (after Penard); B, *Oicomonas termo* (after Bütschli).

of food ingestion in these forms and distinguishes four types, viz.: Ingestion by (1) "circumvallation," (2) "circumfluence," (3) "invagination" and (4) "importation." Food-taking by "circumvallation" is illustrated by *Amoeba proteus* and usually takes place at that portion of the body which, for the time being, is posterior. According to Hofer (1889), Schaeffer (1917) and others, the body becomes anchored to the substratum by the secretion of an ectoplasmic gelatinous substance; then, through the physical stimulus (Schaeffer, 1917) produced by a moving object (even a moving needle point according to Verworn, 1889), walls of protoplasm flow out on either side of the object and meet around it, thus enclosing a rotifer, an

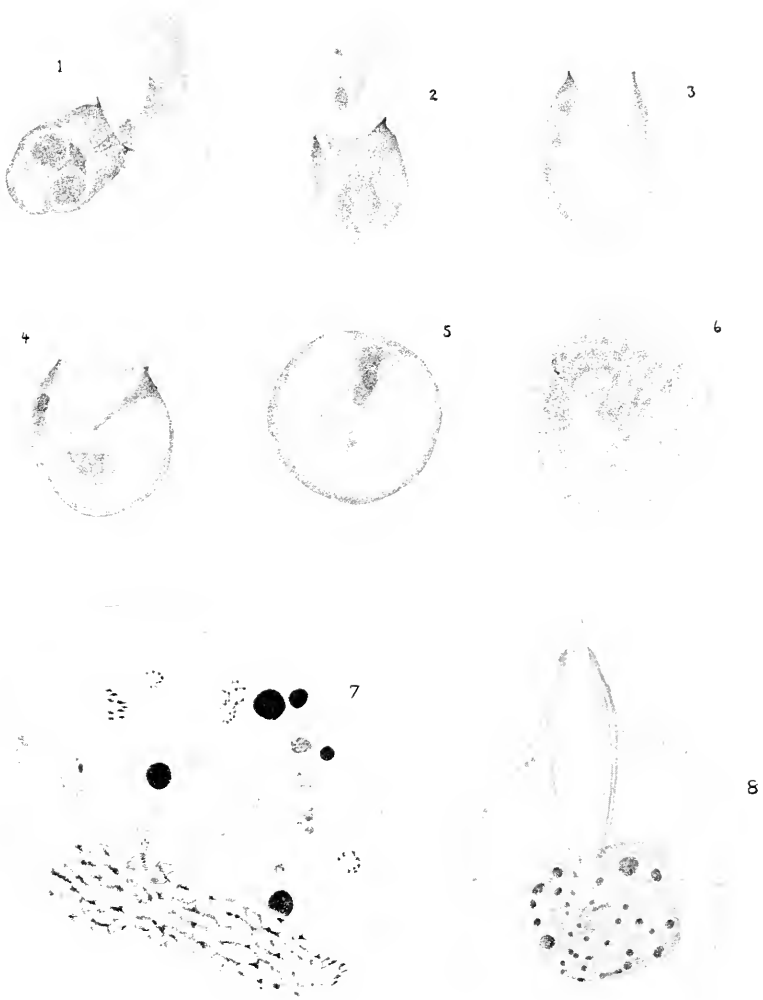


FIG. 98.—*Didinium nasutum* O. F. M. capturing and swallowing *Paramecium caudatum*. 1 to 6, Successive stages in the ingestion of *Paramecium*; 7, section of conjugating form of *Didinium* with spindle-form gastric vacuoles (?), and two micro-nuclei in mitosis; 8, section of *Didinium* just prior to encystment. The seizing organ with zone of trichoecysts is protruded from the mouth; and rhizoplasts run from the membranulae (motile organs) deeply into the cell. (After Calkins.)

Arcella, a diatom or other food body. Ingestion by "circumfluence" appears to be due to a stimulus emanating from a living food body, the effect of which through the motor response (Jennings, 1904) is to cause pseudopodia to flow toward the prey and to entrap it while still at some distance from the body of the captor as in the testate rhizopods, Foraminifera and Choanoflagellates where an endoplasmic projection forms a pseudopodium which engulfs the prey and then withdraws within the endoplasm where the prey is

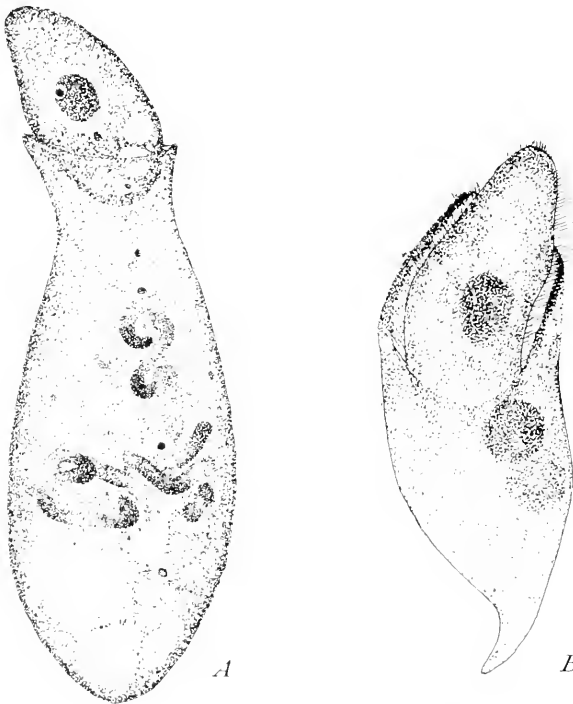


FIG. 99.—Two types of ciliated carnivores. A, *Spathidium spathula* about to ingest a *Colpidium colpoda*; B, *Lionotus fasciola* swallowing a *Colpidium colpoda*. (Original.)

digested (De Saedeleer, 1927 and 1929; Ellis, 1929). "Invagination" occurs in forms having a somewhat resisting periplast-like ectoplasm such as *Amoeba terricola* according to Grosse-Allermann (1909). When a living organism comes in contact with the surface at any point, the local ectoplasm with prey attached sinks into the endoplasm as though "sucked" in, the ectoplasmic walls being transformed into endoplasm, while the ectoplasm about the area of ingestion comes together sphincter-like, and fuses again to a smooth surface. So, too, in *A. proteus* where, according to Mast (1916 and

1923) and Beers (1924), the sphincter-like ingesting area is powerful enough to cut in two organisms like *Paramecium* and *Frontonia*. Ingestion by "importation" finally occurs where a food body, without apparent movement on the part of the *Amoeba*, merely sinks into the protoplasm of the captor as in *Amoeba doffleini* according to Neresheimer.

In most of these types, which grade more or less into one another, the process of food ingestion may be interpreted as due to local liquefaction in the more solid ectoplasm, and to special conditions of capillarity in the more fluid endoplasm. Rhumbler has shown that a filament of *Oscillaria* which enters *Amoeba verrucosa* by "importation" and is too long to be entirely engulfed, becomes coiled up as a result of the physical properties of the protoplasmic mass. In a similar way a filament of shellac may be drawn from water into a chloroform drop in which, by variations in surface tension, it becomes rolled up in a strikingly similar manner.

Some of these methods of food-getting in holozoic types are suggestive of "conscious" activities to a given end. Thus ingestion by "circumfluence" suggests preliminary activities in anticipation of a "square meal." Or traps formed by pseudopodia or by tentacles, or the balloon sails of *Pleuronema chrysalis*, etc., might be regarded as "set" by Protozoa for the purpose of catching food. Such interpretations, however, are more probably evidences of a temperamental imagination on the part of the observer than of purposeful activities on the part of these minute organisms. "Sensing" at a distance has been described for *Amoeba* (Schaeffer, 1912), and for *Spathidium spathula* (Woodruff and Spencer, 1922), and until these phenomena are explained they will continue to serve as a basis for such speculations. Losina-Losinsky (1931) gives good reasons for interpreting all such phenomena as chemiotactic and dependent upon the organizations of captor and prey.

The so-called "selective" activities of some Protozoa in their apparent choice of food or of building materials for their shells are likewise better interpreted as the outcome of physical conditions of the protoplasm than as purposeful actions of the organisms. Schaeffer (1917) attributes the power of discrimination in food-taking to *Amoeba*, as does Metalnikoff (1908) to *Paramecium*, a conclusion vigorously opposed by Wladimírsky (1916), who interprets negative reactions as a result of depression (fatigue?) in their physiological condition. *Actinobolina radians* apparently chooses, from a great number of miscellaneous forms, one particular species to harpoon, paralyze and swallow. "This remarkable organism possesses a coating of cilia and protractile tentacles which may be elongated to a length equal to three times the diameter of the body, or withdrawn completely into the body. The ends of the tentacles are loaded with trichocysts. When at rest the mouth is

directed downward and the tentacles are stretched out in all directions, forming a forest of plasmic processes among which smaller ciliates, such as *Urocentrum turbo*, *Gastrostyla steinii*, etc., or flagellates of all kinds may become entangled without injury to themselves and without disturbing the *Actinobolina* or drawing out its fatal darts. When, however, an *Halteria grandinella*, with its quick, jerky movements, approaches the spot, the carnivore is not so peaceful. The tentacles are shot out with unerring aim and the *Halteria* whirls around in a vigorous, but vain, effort to escape, then becomes quiet, with cilia outstretched, perfectly paralyzed. The tentacle with its prey fast attached is then slowly retracted until the victim is brought to the body and swallowed with one gulp. Within the short time of twenty minutes I have seen an *Actinobolina* thus capture and swallow not less than ten *Halterias*." (Calkins.)

While these observations do not prove that *Actinobolina radians* eats nothing else, it is certainly true that the usual food is *Halteria grandinella*, a fact which may account for the rarity of *Actinobolina*. That it thrives on *Halteria* is proved by the fact that isolation cultures of *Actinobolina* have been maintained for a period of eight months and through 375+ generations by division during which the only food supplied was a daily ration of 1 to 3 dozen individuals of *Halteria grandinella* independent pure "mixed" cultures of which, with bacteria, were maintained at the same time. In these cases it is quite probable that the motor response brought about by some specific chemotactic stimulus is responsible for the apparent "choice" of food by *Actinobolina*, and chemotactic or thigmotactic stimuli for food capture by "circumfluence," "circumnallation" and "importation."

A certain degree of selection is forced upon some Protozoa by the limitations of their mouth parts. Forms like *Didinium*, *Spathidium*, *Lionotus*, etc., with distensible mouths, can handle organisms of various sizes, but forms like *Paramecium*, *Dileptus*, *Spirostomum*, etc., with small inelastic mouths are constrained to "select" small objects for food. Here there is no apparent choice between nutritious and innutritious particles, carmine or indigo granules being taken in with the same initial avidity as bacteria or other useful foods. A certain so-called "hunger-satisfaction," however, leads to the cessation of ingestion in many organisms. Thus *Actinobolina radians* often captures and paralyzes more *Halterias* than it actually eats; on one occasion, for example, an individual was seen to catch 18 *Halterias*, 11 of which were swallowed while a small group of 7 were abandoned uneaten, when the *Actinobolina* swam away.

Amoeba proteus, after a period of eating no longer reacts to the stimulus of living food substances, and apparently ignores types which were previously engulfed (Schaeffer). So, too, in *Paramecium* and *Stentor*, Metalnikoff and Schaeffer describe an apparent selection

of food as illustrated by the rejection of carmine granules after a period during which such granules were actually taken in. It seems probable that such phenomena indicate a type of fatigue involving the temporary loss of irritability through which the organism responds to stimuli produced by the chemical make-up of foreign substances, a period of rest being necessary for the restoration of this form of irritability. Selection in another sense, however, is quite important. All kinds of food substances are not equally suitable for Protozoa any more than they are for individual men. This may be due to the fact that digestive fluids of a given type of ciliate or rhizopod are not adequate to dissolve all kinds of protein; or it may be due to deleterious substances in the protoplasm of the prey. All observers who have attempted to raise Protozoa in pure cultures are familiar with the difficulty of providing the proper food materials and excluding the harmful. Unsuccessful culture experiments indicate that these conditions have not been met. Furthermore, a culture medium is suitable only when the organism under cultivation continues to live during all phases of its life cycle.

Apparent selection of foreign objects used in shell-building may be due to the physical consistency of the protoplasm and to its ability to pick up foreign bodies like sand crystals, diatom shells, etc., or in part to the size of the shell-opening through which such objects must pass for storage in the protoplasm. Mud and other fine particles of inorganic matter, like carmine granules, are engulfed with bacteria and other microorganisms which produce the stimulus necessary for the operation of food-taking. After the useful substances are digested the residue, like castings of worms, may be voided to the outside or they may serve a useful purpose in the construction of shells.

A special kind of holozoic food-getting is illustrated by the Suctoria which, instead of cilia, are provided with suctorial tentacles (Fig. 100). The prey, usually some form of ciliated Protozoa, comes in contact with one of these tentacles and is paralyzed through the action of some kind of poison contained in it. The cortex of the prey is perforated by the end of the tentacle and the fluid endoplasm is sucked into the body of the captor, a stream of granules being visible within the tentacle. In some cases it is said that the endoplasm of the captor flows through the tentacle and into the body substance of the prey where the latter is digested (Maupas, 1883). The body of the victim gradually collapses until nothing remains but the denser walls and the insoluble parts.

Many of the Protozoa, while parasitic in the cavities and cells of different animals, retain the holozoic method of food-getting, feeding upon parts of the protoplasm of the host or upon other living organisms such as bacteria of the digestive tract, or solid detritus of one kind or another. Thus *Endamoeba coli* lives on intestinal

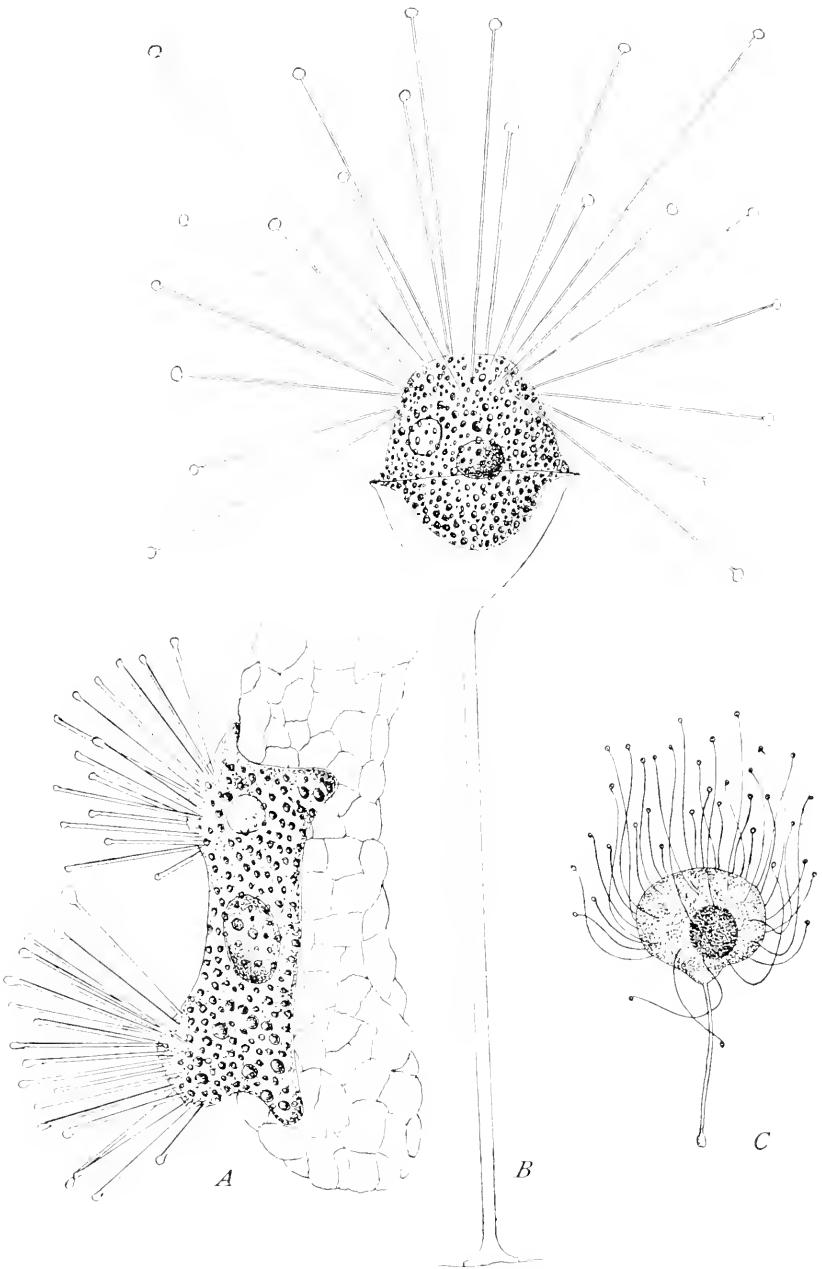


FIG. 100.—Types of Suctoria. A, *Trichophrya salparum* on a gill filament of *Salpa*; B, *Acincta* sp.; C, *Podophrya* sp. (Original.)

bacteria, while *Endamoeba dysenteriae*, *Dientamoeba fragilis*, etc., engulf, with other food substances, red blood corpuscles and digest them. According to Haughwout (1919), the flagellate *Pentatrichomonas* sp. likewise ingests red blood corpuscles. In the majority of protozoan parasites, however, the organisms do not digest the food necessary for the growth of their own protoplasm. They practically live in a huge gastric vacuole and are surrounded by food already digested or partly digested, which is absorbed by osmosis through their body walls. Dofflein thinks that such food substances, if not appropriate for the up-building of protoplasm of the parasite, may be made suitable by the secretion from the parasite of special digestive substances and is ready for absorption after the action of such secretions. He further suggests that the cytolytic action upon cells and tissues of the host may be due to such secretions (for example *Endamoeba dysenteriae*) and that other toxins of pathogenic Protozoa, probably enzymatic in their activity, may be similar digestive secretions from the parasites (see p. 362).

Secretions and Digestive Fluids.—Products of metabolic activity in the form of secretions and precipitations play most important rôles in structure and activities of all kinds of Protozoa. Skeletons, shells and tests, gelatinous mantles, stalks, cyst and spore membranes, and the like are all evidences of the secretory activity of the protozoan protoplasm (see Chapter IV). There is evidence that these activities, like secretory activity of the gland cells in Metazoa, are dependent upon the general function of irritability and that specific secretory response follows a specific stimulus. Thus Bresslau (1921) finds that gelatinous mantles or tubes about *Colpidium colpoda* may be called forth at will by the use of certain chemicals (iodine, fatty acids). If fatty acids are used, the individuals, as in artificial parthenogenesis, must be replaced in a suitable medium before the membranes are formed. Enriques (1919) gives evidence to show that the secretion of stalk material in *Anthophysa vegetans* depends upon the quantity of food available. Stimulation, through the agency of foreign proteins, is without much doubt responsible for the secretion of digestive fluids and ferments in holozoic nutrition, and considerable advance has been made in our knowledge of intracellular digestion. This advance has been due mainly to the application of the method first devised by Gleichen (1778) of introducing into the body with food substances inorganic, usually colored particles which clearly outline the limits of the digestive cavities. These cavities, early termed gastric vacuoles, were recognized as digesting centers of the organisms, and Gleichen's method, employed by Ehrenberg (1833-1838) led to his elaborate and at first widely accepted, but erroneous, conception of the Polygastrica. Modern applications of this method consist in the introduction with the food of delicate chemical substances, or indicators, which change

in color according to the acid or alkaline nature of the fluids in which they lie. The observations of le Dantec (1890), Fabre-Domergue (1888), Metschnikoff (1889), Greenwood (1887-1894), Nirenstein (1905), Khainsky (1910) and Metalnikoff (1903, 1912), together with the study of extractives by Mesnil (1903), Mouton (1902), Metschnikoff (1893), Krukenberg (1886), Hartog and Dixon (1893), etc., have given a fairly comprehensive idea of the processes of intracellular protein digestion in Protozoa. Another group of observers including Meissner, Greenwood and Saunders, Stolç (1900), Wortmann (1884), Celakowski (1892), Nirenstein, etc., have shown the digestive possibilities in relation to carbohydrates and fats.

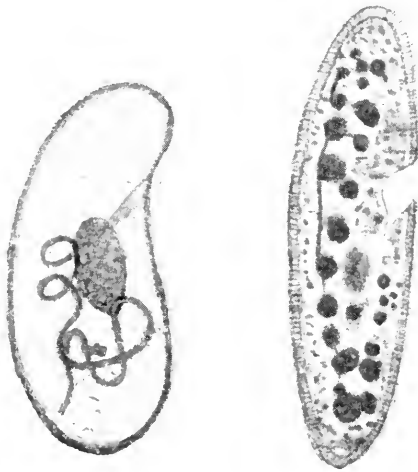


FIG. 101.—*Colpidium colpoda* and *Paramecium aurelia* after feeding with amylo-dextrin and treatment with iodide. (After Cosmovici, courtesy of Annales Scientifique de l'Université de Jassy.)

An interesting conception of the gastric vacuoles in ciliates has been given recently by Cosmovici (1932). Using an ingenious method of dissolving rice starch with saliva and immersing ciliates in the dextrin thus formed, he found, upon treating them at different intervals with iodide, that a canal, colored blue, often convoluted or swollen into "gastric vacuoles," runs from mouth to anus (Fig. 101). Further investigation of this remarkable canalicular system is needed.

The majority of Protozoa which ingest "solid" food take in at the same time more or less water, which forms the gastric vacuole. Thus in trichostomatous ciliates a vacuole is formed at the base of

the cytopharynx which varies in size according to the abundance of food particles present. In *Paramecium caudatum* the vacuole, when formed, becomes spindle-shape as though pulled away from the gullet by endoplasmic force, but it soon becomes spherical as it moves about in the fluid endoplasm (Nirenstein, 1905). With the ingestion of larger food bodies such as infusoria, flagellates of larger size, diatoms, rotifers, etc., comparatively little water accompanies the prey. *Paramecium caudatum* when eaten by *Didinium nasutum*, for example, lies in close contact with the protoplasm of its captor and no water at all can be made out (Fig. 98). In such cases the ingested organism is paralyzed and therefore motionless when swallowed, but it very often happens that resistant food bodies continue to struggle after they have been taken into the protoplasm; rotifers, for example, are usually not motionless when engulfed by *Amoeba proteus*. In such cases a considerable volume of water gives the prey ample room to move without danger to the make up of the captor. In other cases in which water does not appear to be taken in with the food, the latter becomes surrounded by fluids secreted by the protoplasm.

With many types of Protozoa the process of digestion begins before the living prey is taken into the protoplasm of the captor. This is manifested in most cases by the paralysis of the victim when it comes in contact with pseudopodia of many rhizopods and Heliozoa, Ehrenberg (1833) for *Actinophrys sol*; F. E. Schultz (1875-1876) for *Allogromia* and *Polystomellina*; Winter (1907) for *Pencroplis*, etc. In some cases, at least, it is not improbable that this paralyzing killing substance is analogous to, if not the same as, the digestive fluids which kill bacteria and other prey after they are taken into the body protoplasm. Thus bacteria become motionless in about thirty seconds after the gastric vacuole is detached from the cytopharynx of *Paramecium caudatum* (Metalnikoff, 1903 and 1912). The color changes of chemical indicators, for example alizarin sulphate, show that the killing agent is acid in nature; this was early detected by Greenwood and Saunders (1894), who interpreted it as a mineral acid without further specification. Later observers have confirmed this suggestion, Nirenstein, Metalnikoff and others showing that digestion in the vacuole is a process which is divisible into two periods, in one of which the reaction of the vacuole contents is acid, while in the other it is alkaline. The acid reaction lasts for about fifteen minutes, according to Nirenstein and Metalnikoff, in the gastric vacuoles of *Paramecium*, but Khain-sky concluded that the acid reaction is maintained during the entire period of digestion, becoming alkaline only after the dissolution of the protein substances is at an end. In other cases, however, no acid reaction at all can be demonstrated. Thus, Metalnikoff, also in the case of *Paramecium*, found that some vacuoles never give an acid reaction; others much more rarely show an acid reaction

throughout, while still others in the same organism are first acid and then alkaline. Minchin (1912) suggests, in connection with this diverse history of vacuoles in the same species, that different food substances incite different responses on the part of the protoplasm much as different antibodies are formed from cells of the Metazoa in response to toxins from different types of pathogenic parasites. Shapiro (1927) followed the change in pH of the gastric vacuole in *Paramecium* from an initial alkaline stage (7.6) which quickly changed to a maximum acid stage (pH 4) from which it slowly returned to the alkalinity of the surrounding water (pH 7). In Heliozoa, Howland (1928) shows that the initial pH of a gastric vacuole of *Actinosphaerium eichhornii* is about neutral or slightly

acid (pH 7 to 6.6). This lasts for a period of five or ten minutes but changes to $\text{pH } 4.3 \pm 0.1$ in all vacuoles in which active digestion is going on, while old vacuoles containing indigestible remains have a pH range from 5.4 to 5.6.

In view of the number of different ferments which have been isolated from different types of Protozoa, it is quite probable that digestion does not take the same course in all types. Pepsin-like ferments, which dissolve albumins in an acid medium, were isolated by Krukenberg (1886) from the Mycetozoön *Aethalium septicum*, and by Hartog and Dixon (1893) from the ameba *Pelomyxa palustris*, while Metschnikoff (1889) showed that the food vacuoles in the plasmodia of *Aethalium* have an

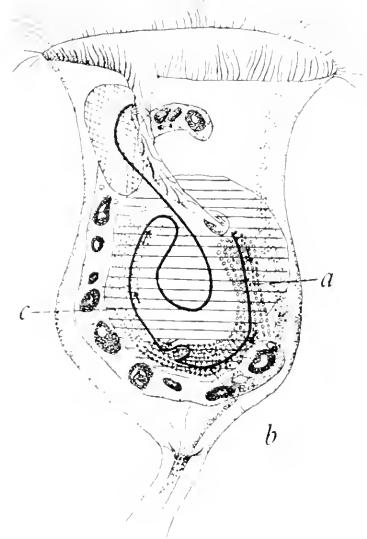


FIG. 102. — *Carchesium polypinum*? History of food vacuole: (a) stage of storage and little change; (b) stage of acid reaction; (c) neutral reaction. (After Greenwood.)

acid reaction favorable to the activity of such ferments. Trypsin-like ferments have likewise been isolated by Mouton (1902), from soil amebae cultivated in large numbers on agar; also diastatic ferments were easily obtained from *Balantidium coli* by Glaessner (1908), and from *Pelomyxa palustris* by Hartog and Dixon (1893).

The typical course of a gastric vacuole through the endoplasm of ciliates has been carefully worked out by Greenwood and by Nirenstein for *Carchesium* and *Paramecium caudatum* (Fig. 102). Prowazek (1897) staining with neutral red found a collection of red granules about the gastric vacuole; similar granules were observed

by him and by Nirenstein (1905) to pass into the gastric vacuole and to mix with the food substances from which circumstance they were regarded by both observers as the bearers of ferments (trypsin-like according to Nirenstein). The so-called Excretperlen (excretory granules) first described by Prowazek (1897) and interpreted by him, by Nirenstein and by Dofflein (1916) as furnishing evidence of excretion through the general cell membrane, with equal justification may be interpreted as secretory granules. If the neutral red staining granules about the gastric vacuoles are bearers of ferments as maintained by Prowazek, they certainly are secretory in nature. There is some uncertainty, however, as to the identity of these with the so-called excretory granules. The experiments of Slonimski and Zweibaum (1922) show that there are two types of these granules which they call A and B, and that the peripheral granules (B) which exude from the membrane vary in number and size according to external conditions of temperature and internal conditions of vitality, being rare or absent prior to conjugation. The nature of these varying granules and their function in metabolism are still unsolved problems.

In connection with secretions we may take into consideration the various poisons produced by Protozoa either in the form of toxins exuded by the individuals and soluble in the surrounding medium, or in the form of endotoxins which are liberated only when the individual is disintegrated. What little is known about these secretions is mainly in connection with parasitic forms and here knowledge is limited to the effects produced upon the host (see Chapter X). In general it may be stated that, if we except the toxins produced by the so-called Chlamydozoa (particularly small-pox and rabies organisms), the poisons of protozoan origin are much slower and indefinite in their action on the host than are bacterial toxins, and the course of the specific diseases caused by pathogenic protozoa is relatively much slower than diseases caused by bacteria. Relatively few toxins of protozoan origin have been extracted and used in experimentation. One such, called sarcocystin, was obtained from sarcosporidia by Pfeiffer and Gasparck and by Laveran and Mesnil (1899). The latter found that rabbits are soon killed by the blood injection of sarcocystin in glycerin solution, also that crushed cysts give rise to characteristic pathological effects in the muscles, whereas no such reaction accompanies the presence of uninjured cysts.

Filtered blood of malaria victims, if taken at the height of paroxysm and injected into a malaria-free individual, produces in the individual a characteristic malarial paroxysm according to Rosenau and his co-workers, and analogous "paroxysm toxins" have been detected in connection with other blood parasites.

Toxins from organisms of amebic dysentery are more regional in their action, causing local ulceration and abscess formation indi-

eating a cytolytic process possibly due to secretions of digestive fluids. There is still some uncertainty, however, in regard to this matter, and the possibility of participation by bacteria in the reactions is not excluded.

Notwithstanding the serious diseases in man and mammals generally due to trypanosomes, there is very little positive evidence that secretions are responsible for the effects produced. Experiments with extractives from *Trypanosoma brucei* by Kanthak, Durham and Blanford, and by Laveran and Mesnil, gave no indication of toxic effects. On the other hand, Novy and MacNeal, injecting dead *Trypanosoma brucei* in guinea-pigs obtained definite fever symptoms, loss of weight and local ulcerations which, however, they did not trace to the effects of a specific toxin.

Somewhat more positive evidence is accumulating in regard to the possibility of endoenzymes locked up in the trypanosome protoplasm and liberated on disintegration. Thus a number of observers, among whom may be enumerated MacNeal, Plimmer, Leber, Martin and others, have interpreted the rise in temperature of organisms with trypanosomiasis as due to the presence of endotoxins, freed in the blood upon death and disintegration of trypanosomes resulting from treatment with medicaments. Also Uhlenhuth, Woithe, Hübener and others have concluded that endotoxins fatal to rats are liberated if blood containing *Trypanosoma equiperdum* is first dried, then dissolved again and injected into rats. Schilling, Braum, Teichmann, on the other hand, got no reaction upon injecting dead pathogenic trypanosomes into the peritoneum or subcutaneously (see pp. 363 and 384).

In all of these cases, with the exception of sarcocystin, the evidence in favor of the secretion of exotoxins or the presence of endotoxins is purely circumstantial and verification by chemical and biological methods with exclusion of other possible contributing factors has not yet appeared.

Digestion of Carbohydrates and Fats.—Specific ferments for the transformation of starch into soluble sugar have not been isolated; nevertheless, the evidence that such action takes place is convincing. Curiously enough, this evidence does not apply to the Infusoria where very little digestion, beyond a slight corroding of starch grains, occurs. In rhizopods, however, especially in the ameboid *Pelomyxa* and in species of *Ameba*, starch grains are entirely dissolved, according to the observations of Stolç (1900) who found that the characteristic refringent granules of *Pelomyxa palustris* have a very definite relation to carbohydrate nutrition. These granules (Glanzkörper) are filled with glycogen, the volume of which increases up to fourfold when the animals are fed with starch, and decreases to entire disappearance when they are starved.

Even cellulose is said by Stolç to be digested by this organism and Schaudinn made the same observation on the Foraminiferon *Calcituba polymorpha*. In Foraminifera generally, according to Jensen, and in myxomycetes, according to Wortmann, Lister and Celakowsky, starch may be similarly digested. The flagellates apparently have in some cases, at least, the same power of dissolving starch. Thus, *Protomonas anyli* and *Phyllonitius augustatus* eat practically nothing but starch, a fact indicating the action of appropriate digestive ferments. The Hypermastigidae which are abundant in white ants (termites) are unusual in their ability to digest cellulose. It has been shown that these flagellates live as symbionts with their termite hosts digesting the wood eaten by them. The termites die if deprived of their protozoan symbionts by heating or by oxygenation; the protozoa die if the wood diet of the termites is stopped (Cleveland, 1923).

In few Protozoa has the actual digestion of fat been observed. Under experimental conditions, ingested fats are usually carried along unchanged in the protoplasm. We cannot state arbitrarily, however, that fats are not emulsified and used as food. On the contrary, it is difficult to account for the presence of oils and fat bodies in varying quantities in all groups of Protozoa under any other assumption, despite the negative results of Stamiewicz (1910) and of Nirenstein (1909). Positive results indeed have been obtained by Dawson and Belkin (1928), who injected oils of different kinds into *Amoeba proteus*; of these 8.3 per cent of cod-liver oil was digested, 8.2 per cent of olive oil, 4 per cent of cotton-seed oil, 3.5 per cent of sperm oil and 1.4 per cent of peanut oil.

Saprophytic Nutrition.—In holozoic nutrition the food substances are in the form of complex proteins, carbohydrates and fats, making up the bodies of the various organisms ingested. In saprophytic and saprophytic nutrition the food substances are less complex chemically, consisting of materials dissolved out of the disintegrating bodies of animals and plants. These are taken in, not through the agency of specialized oral motile organs, nor through a definite mouth, but are absorbed through the body wall. Many of the smaller types of flagellates obtain their nutriment in this way, extracts or infusions of animal or plant tissues containing various salts and organic compounds forming excellent culture media for such Protozoa. Little is known, however, of the chemical make-up of such fluid substances, nor is it known whether they are prepared for absorption by chemical processes due to the activity of the receptive organism; nor is there any evidence to indicate processes of digestion subsequent to their absorption. The general assumption, based upon the thriving cultures in infusions of disintegrating animal and plant matter, has been that dissolved

proteins are taken into the protoplasmic bodies of many kinds of Protozoa by absorption through the general cortex or through some specialized region for the purpose.

From experiments with the green alga, *Euglena gracilis*, by Zumberstein (1900), Ternetz (1912), *et al.*, it appears probable that some saprozoic forms of Protozoa get their main nourishment from amino-acids derived from disintegration of animal and plant matter through the agency of bacteria, and from carbohydrates in solution. The necessary mineral matters are obtained from the surrounding alkaline medium.

Emery (1928), experimenting with *Paramecium caudatum*, found that a measurable quantity of amino-acids is utilized in place of the normal bacterial food. With a mixture of equal parts of ten amino-acids he figured out that 100,000 *Paramecium caudatum* in twelve hours would use 48.3 per cent of a 0.1 per cent solution, while different amino-acids used singly gave differing results.¹

In this connection, it is important to consider the possible interaction of excretion products of different Protozoa upon themselves and upon each other, as well as the effects of products of bacterial action. It has long been known that isolation cultures are frequently threatened by the growth of detrimental bacteria. On *a priori* grounds it is not improbable that excretion products of Protozoa themselves may have such an effect. Woodruff (1912, 1913) has studied this problem in connection with *Paramecium aurelia* and the hypotrichous ciliates, *Stylonychia pustulata* and *Pleurotricha lanceolata*, and found that *Paramecium*, when placed in filtered medium which had contained enormous numbers of *Paramecium* in pure culture, were manifestly weakened in vitality. Similarly the hypotrichs, when placed in filtered medium which had swarmed with hypotrichs, showed a weakened vitality. When, however, *Paramecium* was placed in filtered hypotrich culture medium, the result was an increased vitality. Woodruff concluded that excretion products from *Paramecium* are detrimental to *Paramecium*, and hypotrich products to hypotrichs, while the latter products have a somewhat stimulating effect on *Paramecium*. This may be, as Woodruff suggests, of some importance in determining the sequence of protozoön forms in a limited environment such as hay infusion.

¹ The degree of absorption of specific amino-acids is as follows:

	Per cent.		Per cent.
Mixture of different amino-acids (except arginine)	48.3	Alanine	15.5
Glutamic acid hydrochloride	45.6	Glutamic acid	13.2
Cysteine hydrochloride	26.3	Leucine	12.0
Aspartic acid	25.1	Glycocoll	9.6
Tyrosin	17.7	Tryptophane	9.6
Arginine	15.9	Phenylalanine	7.7

Specific structural adaptations, useful in methods of food-getting, are characteristic. Haustoria-like processes, derived from the epimerites of gregarines, in some cases extend deeply in the tissue cell (*Stylorhynchus longicollis*, *Echinomera hispida*, *Pyrinia moebiuszi*, etc., Fig. 103). The coccidian *Caryotropha mesnili*, according to Siedlecki, shows a significant relation between the nucleus of the host cell and that of the parasite. This organism is a parasite in the spermatozoa of the annelid *Polynnia nebulosa* where the sperm cells are aggregated in bundles in the characteristic annelid fashion, usually about a feeding mass or blastophore. The parasite gets into such a cell as an agamete or sporozoite, one only of the bundle,

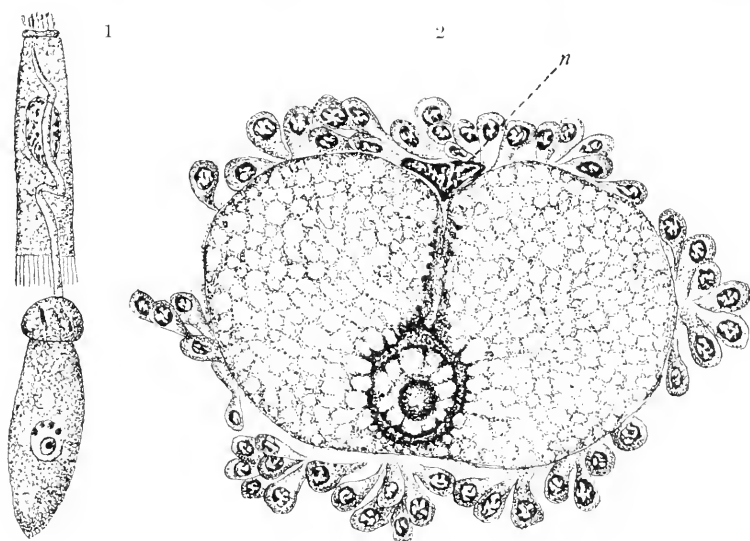


FIG. 103.—Food-getting adaptations of Sporozoa. 1, *Pyrinia moebiuszi* with epimerite deeply insunk in the epithelial host cell (after Léger and Dubosq); 2, *Caryotropha mesnili* with an intracellular canal from the nucleus of the host cell (*n*). (After Siedlecki.)

as a rule, being infected, and as it grows the nucleus of the cell is displaced to one side and the cell loses its characteristic structure, becoming hypertrophied and distorted (Fig. 103, 2). Not only the infected cell but all the other cells of the spermatogonia bundle are affected, and none of them continues the normal development, but they become arranged like epithelial cells about the hypertrophied infected cell.

The specific effect of the young *Caryotropha* on the infected cell consists not only of the enlargement of that cell, but of a definite feeding mechanism by which the parasite is supplied with food. That the nucleus is a center of constructive metabolic changes is well assured at the present day, and the conditions in these para-

sites suggest the peculiar relation which Shibata (1902) has described in the intracellular mycorrhiza, where a mycelium thread is grown straight toward the nourishing cell nucleus of the host, causing marked hypertrophy on the part of the cell. In *Caryotropha*, the nucleus of the host cell is pushed to one side and the parasite assumes such a form that the nucleus lies in a small bay (Fig. 103, 2 *u*). In the cytoplasm of the cell an intranuclear canal is then formed which runs from the host nucleus to the nucleus of the parasite, and Siedlecki holds that the food of the parasite is all elaborated by the nucleus of the host cell, while the other spermatogonia form a protective epithelial sheath around it. When the parasite is full grown the cell is destroyed and the bundle degenerates.

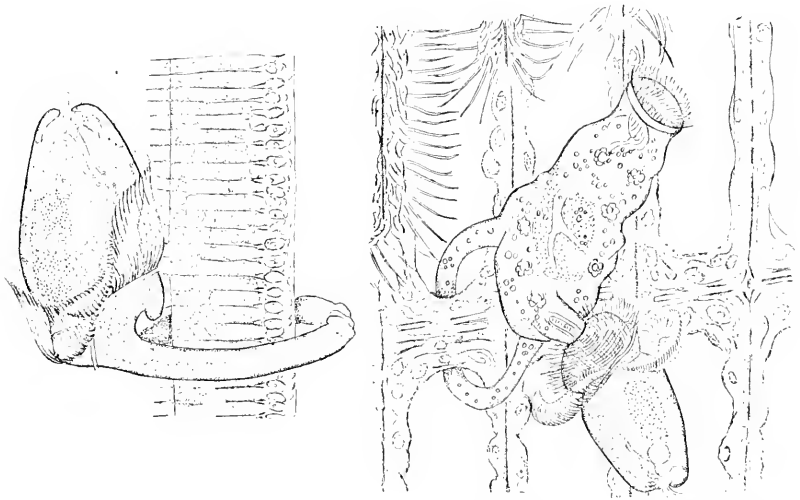


FIG. 104.—*Ellobiophrya donacis*, a peritrich with ring-form attaching organ which passes around the gill bars of the lamellibranch. $\times 800$ and 1350. (After Chatton and Lwoff, Bull. biol. de la France et de la Belgique, 1929; courtesy of Prof. N. Caullery and Les presses Universitaires de France.)

Other special adaptations in the interest of food-getting are frequently spectacular. Thus *Ellobiophrya branchiorum* (Chatton and Lwoff, 1928), a commensal ciliate on the gills of the lamellibranch *Donax* sp., has developed a curious, posterior, ring-form process whereby it is firmly anchored to the gill bars (Fig. 104).

It is difficult to draw the line between symbionts, commensals and parasites. Symbionts are organisms living with a host in such a relation that both are benefited; commensals are organisms which live with a host without benefit or injury to the latter but to their own advantage, and parasites are organisms which, to their own benefit, cause injury in one form or other to the host. Symbiosis

is well illustrated by the harmonious life of some chlorophyll-bearing forms, *Zoöchlorrella*, *Zoöxanthella*, etc., and Protozoa in which the former live (*Paramecium barsaria*, "yellow cells of Radiolaria and Foraminifera," *Stentor viridis*, *Amoeba viridis*, *Vorticella viridis*, etc.), and it is conceivable that some gut-dwelling forms may perform a useful activity for a host by disposing of pernicious bacteria, or by preparing food substances for use by the host as do *Hypermastigidae* in termites (Cleveland). Commensals, such as *Eudamoeba coli*, *Eudamoeba nana*, *Trichomonas* species and other intestinal forms may, on occasions, turn into parasites, as is the case with *Trichomonas* (*Tritrichomonas*, Kofoid), *Giardia* (*Lamblia*), etc.

2. **Products of Assimilation.**—With the majority of forms the products of assimilation vary with the type of food used and are frequently so abundant in the cell as to give a characteristic appearance or color to the animal. Thus the refringent granules of *Pelomyxa palustris* (Stolç) produce a peculiar refringent effect. The brown granules of *Plasmodium* species, characteristic of malaria, are products of hemoglobin assimilation. Similarly the coccidin of *Coccidia*; stentorin of *Stentor coerulesus* and *Folliculina ampulla*; the pink of *Holosticha*; the lavender of *Blepharisma undulans* or the red of *Mesodinium rubrum*, are examples of the great variety of colored cellular substances dependent upon the food that is eaten. In the absence of the specific kinds of food which yield these chromic products the organisms are colorless, and colored or colorless individuals of the same species may appear in the same culture (see p. 134).

CHAPTER VI.

REPRODUCTION.

OF all the marvels associated with the Protozoa there is nothing more staggering to the imagination than the fixity of type which their protoplasm manifests. The genotype, represented by the derived organization, subject to minor variations of a fluctuating character in the course of a normal life history, or subjected experimentally to all kinds of unusual environmental conditions, remains fundamentally unchanged. Types modified through amphimixis or through permanent modifications of the environment may lead to divergent types. This conservatism or fixity of type is a function of the organization which has been continuous in the past and will be continuous in the future. The activities which take place in the organization, the sum total of which constitute vitality, are discontinuous, they have been and will continue to be dependent upon the interactions between organization and environment.

The single individual which we study under the microscope has had no such history in the past and no promise for the future; its span of life as an individual is measured by hours or days only. It is the temporary trustee of a small portion of an organization which has been parceled out among unknown myriads of similar trustees. Its metabolic activities are the interactions within the organization and as a result of these activities the fluctuating variations characteristic of the genotype follow one after another in the form of inevitable differentiations which may or may not be visibly indicated by structural changes (see Chapter VII). Ultimately its possibilities of further vitality as a single individual are exhausted and it undergoes its final manifestation of vitality. The significance of this final act is a function of all genotypes and of all organizations whereby the organization is further parceled out to two or more trustees. It is reproduction by division, which by reason of its universal occurrence is one of the most characteristic properties of protoplasm.

There is no doubt that division of the cell is a phenomenon of deep-reaching significance; we shall endeavor to show that the organization as parceled out to the descendants by division is not a mere equal division of the protoplasm of the individual with its load of metaplastids and other modifications of the organization, but a renewed or purified organization such as the individual received when it was formed. Unlike Metazoa, with the processes of division, the old derived organizations of Protozoa are lost by absorption,

the organization being de-differentiated, and the protoplasm has a renewed potential of vitality.

In order to understand the relations of division to the chain of metabolic activities we should know more about the conditions under which division occurs, and the "causes" of division. There is very little real evidence for conclusions in this matter but there have been many theories. The latter for the most part are based either upon analogies with physical phenomena or upon hypothetical "spheres of influence" of morphological elements of the cell. They have been developed in the main to interpret phenomena of division in metazoan cells, particularly in egg cells, and fall completely to the ground when applied to division of Protozoa. So it is with the contractility hypothesis of Heidenhain, Drüner and others, who see in the spindle fibers and astral rays a contractile system whereby the nucleus and cell are divided in a strictly mechanical manner. The intranuclear spindle and the absence of cytoplasmic rays in the great majority of Protozoa are enough to show that such physical interpretations do not reach to the root of the matter. The "spheres of influence" hypotheses, based upon the kinetic center of the cell and its influence on the cytoplasm, was developed by Boveri in the attempt to associate cell growth and the causes of division. The "energid" theory of Sachs and Strasburger was an analogous effort to trace the causes of cell division to increasing volume of the cell through growth, each nucleus having its sphere of influence in the cytoplasm and dividing when the volume of the cell outgrows the sphere of activity of the nucleus. The *Kernplasmverhältnis* theory of Hertwig was based upon somewhat similar grounds. According to this the volume of the nucleus bears a certain normal relation or ratio to the volume of the cytoplasm in young actively functioning cells, evidence of which in *Frontonia* was given by Popoff (1909) and by Hegner (1920) in the equidistant distribution of nuclei in various species of *Arcella*. With increasing age this ratio is altered to the advantage of the cytoplasm until division of the cell restores the normal ratio. With uninucleate forms such as *Paramecium* or *Frontonia* there is some evidence of change in relative volumes, and careful measurements by Popoff (1909) and other followers of Hertwig are adduced to support the hypothesis. In these forms the volume of the nucleus is proportionally reduced until just prior to division when the nucleus rapidly increases in volume and divides. Looper (1928) more recently, by mechanical stimulation, caused *Actinophrys sol* to fuse with enucleated fragments from other individuals. This led to change in the nucleus-cytoplasm ratio to the advantage of the cytoplasm. Such forms divided from one-half to two times faster than the controls. If, on the other hand, some cytoplasm is cut away, the reduced cells (100 cases) divided on the average in eighty-eight hours, while

the controls divided in twenty-four hours (see Hartmann's similar experiments with *Ameba*, p. 239). In *Uroleptus*, *Uronychia* and similar forms, however, the many nuclei fuse to form one compact and relatively small nucleus prior to division. It would seem that such changes in relative volume of nucleus and cytoplasm are better interpreted as the effects of underlying conditions which lead to division rather than as the direct cause of division.

None of these theories is of much value in analyzing the antecedent phenomena of division. These must be sought in the reactions of different substances constituting protoplasm. Division of the cell itself is a last step in a progressive series of reproductive changes affecting the entire protoplasm, the constituents of which—microsomes, mitochondria, plastids, chromomeres, kinetic elements, etc.—have already divided. It is in the division of these fundamental granules in the make up of protoplasm that we must look for the underlying causes of cell division. The dependence upon growth and metabolism of the succession of division processes which characterize reproduction is clearly evidenced by simple starvation experiments, division ceasing with cessation of metabolic activities. There is a possibility that environmental conditions play a more direct part in reproduction than is indicated by their relations to metabolism. Thus Robertson (1921) concludes that a catalase (X substance) is secreted by the living cell which directly enhances division. He found that two individuals, or more, of *Enchelys farcimen* in a drop of culture medium would divide from four to sixteen times more rapidly than a single individual in a similar drop, the result being interpreted as due to contiguity of individuals. This, however, is a direct contradiction of Woodruff's (1911) results with *Paramecium* and *Stylonychia*, according to which the division rate is reduced by accumulation of products of metabolism in the medium. Nor is Robertson supported by other observers. Cutler (1924) for example, found for *Colpidium colpoda* that the division rate depends upon the number of bacteria present as food, and that increase in number of individuals in a drop means a decrease in the individual division rate. Greenleaf (1924) similarly found that solitary individuals of *Paramecium caudatum*, *P. aurelia* and *Pleurotricha lanceolata* isolated in 2, 5, 20 and 40 drops of medium, gave a highest division rate in five days in the 40-drop test, the lowest in a 2-drop test. Also in *Uroleptus mobilis*, in a sixty-day test in which 1 individual, 2, 3 and 4 individuals were isolated daily in a single drop of medium the highest division rate was shown by the solitary individual in a drop as shown in the following table (see also table on next page):

10 individuals, 1 to a drop, each divided in the sixty days	. .	74.1 times
20 individuals, 2 to a drop, each divided in the sixty days	. .	59.5 "
30 individuals, 3 to a drop, each divided in the sixty days	. .	54.7 "
40 individuals, 4 to a drop, each divided in the sixty days	. .	54.2 "

Environmental conditions which alter the permeability of the cell, thereby enhancing or retarding metabolic activities do, however, have a corresponding effect upon the division rate. Age of individuals, or the protoplasmic organization at different periods of the life cycle likewise has a determining effect on the rate of division, the differences, as shown in the following table, being due to the differences in the reactions of the protoplasm to the same medium under different conditions of organization. Series 111 and 112, for

UROLEPTUS MOBILIS DIVISION RATE.

Experiment from September 21 to November 10, 1924.

Series.	Age, Generation.	No. in drop.	Divisions per individual.						Total, sixty days.
			First ten days.	Second ten days.	Third ten days.	Fourth ten days.	Fifth ten days.	Sixth ten days.	
111	279	1	12	7	10	13	9	9	60
		2	11	7	6	10	5	5	44
		3	9	5	6	7	5	4	36
		4	10	4	3	6	3	5	31
112	263	1	14	14	9	10	7	6	60
		2	11	13	5	8	4	6	47
		3	13	8	4	7	2	4	38
		4	8	11	10	7	2	3	41
114	160	1	11	8	5	9	4	6	43
		2	6	8	3	6	1	6	30
		3	5	4	3	4	2	0	18
		4	8	4	3	2	3	1	21
115	247	1	14	17	9	10	13	10	73
		2	10	13	6	9	10	9	57
		3	14	16	7	8	8	4	57
		4	15	13	7	9	10	7	61
116	189	1	13	14	10	9	7	7	60
		2	9	10	8	10	9	8	54
		3	9	11	5	7	8	7	47
		4	7	7	3	7	6	4	34
117	133	1	16	18	11	10	14	12	81
		2	14	17	7	10	8	9	65
		3	11	17	8	9	10	9	67
		4	13	17	6	8	10	8	62
118	140	1	18	22	12	16	17	14	99
		2	18	14	8	11	13	13	82
		3	15	20	9	12	11	9	76
		4	11	20	7	12	12	12	77
119	110	1	15	19	10	10	10	8	72
		2	15	14	7	7	7	9	59
		3	11	14	7	8	6	6	52
		4	10	14	6	8	7	5	50
120	12	1	18	19	11	13	13	12	86
		2	16	16	6	12	9	10	69
		3	17	15	5	8	13	9	67
		4	16	15	9	9	13	11	73
121	10	1	18	23	13	16	18	19	107
		2	14	24	9	8	15	18	88
		3	15	23	10	11	14	16	89
		4	19	21	10	11	14	17	92

example, were 279 and 263 generations old at the beginning of the experiment, the single individual isolated daily in a drop of medium divided 60 times in sixty days; with 4 individuals in a drop, each divided only 31 times. Series 120 and 121 were 12 and 10 generations old, and each solitary individual divided 86 and 107 times in the same sixty days, and with the same medium freshly made each day. From this table it is apparent that the division rate is reduced by the presence of more than one individual to a drop. Furthermore, the reduction of the division rate under such conditions is much less for "young" individuals than for old.

Substances making up the composition of living protoplasm are constantly manufactured. Such substances, usually in the form of granules, grow to a certain limit of size and each then divides. Evidence for this is apparent only in the more obvious of the protoplasmic elements such as plastids, kinetic elements, chromomeres, etc., the division of which has been mentioned in the preceding pages. Finally the grand aggregate, the cell itself, divides as a last expression of the series of events that have taken place. It is evident that such division of the cell as a whole constitutes only a small part of the phenomena of reproduction and perhaps not the most important part. While most of the elementary granules, apart from those enumerated above, which make up the bulk of protoplasm, cannot be followed from their smallest stages to the stage when they become visible, it is not inconsistent with the idea of continuity from generation to generation to regard even the smallest as retaining its integrity and reproducing itself by division. "For my part I am disposed to accept the probability that many of these particles, as if they were submicroscopical plastids, may have a persistent identity, perpetuating themselves by growth and multiplication without loss of their specific individual type" (E. B. Wilson, 1923).

While the division of a single granule results in the formation of two probably identical granules of the same substance, the division of aggregates of granules of different substance may or may not result in identical daughter aggregates. The nucleus is such an aggregate which, by ordinary equation division, is probably divided into two identical halves, but in meiotic divisions the products of the nucleus are different, visible evidence of which is shown by the history of the sex chromosomes and by the results in modern genetics. It is entirely possible that differentiations may arise from such inequalities in nuclear division (see Chapter IX).

The cytoplasm of the cell, likewise, is such an aggregate, made up of all the different substances variously distributed, which compose living protoplasm. If all the granules were equally distributed at division to the daughter cells, as are nuclei and many kinetic elements, then the products of cell division might be identical. Mor-

phological evidence that all granules are not thus equally distributed is furnished by all budding and spore-forming types, and by forms like *Dileptus gigas* or *Holosticha multinucleata*, where the large chromatin granules, while still in the process of division, are carried bodily to one or the other daughter cell (Fig. 46, p. 92).

Reproduction whereby a type of organism is perpetuated and distributed, is thus preëminently a process of division. In the last analysis cell division is the only kind of reproduction known. Potential individuals are contained in every germ cell, but germ cells, like other cells, are formed by division and it follows that every female reproduces as many potential offspring as eggs. Development of such eggs, however, is usually dependent upon fertilization, which is quite a distinct phenomenon, accessory to reproduction and necessary in most animals, but not itself reproduction. In the present chapter only a summary of the more obvious phenomena of reproduction will be described, leaving the problems associated with fertilization for treatment in a later section (see Chapter VIII).

It is division of the grand aggregate of protoplasmic substances, *i. e.*, division of the cell itself, that is usually described as reproduction of the Protozoa. Such reproductions are usually classified as division, budding or gemmation, and sporulation, the inference being that these are different modes of reproduction. In reality, however, they are different types of reproduction by division, and such modifications would be expressed better by the terms equal division, unequal division, and multiple division.

I. EQUAL DIVISION AND EVIDENCE OF REORGANIZATION.

In the ordinary metabolic processes of an active protozoön there is evidence of a cumulative differentiation which indicates a difference in organization between a young cell immediately after division by which it is formed and the same cell when it is mature and ready itself to divide. Child (1916) mainly from experiments with cells of the Metazoa, came to the conclusion that "senescence consists in a decrease in metabolic-rate determined by the change in, and the progressive accumulation of, the relatively stabile components of the protoplasmic substratum during growth, development and differentiation" (*loc.cit.* p. 333). He further suggested that in every cell division in unicellular animals, with the accompanying processes of reorganization, there is some degree of rejuvenescence and, if such rejuvenescence balances the cumulative differentiation, continued life of the organisms by division alone may go on indefinitely. By proper conditions of the environment it is conceivable that such a balance may be established. On such an hypothesis it is possible to account for the continued vitality of animal flagellates in which fertilization processes are unknown, for

the continued life of many of the higher plants, and for the continued life of the tissue cell cultures in the hands of Carrel and others (see Chapter VII).

In many Protozoa there is unmistakable evidence of such reorganization processes which will be described in the following pages; in many there is no visible evidence, but in such cases and in the absence of other possibilities of reorganization, it is permissible to assume that reorganization processes which escape the most vigilant watchfulness of the observer, do actually occur.

A. Division in Mastigophora.—With very few exceptions cell division in flagellates is longitudinal, beginning as a rule at the anterior or flagellar end, the cleavage plane passing down through the middle of the body. As the halves separate the two daughter cells usually come to lie in one plane, so that final division appears to be transverse. In the majority of forms the individuals divide while freely motile, but this is by no means universal, variations in this respect occurring in the same family and even in the same genus.

As there are few details in the structure of a simple flagellate on which to focus attention, descriptions of division processes are practically limited to the history of the nucleus, kinetic elements and the more conspicuous plastids. Here, in the main, are fairly prominent granules of different kinds which divide as granules, and, save for the chromatin elements of the nucleus, without obvious mechanisms.

In the simpler cases there is little evidence that can be interpreted as reorganization at the time of division, and the little we find is limited to the motile organs. In the more complex forms, however, there is marked evidence of deep-seated changes going on in the cell.

The earlier accounts of cell division in the simpler flagellates described an equal division of all parts of the body including longitudinal division of the flagellum, if there were but one, or equal distribution if there were two. One by one such accounts have been checked up by use of modern technical methods until today there is very little substantial evidence of the actual division of a flagellum. The basal body and the blepharoplast usually divide, but the flagellum either passes unchanged to one of the daughter cells as in *Crithidia*, *Trypanosoma*, etc., or is absorbed in the cell. In some doubtful cases it may be thrown off. If the old flagellum is retained in uniflagellate forms the second flagellum develops by outgrowth from the basal body or the blepharoplast. If the old flagellum is absorbed, both halves of the divided kinetic element give rise to flagella by outgrowths (Fig. 49, p. 95). Similarly, if there are two or more flagella, one or more may be retained by each daughter cell while the other, or full number, is regenerated. In some cases, as in *Herpetomonas musca-domesticae*, the regenera-

tion of a second flagellum occurs before division of the cell is evident, a circumstance which evidently led Prowazek (1905) to conclude that this organism is normally bi-flagellated (Fig. 170, p. 368).

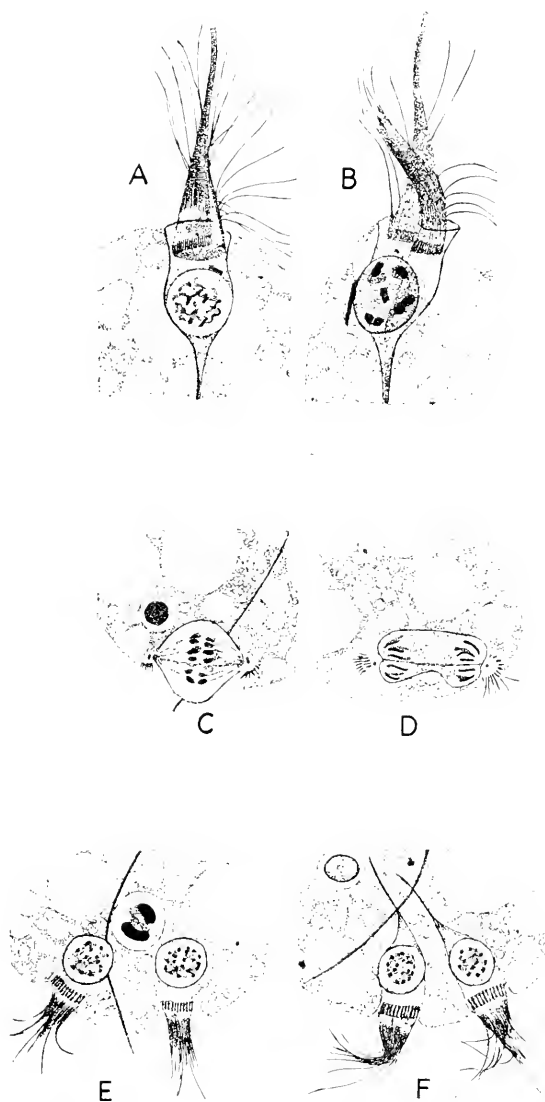


FIG. 105.—*Lophomonas blattarum*. A, flagellar tuft and nucleus in calyx in prophase of division; B, nucleus with chromosomes leaving calyx; paradesmose on side; C–F, stages in nuclear division in the posterior part of the organism and formation of new calyces and flagellar tufts. $\times 1850$. (After Belař, Erg. u. Fortschr. der Zool., courtesy of G. Fischer.)

Reorganization is indicated to some extent by these cases in which the old flagellum is absorbed. It is still better indicated by a number of flagellates in which the cytoplasmic kinetic elements, as well as the flagella, are all absorbed and replaced by new combinations in each of the daughter cells. Thus in *Spongomonas splendida*, according to Hartmann and Chagas (1910) the old blepharoplasts and the two flagella are absorbed and new ones are derived from centrioles of the nuclear division figure (Fig. 49, p. 95). The phenomenon cannot be regarded as typical of the simple flagellates, for in the great majority the kinetic elements are self-perpetuating, even the axostyles according to Kofoid and Swezy (1915) dividing in *Trichomonas* (Fig. 77, p. 145). This, however, has not been supported by later workers.

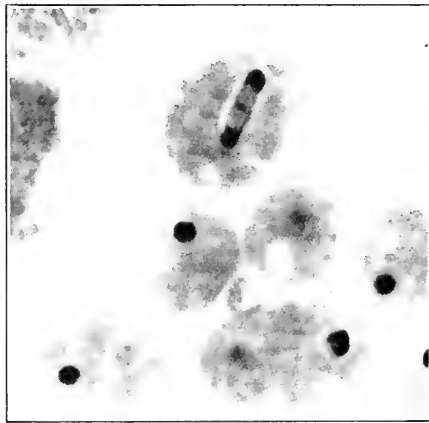


FIG. 106.—*Vahlkampfia limax*. Nucleus in upper cell in full mitosis (promitosis). (From Calkins.)

An extreme case of reorganization is apparent in the two species of *Lophomonas* (*L. blattae* and *L. striata*) first described by Janicki (1915). Here the parental calyx, basal bodies, blepharoplasts and rhizoplasts all degenerate during division (Fig. 105). At division a cytoplasmic centriole first divides with a connecting fibril which is retained throughout as a parademose. The nucleus emerges from the calyx in which it normally lies, and moves with the spindle to the posterior end of the cell. The spindle takes a position at right angles to the long axis of the cell; chromosomes, probably eight in number, are formed and divided, and two daughter nuclei result, each of which is enclosed by a new calyx while new basal bodies and blepharoplasts apparently arise from the polar centrioles (Fig. 105). Thus the old kinetic complex, with the exception of the cytoplasmic centriole, is discarded and entirely new aggregates are formed.

B. Division in the Sarcodina.—It is questionable whether any rhizopod divides in the very simple manner described by F. E. Schultze for *Amoeba polyppodia*. The "limax" types indeed approach this simplicity (Fig. 106) but new discoveries are constantly at hand to indicate that these are not as simple as they have been described. Thus Arndt (1924) quite recently has given creditable evidence of the existence in a simple ameba, *Hartmannella klitzkei*, of a definite centrosome with centriole which is permanently extranuclear (Fig. 58, p. 106). At division of the cell the centrosome divides and the daughter centers with their centrioles, take positions at the poles of the nuclear spindle which originates within the nucleus. The mitotic figure is thus made up of cytoplasmic elements, kinetic elements derived from the nucleus, and chromatin. A similar combination occurs in dividing Heliozoa. The original description of division of *Acanthocystis aculeata* by Schaudinn, a form possessing the characteristic central granule of the Heliozoa, has been considerably modified by later observations. According to Schaudinn the central granule or centroblespharoplast, which is the focal point in the cell of the radiating axial filaments, divides to form an amphia-ster (Fig. 50, p. 95) which becomes the central spindle of a typical mitotic figure. The more recent observations of Stern (1924) indicate that, as in the simpler ameba described above, the central granule of *Acanthocystis* behaves as a cytoplasmic centrosome, forming poles of a mitotic figure which is derived otherwise entirely from the nucleus. Individuals which have been deprived of their skeletons and membranes, which afford resistance to the activities of the enclosed protoplasm, become "sprung," so to speak, and the unusual freedom from restraint results in a separation of the centrosomes from the remainder of the spindle which completes its division without further participation of the centrosomes (Fig. 67, p. 121).

Schaudinn's description of division in Heliozoa was confirmed in the main by Zuelzer (1908) in connection with the aberrant form *Waquerella borealis*. Here the axopodia-bearing portion of the cell is free from the silicious mantle which covers the remainder of the animal, the nucleus being in an enlarged pedal portion attached to the substratum. The central granule is in the geometrical center of the "head" and is the focal point of the axopodial filaments. Each of the latter bears a granular enlargement similar to a basal body. In preparation for division these move centripetally toward the central granule forming a zone about it which divides with the division of the central granule. In the meantime the nucleus migrates from the other end of the body and with the spindle formed by the divided central granule forms the mitotic figure.

Complications in the division process accompany the presence of shells and tests. Where these are chitinous or pseudochitinous, they may also divide with the cell body (*Pseudodifflugia*, *Cochlio-*

podium). In other cases the individual divides within the shell, after which one of the daughter individuals moves out and forms a new shell, while the other one remains in the original test (*Microgromia socialis*, *Clathrulina elegans*, etc., Fig. 107). In most cases, however, a novel method of shell duplication found in no other division of the Protozoa, has been developed. This process, known as "budding division," occurs throughout the group of the testate

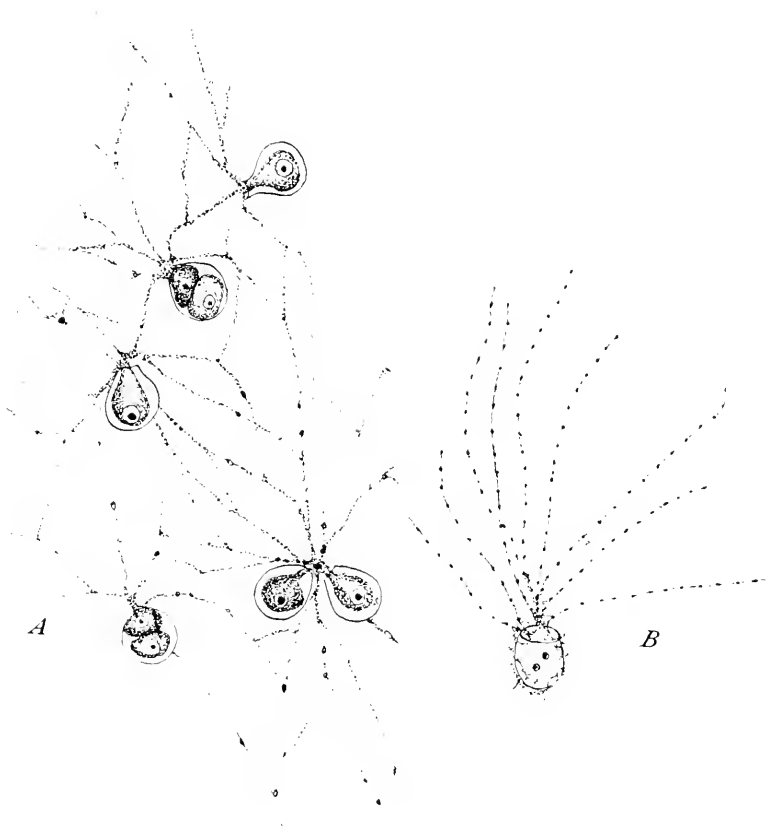


FIG. 107.—*Microgromia socialis* after Hertwig (A), and *Microgromia* sp. (B), original.

rhizopods and is well illustrated by the classical example of *Euglypha alveolata* first described by Schewiakoff (1858). Here after full growth following vegetative activity of the individual, the pseudopodia are drawn in; water is then absorbed whereby the protoplasmic density is greatly reduced and the volume increased. This is followed by a process resembling pseudopodia formation, the protoplasm emerging from the parent shell opening as a ball or dome which

assumes the general form of the parent organism. A new membrane of pseudochitin is formed about the extruded mass and on it the silicious shell plates, preformed in the parent protoplasm, are now cemented. In some forms, *e. g.*, *Arcella* species, the chitinoid membrane becomes the permanent shell of the organism, older shells becoming brown or reddish by coloring due to oxides of iron; in other forms as in the Diffugiinae the chitinoid membrane is covered by foreign objects picked up and stored by the parent organism. In all cases of budding division after the budded individual is fully molded, the nucleus divides and one-half passes into the protoplasm of the new shell. The connecting zone of protoplasm between the old and the new shell breaks out into pseudopodia and the two individuals separate (Fig. 11, p. 33).

The various types of foraminiferal shells, nodosarine, frondicularine and rotaline—may be interpreted as due to a similar budding division, but without actual separation of the parent and bud protoplasm, the type being dependent upon the density of the protoplasm at the time of protrusion from the shell mouth (Fig. 19, p. 38).

There is very little evidence of reorganization of the protoplasm at division in these rhizopods. The frequent withdrawal of pseudopodia and rounding of the body may be an indication of changes going on within, as in *Chlamydomyxa*, *Nuclearia*, etc., but even such questionable indications are absent in many cases of recent investigation (Bělař, Stern, *et al.*), where reorganization, if it occurs at all, must be in the make-up of the protoplasmic and undifferentiated elements.

C. Division in Infusoria.—Here in the most highly differentiated forms of the Protozoa the processes of equal division are complex and the protoplasmic changes far-reaching. With but few exceptions the division plane is through the center of the body and in a plane at right angles to the long axis of the cell. The externals of division are similar to division in other groups, with preliminary division of the plastids and nuclei and final division of the cell body. As in flagellates and some rhizopods the cup- or test-dwelling forms divide within the parent cup, one of the daughter individuals migrating and forming a cup for itself. In some forms the daughter individuals may remain and share the old house (*Cothurnia ingenta*).

Where a tightly-fitting cell-covering is present as in *Coleps hirtus*, it is divided transversely and the missing parts are regenerated by the daughter organisms (Fig. 73, A, B, C, p. 136). In some Infusoria as in the other groups, division in many cases is incomplete, the daughter individuals remaining attached end to end as in *Polyspira delagei* or *Haptophrya gigantea*. Or daughter individuals may remain attached by incomplete division of their stalks, thus giving rise to arboroid colonies of different types (Vorticellidae mainly).

In some forms, probably in the majority of ciliates, there appears

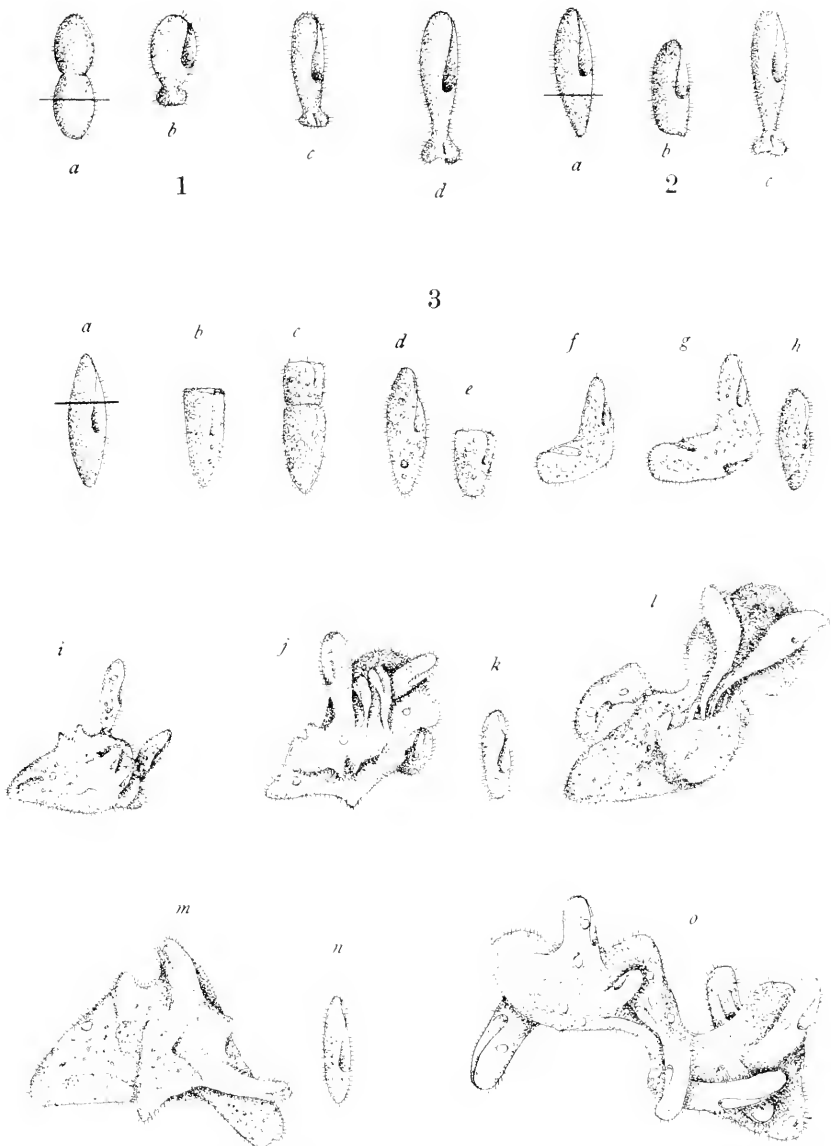


FIG. 108.—*Paramecium caudatum*, merotomy. 1, 2, and 3, different experiments, the straight line indicating the plane of cutting; 3, the history of a monster; an original cell, 3a, was cut as indicated; the posterior fragment (b) divided (c) into (d) and (e), the latter formed a monster (3, f-o); enucleated individuals (b, k, and n) occasionally separated from the parent mass. (After Calkins.)

to be a definite and permanent division zone which indicates the future plane of division and which is not displaced even after diverse mutilations of the body. Thus if *Paramecium caudatum* is cut across either the anterior or the posterior end, the cell ordinarily does not regenerate more than a ciliated surface on the truncated end. It divides like a normal form, but the division plane is not in the geometrical center of the mutilated cell, but in the geometrical center of the cell as it was before the cutting (Fig. 108). The same is true of *Uronychia transfuga* or *U. setigera* (Fig. 113). In daughter cells of dividing *Paramecium* the future division zones appear to be formed at an early period, and if a daughter cell is cut in such a manner that the geometrical center is destroyed without, however, destroying the nuclei, monsters of various types are produced indicating a complete upset of the organization (Fig. 108, *f-o*). In some cases, *e. g.*, *Frontonia leucas*, the geometrical center, or division zone, has a different physical appearance from the remainder of the cell (Popoff, 1908, also mentioned by Hance, 1917, as occurring in *Paramecium*), but in the majority of cases there is no morphological evidence of the plane of division during inter-divisional stages.

(a) **Evidence of Nuclear Reorganization.**—The two types of nuclei, macronucleus and micronucleus, complicate the nuclear phenomena at division. The macronucleus is more like a huge plastid of the cell with active functions in metabolism, while the micronucleus is generally interpreted as a germinal or racial nucleus, functioning at division and particularly at conjugation.

Reproduction of the macronucleus in the majority of ciliates is analogous to that of a plastid. Division is direct with only a few isolated cases showing evidences of spindle formation or of indefinite chromosomes. In preparation for division, however, there is evidence in many forms of profound changes in the make-up of the nucleus destined to divide and some of these afford evidence of a clear-cut reorganization of this important element of the ciliate (see p. 93).

In the less complicated types division of the macronucleus is relatively simple. In *Dileptus gigas*, for example, the nuclear material is in the form of many scattered chromatin and plastin spheres, each of which divides prior to cell division (Fig. 46, p. 92). There is no equal distribution of this chromatin to the daughter cells but the daughter halves may go together to the daughter cell in whose protoplasm they happen to lie. Some of the granules, however, those in the region of the division zone, may be represented in each of the progeny.

In forms with a single ellipsoidal macronucleus as in many of the commoner types (*e. g.*, *Paramecium*, *Colpoda*, *Frontonia*, *Glancoma*, etc.), the macronucleus simply elongates and constricts to form

two equal portions, one passing to each daughter cell (Fig. 35, p. 67). Band-form nuclei characteristic of *Blepharisma*, *Spathidium*, *Didinium*, *Vorticella*, *Euploes*, etc., condense into spheroidal or ellipsoidal bodies before dividing. Where two macronuclei are present in the usual vegetative cell, as in *Oxytricha*, *Stylonychia*, *Gastrostyla*, etc., each divides independently of the other but synchronously. As with band-form nuclei the beaded macronuclei likewise form short rods as in *Stentor*, *Spirostomum ambiguum*, etc., the beaded character in all cases being lost. Here the separate beads are usually enclosed in a common nuclear membrane which is constricted at intervals, the contained chromatin massing together at the period of division. This is the condition in *Uronychia transfuga*, also, the twelve to fourteen apparently separate macronuclei are all connected, and the chromatin fuses prior to division to form a relatively short ellipsoidal nucleus (Fig. 113).

In other types, however, the multiple macronuclei are independent and entirely disconnected. They arise by division and retain their independence during vegetative life. Thus in *Uroleptus mobilis* and *U. halseyi* the eight or more macronuclei are formed as a result of a fourth division of the single parental nucleus from which they came (cf. p. 93 and Fig. 110). In preparing for division of the cell each of these eight nuclei of *Uroleptus* undergoes a remarkable transformation. A nuclear cleft (Kernspalt) appears in each, and in the cleft is a single large granule. The major part of the nucleus lies below the cleft and is filled with densely-staining chromatin; the other part lying above the cleft contains much less chromatin in the form of fine granules (Fig. 47). This latter part, together with the granules in the cleft, is thrown off and the chromatin contents are distributed in the cytoplasm. When each of the nuclei is thus freed from its distal portion the eight remaining parts fuse, forming first a long banded nucleus, and later, by condensation, a relatively small ellipsoidal and single nucleus. This divides twice or three times before the division of the cell is completed, the fourth division always occurring after the daughter cells have separated (Fig. 110).

The micronuclei show no such complicated histories. If they are multiple in the cell there is no fusion, nor is there any elimination of micronuclear material. Each divides with the formation of an unmistakable, but very minute, mitotic figure (Fig. 23, p. 50). They are all represented furthermore by daughter halves in each of the daughter cells.

(b) **Evidence of Cytoplasmic Reorganization.**—Not only is there evidence of change in the cytoplasmic makeup at division through the distribution and absorption of nuclear material as in *Uroleptus mobilis*, but the entire cytoplasm shows other evidence at this period. In all ciliates there is a more or less clearly marked antero-

posterior differentiation, the anterior part usually bearing the mouth and the more or less specialized motile organs for the capture of food



FIG. 109.—*Uroleptus mobilis*. Stages in the fusion of the macronuclei prior to cell division; micronuclei in mitosis. (After Calkins.)

or the directing of food currents, while the posterior part is usually much less specialized. Should such a specialized ciliate be cut through the center as Balbiani (1888) did for the first time, the two

fragments would be different. The anterior fragment of a *Stylo-nychia* or *Uronychia*, for example, would retain the highly differentiated parts about the mouth while the posterior part would be

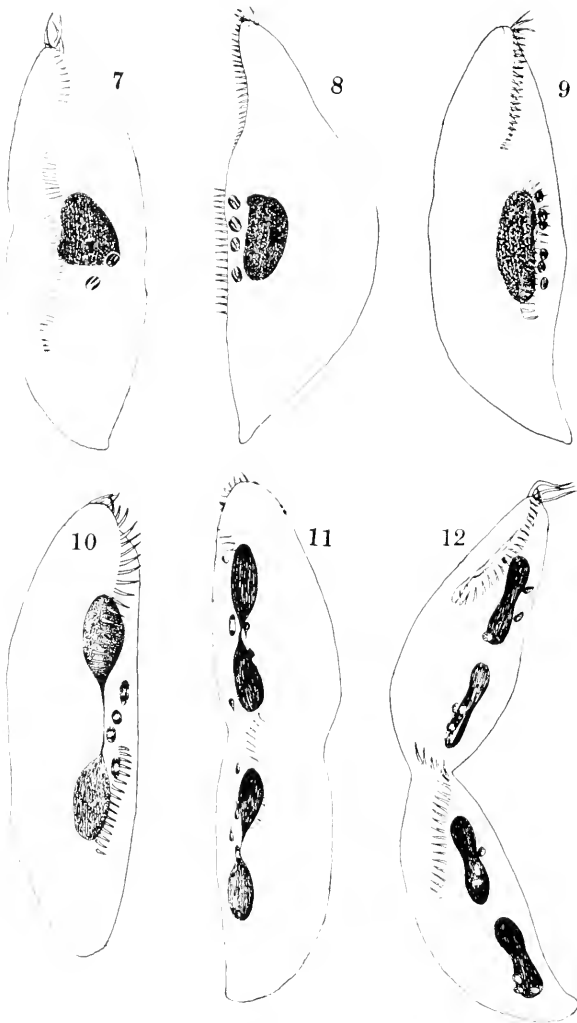


FIG. 110.—*Uroleptus mobilis*. Division stages after fusion of the macronuclei. (After Calkins.)

relatively undifferentiated. The finer organization or genotype, however, is represented by all of the protoplasm of the cell, and that organization has the ability under proper stimulation, of form-

ing all of the differentiated parts of the entire adult organism. By regeneration, therefore, such a cut individual replaces the characteristic structures of the posterior end by the anterior fragment and the characteristic structures of the anterior end by the posterior fragment (Fig. 113). By their usual method of transverse division the ciliates have quite a different inheritance than do flagellates which divide longitudinally. In the latter the highly differentiated anterior ends and the less differentiated posterior ends are equally divided so that the daughter cells have a like inheritance (p. 95).

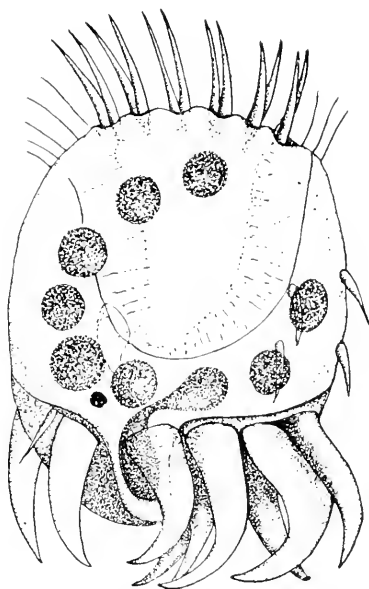


FIG. 111. —*Uronychia transfuga* with giant cirri, membranelles used in swimming, ten macronuclear segments, and single micronucleus. (After Calkins.)

The processes through which the ciliate cell passes during division indicate that the organism is restored to a generalized condition practically equivalent to an encysted cell. Except for the cytostome the entire array of complex cortical organs is withdrawn and a new set is formed from the cortical protoplasm. This significant process first described by Wallengren (1900), later by Griffin (1910) in hypotrichous ciliates, has been observed in many forms and is probably characteristic of the entire group. It is most clearly established in the Hypotrichida where the highly specialized and conspicuous motile organs furnish suitable material for study. According to Wallengren's description the membranelles of the adoral zone slowly decrease in length as the process of absorption

continues and at the same time minute buds of protoplasm appear at the bases of these disappearing membranelles. These buds grow *pari passu* with the dwindling motile organs until finally the latter are entirely absorbed and the buds have developed into functional membranelles. In the same way each cirrus is replaced by a new growing bud quite regardless of the position in anterior or posterior half. Undulating membranes are similarly withdrawn and replaced by new ones so that the young cells formed by division of the metamorphosing parent cell receive a full set of new motile organs commensurate with the size of the young organisms. The phenomenon

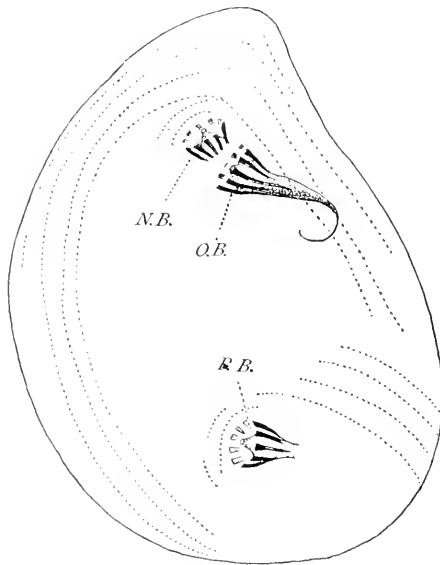


FIG. 112.—*Chilodon uncinatus*. New mouth and basket replacing the old ones prior to cell division. (N.B.) New mouth and basket; (O.B.) old mouth and basket before degeneration and disappearance; (P.B.) new mouth and basket for the posterior individual after division. (After MacDougall.)

is very striking in forms with giant cirri such as the jumping types of Euplotidac—*Diophrys* or *Uronychia*. In the latter genus the great posterior cirri are the most conspicuous organs of the cell (Fig. 111). The buds which are to grow and replace them are apparent before there is other external evidence of the approaching division and even before the nucleus has concentrated into its division form. At the same time similar buds appear in the division zone, that which is destined to form the giant-hooked cirrus appears first and is always larger than the others which appear one after the other according to ultimate size. Owing to their minute size it has not been determined whether or not the individual cilium is

withdrawn in like manner and replaced by new ones. In some, at least, according to the observation of MacDougall on *Chilodon uncinatus* (1925) such substitution does take place and it is quite probable that it is universal. The interesting experiments of Dembowska (1925) show that removal of a single cirrus of *Stylo-*

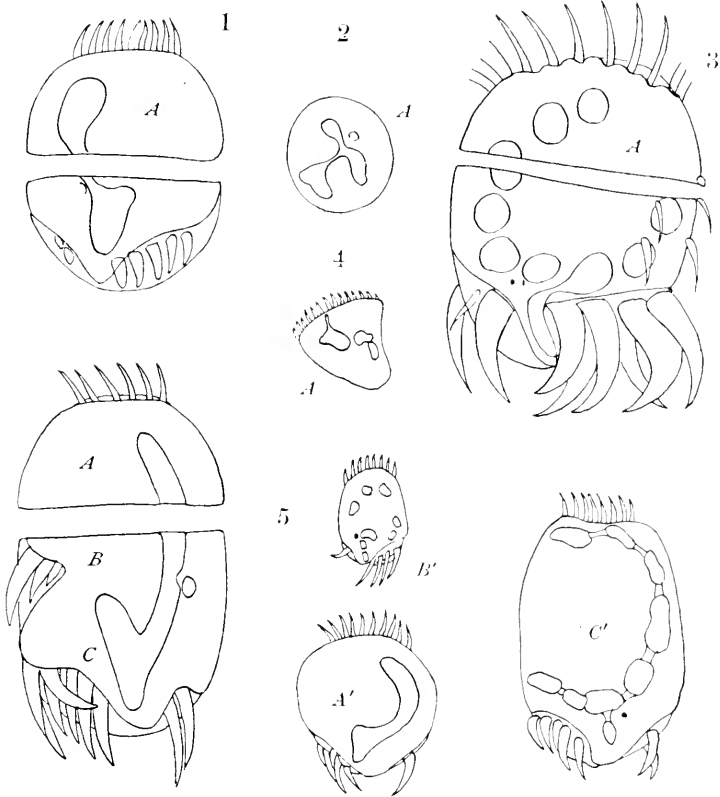


FIG. 113.—*Uronychia transfuga*, merotomy and regeneration. 1, cell immediately after division, cut as indicated; 2, fragment A of 1, three days after the operation; no regeneration; 3, cell cut five hours after division; 4, fragment A of 3, three days after operation, no regeneration; 5, cell cut at beginning of division as indicated into fragments A, B, and C; A', B', C', fragments A, B and C, twenty-four hours after the operation: fragment A regenerated into a normal but amiconucleate individual (A'); B, C divided in the original division plane forming a normal individual (C') and a minute but normal individual (B'). (After Calkins.)

nychia mytilus causes regeneration of the entire motile apparatus, but no such result follows extirpation of any body region that is free from cirri or cilia.

The phenomenon is obviously analogous to the absorption and renewal of flagella in the flagellates. Whether or not there is a

similar division of the basal bodies of the cilia and granules of the silver line system has not been fully established.

Other evidence of protoplasmic reorganization at division is furnished by the history of some of the functional metaplastids of the cell. Trichocysts are apparently handed down without change

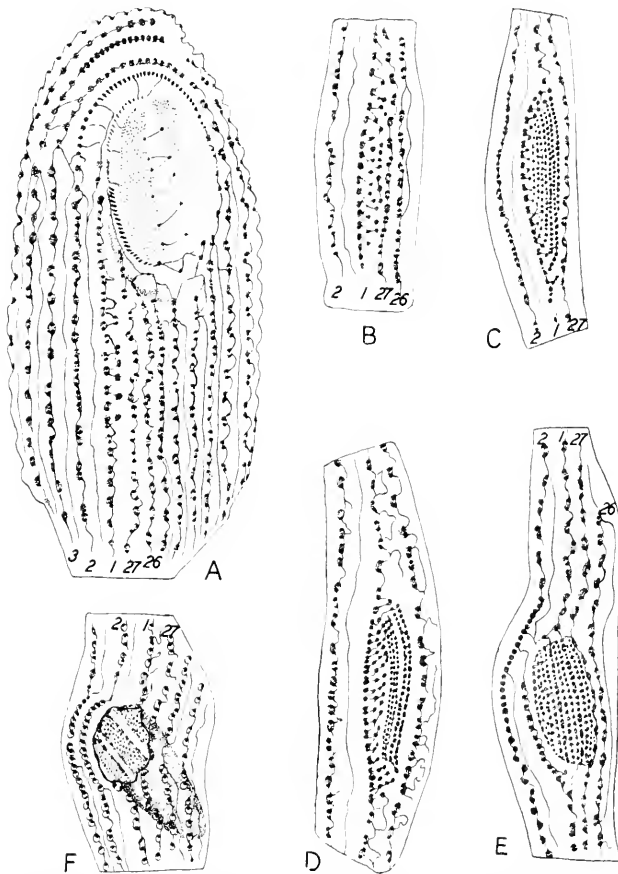


FIG. 114.—*Glaucoma scintillans*. A, individual at beginning of division with silver line system. The beginnings of the mouth of the posterior daughter cell are seen on striation No. 1. B-F, successive stages in formation of the posterior mouth. (After Chatton, A. and M. Lwoff and Monod, *Compt. rend. Soc. biol.*, 1931, courtesy of Masson et Cie.)

(Fig. 35, p. 67), but there is good evidence that the more complicated aggregates of trichites are absorbed and replaced by new ones. This is the case for example in the Chlamydomontidae, where the complex oral baskets are replaced by new ones at each division (Enriques, Nägler, MacDougall, *et al.*, Fig. 112).

From this brief survey it is quite evident that far-reaching changes of the protoplasmic organization take place at periods of division. Both nuclei and cytoplasm are necessary but the micronucleus apparently may be lost without destroying the power of the cell to divide. Amicronucleate races of ciliates, arising possibly through defective reorganization and division after conjugation (see Moore, 1924), have been maintained in culture for many generations by division, although they are ultimately lost (see Chapter VII). On the other hand, the power to regenerate is connected in some manner with the micronucleus. Thus young cells of *Uronychia transfuga*, when transected with a scalpel, will regenerate only that fragment which contains the micronucleus (Calkins, 1911, Fig. 113; Young, 1923). In old cells, however, both fragments regenerate regardless of the presence or absence of a micronucleus, a fact indicating a change in organization with advancing age (Fig. 113, 5).

The fate of the motorium and of the coördinating fibrils both endoplasmic and those of the silver line system, at division is still unknown. It is a significant fact that the peristome and the peristomial organs appear first in the more specialized anterior half of the ciliate cell, and from this position gradually shift to the region immediately posterior to the division zone (Figs. 109, 110). The relation of the posterior mouth to the silver line system in a dividing form of *Glaucoma scintillans* is clearly shown by Chatton, Lwoff (A. and M.) and Monod (1931). The complicated oral membranes of this organism are formed as a result of division of the blepharoplasts at a localized region of certain lines of the silver line system (Fig. 114). In *Vorticella* according to Bütschli (1888) after Fabre, the peristome and adoral zones are reversed in the daughter cells.

II. UNEQUAL DIVISION (BUDDING OR GEMMATION).

In reproduction by budding or gemmation, one or more minute fragments of the cell are produced by unequal division of the organism. Parent and offspring are thus distinguished, their relative sizes varying in different cases. In many instances both parent and offspring continue to live after such reproduction. In many other instances the residual parental protoplasm is no longer able to carry on metabolic activities and dies. Illustrations of both types abound in all groups of the Protozoa, the buds being formed either on the periphery of the parent in so-called exogenous budding, or within the protoplasm of the parent in so-called endogenous budding. The minute cells that are formed by budding always contain a portion, sometimes one-half, of the nuclear structures of the parent and may develop asexually into organisms similar to the parent, or they may be differentiated as gametes requiring fertilization before development.

A. Exogenous Budding.—In *Acanthocystis aculeata* according to Schaudinn (1896) and in *Wagnerella borealis* according to Zuelzer (1909) the nucleus of the cell divides one or more times by simple constriction and without the formality of mitosis or participation of central granule. The minute nuclei thus formed wander to the periphery of the cell where they are pinched off in minute cells. In *Acanthocystis* these buds form minute amebae which after four

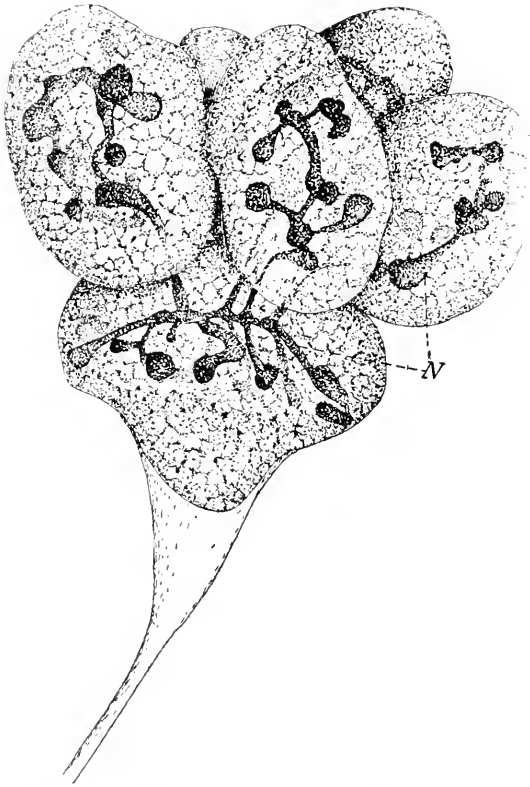


FIG. 115.—*Ephelota bütschliana*, a suctorian. Budding individual with five exogenous buds. N, branching macronucleus. (After Calkins.)

or five days of activity settle down and metamorphose into young Heliozoa (Schaudinn). The buds have no central granule, but during metamorphosis a kinetic element emerges from the nucleus and this becomes the central granule of the adult *Acanthocystis* (Fig. 50, p. 95). In *Wagnerella borealis*, according to Zuelzer, the buds which are formed in a similar manner are flagellated, but her description in other respects follows that of Schaudinn.

In Infusoria, particularly in Suctoria, exogenous budding is not

uncommon. In Ciliata it is comparatively rare and limited apparently to the Conotrichida and some parasitic forms. In *Spirochona gemmipara* according to Hertwig a swelling appears at one side of the base of the peculiar funnel-like peristome. The nucleus divides equally, one-half passing into the swelling which, with only partial peristomial development, breaks away from the parent and then completes its peristomial differentiations.

In Suctorina similar exogenous buds, either single or multiple, are formed from the oral extremity of the cell (Fig. 115). Such buds are dissimilar to the parent which they come to resemble only after a period of metamorphosis and development.

In Sporozoa, with the exception of some Cnidosporidia, exogenous budding is limited to unequal division in gamete-forming processes. Thus, in Gregarinida and in microgametocytes of Coccidiomorpha the nucleus of the cell undergoes several divisions, the final products arranging themselves about the periphery from which they become nuclei of variously formed gametes budded out from the surface (Fig. 173, p. 493). In all such cases the parent protoplasm dies after giving rise to the buds. In some Cnidosporidia, on the other hand, budding processes appear to be normal activities carried on during the vegetative life of the organisms. According to Cohn (1895) large numbers of buds, each containing several nuclei, may be formed from the periphery of *Myxidium lieberkühni*. The phenomenon appears to be an exaggeration of the peculiar process of division termed plasmotomy by Doflein, whereby a multinucleated cell divides spontaneously into two more or less equal parts as in *Chloromyxum leydigi* according to Lühe and Doflein, or into several parts, as in the Coccidian *Caryotropha mesnili* and *Klossiella muris* and termed "schizontocytes," or "cytomeres" by Siedlecki (1902).

Terminal exogenous budding is characteristic of some parasitic ciliates and a chain of posterior reproductive bodies is formed as in *Radiophrya limnodrilli* (Fig. 116).



FIG. 116. — *Radiophrya limnodrilli*, astomatous ciliate with terminal budding. (After Cheissin, Archiv f. Protistenkunde, courtesy of G. Fischer.)

B. Endogenous Budding.—This type of unequal division is not so widely distributed amongst Protozoa as is exogenous budding and is apparently not represented at all in flagellated forms. It does occur, however, in all of the other groups.

In Sarcodina endogenous budding has been described mainly in connection with the testate rhizopods. In *Centropyxis aculeata* according to Schaudinn (1903) it leads to gamete formation, but in *Areolla vulgaris*, according to Szwarczewski (1908) and Elpatiewsky (1909) it is a form of asexual reproduction.

In Infusoria internal budding is characteristic of many types of Suctoria, but is apparently not represented in the Ciliata. In the simplest cases the budding area at the anterior end becomes internal by insinking of the anterior surface and constriction of the body walls on all sides, so that the reproducing area is enclosed by living

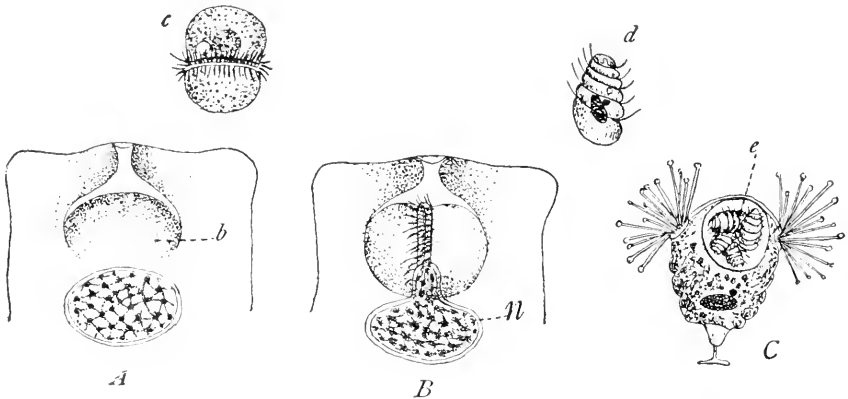


FIG. 117.—Endogenous budding in Suctoria. A, B, two stages in the formation of a bud (b) and (c), of *Tokophrya quadripartita*; C, *Acinetia tuberosa* with endogenous buds (c) and (d). (From Calkins after Bütschli.)

protoplasm which thus becomes a potential brood chamber within which the buds develop. Such buds may be single, as in *Tokophrya quadripartita* (Fig. 117, A, B), or multiple as in *Metacinetia* (Fig. 117, C), and are always provided with cilia either as girdles or otherwise. Through the activity of these cilia the buds swim freely about in the brood chamber until they finally emerge through a "birth-pore" and after a variable period as free swimmers or as parasites in other Infusoria, they develop into adult forms of Suctoria. Cilia in Suctoria are thus confined to the embryonic stages and their various arrangements on the buds of different species recall the types of ciliation in the other branch of the Infusoria.

A biologically interesting phenomenon of internal budding is described by Collin (1911) in the case of *Tokophrya cyclopuum*. Here a brood pouch is formed by the cortical protoplasm within which

the rest of the protoplasm becomes metamorphosed into a single bud with cilia. When mature this bud leaves the parent membrane on its old stalk and swims off as an embryo (Fig. 118).

In Sporozoa endogenous budding is manifested in a number of different ways. In some it is apparently a method of multiplicative reproduction, in others it is associated with gamete formation or with sporulation. Asexual reproduction by internal budding is illustrated by some of the Schizogregarinida where a typical brood pouch is formed through which the internal buds escape through a birth opening as in Suctorina. The *Eleutheroschizon dubosqui*, according to Brasil (1906), the nucleus divides repeatedly until many are formed (Fig. 119, A-D). Each is then surrounded by a small portion of the parent protoplasm cut off from the rest of the cell.

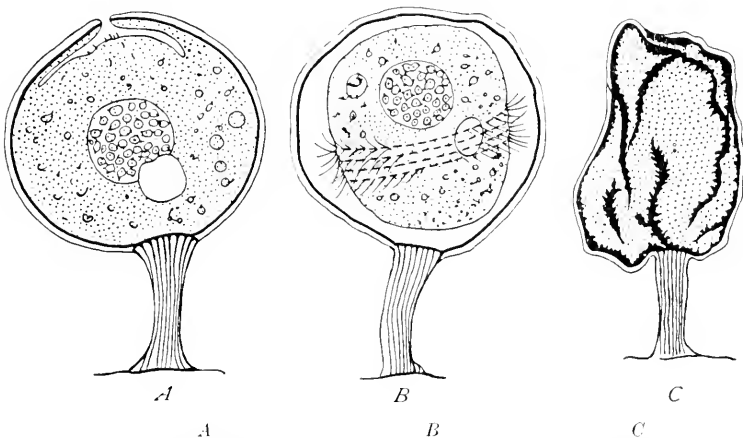


FIG. 118.—*Tokophrya cyclopum*, the entire cell, except the membrane, is used in the formation of a single bud which develops cilia (B) and swims off, leaving the old membrane to shrivel up on its stalk (C). (After Collin.)

The central portion becomes vacuolated and opens to the outside, the agamonts making their way through the opening, leaving the remnants of the parental protoplasm to degenerate. Similarly in *Schizocystis sipunculii*, Dogiel (1907) described the formation of a brood pouch becoming filled with agamonts derived by internal budding from the parent protoplasm (Fig. 119, E-G). Gametes formed by internal budding are described by Léger (1907) in connection with the life history of *Ophryocystis mcsuili*. Here after two "maturation" divisions of the nucleus in each of the gamonts united in pseudoconjugation, a single free cell is formed in each gamont by internal budding (Fig. 120). Each bud here is a gamete and the zygote is formed by union of the two in the parental brood chamber.

The phenomena of internal budding in the ameiboid Myxosporidia of the Cnidosporidia, are still different in character and fate of the buds. Here in the endoplasm local islands of protoplasm are quite

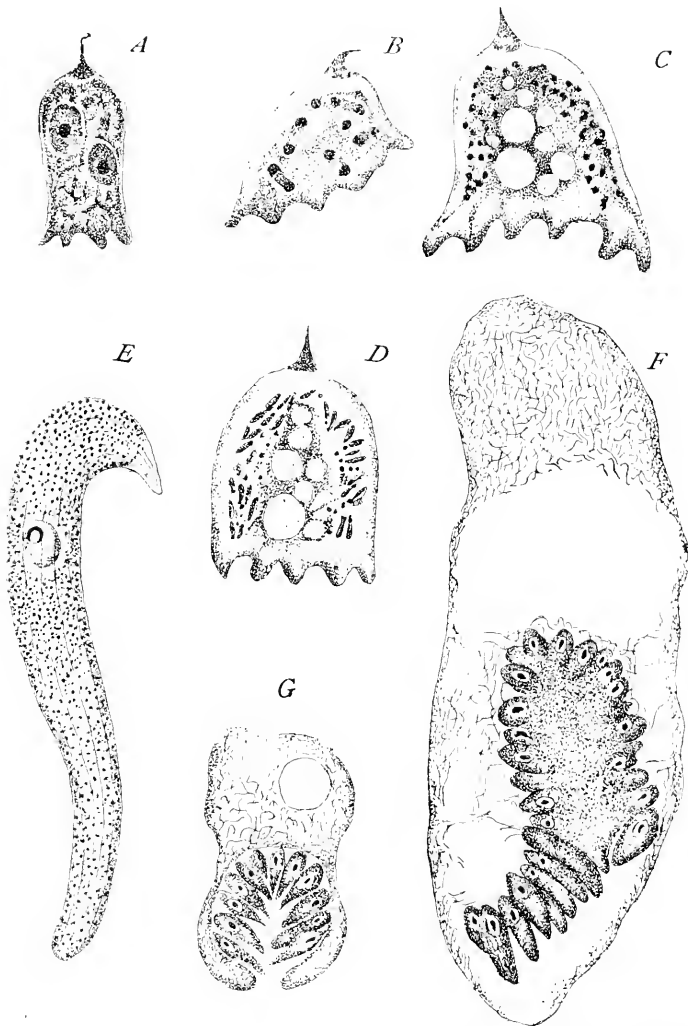


FIG. 119.—Endogenous budding in Gregarinida. A to D, *Elutheroschizon dubosqui* and formation of endogenous agametes. (After Brasil.) E to G, *Schizocystis sipunculi* and similar formation of agametes. (After Dogiel.)

separated from the surrounding protoplasm of the parent. Such islands, called pansporoblasts by Gurley (1893) or internal "cells" by Davis (1916), are specialized reproductive centers in each of

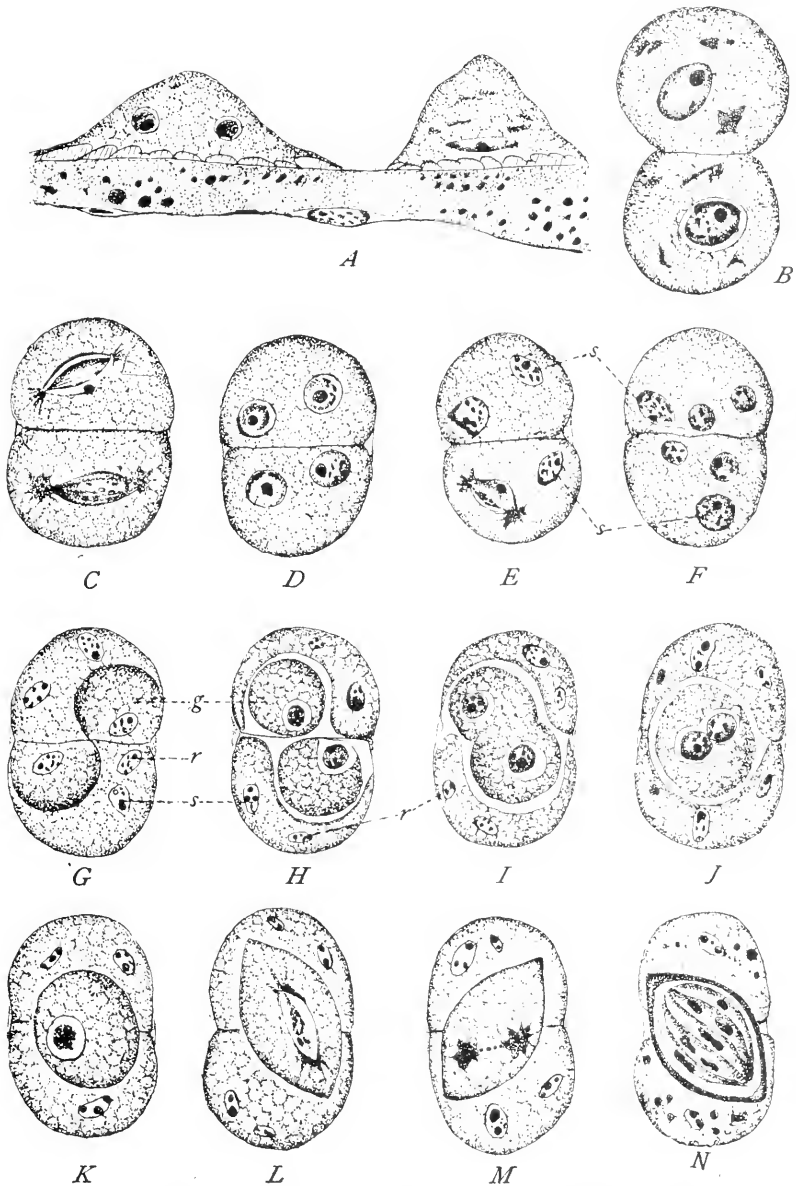


FIG. 120.—Gamete formation and fertilization in *Ophryocystis mesnili*. A, two individuals attached by processes to ciliated cells of a Malpighian tubule of *Tenebrio molitor*; B, union of gamonts in pseudoconjugation; C, D, E, probable meiotic divisions of nuclei of the two gamonts; G to K, formation of two gametes and their union in fertilization; L to N, metagametic divisions resulting in eight sporozoites in the single sporoblast. (After Léger.)

which one or more sporoblasts are formed (see p. 545). In the same living parent organism internal buds in various stages of maturity may be present and in some cases the ameoboid parent organism may

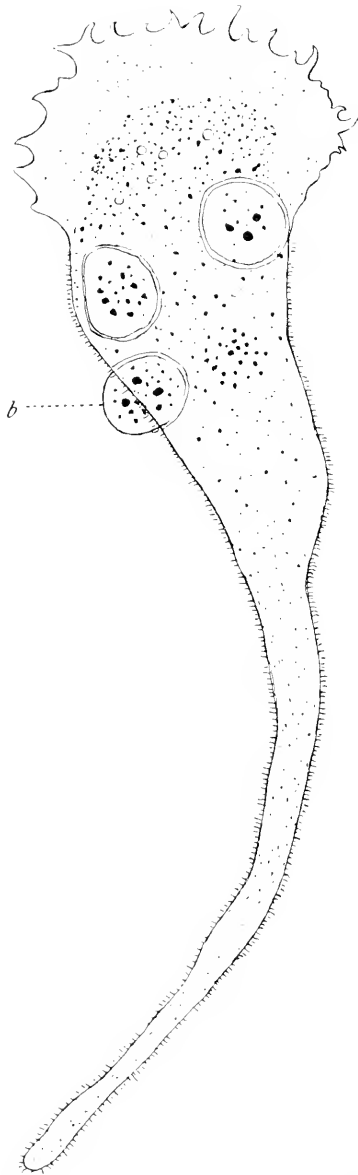


FIG. 121.—Internal buds or "gemmules," *b*, of *Sphaerospora dimorpha*, a myxosporidian. (After Davis.)

ultimately become a mere cyst wall containing large numbers of encysted young. A quite different type of internal bud called a "gemmule" is formed in *Sphaerospora dimorpha* according to Davis (1916). These correspond to the agamont buds of the gregarines (Fig. 121).

III. MULTIPLE DIVISION (SPORE FORMATION).

In reproduction by multiple division the entire protoplasm breaks up simultaneously into a brood of minute young, a mere fragment with perhaps a residual nucleus, may be left unused. Although the end-product may be the same there is a difference in principle between rapidly following divisions of cells within a cyst (as in *Colpoda cucullus*) and the fragmentation of a cell into many minute cells. There is less difference between sporulation and multiple endogenous budding as in *Schizocystis* or *Eleutheroschizon* described above.

Multiple division in many cases results in the formation of a brood of smaller cells which develop directly into organisms similar to the parent. In other cases the representatives of the brood are differentiated as gametes, and fertilization is necessary before development begins. We thus distinguish between sexual and asexual generations of spores, a distinction mainly characteristic of parasitic forms, but typical of many free-living types as well. In still other cases multiple division may follow immediately after fertilization, a phenomenon which is highly developed in the Sporozoa where the ultimate products of division—sporozoites—have a renewed potential of vitality.

Multiple division or spore formation thus may occur either in the agamont (asexual) phase, or in the gamont and zygote phases (sexual) of the life cycle. Division, budding or sporulation in the asexual phase is called agamogony (=schizogony); in the sexual phase gamogony (=sporogony). In the great majority of Protozoa the two phases together in an alternation of generations, make up a complete life history.

In Mastigophora sexual processes have in no case been safely established, multiple division when it occurs being agamogony. In animal flagellates, however, particularly the parasitic forms, a highly characteristic method of multiple division is widely distributed. Here in certain phases or under conditions not yet well understood, trypanosomes, trichomonads, lophomonads and other parasitic flagellates undergo a process of asexual sporulation to which the specific term "somatella formation" has been applied. It is well described by Minchin and Thompson (1915) in the case of *Trypanosoma lewisi* (Fig. 122) as follows:

"The parasites when taken up by the flea (*Ceratophyllus fasciatus*)

pass with the ingested food into the stomach (mid-gut) of the insect. In this part they multiply actively in a peculiar manner, not as yet described in the case of any other trypanosome in its invertebrate host; they penetrate into the cells of the epithelium, and in that situation they grow to a very large size, retaining their flagellum

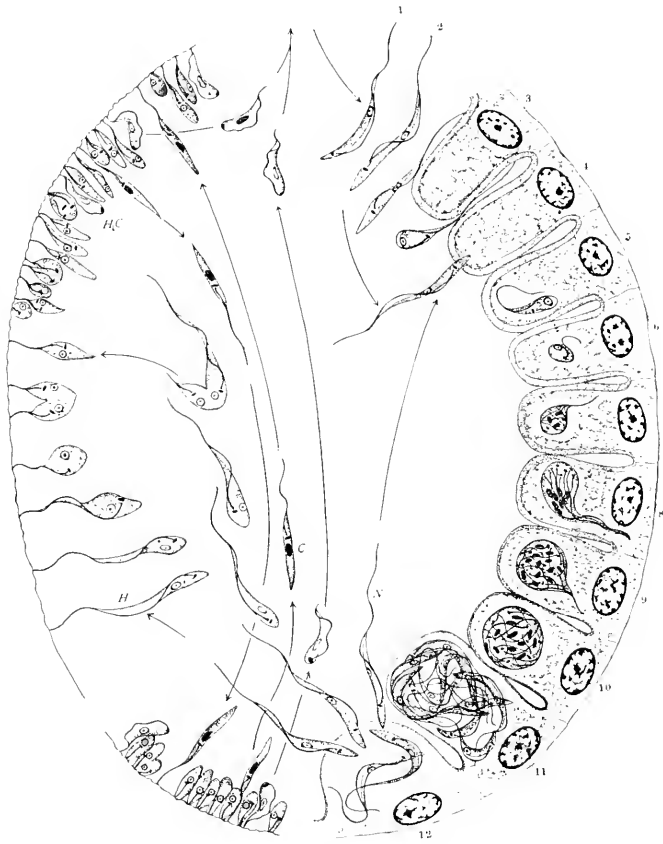


FIG. 122.—*Trypanosoma lewisi*. Cycle in the rat-flea *Ceratophyllus fasciatus*. 1, 2, blood trypanosomes entering the stomach; 3, 4, entering epithelial cells; 6-10, intracellular somatella formation; 11, 12, adult trypanosomes leaving cell; N, young trypanosomes repeating intracellular phase; C, Crithidial forms; H, haptomonads reproducing by division. (After Minchin and Thompson.)

and undulating membrane, and exhibiting active metabolic changes in the form of the body, which in early stages of the growth is doubled on itself in the hinder region, thus becoming pear-shaped or like a tadpole in form, but later is more block-like or rounded. During growth the nuclei multiply, and the body when full-grown approaches a spherical form, and becomes divided up within its

own periplast into a number of daughter individuals, which writhe and twist over each other like a bunch of eels within the thin envelope enclosing them (Fig. 122, 11). When this stage is reached, the flagellum, which hitherto had been performing active movements and causing the organism to rotate irregularly within the cell,

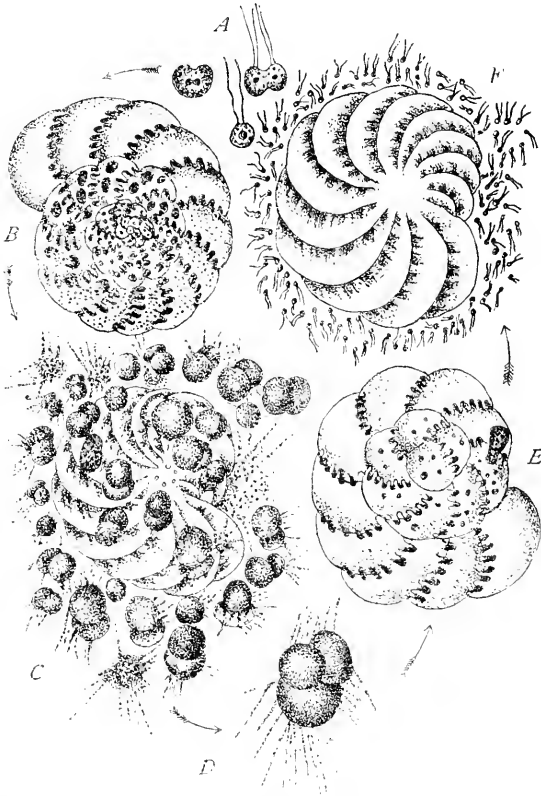


FIG. 123.—*Polystomellina crispera*. A zygote (A) develops into an organism with a microspheric type of shell (B) in which the nucleus divides by mitosis until many nuclei are present which form chromidia. The protoplasm fragments into reproductive bodies or agametes, each having several granules of chromidia (C). Each agamete develops into an adult with a macrospheric type of shell (D, E); when adult these fragment into hundreds of flagellated gametes (F) which fuse in fertilization and so complete the cycle. (From Lang and Schaudinn.)

disappears altogether, and the metabolic movements cease; the body becomes almost perfectly spherical, and consists of the periplast envelope within which a number of daughter trypanosomes are wriggling very actively; the envelope becomes more and more tense, and finally bursts with explosive suddenness, setting free

the flagellates, usually about eight in number, within the host cell (Fig. 122, 12). The products of this method of multiplication are full-sized trypanosomes, complete in their structure, and differing but slightly in their characters from those found in the blood of the rat. They escape from the host-cell into the lumen of the stomach." (*loc. cit.*, p. 299).

Similar multiple division phases have been described for *Trypanosoma cruzi* (Chagas, Hartmann), for *Eutrichomastix serpentis*, and *Tetrahymonas prowazeki* (Kofoid and Swezy), *Lophomonas blattae* (Janicki) and others. In these cases, as in *Trypanosoma lewisi*, the number of individuals formed is usually eight.

In Sarcodina there is a typical alternation of generations combined with multiple division best illustrated in the Foraminifera. According to the independent observations of Schaudinn (1903) and Lister (1905) the zygote develops into an agamont characterized by an initial central chamber of relatively minute size (microspheric shell, Fig. 123, B). When fully grown the chromidia-laden protoplasm breaks up by multiple division into a great number of ameboid agametes (pseudopodiospores) each with a number of chromidial granules which fuse to form a nucleus. Each agamete develops into a gamont or individual of the sexual phase, characterized by a large initial central shell-chamber (macrospheric shell, Fig. 123, D, E). When these gamonts are mature, they also break up by multiple division into myriads of flagellated gametes (flagellispores, F). These are isogametes which fuse two-by-two forming zygotes, and these zygotes repeat the cycle by developing into microspheric individuals (Fig. 123, A). Similarly in *Arcella vulgaris* there is an alternation of generations which is even more complicated than that of the Foraminifera according to the descriptions of Swarczewsky (1908) and Elpatiewsky (1909). A zygote (amebula) develops into a typical adult *Arcella* agamont. This reproduces by agamogony in no less than four ways if these observers are correct.

A first method is by exogenous budding whereby agametes (amebulae) are liberated to develop again into agamonts. Another method is by multiple endogenous budding whereby many agametes are formed each of which develops into an agamont. A third method involves the desertion of the parent shell and of the primary nuclei by the bulk of the protoplasm and secondary nuclei formed by chromidia, and breaking up of this mass into agametes which likewise develop into agamonts. Ultimately these agametes develop into gamonts which become either macrogametocytes or microgametocytes, or gamonts which conjugate as do the ciliates with an interchange of chromidia (chromidiogamy). The macrogametocytes by multiple division give rise to macrogametes, and microgametocytes to microgametes. A macrogamete is fertilized by a microgamete, and the resulting zygote repeats the cycle.

Multiple division is safely established for a number of Radiolaria although it is not yet determined whether the products are agametes or gametes. In many cases the flagellated swimmers which are thus formed by one individual are large, while those formed from another individual are smaller. This has led to the view that the swimmers are anisogametes, but actual fertilization has not been safely established. They are formed from the materials of the central capsular protoplasm which, at first uninucleate, becomes multinucleate by repeated divisions of the nucleus. Comparatively little cytological work has been done on these forms which offer a promising field for further research. According to Brandt (1885) the nuclear material is distributed about the endoplasm in the form of many clumps of chromatin which later become vesicular nuclei and undergo mitotic divisions. Hertwig (1879) describes the nucleus of *Acanthometra* as composed of a large endosome and a massive peripheral zone of chromatin which metamorphoses into a great number of small nuclei. In *Aulacantha scolymantha* according to Borgert (1900) the great primary nucleus gives off minute chromatin vesicles until the entire substance of the original nucleus is thus distributed in the endocapsular plasm and these become minute nuclei which now divide by mitosis. Ultimately the central capsule is dissolved, the pseudium disappears and the protoplasm breaks up into many small spheres each with several nuclei. Differences in these spheres indicate the later differences in the resulting swimmers. A somewhat similar history has been described for the giant nucleus of *Thalassicola*, but despite the observations of Brandt (1885), Hartmann and Hammer (1909), Huth (1913), Moroff (1910) and others, the significance of the peculiar processes is not clear. A rather unusual phenomenon is described by Haecker (1907) in *Orosceua regalis*. Here the huge single nucleus of the central capsule divides into two nuclei of which one remains as a functional nucleus of the organism, the other is interpreted as giving rise to gametocyte nuclei. There is also some evidence, not conclusive indeed, that an alternation of generations occurs, somewhat as in Foraminifera. Some types give rise by multiple division to isospores, e. g., *Aulacantha*, which are biflagellated cells with characteristic crystalloid structures interpreted by Brandt as the product of an asexual generation. Other individuals of the same species give rise to broods of anisospores which are interpreted as microgametes and macrogametes representing the sexual generation.

In Mycetozoa multiple division is characteristic but complicated by the typical plasmodium nature of the organisms. Such plasmodia are formed usually by the plastogamic union of amebae arising from spores, the nuclei remaining separate and thus forming a multinucleated protoplasmic aggregate. Many of these nuclei degenerate (Kränzlin, Jahn); some become active agents in the

formation of specialized structures of the fruiting bodies (elaters, etc., Kränzlin, 1907); others divide by mitosis to form nuclei of the spores contained with the elaters in the spaces of a meshwork formed by a special protective and supporting part of the fruiting bodies called the capillitium (Fig. 184, p. 447, see also p. 446).

Multiple division in the Sporozoa is characteristic of practically all Coccidiomorpha, particularly in agamogony. The nuclei divide repeatedly by mitosis until many are formed, after which the body plasm breaks up into as many agametes as there are nuclei. In many cases a portion of the old cells is left unused or not included in the protoplasm of the offspring. Thus in *Plasmodium vivax* and other malaria organisms, the pigmented granules (melanin) are left behind when the agametes separate (Fig. 124); in many coccidia the agametes are oriented in respect to such residual products. Multiple division is also characteristic of the developing zygotes of gregarines and hemamebidae, the eight sporozoites of gregarines and the multitude of Sporozoites of *Plasmodium* being formed in this manner.

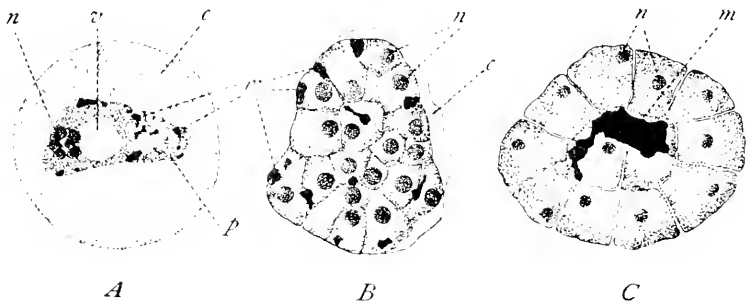


FIG. 124.—Malaria organisms. A, *Plasmodium vivax* in blood corpuscle; B, same in agamete formation with distributed melanin (*m*). C, *Plasmodium malariae*, agamete formation with concentrated melanin. *c*, red blood corpuscle; *m*, melanin; *n*, nuclei; *p*, parasite; *v*, vacuole. (After Calkins.)

In the above account of the reproductive activities of the Protozoa no attempt has been made to give an exhaustive treatment, but other examples will be given in the following chapters on classification.

In many cases in the above description there is evidence of reorganization of the protoplasm and evidence that may be interpreted as supporting Child's view of de-differentiation as an offset to the accumulation of products of metabolism which hamper further metabolic activities. Some of this evidence is given in connection with the phenomena of equal division, particularly in division of the ciliated forms and the conclusions reached are in agreement with Child's. Hartmann, also, comes to a similar

conclusion in connection with merotomy experiments on *Amoeba polyppodia* (1924). In the latter an individual was cut in two fragments; the nucleated part regenerated, but instead of permitting it to divide it was cut again when fully grown. This process was repeated until the original ameba had been cut 32 times in forty-two days and without an intervening division. The control amebae from the same clone divided 15 times in the same period. This experiment would appear to confirm Child's argument that amputation of a part of the differentiated protoplasm would effect a partial rejuvenescence, and Hartmann interprets it in this way: "Reproduction," he says, may rightly be interpreted as a process of rejuvenation. Our continued amputations in these experiments provide a substitute for the rejuvenating effect of reproduction (1924, p. 458). His further conclusion that his results "indicate experimentally, a potential immortality of the protozoan individual" (p. 456) can scarcely be allowed on the basis of forty-two days' experience. A single individual of *Uroleptus mobilis* has lived for more than ninety days without dividing, and similar but younger individuals have been cut as in Hartmann's experiments, to find out if ciliates would sustain Child's conclusion. The results (not published) were invariably negative, although *Uroleptus* is an excellent type for this kind of work and invariably undergoes rejuvenescence after conjugation and after endomixis (see Chapter VIII).

With unequal division by budding and multiple division there is further evidence of reorganization with reproduction. The small cells that are budded off contain none of the differentiated cellular elements of the parent organism. The spores are likewise provided with protoplasm whose activities are unhampered by accumulated products. This is clearly evident in the asexual reproduction of *Plasmodium vivax* (p. 238), and is well illustrated in forms where specialized structural elements are indications of the differentiations which the old protoplasm has undergone. Thus in Mycetozoa some of the hundreds of nuclei degenerate and give rise to spiral elaters which with their spiral walls are made up of microsomes and kinetic elements (Strasburger, Kränzlin), while parts of the protoplasm become differentiated into encrusting peridia and supporting capillitia. All of these differentiations are left behind when the spores are formed and distributed. Analogous somatic structures are also characteristic of the spore-forming stages of some types of Gregarinida and Myxosporidia. In the former the spore-containing organs are either relatively simple spore cysts as in *Monocystis* types (Fig. 213, p. 531) or more complicated structures—sporangia—of some polycystid gregarines (*e. g.*, *Echinomera hispida* or *Gregarina cuneata*). In the former the spores are dispersed by the formation of gas which bursts the cyst membranes. In the latter, finger-formed tubes are developed from the peripheral protoplasm

of the cyst. These are formed from residual "chromidia" which collect in rings about the periphery and from which the finger-formed tubes grow into the mass of developing zygotes (Fig. 125).

When the cysts are mature absorption of water causes the rupture of the cyst walls, the tubes are forced out and evaginated as an intumed glove finger may be blown out. The spores then are distributed through these hollow tubes or sporoducts.

In Myxosporidia still more complicated structures recalling the capillitia of Mycetozoa, are characteristic of the spore-forming stages. In *Sphaeromyxa sabrazesi* according to Schröder (1907) and in *Myxobolus Pfeifferi* according to Keysseletz (1908) the internal

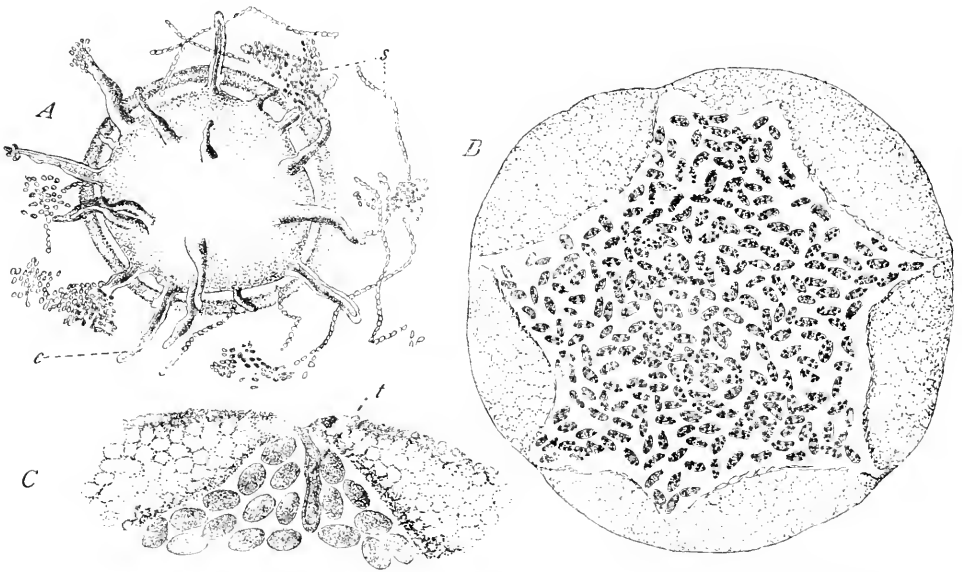


FIG. 125.—*Gregarina cumata*. A, surface view of sporocyst with ripe sporoblasts issuing from sporoducts (c). B, C, sections of sporocyst with ripening spores and developing sporoduct (d). (From Calkins after Kuschakewitsch.)

bud (pansporoblast) which is destined to form the spores, contains two nuclei, one of which is smaller than the other. These nuclei increase by division until there are 14 altogether; 2 of these degenerate without further function, and the remaining 12 are divided into two groups of 6 each, the protoplasm dividing with them to form two protoplasmic multinucleated bodies which will develop into sporoblasts (Fig. 164, p. 325). Of the 6 nuclei in each cell, 2 are "somatic" and take part in the formation of the shell or capsule of the sporoblast; 2 others are also "somatic" and participate in the formation of the polar capsules and threads characteristic of the Cnidosporidia; the remaining 2 nuclei persist as germinal

nuclei which, according to observations of several different authorities, later fuse into one (p. 546).

In all of these cases the specialized structures accompanying spore formation are formed only at one period in the life cycle and a period which comes at the end of long-continued metabolic activity. They represent therefore, a differentiated protoplasm which is not evident in the protoplasmic make up of the progeny. What is true of these visible differentiations is also probably true of analogous differentiations which are not visible, and we have reason to believe that the products of unequal division and of multiple division are not encumbered by protoplasmic conditions which hamper vitality—in other words, that they have undergone reorganization. Such young forms have again the potential of vitality of the genotype and are able to go through the series of differentiations which are characteristic of the life of the genotype.

IV. DEVELOPMENT.

In Metazoa, development starts with the fertilized egg and consists in the progressive formation of organs and organ systems by differentiations, and grouping of differentiated cells. A strict comparison of Protozoa with Metazoa in development would involve the history of a fertilized cell through all phases of asexual reproduction (comparable with somatic cell division) to the gamont stage. Only by a fanciful interpretation, however, can the entire progeny of a single fertilized cell of Protozoa be regarded as an individual similar to a metazoön, although there are similar phases of vitality which may be indicated in common by the terms youth, maturity and age (see Chapter VII). The protozoan "individual," however, is a single cell and as usually seen is in the agamont stage. In the majority of Protozoa little or no development is necessary, the daughter cells being almost perfect individuals when formed and similar enough to the parent to be mistaken for nothing else. Here the only processes that can be regarded as development are those which have to do with the formation of shell structures, as in *Coleps hirtus*, etc., and the new development of anterior parts of posterior daughter cells and posterior parts of anterior cells.

It is quite different, however, with the products of multiple budding or of multiple division. Here the young forms are unlike the parent, and during growth undergo changes which may properly fall under the heading of development. In some cases, for example in Foraminifera, Mycetozoa, and Sporozoa, the small fragments produced by a parent may or may not require fertilization in order to develop. The zygote of *Polystomellina crispa* or of *Trichosphaerium sieboldi*, formed by the fusion of flagellated gametes (flagellispores) develops into the asexual generation by protoplasmic growth and

nuclear division, but without cell division, development of the former being indicated externally by the formation of a many-chambered shell. Similarly in the Mycetozoa the zygote formed by aneuroid or flagellated gametes develops into a plasmodium by cell fusions and nuclear divisions.

In the Sporozoa the zygotes, formed by union of similar gametes (isogametes) or of dissimilar gametes (anisogametes) undergo a variable number of metagamic divisions, three in the majority of Gregarinida and two or more in the Coccidioromorpha. The end-result of such metagamic divisions is the formation of two or more similar sporozoites which are entirely different from the adult individuals and undergo a more or less complex development. When they are introduced into a new host the sporozoites are liberated

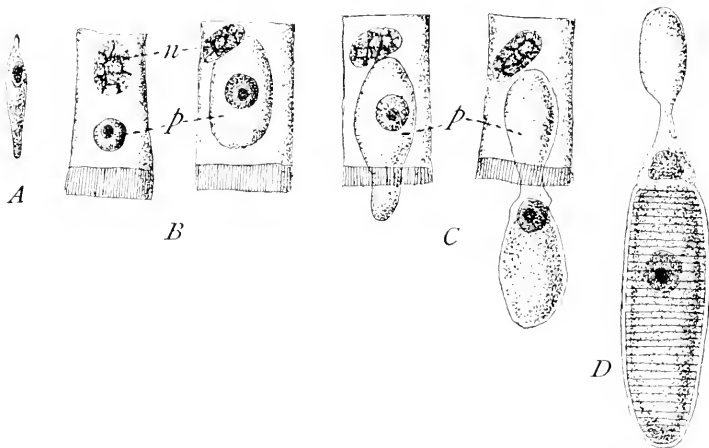


FIG. 126.—Development of a polycystid gregarine (schematic). *n*, nucleus of host cell; *p*, parasite. (After Wasielewsky.)

from their capsules, or introduced naked into the blood by some intermediate host. They make their way to the definitive site of parasitism, penetrate into cells and begin their development. In the simpler gregarines only the young stages are passed in such host cells and growth is not accompanied by any marked structural differentiations. In the polycystid gregarines the parasite never becomes entirely detached from its host cell until it is fully mature and de-differentiation begun by the loss of the attaching organ (epimerite). With its growth the body becomes differentiated into an anterior chamber (protomerite) and a nucleus-holding posterior chamber (deutomerite) and in the different species these three portions of the cell become variously ornamented and specialized. The epimerite particularly becomes modified in different ways that are useful for purposes of anchorage (see p. 536). It may be a mere

ball of protoplasm as in *Gregarina longa*; a spade-shaped structure as in *Pileocephalus herri*; a long knobbed proboscis either simple or provided with spines as in *Stylorhynchus longicollis* or *Geniorhynchus monnieri*; or there may be many finger-form processes as in *Echinomera hispida* or thread-like processes as in *Pterocephalus giardi*. In *Corycella armata* it becomes a single crown of hooks; in *Beloides firmus* hooks combined with a lone spine. While these epimerites serve primarily for attachment, they also serve, in some cases at least, as food-getting organs. In *Pyrcinia moebiuszi* the epimerite forms a long haustoria-like process which extends through the epithelial cell of the gut and into the blood lacunae of the submucosa (Fig. 103, p. 201) and in *Stylorhynchus longicollis* a canal is said to extend from the tip of the epimerite through the protomerite and into the deutomerite of the parasite serving for the passage of food (Léger).

The buds of Suctorina have a rather complicated developmental history, especially in forms whose "embryos" are parasitic in other Protozoa (*Sphaerophrya* species). The buds possess cilia which are arranged in different patterns in the various species, and by which they swim actively about until they finally settle down for development. They also possess, as a rule, some longer cilia at the anterior end which have been homologized with the adoral zone of the ciliated Infusoria, and at the posterior end they possess a sucking disc by means of which the buds attach themselves to some solid object either living or lifeless, and from which a stalk is developed. With growth of the stalk the cilia are absorbed and tentacles—suctorial, piercing or seizing—are developed. In the parasitic forms the ciliated embryos may develop tentacles while in the motile condition, but on coming in contact with a quondam host, cilia and tentacles are absorbed and as an ectoparasite the young form makes a pit in the cortex of the host. It may then reproduce by cell division in this pit until as many as 50 or more are produced, and these escape through a slit-like birth opening of the improvised brood pouch.

In some types of Protozoa finally, especially in the colonial flagellated forms, the single cell undergoes a series of cleavage stages the sequence of which is similar to that of many types of eggs of Metazoa. This is particularly striking in forms like *Epistylis*, *Zoothamnium* and other colonial ciliates, which, as adults, consist of more or less definite numbers of cells arranged in definite patterns.

CHAPTER VII.

VITALITY.

A NORMAL active protozoön is a bit of protoplasm in which the vital activities are perfectly balanced, correlated and coördinated in response to internal and external stimuli. If the physiological balance is disturbed by abnormal activity or inactivity in one or other function the result is evident in the general vitality of the organism. The organization, however, is not rigidly fixed and undergoes adaptive changes in response to the new conditions until activities are again coördinated. The Protozoa thus agree with all protoplasm in having the power of adaptation or ability of the protoplasmic substances to react within limits to unusual stimuli in such a way as to maintain perfect correlation and coördination under the new conditions.

An interesting case of orderly response to unusual conditions was the fusion of two conjugating individuals of *Uroleptus mobilis* (Calkins, 1924). Instead of separating at the end of twenty-four to twenty-six hours as in ordinary conjugation, these two individuals remained attached for six days during which time the usual reorganization processes occurred in each. On the seventh day they fused along the entire ventral side, forming a bilaterally symmetrical individual with two oppositely placed mouths and peristomes, two contractile vacuoles and two independent sets of macro- and micronuclei (Fig. 127). On the eighth day this remarkable creature divided three times, giving eight double individuals all similar to the original bilaterally symmetrical one from which they came. They continued to divide at the rate of approximately one division per day on the average for a period of four hundred and five days and through three hundred and sixty-seven divisions. The interesting fact here is the correlation of two distinct sets of structures and functions so as to act harmoniously and synchronously as one individual, and the setting up of an entirely new organization. Had the two individuals separated as in normal conjugation their metabolic processes would not have been synchronous, the periods of division would have been more or less similar but not identical. In the double individuals the two sets of eight macronuclei behaved differently in different individuals. In one case each set would fuse prior to division to form a single ellipsoidal macronucleus (Fig. 128), behaving thus like two normal individuals when ready to divide

(p. 218). In the other case the sixteen macronuclei would all fuse to form one single macronucleus which would divide and form two groups of eight each (Fig. 129). In the latter case there was not

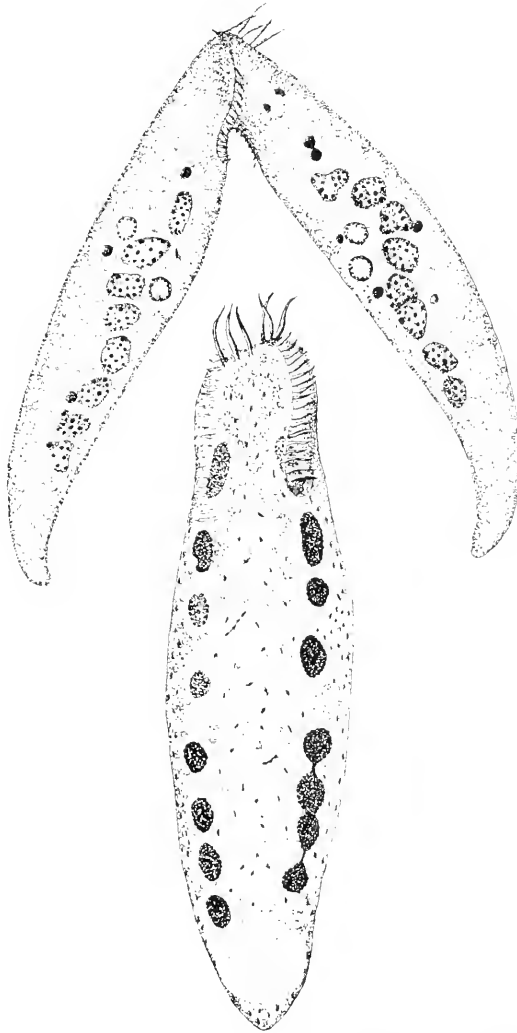


FIG. 127.—*Uroleptus mobilis*; origin of double individual. Above, two conjugating cells; below, the double individual which was formed by the fusion of two such conjugating individuals. (Original.)

only a definite adaptation to the new conditions but a further advance toward a composite animal of a new type and with a novel organization. The synchronous activities indicate that common

responses to common stimuli were operating and that a perfect equilibrium was established throughout.

Vitality, as the sum total of all the protoplasmic activities set up in response to internal and external stimuli, is variable. Variations due to external conditions may be readily seen in the effects of heat and cold. Increased temperature increases oxidation leading

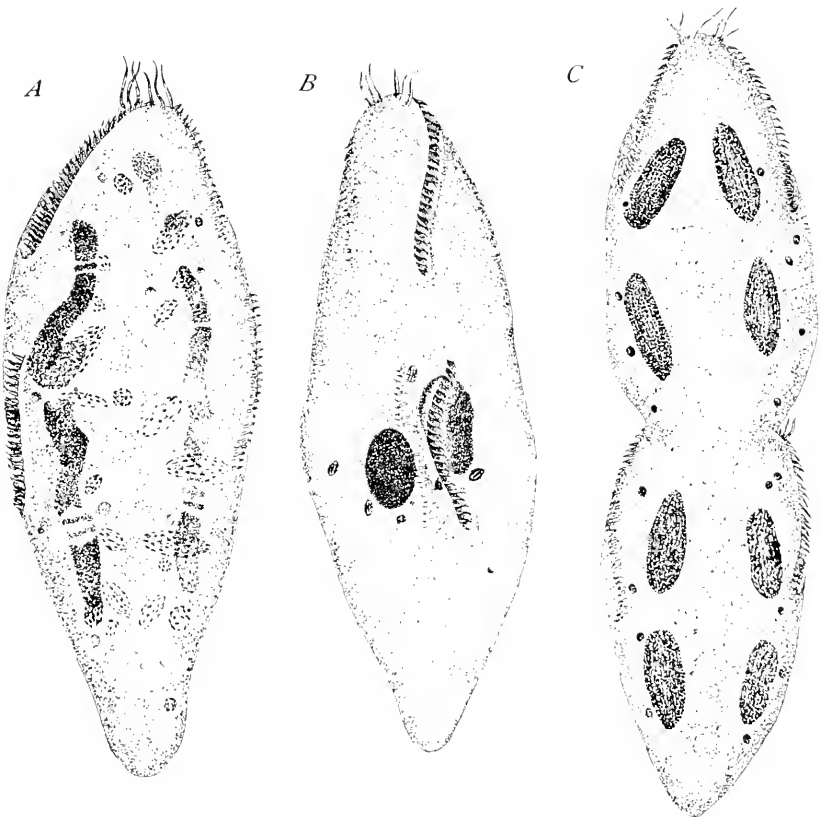


FIG. 128.—*Uroleptus mobilis*. Division of double individual: type with two division nuclei. A, stages in the fusion of the two sets of macronuclei independently; B, two division nuclei and two new peristomes; C, division of the cell, each half with two sets of nuclei. (After Calkins.)

to more rapid movements including food-taking activities, more active digestion, assimilation, growth and reproduction. It involves more waste and more active pulsation of the contractile vacuole. Conversely, decreased temperature slows up the entire series of activities and vitality is reduced. In like manner any condition of the environment which tends to quicken, to weaken, or to nullify

any one link in the chain of vital activities will have its effect on the general vitality.

It is not improbable that internal reorganization, or disorganization, with increase or decrease of activity in all or in some part of the protoplasmic make-up may bring about similar variations in vitality. Thus changes in organization may be effected by amphimixis or by long-continued metabolic functioning with correspond-

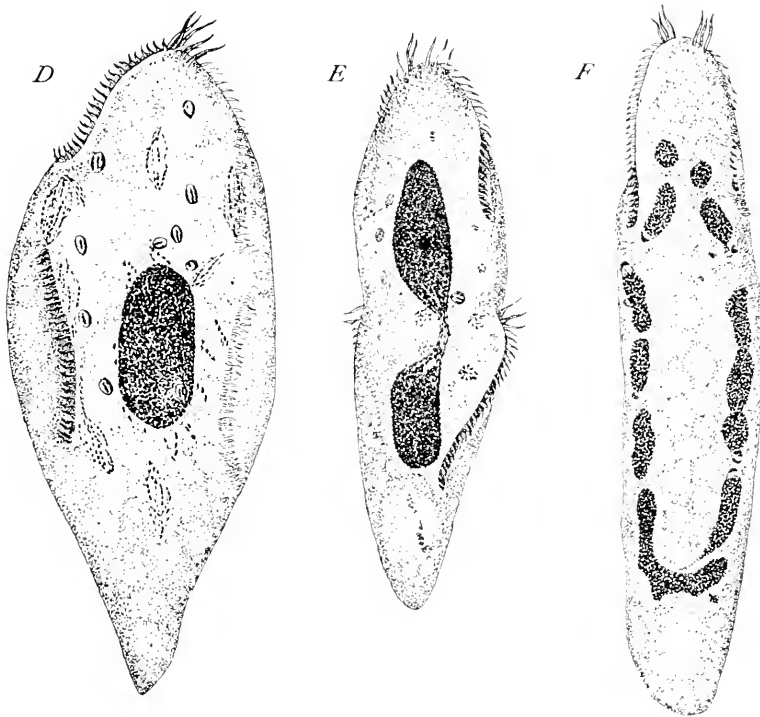


FIG. 129.—*Uroleptus mobilis*. Division of double individual; type with one division nucleus. *D*, the single nucleus formed by fusion of the two independent sets of macronuclei; *E*, first division of the single nucleus; *F*, reconstruction after division with a new type of macronucleus formed from the single division nucleus. (After Calkins.)

ing effects upon the general vitality. The chemical and physical make-up of the protoplasm of an individual may change with continued metabolic activities and lead to a change from what is termed a labile condition when actions, reactions and interactions are perfectly balanced and at a maximum of activity, to a more stable condition when these activities become increasingly unbalanced or cease altogether.

I. ISOLATION CULTURES.

The study of protozoön protoplasm by the isolation culture methods has thrown considerable light on these problems of general vitality. If a bit of such protoplasm in the form of a single individual organism, and its progeny by division, is maintained under conditions of food and temperature as constant and uniform as possible, then variations in vitality may be measured and compared in relation to phenomena in the life cycle which are suspected of playing a rôle in connection with the lability of that protoplasm.

In order to study protoplasm in this manner it is necessary to adopt some measure of vitality which will be an expression of the sum-total of all vital activities. Since every function is a link in the chain of vital activities any one function would do were it possible to measure it accurately, but the difficulty comes with the inability to measure excretion, or nutrition or irritability in any complete and definite manner. Reproduction, however, can be readily measured and being dependent upon the general functions of metabolism, becomes an excellent measure of vitality in a relative and comparative sense. In one way or another the division-rate has been used as a measure of vitality ever since Maupas, in 1888, first attacked the problem of age and natural death in Protozoa by the isolation culture method.

In practically any free-living form of Protozoa if proper conditions of food and temperature are provided, the general vitality or sum-total of functional activity as measured by the division-rate, continues more or less uniformly for long periods. The single individuals thus watched appear to be self-sufficient and able to continue their vital activities indefinitely. The question may be raised as it has been raised repeatedly, does the protoplasm of such an individual retain this constant potential of vitality indefinitely, or like a machine, does it wear out sooner or later, and will it ultimately stop altogether?

The problem thus worded is only a partial restatement of the old problem concerning life and death of unicellular organisms which Weismann raised more than fifty years ago. He took the ground that Protozoa do not grow old and do not die a natural death both of which are prevented by an individual dividing into two while in full vigor. The two young ones thus formed by division leave no parental corpse but share the old protoplasm between them and they in turn grow and similarly divide, so that old age is impossible and natural death inconceivable. Weismann further maintained that these fateful phenomena—age and death—are penalties which the Metazoa must pay for their privilege of specialization and differentiation into somatic and germinal protoplasm. Protozoa he compared with the germinal protoplasm of Metazoa in common

with which they have the potential of an indefinitely continued existence.

The experiments of Maupas (1888) to determine by isolation culture experiments whether Infusoria do actually grow old were not convincing. He found, indeed, that a bit of protoplasm in the form of a single infusorian cell if isolated in a suitable culture medium would live, grow and divide. One individual cell formed by such division, if similarly isolated, would repeat the process, and from its progeny another representative bit of protoplasm would continue the race. Maupas found that, ultimately, such protoplasm

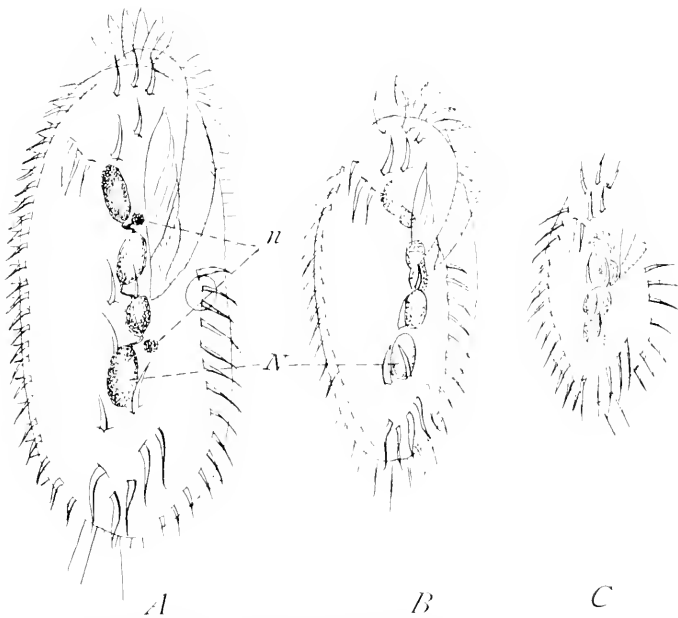


FIG. 130.—*Stylonychia pustulata*, senile degeneration. B, C, degenerated individuals without micronuclei. (After Maupas.)

would lose its vitality and the race would die after morphological and physiological evidences of degeneration (Fig. 130). In this manner he followed the history of *Stylonychia pustulata* through 316 generations by division when the race died. Another species, *Stylonychia mytilus*, died out after 319 generations; *Leucophrys patula* after approximately 660 generations, etc. The single individual was isolated in culture medium under a cover-glass and kept in a moist chamber. Here it divided repeatedly during a period of from two to six days until many individuals were present (in one case 935) all descendants of the original *c. e.* One of these was then isolated and the process repeated. From these experiments

he concluded that Infusoria die a natural death after a typical life cycle and after a definite number of generations by division.

The criticism was soon advanced that adverse conditions and bacterial products were responsible for death of his organisms, or, that instead of dying from old age they were slowly killed. There certainly was some justification for this criticism for not only was the covered medium abnormal but the accumulation of bacterial and protozoan products of metabolism might well have been detrimental, particularly if certain types of bacteria gained supremacy. Woodruff (1911), furthermore, has shown that excretion products of *Paramecium* are detrimental to *Paramecium*, and *Stylonychia* products to *Stylonychia*, and the implication is that any type, if continued for long intervals in an unchanged medium, will slowly weaken in vitality and ultimately die.

Such criticisms, continued even to the present time in connection with isolation culture work, do not minimize the value of the splendid contribution of Maupas in these pioneer studies on vitality. The present day scepticism in regard to his general conclusion is based upon diverse results obtained by various experimenters with mass cultures as compared with isolation cultures, the great majority of the latter giving results which confirm Maupas. In these the criticism that an unfit environment gradually killed the organisms has been met by the use of carefully prepared culture media and by daily transfers of the experimental organisms to freshly prepared media. In this manner the undue accumulation of bacteria and their products is prevented while the organisms under observation are never present in large numbers.

By use of this method of study the life cycles of many different kinds of ciliates have been established and with the exception of the results obtained by Enriques (1913, 1915, 1916), Chatton (1923) and of Woodruff (1908-1921), they all agree in demonstrating a gradually waning vitality and ultimate death of the protoplasm under observation. The method now generally employed is to start with an ex-conjugant, or individual which has just emerged from conjugation and allow it to reproduce by division three times. Four (Woodruff) or five (Calkins) of the eight resulting individuals are then isolated and continued in daily isolation cultures as "pure lines," four or five pure lines to a "series." For vitality comparisons the daily division-rates of all lines of a series are averaged for periods of five days (Woodruff) or ten days (Calkins), and when the cycle is completed the consecutive five- or ten-day division-rates may be plotted to give a graph in which the ordinates represent the average rates of division, the abscissas the consecutive periods. By this method the history of the vitality of the protoplasm under observation is summarized in a graphic and effective manner (Figs. 131, 132, 133).

The above method was first used in connection with the life history of *Paramecium caudatum* (Calkins, 1904), and many other experiments of similar nature were made on this genus by later

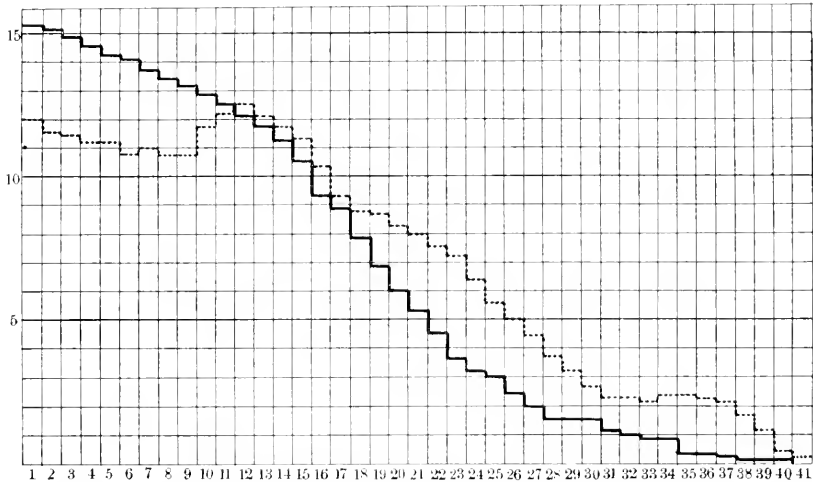


FIG. 131.—Composite graph of vitality of twenty-three series of *Uroleptus mobilis*, each having vitality of more than 85 per cent (solid line). The ordinates represent the average numbers of divisions in ten-day periods. The dotted line is the vitality graph of the double organism. (After Calkins.)

observers. It turned out to be an unfavorable subject in some respects for the study of this particular problem of vitality, for in 1914 Woodruff and Erdmann announced the discovery of a periodic

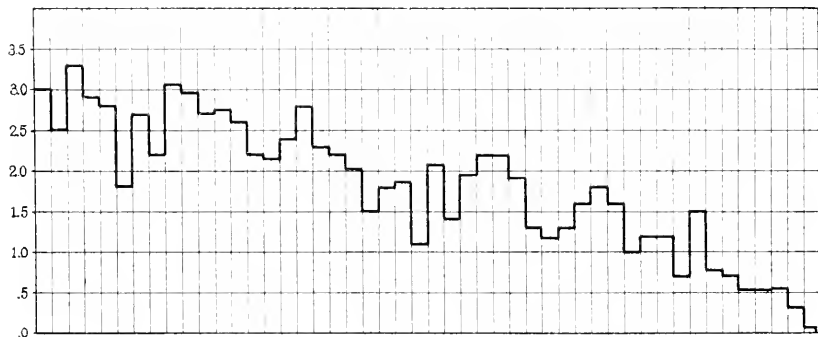


FIG. 132.—Vitality graph of *Pleurotricha lanceolata*. (After Baitsell.)

reorganization process without conjugation or encystment in *Paramecium aurelia* which is exactly comparable with one type of parthenogenesis occurring in Metazoa (see p. 316). The discovery

of this reorganization process which they called "endomixis" was the culmination of Woodruff's brilliant and long-continued study of the life history of *Paramecium aurelia* which he began in 1907, and which had been generally hailed as giving positive proof of the correctness of Weismann's point of view. Parthogenesis, however, has the same effect upon organization and upon vitality that conjugation has, and as Woodruff and Erdmann showed that "endomixis" occurs approximately once in thirty days in *Paramecium aurelia* and about once in sixty days in *Paramecium caudatum*, any experiments and observations on vitality are valuable only as they lie within these limits of time. For this reason many of the conclusions of Hertwig (1889), of Joukowsky (1898), of Calkins (1903, 1904, 1913) and of Jennings (1909, 1913) drawn from



FIG. 133.—Vitality graph of *Spathidium spathula*. (After Woodruff and Spencer.)

observations on *Paramecium* are of questionable value, and should be used cautiously in connection with the present problem. In other forms, however, analogous reorganization processes occur during encystment and are thus advertized in cultures whereas *Paramecium* does not encyst under such conditions but continues with low vitality to live and move during such periods of depression when "endomixis" is taking place.

While the list of recent experimenters with the Infusoria is rather a long one, the actual number of different organisms studied is comparatively small, but different experimenters working with the same species obtained strikingly similar results. Thus *Pleurotricha lanceolata* has been studied by Joukowsky (1898) and by Woodruff (1906), the former following out four series, three of which died out after approximately 220, 250 and 442 generations without conjuga-

tion while a fourth was abandoned after 458 generations. Woodruff, using the daily isolation method, found a gradually waning vitality with ultimate death. Baitsell (1914) also carried out isolation cultures with this organism, obtaining a vitality curve similar to that found by Woodruff (Fig. 132). *Oxytricha fallax* has been similarly studied by Enriques (1905), by Woodruff (1906) and by Baitsell (1914). The first gives no detailed account of his cultures but makes the general statement that this and other organisms cultivated by him are capable of multiplying asexually *ad infinitum*. Woodruff, however, finds a definite curve of vitality similar to that of *Pleurotricha* with a waning vitality and ultimate death after 860 generations by division, and Baitsell followed the history of three cultures all showing the typical life history, one dying out in the 131st generation, a second in the 159th, a third in the 150th, while a fourth culture in test-tubes lived for a longer period but it also finally died, none of these cultures approaching the long history of Woodruff's strain. *Stylonychia pustulata* also has been cultivated by Enriques (1905) and by Baitsell (1912), the former giving no statistical data but maintaining that division can go on indefinitely without degeneration or conjugation if the conditions are right. The latter follows out the history in isolation cultures and finds a typical curve of vitality with waning vitality ending in death, in the longest line after 572 generations. In other organisms Woodruff (1905) found waning vitality and death in *Gastrostyla steinii* after 288 generations, and Gregory (1909) a similar result with *Tillina magna* after 548 generations, and Calkins (1912) a similar result with *Blepharisma undulans* after 224 generations.

In all the cases cited above the organisms under investigation are bacteria feeders, and despite the daily change of medium and care in maintaining the isolation cultures the old criticism of bacterial poisoning or deleterious effects of the medium has been repeatedly advanced. Woodruff, however, has kept *Paramecium aurelia* continuously living for seventeen years on the same bacteria diet, "endomixis" occurring at stated intervals and the same observer using the same methods has followed other organisms through periods of waning vitality and death. Metalnikov (1919) similarly has continuously cultivated *Paramecium caudatum* without conjugation. It seems highly probable, therefore, that the prevention of death has little to do with the environment in these experiments but lies in the organisms themselves—with *Paramecium* in the phenomenon of "endomixis."

More direct evidence that bacteria contamination is not responsible for the ultimate death in isolation cultures is afforded by similar experiments with carnivorous ciliates. With these it is possible to use bacteria-free culture media in which the food organ-

isms are introduced with the experimental individual. Again in the majority of cases the ultimate result has been the same as with bacteria eaters. Thus *Actinobolina radians* was followed through 448 generations in isolation cultures in sterile spring water with *Halteria grandinella* as food (Calkins, 1912) and *Spathidium spathula* through 218 generations with *Colpidium colpoda* as food (Moody, 1912), the organisms finally dying in both cases.

Further and very complete evidence that environmental conditions are not responsible in any direct way for waning vitality and death is afforded by a long-continued study of the protoplasm of *Uroleptus mobilis*, an hypotrichous ciliate (Calkins, 1918, 1919, 1920, etc.). This rare organism found and isolated in 1917 is a bacteria eater and was cultivated on a medium consisting of flour and timothy hay boiled in spring water and allowed to stand for twenty-four hours before using. Individuals were transferred daily to such fresh medium in order to avoid an excess of bacteria. For each series of five lines the division rates were figured in ten-day unit periods which were then averaged for sixty-day periods at ten-day intervals. The vitality history of twenty-three series averaged for sixty-day periods and the history of the double *Uroleptus* are shown in Fig. 131. The average division-rate here for the first sixty days was 15.4 divisions per ten days from which it descended regularly in successive sixty-day periods at ten-day intervals until death. A single series by itself would be no evidence that slow killing had not occurred. But when two of the progeny of a series are allowed to conjugate with one another at any time after the first 75 generations, the ex-conjugants repeat the history of the parent series but do not die when the parent series dies. In this manner the protoplasm of the original *Uroleptus* which was isolated November 17, 1917 was still under observation twelve years later, although any single series lived from ten months to a year only. The life of the progeny overlaps that of the parent; its progeny overlaps it, etc.; the daily treatment of parents and offspring was identical throughout; both were subject to the same deleterious conditions if present but parents died and offspring lived, a history which was repeated more than 140 times with as many series during a period of twelve years.

From these clear-cut experimental results with *Uroleptus mobilis* the fact is obvious that under these experimental conditions a fairly uniform life cycle is the rule. The 140 completed life cycles upon which this conclusion is based were all characterized by the same phenomena, viz.: (1) A high initial vitality of the ex-conjugant lasting for a limited period; (2) gradually waning vitality ending in complete exhaustion and death; (3) a period of sexual "immaturity" lasting from the first thirty to ninety days during which encystment occurred if appropriate external conditions were pro-

vided but conjugation did not occur; (4) a period of maturity beginning after the first thirty to ninety days approximately and lasting until the ultimate depression when conjugation, under appropriate external conditions did occur; and (5) a period of old age indicated by morphological degeneration with accumulating physiological depression which ended in death.

The many different series studied furnish ample opportunity for the comparison of vitality in different series. In some there is a greater intensity of vitality, *i. e.*, the average division-rate is higher throughout the cycle; in others the endurance factor is greater, *i. e.*, the individuals live for longer inter-divisional periods without division and the cycle is correspondingly lengthened (see Chapter VIII).

On the basis of such consistent experimental results one is tempted to generalize and to hold that all Protozoa pass through a similar life history. The temptation is increased by the confirmation of the main results in connection with an entirely different ciliate, *Spathidium spathula*, in the hands of a no less competent observer than Woodruff (Woodruff and Spencer, 1924). *Spathidium* is carnivorous and feeds normally on *Colpidium colpoda*. Woodruff and Spencer's isolation cultures were carried on in a basic medium of standardized beef extract to which a few individuals of *Colpidium* were added. The individuals were transferred daily to fresh medium and new food. Many complete series were followed from ex-conjugants, four lines to a series until the protoplasm died a natural death. A typical example is illustrated in Fig. 133, representing the division-rate averaged for five-day periods (solid line) and one offspring series. "The data presented show that in the great majority of cases the cultures died out sooner or later after a somewhat gradual decline in the division-rate" (*loc. cit.* p. 178). Seventy-nine series ran synchronously with their parent series for at least fifteen days; some of these were then discarded but enough were followed through to afford a justifiable basis for conclusions. Here then we have again a large number of series carried on in isolation cultures, all derived from the same ancestral single ex-conjugant, and dying out "after a somewhat gradual decline in division-rate."

Woodruff, however (*loc. cit.*), does not grant that the decrease in vitality is due to any intrinsic ageing tendency in the protoplasm, but believes that both in *Uroleptus* and in *Spathidium* the proper milieu for continued life was not provided in the culture methods used, and implies that when a series dies in the absence of conjugation or of endomixis, it is *ipso facto* evidence of a faulty environment. The matter is important for, if Woodruff's conclusion is correct, it brings us to an *impasse* in the subject under discussion. He supports his argument with the citation of cases on record in which there is no evident diminution in the division-rate under the condi-

tions of culture, and in such cases he believes that natural environmental conditions have been supplied. He obtained some cases of greater longevity in a few series of *Spathidium*, and although the methods and the culture medium supplied did not differ in any way from those used in the series that showed decline and death, he concludes that somehow the conditions were more suitable, and that when suitable the ciliate has the ability or potential for an indefinitely continued existence without the necessity of conjugation (fertilization) or of an equivalent process.

Chatton (1921) shares this scepticism: "One may even conclude," he says, "that the more the facts accumulate, especially those of an experimental nature, the more nebulous does this conception of a life cycle (in ciliates) become" (*loc. cit.* p. 128). The "facts" thus mentioned include the exceptional results with experimental culture methods by Woodruff as above, by Baitsell, Dawson, Enriques, Mast and others, these being the most prominent, in connection with the Infusoria. It is quite possible, as M. Robertson (1929) brings out, that conditions of the milieu are such that stimuli from the environment which ordinarily call forth adaptive changes in the organization are not developed.

In a similar manner Dawson (1919) found that an amieronmeleate race of *Oxytricha hymenostoma* presents a typical cyclical curve of vitality, and death follows a gradually decreasing vitality, if the organisms are cultivated in isolation cultures. If maintained in mass cultures they were found to live for a considerable period longer than the isolated forms, and Dawson concludes that if a suitable medium is provided an indefinite life is possible without conjugation, endomixis or encystment. It is conceivable that environmental media may induce different protoplasmic reactions at different periods of the life cycle, as shown by Gregory's (1925) experiments with *Uroleptus*, and that proper salts in the medium at appropriate periods would enable the protoplasm to maintain its youthful labile condition. Individuals might thus be "doctored" at intervals with a resulting repression of cumulative differentiations and a corresponding maintenance of youth. This was the underlying principle of Woodruff's cultivation of *Paramecium aurelia* on a variable diet, the medium being changed at intervals but in this case without difference in his results. Austin (1927) likewise, subjecting *Uroleptus mobilis* to different media throughout entire cycles, was unable to alter the usual history. It is possible that old protoplasm might be reorganized by increasing the permeability and with proper interaction between protoplasm and medium, restored to its original labile condition.

In other groups than the ciliates, exceptions to the type of life history shown by *Uroleptus* are true of the few cases known. In the animal flagellates for example there is no case of indubitable

proof of fertilization in the entire group. On the other hand, there have been no successful attempts to cultivate such flagellates by the isolation culture method so that we are entirely uninformed as to the relative vitality in a life cycle. It is possible that processes analogous to endomixis in ciliates take place during encystment stages but as to this we are also ignorant. With these exceptional cases, therefore, we must wait for further information.

Exceptional cases are increased through Bělař's observations on *Actinophrys sol*, a heliozoön (1924). A single line of his main culture was followed through 1244 generations by division during two years and eight months. Fertilizations were obtained from time to time in mass cultures, but these were prevented in the isolation cultures, the latter showing no indication of reduced vitality with continued life (Fig. 134). Bělař also concludes that, given proper conditions, the protoplasm of *Actinophrys* has the possibility of indefinitely continued life and reproduction by division.

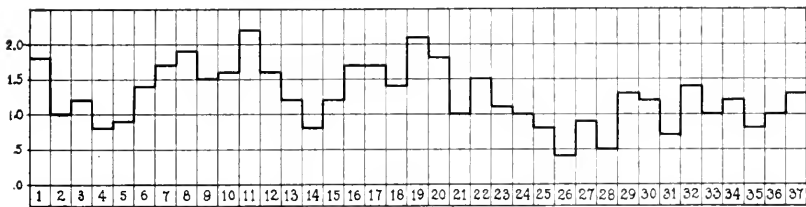


FIG. 134.—Vitality graph of *Actinophrys sol*. (After Bělař.)

In these exceptional cases we meet indeed with diverse experimental results and diverse conclusions. Granted that the experimental work in all cases is done with an equally conscientious regard for controls and pitfalls of all kinds, it is necessary to accept the conclusions on their merits and endeavor to find an explanation which will bring them all into harmony. The first difficulty comes in connection with the popular conception of an abnormal condition of the environment. It is obviously impossible to study the life history of an organism under normal environmental conditions in Nature—in all probability there is no constant "natural" environment. To Enriques, Baitsell, Dawson, Bělař, Chatton, Jollos, and Woodruff in part, the culture methods employed for ciliates are "abnormal" and death is a result of these conditions. With *Uroleptus mobilis* in mind it is difficult to understand by what process of reasoning the conditions of the environment are responsible for the decline of vitality and death when two individuals from such cultural material are restored, upon conjugation, to full vitality in the same medium. The conditions are identical for parent protoplasm and offspring protoplasm and yet the former dies, the

latter lives until a corresponding age, and dies in turn. The more than one hundred and forty series that have followed one another since 1917, in the same medium and under the same conditions, in the same rhythmical cycles and with surprising uniformity, furnish strong evidence that the environmental conditions have been suitable or "normal." For each series there has been the same sequence of physiological conditions—high vitality and sexual immaturity, encystment power, sexual maturity, decline in vigor and ultimate death. If these phases of vitality are normal, if encystment and reorganization, and conjugation are normal phenomena in the life history of a ciliate then the conditions under which they occur must likewise be normal. A hypercritical mind may deny the existence of conjugation in Nature and maintain that conjugation occurs only under the abnormal conditions introduced when the samples are collected and transferred to small holders in the laboratory. With such an individual convincing proof is apparently impossible and we can only ignore the implication that conjugation is a phenomenon which did not occur under "normal" conditions in Nature but manifested itself only when man began to collect material. I have no sympathy with such a point of view; I regard conjugation as an entirely "normal" process in ciliates as gamete formation and fertilization are "normal" processes in Sporozoa and Sarcodina. When the conditions of the environment are such that this phenomenon does *not* occur, then we may justly look for the unusual at least. In a similar connection M. Robertson (1929) states: "As the outcome of all the experimental work discussed above, the American workers (*i. e.*, the Woodruff school) deny the existence of a life cycle in ciliates. To the present writer this seems an erroneous attitude. . . . The result of this series of investigations is to show that the cycle is not a rigidly internally conditioned sequence but is the response of an internally adaptable organism to the external stimulus of the environment" (p. 163). The limits of adaptation of protoplasm are unknown to us; it is quite conceivable that conditions may be so arranged that for long periods the normal sequence of phenomena in a life cycle are in abeyance and the impression is gained that protoplasm under such conditions has the possibility of indefinitely continued existence. But can this be considered a normal environment? Here the conditions which lead to conjugation are not offered and such conditions, if any, might reasonably be regarded as abnormal; if conjugation is needed the need is met by the artificial conditions and the organism is more or less adapted to them. No one can maintain consistently that Carrel's long-continued tissue cultures are normal, yet here we have artificial conditions under which these vertebrate tissue cells continue, apparently indefinitely, to live and divide. Death of cells occurs when the transfers are not made at appropriate intervals;

they have become adapted to the artificial conditions of cultivation and continue to live and divide so long as these conditions are maintained *but they must divide*.

The question of "normal" or "abnormal" environment after all appears to me to be of an academic nature, and I cannot agree with Woodruff and his followers in their belief that natural death is not inherent in ciliates under natural, or, as he calls it, "normal" conditions. Nor can I accept his further conclusion that the life cycle of a ciliate is a "myth." It is quite evident that the cycle may be greatly varied by reason of external conditions and it is plainly obvious that it has no definite or fixed limits such as postulated by Maupas. Chejfec (1929), for example, found that the life of a single individual of *Paramecium caudatum* may be prolonged up to one hundred and twenty days by appropriate regulation of the number of *Bacterium coli* supplied. If fertilization is an almost universal phenomenon we should be able to determine the conditions both within the protoplasm and in the environment which bring it about. If fertilization satisfies a protoplasmic need we should be able to find out what the need is. When that explanation is forthcoming we shall probably be able to understand why the animal flagellates continue to live so successfully without it.

In regard to the life cycle of Protozoa we are apparently all agreed on some cases. Since the classical work of Schaudinn (1900) on *Eimeria (Coccidium) schubergi* no one doubts the general facts of the life cycle in Sporozoa; his work has been confirmed by scores of investigators and upon an enormous number of representative species. A sequence of vital phenomena intervening from fertilization to ultimate gamete formation and fertilization is characteristic of all such cycles and in all cases the race comes to an end with the formation of gametes, when without fertilization, the gametes die. Similar cycles are characteristic of Foraminifera and wherever gametes are formed the ultimate fate is the same. With ciliates, except in rare instances, gametes are not formed but the organization of the protoplasm undergoes changes at maturity when fertilization processes (conjugation) occur, and in the great majority of pedigreed cultures, the race, like unmated gametes, comes to an end by natural death (see p. 282). The life cycle in all Protozoa signifies the series of events between fertilization and fertilization again or natural death. It involves characteristic changes in organization of the protoplasm and equally characteristic manifestations of vitality.

I have dwelt at some length upon these experimental results, and on the diverse conclusions based upon them because I believe that the principle of the life cycle in Protozoa is a fundamental biological concept involving changes in protoplasmic organization as a result of continued metabolism. I have reason to believe,

furthermore, that such changes or differentiations from the fundamental organization underlie the phenomena of cell division, of endomixis, of sex differentiations, fertilization and protoplasmic age followed by natural death. In the following section an attempt is made to correlate these characteristic phenomena in a life cycle with progressive changes in the organization of the protoplasm.

II. ORGANIZATION AND DIFFERENTIATION.

It is evident to any one who has made a study of Protozoa that forms and structures are practically unlimited. It is equally evident that these characteristics are specific for each species. Regeneration experiments show, furthermore, that these specific characteristics are carried in all parts of the protoplasm of an individual, a small part of a *Stentor* becomes a perfect *Stentor*, a small part of a *Uroleptus* develops into a fully differentiated *Uroleptus*, etc. The structure of the adult by which we recognize the species in any particular case is the product of the finer make-up of the protoplasm as it exists in a cyst for example or in a rounded-out fragment cut from the body of an adult. What this finer make-up is is purely conjectural, but the idea is carried by the non-committal term "organization" as used in the preceding chapters. In this term we include both the adult structures of the fully formed individual and the undifferentiated protoplasm which has the ability to produce them. There is reason to believe that the differentiations which characterize the adult are brought about as a result of metabolic activities constituting vitality, and these may be induced by changes in environmental conditions as when an organism emerges from a cyst, or regenerates at division periods (p. 224); or they may require a longer period of metabolism and be combined with growth; or they may appear only as a result of cumulative differences representing a gradual change in organization. In general the facts at hand warrant the statement that differentiations always involve changes in organization, and for purposes of description it is convenient to describe them as: (1) Inter-divisional or Ontogenetic Differentiations, and (2) Cyclical Differentiations.

1. **Inter-divisional Differentiations.**—In the development of a Metazöön differentiated structures are never present in the initial egg cell but appear in orderly sequence as a result of metabolism, growth and division of cells. A protozöön about to emerge from its cyst is comparable with such an egg cell. The cyst wall becomes permeable, water and oxygen are admitted and metabolism begins. Soon the characteristic motile organs make their appearance differentiated from the apparently homogeneous protoplasm. The oral apparatus, anal aperture and contractile vacuole appear and the

organism emerges apparently complete from its cyst. This is a rapid differentiation accompanying the onset of metabolism.

Analogous processes of differentiation accompany the regenerations associated with division of the cell. In ciliates a new oral apparatus and specialized motile organs are formed at appropriate positions by the dividing organism (see Chapter VI), and differentiation is rapid and complete. The organization under which this differentiation occurs is evidently a result of metabolic activities prior to division (see below).

Differentiations accompanying growth of the cell are characteristic of Protozoa which reproduce by unequal or by multiple division. Here the protoplasm is parcelled out amongst many offspring and each bit of protoplasm, like an encysted cell or a cut-out fragment, possesses the fundamental organization characteristic of the species, but undifferentiated. Thus a bud of *Acanthocystis* or of a *Suctorian* has none of the adult characters but develops them gradually during a period of some days. Or the sporozoite of a polycystid gregarine slowly acquires, with growth, the particular epimerite, protomerite and deutomerite of its species (Fig. 126). Differentiation occurs here, but more slowly than in the case of a ciliate, and is apparently more directly associated with metabolism. Arrested stages in development are not uncommon and frequently lead to puzzling complications in the life cycle. *Trypanosoma lewisi*, for example, passes through stages resembling *Leptomonas* and *Crithidia* (Fig. 122) or *Leishmania douorani* through a flagellated *Leptomonas* stage to an adult quiescent intracellular phase. Similarly the young ciliated bud of a Suctorian which may be either parasitic or free-living gradually loses its cilia develops tentacles and a stalk before it becomes the adult form of the specific description.

The changes in form and structure with growth are to be traced to changes in the protoplasmic organization which in turn are doubtless due to metabolic activities, and there is evidence that analogous changes are responsible for the differentiations which accompany regeneration in the more actively developing ciliates. In this connection the merotomy experiments of Calkins (1911) and Young (1922), patterned after the original merotomy experiments of Balbiani (1891), are suggestive; in Chapter VI it is shown that anticipatory changes in the cell precede the nuclear changes. This was first demonstrated by Wallengren (1900) for *Stylonychia* and *Euplotes*, and is clearly shown in *Uronychia transfuga* in which the new posterior giant cirri are formed sometime prior to the nuclear changes in preparation for division. The new cirri appear in a region of the cell previously free from cirri, as well as at the bases of the old cirri. Similarly there is a complete new formation of the peristome with membranelles in the posterior half and a new series of membranelles which replace the old ones in the anterior

region. Except for mutilations these regenerations and replacements occur only at periods antecedent to cell division and indicate some far-reaching change in the constitution of the protoplasmic make up. The ability to undergo such a change furthermore is progressive as shown by experiments in cutting *Uronychia* (Calkins,

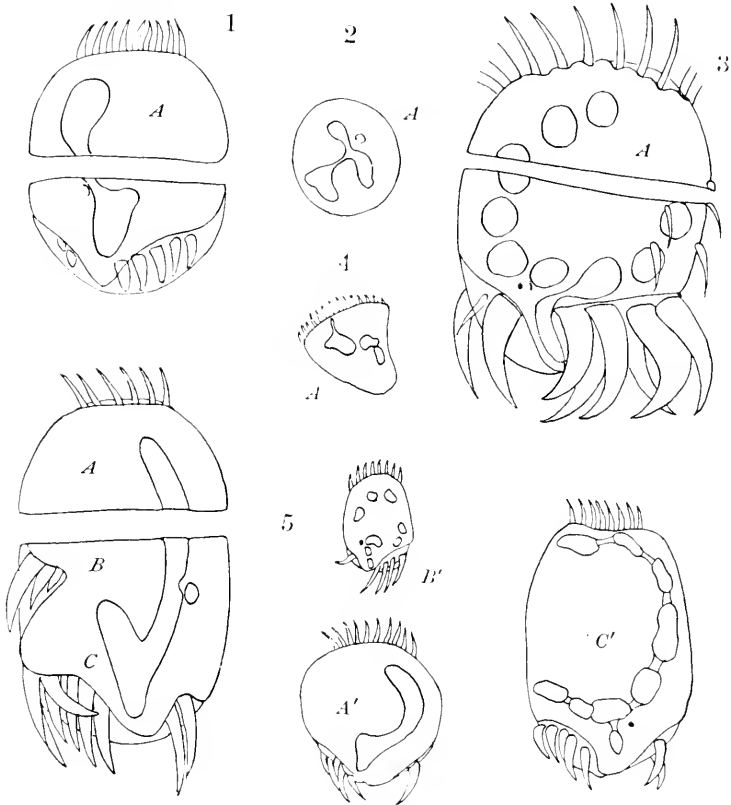


FIG. 135.—*Uronychia transfuga*, merotomy and regeneration. 1, cells immediately after division, cut as indicated; 2, fragment *A* of 1, three days after the operation, no regeneration; 3, cell cut five hours after division; 4, fragment *A* of 3, three days after operation, no regeneration; 5, cell cut at beginning of division as indicated, into fragments *A* and *BC*; *A'*, *B'*, *C'*, fragments *A*, *B*, and *C*, twenty-four hours after the operation; fragment *A* regenerated into a normal but amiconucleate individual *A'*; *B* *C* divided in the original division plane forming a normal individual *C'*, and a minute but normal individual *B'*. (After Calkins.)

1911). In these experiments the cell if cut immediately after division in a plane indicated by the section line (Fig. 135) is divided into two fragments, one of which, the posterior with giant cirri, contains the single micronucleus, while the anterior portion, with peristome, contains a part of the macronucleus but no micronucleus.

In such cases the anterior portion may live for four or five days as an amorphous fragment, but it never regenerates the giant cirri. The posterior part, however, regenerates the missing anterior region within a few hours and becomes a perfect cell. Exactly the same result invariably follows if an individual is cut when five to eight or ten hours old after division (Fig. 135, 3). At this time the normal individual is fully grown and active. At the age of sixteen to eighteen hours different results are obtained. If a number of individuals are cut at this age a small percentage of the anterior parts without micronuclei will regenerate into perfect individuals save for absence of the micronuclei; the posterior parts always regenerate. This percentage rises to 100 per cent of cases when individuals twenty-four hours' old are cut. Under the conditions at the time the experiments were made divisions occurred in normal animals at intervals of twenty-six hours. Older cells, when cut, frequently resulted in the formation of three perfect individuals; one from the transected anterior portion without a micronucleus and two from the normal division of the posterior portion. One of the latter, the more anterior part, although perfect is of minute size owing to the fact that division of the cell takes place through the original geometrical center, or the "division zone" of the cell. This minute cell grows to normal size and ultimately divides, although its division is delayed. The original anterior fragment is perfect as far as external appearances are concerned, but it has no micronucleus and after seven or eight days it dies without dividing.

This experiment, fully confirmed in the essential points by Young (1922), indicates a progressive change in the protoplasm in the inter-divisional period. Except when a micronucleus is present, young cells when cut are unable to regenerate the missing parts. Fragments of old cells have the power to regenerate missing parts even in the absence of a micronucleus. Such regeneration is characteristic of cells in preparation for division and occurs with every division. It follows, therefore, that the formation of cirri in these regeneration experiments is due to some condition of the protoplasm in old cells which is not apparent in young ones and illustrates one type of inter-divisional differentiation.

These experiments also indicate another significant phenomenon, viz.: the reorganization (de-differentiation) of the protoplasm with every division of the organism, a phenomenon fully confirmed by Taylor (1928). When division is nearly completed the power to regenerate without a micronucleus which was possessed by the organism two hours before is entirely lost and fragments without a micronucleus remain as they were when cut (Fig. 135). As stated above a young cell is unable to regenerate unless the micronucleus is present and this possibility does not appear in the protoplasm until after some hours of metabolic activity. This strongly indicates

the reorganization of the protoplasm or a restoration to a labile and undifferentiated condition. Other evidences of de-differentiation are shown by the loss through absorption of the old membranelles, cirri, undulating membranes, oral baskets of the Chlamydidontidae and kinetic elements of different kinds (see Chapter VI) while new elements replacing them are developed from the protoplasm. In this way there is a more or less complete reconstruction or reorganization of the organization at each division. (See also Herzfeld, 1925, and Schmahl, 1926.)

Another characteristic evidence of inter-divisional differentiation is shown by the polarization of the cell immediately prior to division whereby "division zones" are set up through which division of the cell takes place. Such division zones first described by Popoff (1907) are quite evident morphologically in *Frontonia leucas* and physiologically in *Paramecium caudatum* or *Uronychia transfuga* (Fig. 136). *Paramecium caudatum* when cut near the anterior or posterior end, as indicated in Fig. 136, does not regenerate the lost part (Calkins, 1911; Peebles, 1912). A membrane is formed over the cut surface and cortical differentiations in the form of coördinating fibrils, basal bodies, cilia and trichocysts are produced. The result is a characteristic truncated cell. When this divides, division occurs in the geometrical center of the organism as it was before cutting and not in the center of the truncated cell (Fig. 136, 3c). Two diverse cells result from division; one is normal and full-sized, the other small and truncated. It very often happens that cutting in this manner induces deep-seated changes in the organization and such that the precision of division phenomena in the truncated cell is destroyed and incompletely divided cells or monsters result. (Such a monster, one with 16 mouths, is illustrated in Fig. 136, o). See also Herzfeld (1925) on the occurrence of abnormalities and monsters in *Paramecium*. Similar monsters may be produced experimentally by use of drugs (*e. g.*, KCN) as shown by de Garis (1927).

Still further evidence of inter-divisional differentiation is shown by the antecedent nuclear changes preparatory to division whereby, in ciliates, macronuclear elements discard part of their substance into the cytoplasm and fuse to form a single, usually ellipsoidal macronucleus which then divides (*Uronychia*, *Stentor*, *Uroleptus*, *Spirostomum*, etc.). Or in flagellates the entire kinetic complex is absorbed in *Lophomonas* and several other types of flagellates (see Chapter VI).

It thus appears that well-marked changes of the nature of differentiations in the organization are taking place during the inter-divisional metabolic period, and that transformations of the nature of de-differentiations whereby the protoplasm is restored to the labile condition of a young organism occur with each division of

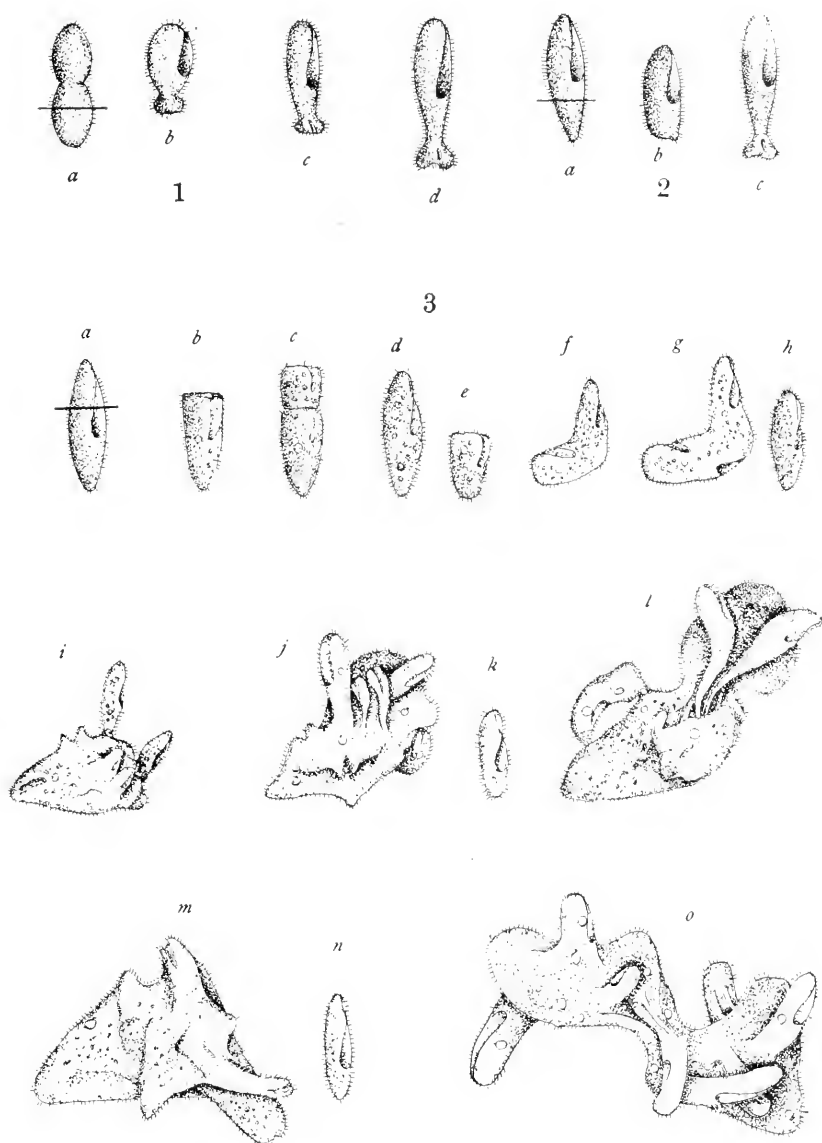


FIG. 136.—*Paramaecium caudatum*, merotomy. 1, 2, and 3, different experiments, the straight line indicating the plane of cutting; 3, the history of a monster: an original cell (3a) was cut as indicated; the posterior fragment (b) divided (c) into (d) and (e), the latter formed a monster (3, f to o); enucleated individuals (h, k and n) occasionally separated from the parent mass. (After Calkins.)

the cell. It is quite possible that this divisional reorganization is adequate for the preservation of the protoplasm through long periods of activity and may be the explanation of the long-continued life in certain cultures of ciliates, or continued life of animal flagellates in which fertilization processes are unknown.

Other differentiations occur in Protozoa which cannot be regarded as inter-divisional in character. These are rather of a cumulative nature and are not lost with the de-differentiation which occurs at division.

2. Cyclical Differentiations.—This second group of differentiations is not manifested in every cell of a species but appears at certain phases in the life history of the protoplasm composing any series of individuals. They are racial, therefore, and correspond roughly with periods in metazoön development such as youth, adolescence and age. Some of these differentiations are characteristic of very young forms, occurring immediately after fertilization and at no other time in the life cycle. Others make their appearance later in the cycle and often after many generations by division. These lead to and accompany the phenomena of fertilization and include maturation stages and gamete formation. Still others occur only at the end phases of the life cycle and are specific characteristics of age. We find justification, therefore, for purposes of description at least, in presenting facts concerning differentiations of youth, of maturity and of age, but we have no intention of setting limits to these phases.

(a.) **Cyclical Differentiations Peculiar to Youth.**—Intensity of metabolic activities is one of the characteristic features of young organisms, but with Protozoa exact data are difficult to get except from isolation cultures. In such cultures intensity is indicated by the division-rate and the great majority of ciliates show a higher division-rate in the early periods of vitality (see p. 250 and Figs. 131 to 133). In *Uroleptus mobilis* this intensity lasts for approximately sixty days (Fig. 131) and in *Spathidium spathula* for about forty days (Fig. 133). The evidence is not consistent, however, if all isolation cultures are considered, and in exceptional cases of *Uroleptus* and of *Spathidium* there is no indication of this relative intensity. Nor does Bělař give any evidence of it in his isolation cultures of *Actinophrys sol*; nor does Hartmann (1921) for *Eudorina elegans*, nor E. and M. Chatton (1923–1925) for *Glaucoma scintillans*. In such cases it is quite possible that the conditions of the cultures are such that differentiations are offset and reorganization at division periods is adequate for continued vitality. With parasitic forms exact data in this matter are wanting and general impressions are of little value.

Young organisms show the effects of abnormal conditions of the environment more quickly and more intensely than do older ones.

Gregory (1925) for example has shown that salts and change of medium are deleterious to very young forms of *Uroleptus mobilis* while older forms are not affected. This is in line with Child's results in connection with the action of potassium cyanide on many kinds of organisms, those parts which have the highest metabolic rate being first to succumb.

The differentiations indicated above are physiological in nature and are rather intangible. Other differentiations characteristic of youth while also physiological are indicated by morphological or structural modifications. Of these the most noteworthy are the different types of cysts which are secreted by all kinds of Protozoa. Some are temporary cysts in which no endomictic phenomena occur (e. g., division cysts of *Colpoda*, *Tillina* and many flagellates). Experimentally produced cysts are presumably of this kind (see Lwoff, 1927; Wolff, 1927; Garnjobst, 1928; Bresslau, 1921, etc.). Encystment has been generally regarded as a means of protection for the organism against adverse conditions of the environment. This is probably more traditional than accurate, for very few Protozoa are actually known to encyst when the external conditions are unfavorable. Mast (1923) for example finds that food and temperature have little effect in causing *Didinium nasutum* to encyst, but encystment takes place under the best conditions. It is more probable that organisms which have had the power to encyst persist under such conditions while the great majority are killed. Cutler (1919), however, gives evidence to show that skatol induces encystment in *Endamoeba dysenteriae*, and Cleveland (1927) that encystment of *Paramecium* occurs when injected into the rectum of frogs. This power to form reorganizing and "permanent" cysts appears to be a factor of young organisms induced possibly, as Mast (1923) suggests, by the accumulation of waste materials.

The sporoblast capsules of all Sporozoa, with the exception of the Cnidosporidia (p. 552), are formed as a result of the first activities of the young fertilized cell and they do not occur again. The same phenomenon is characteristic of zygotes in Sarcodina. With Infusoria where fertilization is accomplished through conjugation such zygote cysts are practically unknown, but encystment, with reorganization processes, is possible during the early period of the life cycle until maturity, when it is apparently replaced by conjugation. Thus in *Uroleptus mobilis* in connection with which this phenomenon has been carefully studied, encystment may occur within three days after fertilization but usually after a longer period has elapsed. Such encystments occur under the same external conditions as do conjugations later in the cycle. So-called "conjugation tests" are made every week or ten days. For these, all of the individual cells of a series left over a daily isolation has been made are placed in a large container with fresh medium. Here they are allowed to

accumulate until thousands of individuals are present. The food medium is not replenished and such mass cultures are watched daily until the individuals die. After five or six days conjugations will

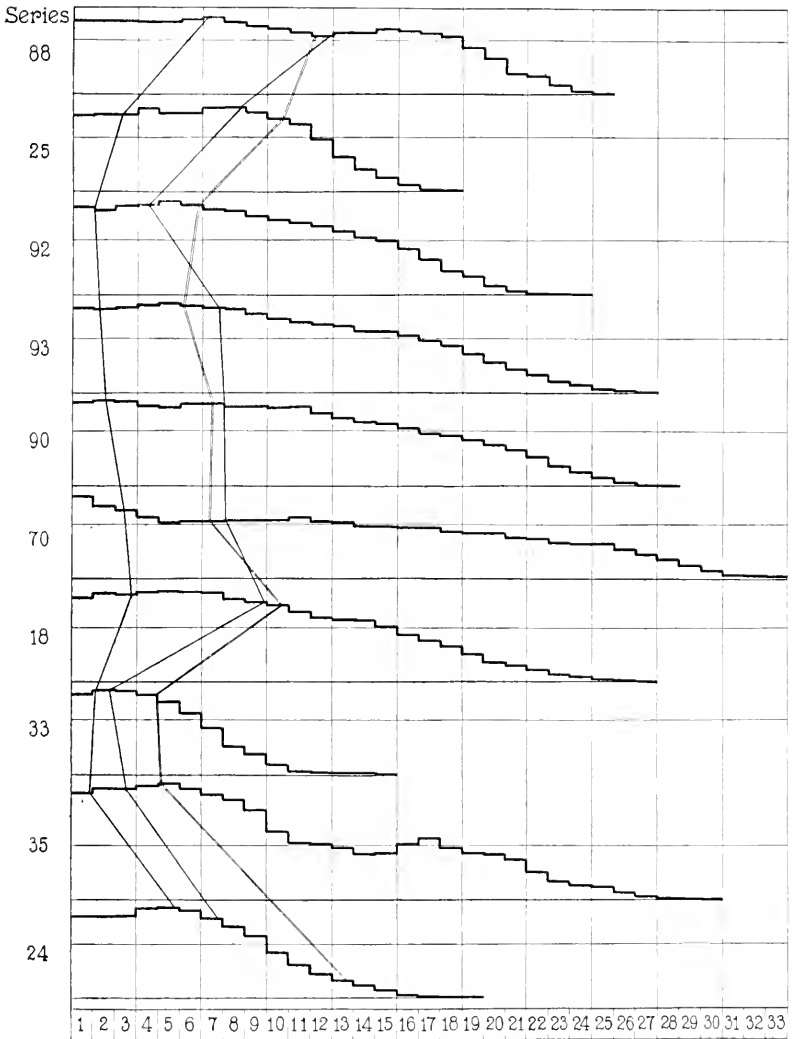


FIG. 137.—Vitality graphs showing the limited period of encystment (between the two irregular vertical single lines), and the periods at which conjugation begins (double line) in ten different series of *Uroleptus mobilis*. (Original.)

take place provided the organisms are mature; if they are not mature encystment takes place and it frequently happens that thousands of cysts are present in one container. From the records

made during the experiments it is possible to work out the incidence of encystment and of conjugation for each series. Fig. 137 shows the vitality curve of ten different series. The periods of the first encystments observed and the last encystments in the different series are connected by vertical lines. The first appearance of conjugation is indicated in the same manner but with double lines. In some series it happens that both encystments and conjugations occur in the same container but tests of the same series made later give only conjugations. With *Uroleptus* at least it appears, therefore, that encystment is a characteristic phenomenon of young organisms comparable with the Dauersporen of phytoflagellates, and lower plants generally, after fertilization; and that the power to form reorganization cysts disappears with the advent of maturity. It is highly desirable to have similar data for other types of ciliates and to determine whether or not endomixis occurs in each case.

(b.) **Cyclical Differentiations Peculiar to Old Age.**—Toward the end of the life cycle even more characteristic differentiations occur than at the outset. In many cases these are coincident with the fertilization phenomena and will be discussed in connection with differentiations at maturity. The most significant of these age differentiations are: (1) Greatly reduced vitality; (2) structural degeneration; (3) abnormal divisions leading to monster formation; (4) special structures appearing at no other time in the life cycle.

The best evidence of reduced vitality toward the end of the cycle is afforded by *Uroleptus mobilis* and *Spathidium spathula*. In the former, series after series have been followed from high initial vitality after fertilization until death occurred. In more than one hundred and forty such series the history has been the same but with variations in time and in number of generations well illustrated by the series selected from the records of different years and shown in Fig. 131. The last individuals of such series may show a remarkable tenacity in vitality but without the power to reproduce. Of 283 such "last individuals" 1 lived more than ninety days; 2 lived more than sixty days; 7 more than forty days; 15 more than thirty days; 26 more than twenty days; 88 more than twelve days; while the remainder lived from one to ten days. In all of these cases the old individuals were transferred daily to fresh medium from the same source as that in which other, younger, individuals were dividing from one to three times per day. In most of the old specimens apart from the reduced division-rates, there is little evidence of physiological weakness. They move with the usual vigor and apparently maintain an equilibrium between income and outgo for many days. This condition is the outcome of a gradually waning vitality which in turn may be due to a slowly increasing stability of substances in the protoplasmic organization, or as Robertson (1921) suggests, to accumulation of substances

which can no longer be discharged from the cell. This I interpret as evidence of old age differentiation with the same fatal termination as that which follows highly differentiated gametes which fail to unite in fertilization.

In many organisms this physiological deterioration is accompanied and manifested by structural degenerations. Maupas (1888) noted the loss of micronuclei in old age ciliates as well as other degenerations involving the motile organs (Fig. 130). The observations have been fully confirmed with *Uroleptus mobilis*, particularly in regard to the loss of micronuclei, but also noticeable in the extreme vacuolization of the protoplasm (Fig. 7, p. 28). In *Paramecium caudatum* and in individuals which have not conjugated for a long period, old individuals are characterized by hypertrophy of the micronucleus and by the loss of trichocysts in the cortex.

Still another outcome of the physiological weakness is the tendency to divide abnormally, thus leading to monster formation.

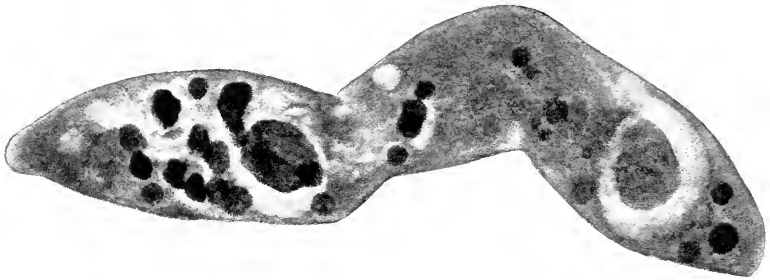


FIG. 138.—*Paramecium caudatum* monster, a type common at periods of old age. (After Calkins.)

This has been typical of all old age cultures which have come under my observation. Such monsters are strikingly like those formed as a result of cutting *Paramecium* (see *supra* p. 264), but they never grow into large amorphous masses of protoplasm which frequently develop from mutilated *Paramecium* individuals (Fig. 138).

The old age phenomena discussed above all involve a physiological weakness or reduced vitality which may well be traced back to increasing stability of protoplasmic substances, and lead to a break-down in the protoplasmic organization. A fourth type has to do with protoplasmic differentiations of a formative character and involves structures which appear for the first time, and only, when the protoplasm is old, probably as a result of the cumulative differentiation which has taken place. The sporoducts of gregarines furnish a good illustration of this phenomenon. Here in *Gregarina cuneata*, according to Kuschakewitsch (1907), the old nucleus gives rise to a minute germinal nucleus while the remainder is distributed as chromidia throughout the cell. The characteristic sporoducts

grow into the brood cavity of the gametocyst in the form of tubules at the bases of which the observer found collections of chromidia (Fig. 125, p. 240). Similar observations have been made upon other sporoduct-bearing forms (*Clepsidrina*, *Gregarina orata*, etc.). These are final products of protoplasmic activity with the prospective function of sporoblast elimination and have nothing at all to do with fertilization (see Chapter XIV). Also in the Cnidosporidia some of the residual nuclei and protoplasm become differentiated into sporoblast capsules while others give rise to the peculiar polar capsules and the threads characteristic of these Sporozoa (p. 324).

In a number of Sarcodina, as in Gregarinida, there are special morphological structures for the purpose of distributing the mature products of multiple division. These are frequently quite complex, the elaters and capillitia of Mycetozoa for example, recalling the spore-disseminating elements of the higher plants. The life history is varied, the complications being due mainly to the formation of multinucleated plasmodia by fusion of numerous multinucleated cells and to fruiting or spore structures which arise from the plasmodium. According to the later observations of Jahn (1911) the plasmodium begins as a single zygote in the form of an aneuboid cell with one nucleus. This nucleus divides repeatedly, resulting in a multinucleated cell and plasmodia are formed by fusion of such cells. When mature the plasmodium gives rise to the elaters through the activity of nuclei which degenerate with the process. In some forms the old plasmodium loses water, dries and forms a hard indurated crust called a sclerotium. In the majority of forms the protoplasm becomes transformed into a tough skin or membrane, termed the peridium, which may be strengthened by deposits of lime. Other parts of the protoplasm become modified into felted spore capsules or capillitia through which the elaters ramify.

In all of these cases of old age protoplasm the evidence justifies the conclusion that the organization has become profoundly changed, the change often resulting in useful morphological and physiological differentiations. The changes are of a character, however, which prevents any recovery of vitality and death of the protoplasm results unless gamete formation and fertilization supervene.

(c.) **Cyclical Differentiations Peculiar to Maturity.**—Sexual maturity in Protozoa is not a theory but a fact demonstrated in many different kinds of Protozoa. In many cases the young form slowly grows to its adult condition; differentiations appear with continued metabolism until the individual becomes a gamont and gives rise to gametes. Thus in polycystid gregarines the sporozoite slowly grows to its definitive size and differentiations appear with that growth. The protoplasmic conditions leading to gamete formation may, with equal reason, be regarded as evidence of still further differentiation in the protoplasmic organization. In Schizogre-

garinida and in Coccidiomorpha an asexual reproductive cycle intervenes between the sporozoite and the gamont and the same is true in the Foraminifera. In Infusoria, as Maupas long since demonstrated, fertilization is possible only after a period of vegetative metabolism and reproduction. Sexual maturity in general therefore, like other conditions of protoplasm, may well be interpreted as evidence of specific differentiations of the protoplasmic organization.

Few problems in biology have attracted more attention than those associated with sex, and attempts to interpret the phenomenon have been as varied as they are sometimes ingenuous. The very definition varies with different interpreters, the usual definition involving association of the concept sex with peculiarities of structure and function which enable an observer to distinguish males from females. Others regard sex as evidence of a fundamental difference in protoplasm, one type giving rise to males, another type to females as in Weininger's arrhenoplasm (male-producing) and thelyplasm (female-producing). Or the differences of sex, according to Minot (1882) and Schaudinn (1904), are due to specific types of chromatin both of which are present in all individuals derived from a fertilized cell, but male chromatin predominating in males, female chromatin in females. Still others interpret sex differences as originating through metabolic activities, segregation of protoplasm thus differentiated, and distribution by inequalities in division of the cell as Bütschli first suggested.

Not only somatic differentiations with their specific functions, but products of such differentiation in the form of gametes together with the causes which bring about the attraction and fusion of gametes, are all bound up in the ultimate significance of sex. Somatic differentiations indicating male or female types are extremely rare in Protozoa, but problems of gamete formation and fusion are presented by Protozoa of all kinds and, so far as it applies to such problems, the term sex and its connotations apply to the unicellular animals.

There is little reason to doubt that a fundamental effect of sex is the perpetuation of the species through union of gametes; and there is equally little reason to doubt that the same function underlies conjugation and fertilization generally in Protozoa. It is tacitly understood by biologists that the sum total of conditions leading to the production of eggs or of spermatozoa is typical of the female or of the male, hence egg-like gametes in Protozoa are regarded as the result of female activities, while spermatozoa-like gametes come from males. This line of thought has led to the widespread custom of describing macrogametes in Protozoa as female and microgametes as male organisms. A difficulty has arisen, however, in connection with the entire absence of visible differences

between the gametes of many species distributed amongst all groups of Protozoa, and here, obviously, the attempt to apply any definition of sex fails completely. Yet such fertilizations are as fruitful and as important for the species as are those in which genetic differences are well-marked.

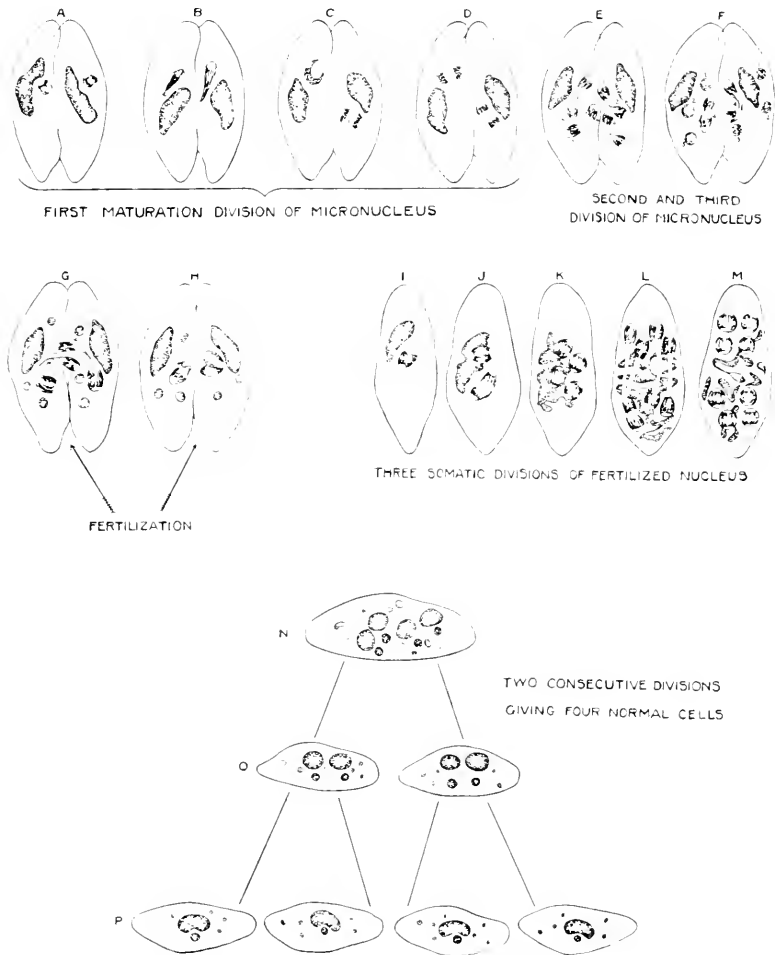


FIG. 139.—*Paramecium caudatum*. Diagram of the fertilization processes. (After Calkins.)

There are two fundamental biological problems associated with the formation and fusion of gametes. These are: (1) The explanation of the origin of gametic differences, and (2) explanation of the phenomenon of attraction of gametes followed by their tem-

porary or permanent fusion. It would be mere presumption to claim that our present state of knowledge permits an explanation of these phenomena, but there is an abundance of data from which working hypotheses may be deduced.

Gametic Differences.—In Metazoa differences in gametes are reduced to practically those between egg and spermatozoön. In Protozoa there is no common type of difference but all gradations may be found here, from apparently similar individuals to differentiated eggs and spermatozoa. This has led to attempts to classify gametes for purposes of description, into those which are similar (isogametes) and those which are dissimilar (anisogametes). Similar gametes, however, may be minute derivatives of adult individuals—microgametes—or they may be adult individuals which cannot be distinguished from ordinary asexual, vegetative individuals. The latter type is represented by the vast majority of Infusoria, and, as Minchin maintained, there is very little justification for calling them gametes at all; yet they come together for purposes of fertilization and to this extent at least resemble gametes. In the majority of Protozoa fertilization involves the permanent fusion of cell bodies as well as of cell nuclei and the term copulation is applied to such cases. In the Infusoria fertilization involves the permanent fusion of nuclei only, while the cell bodies, with few exceptions, are incompletely fused and this is only temporary (Fig. 139). To this phenomenon the term conjugation is given. A conjugating ciliate, however, is physiologically different from a vegetative individual and may be distinguished by the general designation gamont. These considerations lead to the following classification:

(a) *Conjugation.*—Temporary cell fusion of gamonts; permanent nuclear fusion.

(b) *Copulation.*—Permanent fusion of cell bodies and cell nuclei of gametes.

Gametes	{	A. Isogametes	{	(a) Similar macrogametes or gamonts (hologametes).
				(b) Similar microgametes.
		B. Anisogametes	{	(a) Dissimilar microgametes.
				(b) Macrogametes and microgametes.
				(c) Egg and spermatozoa (oögamogamy).

(a) *Hologametes and Conjugants.*—The nearest approach to conjugation of the ciliates is to be found in the fertilization phenomena (pseudo-conjugation) of the Sporozoa, particularly in the Gregarinida. Here, two gamonts (gametocytes) come together but do not fuse; after the formation of a common gametocyst each cell

proceeds to form a number of gametes which may be isogamous or anisogamous. After the gametes are formed the gametocytes degenerate and disappear while the gametes fuse two by two in copulation. In the coccidian *Adelina* the phenomena are more nearly like those of the ciliates. Here a microgametocyte and a macrogamete become associated in conjugation and without the formation of a cyst membrane (gametocyst). The former produces four or more microgametes by division and one of these penetrates the macrogamete and fuses with its nucleus (Fig. 140). One of the conjugants thus resembles a ciliate while the other one, the microgametocyte, resembles a gregarine in that it degenerates and disappears. In ciliates there is a mutual formation of gametic nuclei, a mutual interchange and a mutual fertilization. Here both individuals correspond to the macrogamete of *Adelina* and fertilization is mutual.

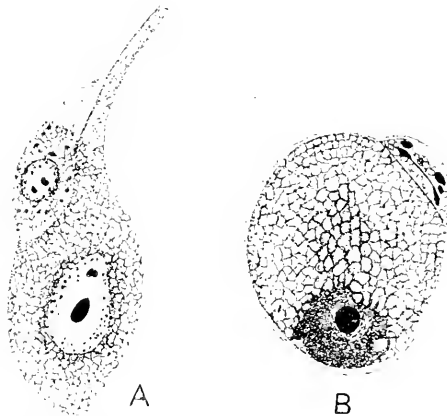


FIG. 140.—*Adelina dimidiata* A. Schn. A, association of macrogamete and smaller microgamete. B, nuclear divisions in microgamete and formation of gametic nuclei. $\times 1400$. (From Dofflein after Shellack, Arbeit, aus d. kaiserlichen Gesundheitsamt, courtesy of J. Springer.)

It is possible that the peculiar conditions existing in present-day ciliates may have resulted from conditions of pseudo-conjugation as illustrated by the present-day gregarines, and that originally, a group of gametes were formed which united to form zygotes outside of the parent cells, or inside as in the case of *Ophryocystis mesuili*¹ (Fig. 120, p. 231). On this hypothesis which has been very generally accepted by protozoologists, the fusing nuclei of conjugating ciliates are interpreted as the nuclei without cell bodies

¹ Some of the parasitic ciliates suggest the gregarines in their conjugation phenomena. Thus in *Balantidium coli*, according to Brumpt (1909), two individuals come together and form a common enveloping cyst membrane within which the two cells now completely fuse.

of gametes, such as those of *Ophryocystis*. An interesting observation by Dogiel (1923) on the parasitic ciliate (*Cycloposthium bipalmatum* and in other Ophryoscolecidae as well (Dogiel, 1925) lends some support to this theory. Here gametic nuclei are formed as in other ciliates; one of these nuclei, the migrating nucleus, develops a tail and, like a spermatozoön, makes its way through the membrane of the peristomial region of the mother-cell, and into the external chamber formed by the mode of fusion of the two gamonts (Fig. 141). From this chamber it enters the other gamont by way of the mouth and ultimately meets and fuses with the stationary nucleus of this gamont.

(b) *Isogametes and Anisogametes*.—The term copulation as used in connection with the Protozoa refers to total and permanent

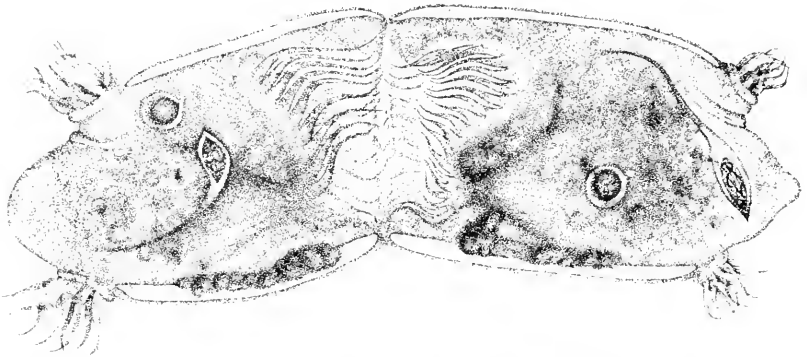


FIG. 141.—*Cycloposthium bipalmatum*. Conjugating individuals with spermatozoön-like wandering nucleus. (After Dogiel.)

fusion of gametes. Of these there is the greatest variety of structures and differences in different types of Protozoa. In very few cases of isogametes do we find copulation between individuals whose differentiations are not expressed by morphological characteristics. In such types the individuals differ little if at all from the ordinary vegetative forms except in a physiological sense. Plastogamy or casual cell fusion is easily mistaken for such hologamic copulation and descriptions of so-called fertilization processes in testate and in naked rhizopods, in Heliozoa and in different types of flagellates are open to criticism on this ground. In the case of *Helkesimastix faecicola* and *H. major* (Woodcock and Lapage, 1915, and Woodcock, 1921) the evidence, from observations on living cells, seems to indicate that copulation of these flagellates does occur, but even in these cases the interpretation is not above criticism in the absence of cytological confirmation.

The majority of isogametes show morphological characteristics

which easily distinguish them from agametes or vegetative individuals. In many cases the physiological differences at maturity are expressed by a change in the type of division whereby binary fission is replaced by multiple division. Many daughter cells are thus formed from one gametocyte and the term microgametes has been applied to such a brood. The copulating gametes, however, show no distinguishing morphological characteristics and the differences between them if there are any must be of a chemical or physical nature. In Foraminifera such isogametes are the rule and their formation indicates a well-defined cyclical differentiation of the parental protoplasm. Thus in *Polystomellina crispa* according to Schaudinn (1903) and Lister (1905); in *Peneroplis pertusus* according to Winter (1907); in *Trichosphaerium sieboldi* according to Schaudinn (1899) and in Foraminifera generally, the young protoplasm after fertilization forms one type of organism termed the microspheric generation which after nuclear fragmentation and chromidia formation reproduces by agamete formation (Fig. 123, p. 235). Such agametes develop without fertilization into organisms of a different type, the difference being shown by the larger size of the initial shell chamber, hence a macrospheric generation. After metabolic activities and full growth the macrospheric organism breaks down into a multitude of isogametes which have an entirely different organization from that of the agametes. Whereas the latter are pseudopodiospores, the isogametes are flagellispores, each bearing two similar flagella, and copulation occurs by union of two of these similar flagellispores (Fig. 123, A, C).

According to Schaudinn's interpretation of the fertilization processes in *Actinophrys sol* (1896) there is a permanent fusion of similar adult cells (hologametes). But the recent investigations of Bělař (1922) show that one of the apparent hologametes develops a pseudopodial process which is the first to unite with the other gamete and undergoes its meiotic divisions more quickly than does its mate (Fig. 142). Similar minute differences in microgametes are characteristic of *Monocystis rostrata* but the differences become more pronounced in *Pteroccephalus nobilis*, *Schaudinella henleae*, or *Stylorhynchus longicollis*. In Sarcodina, apart from *Actinophrys sol*, there are few cases in which the full development and fusion of anisogametes have been convincingly demonstrated. Schaudinn (1903) described the formation and union of anisogametes in *Centropyxis aculeata* but the confirmation of his arcelliform gametes has not yet appeared. Elpatiewsky (1909) described the fusion of anisogametes in *Arcella vulgaris* as a part of a very complex life cycle. In both of these testate rhizopods the nuclei of the gametes are derived from chromidia formed in the gametocytes while the cell bodies are formed by multiple division of the protoplasm. In Radiolaria, according to Brandt (1885) and Borgert (1900), the

same central capsular protoplasm gives rise to anisogametes in the form of two types of flagellated swimmers, but fusion of gametes was not observed.

Knowledge of the life cycle in *Radiolaria*, however, appears

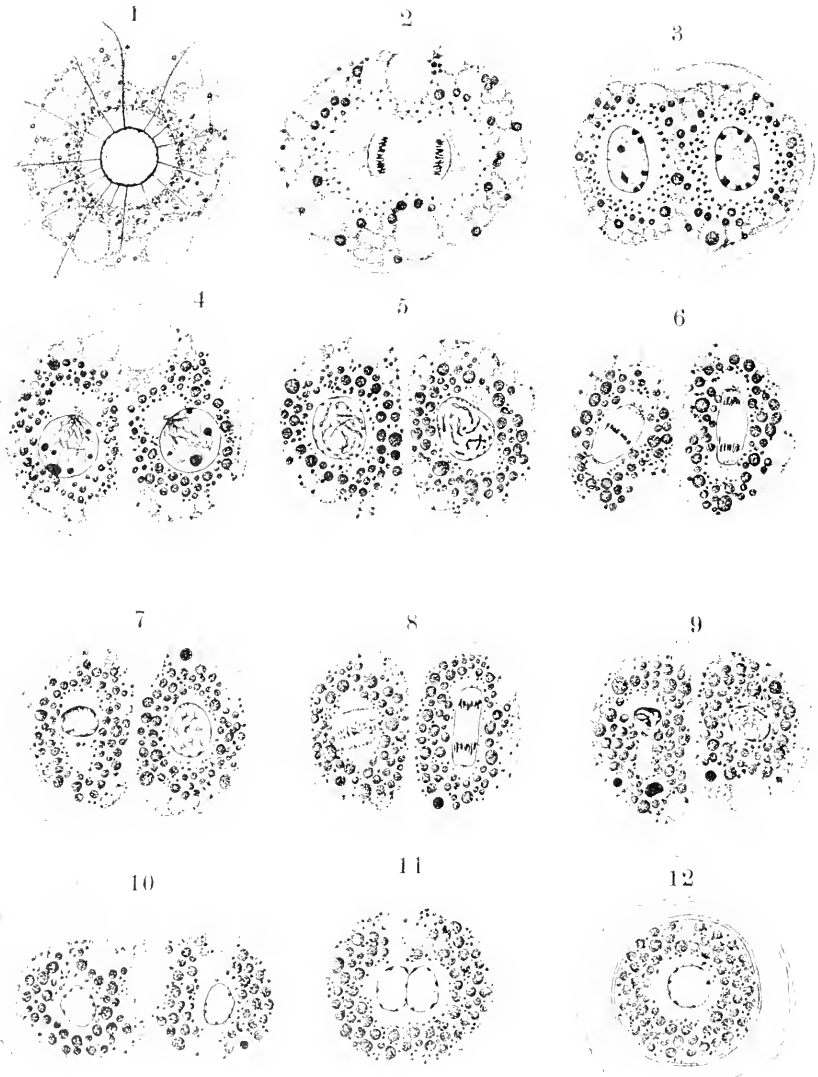


FIG. 142.—*Actinophrys sol*, maturation and copulation of gametes. 1, section of individual prior to fertilization; 2, 3, division of nucleus and cell to form two gametocytes; 4, 5, 6, first meiotic division of the two gametocytes; 7, 8, 9, second meiotic division and formation of gametes; 10, differentiation of the gametes; 11, 12, fusion of cell bodies and nuclei. (After Belar.)

to be inversely proportional to the numerical importance of the group. Division of the complex organization occurs in some cases, *e. g.*, Aulacantha—the nucleus dividing first, then the central capsule, after which the extracapsular plasm with the skeleton divides, so that each daughter cell retains one-half the skeleton and regenerates the other half. In species with a firmly-knit skeleton, if a special mouth opening is present, one of the daughter cells emerges and builds a new skeleton (see *Gromia*). In some cases (Thalassicollidae and Tripylea) divisions of the nuclei and central capsules outrun divisions of the extracapsular plasm so that individuals often remain for considerable time with two, four or eight central capsules recalling the permanent condition of colonial Radiolaria (*Collozoum*, etc.). In some species, representing Spumellaria, Acantharia and Tripylea, multiple division occurs, resulting in broods of isospores (*e. g.*, Polycyttaria) or in some cases anisospores which may be formed by the same parent, or by different parents. Isospores are generally regarded as agametes while anisospores are usually interpreted as macrogametes and microgametes, a conclusion confirmed by Hartmann's observation of their copulation. Chatton (1923), however, holds that, in some cases at least (Polycyttaria and Collodaria), these anisospores are derived from intracellular dinoflagellate parasites (genus *Merodinium*). Both Hartmann and Bělař contend that this is a case of parallelism which may indicate some phylogenetic relation between Radiolaria and Dinoflagellida, for isospores, which undoubtedly are normal stages in Radiolaria life histories, have Dinoflagellate characters in their nuclear division figures and in their body form. Further information on the life history of Radiolaria is very much needed.

A further stage in the manifestation of differentiation at times of maturity is shown by those Protozoa in which the form, character and size of the fusing gametes are widely different. Here progressive differentiation has followed two general directions resulting, in one direction, in the formation of large, usually quiescent, food-stored cells, the macrogametes, in the other direction, in minute highly motile cells, the microgametes. In these cases furthermore the differences in the gametes may be followed back through the gametocytes for several generations so that cells destined to give rise to macrogametes or to microgametes may be distinguished at an early period.

Examples of this type of anisogamy are practically limited to the Coccidiomorpha. In the Ciliata, however, there is a partial differentiation in this direction in the Vorticellidae where a larger and attached individual—the macrogamete—is scarcely distinguishable from vegetative agamonts, while the microgametes are one-eighth as large and are formed by three successive divisions of the microgametocytes (Fig. 143). The microgametes always become

detached and swim about actively until they perish or meet and fuse with a macrogamete.

A complete differentiation, or oögamry, is shown by the majority of Coccidiomorpha amongst the Sporozoa. In some cases, however, notably in the genus *Adelina*, gamete differentiation is of the same general type as in the Vorticellidae. In other cases a multitude of minute sperm-like gametes are formed from the microgametocyte while the macrogamete appears like a slightly modified vegetative individual (Fig. 144). In *Cylospora karyolytica*, Schaudinn (1905) maintained that differences shown by the mature gametocytes could be followed back to the sporozoites from which they came.

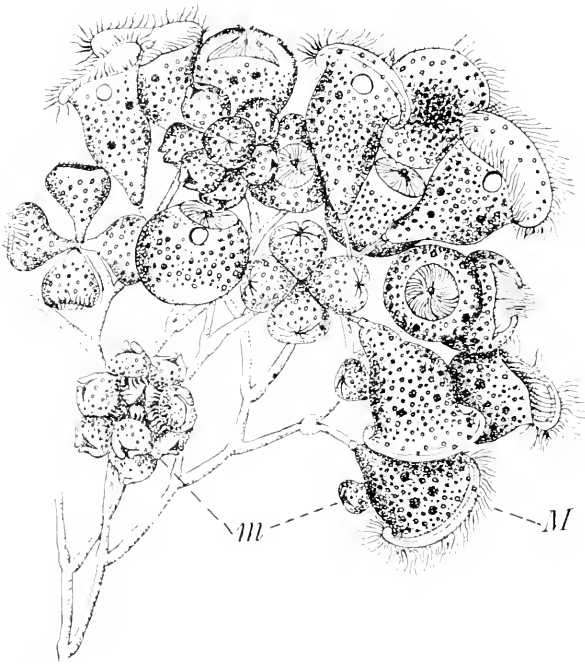


FIG. 143.—*Epistylis umbellaria*; colony with mature macrogametes and microgametes and their fusion (*m*) and (*M*). (After Greeff.)

In these various cases we find quite variable expressions of differentiation in the protoplasm of a given species. This differentiation appears to be cumulative in the life cycle and the same initial protoplasm through differentiation in two directions may, at maturity, give rise to both types of gametes. Anisogametes illustrate not only the cyclical differentiation resulting in a different type of reproduction from that of the usual vegetative type, but they also illustrate the two divergent effects which such differentiations may

bring about, one leading to relatively greater stability, storage of metabolic products and relative inactivity, the other leading to a more kinetic organization with freedom from metabolic products. As one would expect there is every gradation in the relative differentiation of anisogametes, from hologametes to egg and spermatozoon.

If the differentiation in two directions is manifested at the very outset of a life cycle in organisms developing from zygotes, one

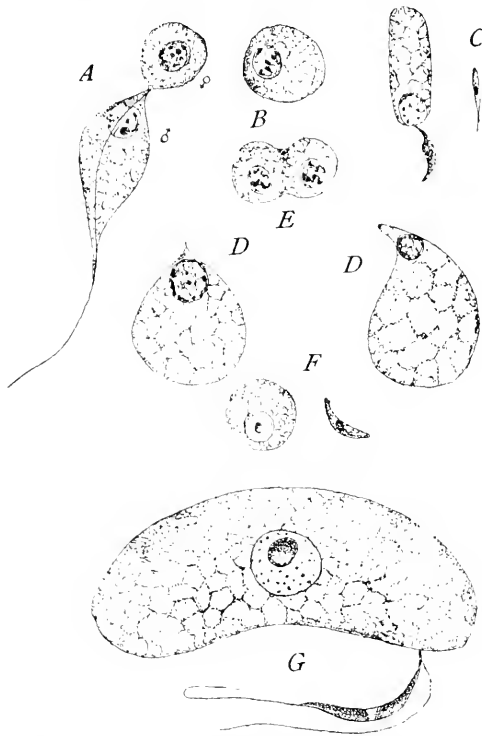


FIG. 144.—Gametes of Gregarines and Coccidia. A, male and female gametes of *Stylorhynchus longicollis*; B, *Monocystis* sp.; C, spermatozoid of *Echinomera hispida*, to the left the two gametes of *Pterocophalus nobilis*; D, gametes of *Uraspora lagidis*; E, of *Gregarina ovata*; F, of *Schaudinella hubeae*; and G, of *Eimeria schubergi*. (From Shellack after Léger, Cuñot, Brasil, Schmitzler and Schaudinn.)

ultimately giving rise only to macrogametes, the other only to microgametes, then we are dealing with a matter of inheritance or fundamental organization and not with progressive or cumulative differentiation through metabolic activities. In such instances, particularly if the differentiations are manifested by structural features whereby one type can be distinguished from the other we are justified in using the term sex in the same sense as used for Metazoa.

Résumé.—In the preceding pages an hypothesis has been developed for the purpose of bringing together a large array of disconnected facts in one comprehensive biological generalization. The underlying principle is the irritability of protoplasm as manifested by the phenomena of adaptation. The fundamental organization or particular type and arrangement of the proteins, carbohydrates, salts and other constituents of living substance is specific for each kind of organism. Vitality is interpreted as the aggregate of chemical and physical reactions going on between and among the diverse parts of the organization and between these and the environment. Adaptation is the response of the organization to unusual conditions. It involves somewhat changed reactions and these in turn may involve new substances which may or may not be the basis of new morphological elements, but the fundamental organization becomes at least somewhat modified. The inciting causes of such changes may be of environmental or of internal origin. Among the latter are new combinations which occur with amphimixis. Here, also, are the substances which are formed as a result of metabolism, particularly of oxidation. These may or may not be labile, *i. e.*, subject to reversal of phase in a physical sense, or to participation in the vortex of vital activities generally. If not labile they become metaplastids and may or may not serve some useful purpose for the organism. If such products of activity are labile, new combinations with other substances in the protoplasm are possible and the results are manifested as differentiations.

On this basis we interpret the differentiations which appear with the intake of water and oxygen by an encysted organism or the various activities characteristic of Protozoa during the early phases of the life history. On the basis of changes due to general metabolic activities and due to the specific organization of any particular form, we interpret the drastic alterations which accompany and characterize cell division. These involve the changes in physical condition of the various colloidal substances, such for example, as the increase in permeability due possibly to the accumulation of hydrogen ions, and the absorption of water. They also involve cytolytic activities as indicated by the disintegration and absorption of kinetic elements, of eliminated nuclear chromatin and division of all the substances active in vitality. The conditions under which these divisional activities are manifested represent inter-divisional differentiations which are reduced or cast out through protoplasmic activities at division, leaving the organization in a labile state characteristic of the early inter-divisional period. If the reorganizations effected by these divisional activities are always the same generation after generation, then, on the hypothesis, there is no *a priori* reason why under appropriate environmental conditions, metabolic activities or vitality, should not

continue indefinitely (see Child, Hartmann, Bělař, Jollos, etc.). Such is the explanation that I would give of continued life without fertilization of animal flagellates, aided here possibly by changes which may take place during the periods of encystment. On the same basis we find an explanation of the long-continued isolation cultures without fertilization of organisms which, under usual conditions, undergo fertilization. Some types of organization are evidently able under appropriate conditions of the environment to return to the same labile organization after each division. Such types would thus have a prolonged asexual cycle, possibly, as Enriques asserts, as long as the observer cares to continue the culture. In such cases it is not improbable, as M. Robertson (1929)



FIG. 145.—*Paramecium caudatum* in a period of depression and recovery by treatment with salts. (After Calkins.)

concludes, that the environment is so stabilized that its stimuli do not call out the cyclical changes which might be expected with an irritable and adaptable protoplasmic organization.

If, however, reorganization as effected by division does not leave the protoplasm in its original labile condition, then inter-divisional activity of the progeny starts with a different organization than did the previous generation and this, continued generation after generation produces an accumulative effect. This is manifested by physiological activities and by structural modifications not shown before. The decline in the division-rate for example may indicate that the living substances are becoming relatively stable and more and more irreversible in phase, as was the case with one race of *Paramecium caudatum* in which the individuals became homogeneous

and black in appearance with complete loss of the usual vesicular character (Fig. 145). This particular condition was relieved by the use of electrolytes (K_2HPO_4 , KCl , etc.) added to the usual medium. In extreme old age in ciliates there is apparently a cessation of the intricate activities involved in cell division. Evidence of this is the tendency to form monsters and the tendency of parts to undergo degeneration, nuclei, motile organs, kinetic elements, etc., in particular.

Between the extremes of youth on the one hand and old age on the other is a condition of cumulative differentiation termed sexual maturity. In this condition phenomena occur which do not occur earlier and the organization may become visibly altered. Thus gregarines lose their attaching organs and become gamonts; the physical condition of *Paramecium* changes to such an extent that two individuals will fuse on contact at any part of the cortex (the author has observed an amorphous group of nine such partially fused individuals); or the phenomena of plastogamy in general are possible under such conditions of differentiation.

With the protoplasm in this latter condition due to continued metabolism further differentiations are possible and, carried out in different directions, lead to specializations characteristic of gametes. As Bütschli first suggested, inequalities in division may account for the differences in gametes, a possibility indicated by the more irritable anterior region of the ciliates, or by the more active pulsations of the anterior contractile vacuole in *Paramecium caudatum*, or posterior vacuole in *P. aurelia* (Unger, 1926).

When such differentiation progresses to the point of isogamete and anisogamete formation further constructive activities and reproduction are no longer possible, and if fusion is prevented, the gametes die. With the ciliates this is true only of the Vorticellidae. In other ciliates, differentiations at sexual maturity have not proceeded far enough to seriously affect the general metabolism and power of reproduction. This is demonstrated by experiments with "split" pairs, or separation of two individuals recently united in conjugation, an experiment first performed by Hertwig (1889) and later by Calkins (1904, 1919) and by Jennings (1909). Here an individual, thus separated, continues with the same division-rate that it would have had had it not conjugated. Yet the history of isolation cultures with exceptions noted above shows that ultimately if conjugation and parthenogenesis are continually prevented, the race, like anisogametes, will die.

CHAPTER VIII.

PHENOMENA ACCOMPANYING FERTILIZATION.

IN the preceding chapters we have endeavored to show that continued metabolism leads to changes in the organization of Protozoa whereby phenomena of a cyclical nature in the life history are possible. Among such changes are those which underlie activities at periods of sexual maturity including gamete formation. In the present chapter we will consider the activities which take place immediately before, during, and immediately after fertilization, phenomena which are involved in any attempt to interpret the effects of fertilization. Here we have to do both with protoplasm which has become so changed in organization that further metabolism is impossible, as in highly specialized gametes, and with protoplasm which is so little changed that metabolic activities are still possible. The special problems to be considered in this connection are: (1) The protoplasmic and the environmental conditions under which fertilization occurs; (2) fertilization types; (3) the internal phenomena of maturation and reduction in number of chromosomes; (4) the immediate metagametic internal activities involved in reorganization; (5) parthenogenesis.

I. THE ENVIRONMENTAL CONDITIONS OF FERTILIZATION.

(*a*) **Ancestry.**—Attempts to analyze the conditions under which fertilization by fusion of gametes, or by conjugation, takes place have been made in relatively few cases. Since the first of such attempts, and the majority of later ones, have to do with the conditions of conjugation in ciliates we may consider these first. Of the three conditions cited by Maupas (1889) as necessary for fruitful conjugation—sexual maturity, diverse ancestry, and hunger—the last one only has to do with environmental conditions. The second condition, however—diverse ancestry—was considered so important by Maupas and has been so frequently called upon in explanation of results obtained by many subsequent investigators, that it cannot be ignored. Maupas found that individuals of the same ancestry either would not conjugate at all among themselves, or if they did the ex-conjugants were weaklings and soon died. He also found that, with other evidences of degeneration, closely related individuals of extreme old age showed a tendency to conjugate and that such conjugations always lead to sterile results or to abnormal ex-conjugants which quickly die.

Largely as a result of these conclusions of Maupas an unwarranted

importance has been attached to the relationship of gametes, and fertilizations have been described as exogamous, endogamous, autogamous, or pedogamous. Of these the third refers to self-fertilization and the second and fourth to union of closely related individuals. Such terms serve a useful purpose for descriptions but are without significance in the matter of effective fertilization. It is quite possible, however, that a brood of gametes from the same gametocyte will have a common physical and chemical make-up and will not be attracted to one another but will meet and fuse with apparently identical gametes from another gametocyte. This appears to be the case with *Polystomellina crispa* according to Schaudinn (1903) and also of gregarines. The significance of ancestry however, appears to be in the matter of mating rather than in that of effective fertilization and belongs to the same group of phenomena as the fact that sperm cells do not unite with sperm cells or eggs with eggs. With Infusoria Maupas' conclusion has not been supported by later observers. Calkins (1904) found that fully as many conjugations between closely related forms of *Paramecium caudatum* were fruitful as between forms of diverse ancestry, and one such ex-conjugant from a closely-related pair, was followed through 379 generations by division. Similar evidence has been furnished by isolation cultures of *Didinium nasutum*, *Paramecium aurelia*, *Paramecium bursaria*, *Stylonychia* sp., *Blepharisma undulans*, *Spathidium spathula*, *Oxytricha fallax*, and *Chilodon cucullus*. With *Uroleptus mobilis* the protoplasm of one individual gave rise to progeny which would conjugate whenever the proper conditions were provided, and the 140 series derived from ex-conjugants from such unions furnish ample proof that the conjugations were fruitful. Such results indicate that Maupas' conclusion regarding the necessity of diverse ancestry was incorrect.

(b) **Environment.**—One unmistakable conclusion can be drawn from the many diverse observations and interpretations of the conditions under which fertilization occurs in ciliates, viz., the protoplasmic state with which conjugation is possible is induced in large part, but not wholly, by environmental conditions.

In practice the simplest way to obtain conjugations in ciliates is the method adopted by Maupas. A pure culture of the organism to be tested is allowed to multiply freely in a rich culture medium; a large number of these are then transferred to a smaller container with enough of the original medium in which they had developed to make it unnecessary to add fresh medium. In this second container, conjugations will appear in from twelve to thirty-six hours provided a mixed population is present or if the organisms are mature. In a similar way conjugation tests are made at regular intervals in all complete isolation culture work. Such tests have been made with *Uroleptus mobilis* approximately every ten days.

The usual interpretation of this result is not very enlightening; it runs somewhat as follows: After abundant feeding and active division the large numbers of individuals produced soon exhaust the food, and hunger follows; conditions thus due to hunger result in conjugations provided the organisms are mature. Jennings (1910) qualified this general statement by emphasizing the necessity of a preliminary period of active multiplication in a rich food medium. "The cause of conjugation," he states, "is a decline in the nutritive conditions after a period of exceptional richness that has induced rapid growth and multiplication" (*loc. cit.*, p. 292). All experimenters since Maupas have used this method with more or less success and it appears to be empirically sound. Some observers, however, interpret the phenomenon as due exclusively to such purely environmental conditions. Thus Chatton (1921) argues that inanition is indeed an "internal condition" but the lack of food which causes it is an external factor. "Inanition," he says, "is a condition which is practically all that is needed for conjugation; it is an almost certain means of obtaining conjugations in no matter what wild culture, and becomes the chosen technical means of producing them. In current theories, however, conjugation is regarded as independent of the external conditions, inanition playing only an occasional rôle" (*loc. cit.*, p. 131). Yet, in a footnote (p. 135), Chatton very properly calls attention to the fact that conditions which call forth conjugations in nature do not cease after conjugation is ended. Indeed it is an unwarranted assumption to explain conjugations in nature as induced by a period of rich feeding followed by a period of lack of food, and this in turn replaced by a rich nutrient medium useful to the ex-conjugant. To this extent the method employed in the laboratory to obtain conjugating pairs is entirely artificial. Chatton's reflections and conclusions supporting the view that external conditions are alone responsible for conjugation are included in his excellent description of the structures, division, and conjugation of parasitic ciliates of the family *Nicollellidae*, particularly *Nicollella* and *Collinella*. In the former the conjugating individuals measure approximately one-fifth of the vegetative forms; in the latter approximately one-half, in both types the conjugating individuals differ in morphological details from the vegetative forms. He interprets these changes as due to the particular part of the digestive tract to which the parasites are carried. Chatton's perplexity and call for further experimental evidence in solving the *raison d'être* of conjugation is justified and the problem will probably remain perplexing so long as external conditions alone are regarded as the controlling factors. In more recent work (Chatton, E. and M., 1927) on *Glaucoma scintillans*, both internal and external factors are regarded as necessary for conjugation.

Of these external conditions other factors than the supply of food may, and apparently do, play a part. Enriques (1903, 1905, 1909, etc.) has long maintained that the phenomena of degeneration and senescence are caused at bottom, not by internal conditions but by external causes, apparently by the accumulation in the medium of bacterial products which poison the organism. Hance (1917) held that they are caused by the concentration of katabolic products derived from the organism and accumulate in the medium. Enriques also makes the statement that upon filtering the liquid in which conjugating forms are present and adding non-conjugating individuals to it, the latter will conjugate; on the other hand a similar liquid with non-conjugating individuals if filtered and used as medium for conjugating individuals, will act as a deterrent to conjugation. Repeated attempts on our part with *Didinium nasutum*, *Paramecium caudatum* and *Uroleptus mobilis* have failed utterly to confirm these results. There is more evidence for his conclusion that salts in the medium are necessary for conjugation, a conclusion based upon his experiments with NaCl, NaBr, and NaI in certain concentrations, on the ciliate *Cryptochilum nigricans*. These particular salts together with strong solutions (1 to 10,000) of CaCl₂ and Fe₂Cl₆, produced epidemics of conjugations, while weak solutions of the last two salts inhibited conjugations. Still more extensive experiments along the same line were made by Zweibaum (1912) on *Paramecium caudatum*. Dilute salts, AlCl₃ in particular, added to the medium after a long period of rich feeding, followed by a period of hunger of five to six weeks (sic) produced almost complete epidemics. No salts at all, or very strong salts added to the medium caused no conjugations. These results are certainly suggestive but the experiments should be repeated with carefully controlled material and with some other type than *Paramecium*. With this organism Hopkins (1921) failed to confirm these results. Some rather incomplete and unconvincing experiments by Baitsell (1912) may also be cited in this connection. Two lines of *Stylonychia* from the same ancestral cell, were cultivated on different media; one line on hay infusion, the other on beef extract. Individuals of the former line refused to conjugate while those of the latter line conjugated. From this Baitsell concluded that the determining condition was the medium used. Chatton (E. and M., 1931) concludes that certain types of food will induce conjugation in *Paramecium* while other types will not. Calkins and Gregory (1914) found that in the same medium some lines would conjugate regularly while other lines from the same ancestral cell would not conjugate at all or conjugate only after nine months of continued culture (see also Hopkins, 1921).

A full consideration of the evidence that has accrued in support of the thesis that external conditions are alone responsible for the

onset of conjugation leaves one with the same perplexity that troubles Chatton, Woodruff, and others and calls forth the same demand for further experimental evidence. Indeed some embarrassing questions based upon what we already know must be answered: If it is environment alone, what are the external conditions responsible for the formation of the gametes in Coccidiomorpha, Gregarinida, Foraminifera and Phytomonadida? Or in the ciliates what is the explanation of the failure of external conditions to induce conjugations in some lines and not in others? Or why will the same external conditions fail with youthful forms when they are successful with older (mature) forms?

In practically any epithelium deeply infected with coccidia adjacent cells contain vegetative stages of the organism, agamont stages in reproduction, gametocyte stages of both kinds, and nearby are zygote stages. If conditions of the infected host cell are responsible for the different phases it must be a very delicate difference that calls out asexual reproduction in one and gamete formation in another, and all within the radius of a single field of the microscope. If products of degeneration of an infected host cell cause gametocyte differentiation in one organism why do not the products of the cell next to it produce a similar effect on its contained organism instead of which we find the latter reproducing asexually? The conception of external factors as the sole cause of protoplasmic changes leading to fertilization must be very elastic to cover such cases. Why are not all malaria parasites transformed into gametocytes if the blood is the determining factor? *Plasmodium vivax* taken into the gut of the mosquito should be transformed into gametocytes producing gametes instead of which only gametocytes already formed produce gametes while agamonts are apparently digested; and in the blood of man or birds these gametocytes circulate with the vegetative forms and with agamonts. Surely in these parasitic forms, granted that external conditions may be provocative, some internal condition of the organism nevertheless predetermines the action of the environmental stimuli.

With ciliates every experimentalist knows that in pure line work conjugation tests are sometimes successful, sometimes not. Jennings (1913) noted this in different races of *Paramecium*; Woodruff for several years was unable to obtain a single pair from his famous culture of *Paramecium aurelia*, although ultimately they did conjugate; Calkins and Gregory (1914), cultivating the first eight individuals from an ex-conjugant of *P. caudatum* in pure lines, found that conjugations were abundant in certain lines whenever a test was made, while other lines remained negative at every test until the race was many months old. Similar tests made with any series of *Uroleptus mobilis*, and by test we mean a period of rich feeding followed by hunger, is negative if the organisms are young,

positive if the organisms are mature (Fig. 137, p. 268). All of these facts, and the literature contains many other similar cases, indicate that environmental stimuli are without effect in producing conjugations unless the protoplasm is in a condition where such conjugations are possible. Indeed, when fully mature, *i. e.*, when the protoplasmic conditions are just right for conjugation, union will take place in a rich food medium and without the transition from full nourishment to hunger. This phenomenon is abundantly illustrated in the records of *Uroleptus mobilis* and in my records of *Paramecium caudatum*, *Blepharisma undulans*, or of *Didinium nasutum*. There is little information as to the exact nature of these protoplasmic conditions prior to conjugation. Zweibaum (1922) gives good evidence to show that the quantity of glycogen in the cell is reduced to a minimum at this period, the large drops of neutral fat disappear while small droplets of another type make their appearance together with some cholesterine ester and large quantities of what was interpreted as fatty acids. These are probably effects of inadequate food material, for the observer obtained similar results with *Paramecia* under conditions of starvation which were not followed by conjugation.

II. INTERNAL CONDITIONS AT THE PERIOD OF FERTILIZATION.

In the last analysis both internal and external conditions play their respective parts in protoplasmic preparations for conjugation. Without external stimuli, without oxygen and food, vitality would soon cease; with them, vitality manifested by metabolism and reproduction will continue. With metabolism, however, the protoplasmic make up is constantly changing and these changes are shown by the general reactions and by the organization (see Chapter V). According to Hertwig (1908), Popoff (1908), and Rautmann (1909), the changes thus brought about lead to disturbances of the normal ratio of nucleus to cytoplasm (*Kernplasmaverhältnis*) and lead to conjugations whereby the normal relation of nucleus to cytoplasm is regained. Whatever the changes due to metabolism are in a given case the conclusion is forced upon us by the mass of evidence that given external conditions will provoke conjugations at one period of the life cycle and will have no effect in producing them at another period, while at the critical period of maturity external conditions may be entirely negligible as they appear to be in the *Coccidiomorpha* and in gamete-forming organisms generally. Here protoplasmic and not external conditions control the issue. There is some significance in the fact that encystment (with endomixis) is induced by the same external conditions as is conjugation. Mengheni (1913) found that *Stylonychia* will not encyst if food is abundant but that hunger and low temperature are necessary con-

ditions. With *Uroleptus mobilis* conjugation and encystment tests are made in exactly the same way and in some tests conjugating pairs and encysted forms are present simultaneously.

In the case of *Uroleptus mobilis* a mass culture of young individuals shows no tendency to agglomerate, the cells are distributed more or less uniformly in the culture. In similar mass cultures of individuals approaching maturity agglomeration in dense groups is highly characteristic. Such cultures may show no conjugations, but a mass culture made with the progeny of the same individuals a week later will show not only the initial agglomerations but epidemics of conjugation as well (Calkins, 1919).

This phenomenon of agglomerations indicates something of the nature of an attraction that increases in intensity as the organisms approach maturity and have a bearing on the problem of mating. What is it that brings two gametes together or two apparently similar ciliates? There is some evidence that the attraction is of a chemiotactic nature as illustrated by the often quoted experiments of Pfeiffer with malic acid and fern spermatozoids. Two citations from Engelmann (1876) illustrate this phenomenon with ciliates of the genus *Vorticella*: "The buds, at the beginning, swarmed about with constant and considerable rapidity rotating the while on their axes but moving more or less in a straight line through the drop. This lasted from five to ten minutes or even longer without any special occurrence. Then the scene suddenly changed. Happening in the vicinity of an attached *Vorticella* a bud quickly changed its direction with a jerk and approached the larger form, fluttering about it like a butterfly over a flower and gliding over its surface here and there as though tasting. After this play, repeated upon several individuals, had gone on for several minutes, the bud finally became firmly attached." Again: "I observed another performance still more remarkable. A free-swimming bud crossed the path of a large *Vorticella* which had become free from its stalk in the usual manner and was roaming about with great activity. At the instant of the meeting, there was no trace of a pause, the bud suddenly changed its direction and followed the *Vorticella* with great rapidity. It developed into a regular chase which lasted about five seconds during which time the bud remained about $\frac{1}{15}$ of a millimeter behind the *Vorticella* although it did not become attached for it was lost by a sudden side movement of the larger form" (*loc. cit.*, p. 583). Another illustration taken from the observations of Schaudinn (1900) on the mating of gametes of *Eimeria schubergi*, suggests an action analogous to that of attractin as described by F. R. Lillie in sea-urchin eggs. During the maturation of the macrogamete of *Eimeria schubergi*, the "karyosome" is cast out of the nucleus, breaks into fragments and the fragments are extruded from the cell, remaining, however, attached to the

periphery. The microgametes swim aimlessly about and are not attracted to the macrogamete until after these fragments are eliminated, but as soon as the granules appear on the surface the microgametes move toward them in the most direct path (*loc. cit.*, p. 257), Zweibaum (1922) observed that the glycogen content is fundamentally different in the two individuals of a conjugating pair of *Paramecium*, which may be significant in this connection. Joyet-Lavergne (1923, 1925) finds that mitochondria and lipoids in gregarines are different in quantity and in distribution in the two individuals of a pair (see p. 76).

While chemotaxis may underlie the phenomena described above, an equally intelligible interpretation might be drawn on the basis of differences in potential of a magnetic nature. Two individuals of *Uroleptus mobilis*, about to conjugate, circle about one another, twist and turn but do not become separated; finally they become lightly fused by the extreme anterior parts of their peristomes and the zone of fusion ultimately extends about half way down the peristomes. In the early stages, as with *Paramecium*, the two individuals can be separated without injury to either ("split pairs") but later the two protoplasts are welded into one, forming a protoplasmic bridge between the individuals. Experiments in cutting apart the two fused individuals have shown that immediately after contact and initial fusion the complete series of maturation divisions proceeds as though the separated individuals were still in conjugation (Calkins, 1921), and similar cutting at any time during the period of conjugation does not alter the course of the internal and consecutive processes (Fig. 155, p. 306). Ultimately reorganization of the individual follows in due course and the subsequent happenings are exactly like those of an ex-conjugant. These experiments indicate that the phenomena of maturation and of reorganization which characterize fertilization in *Uroleptus mobilis* are of the nature of an "all or none" series of reactions and when once started they go through to the end without deviation. It also appears that the stimulus which sets in motion this chain of processes is received at the time of initial contact and is mutually received by both conjugating individuals. It thus appears to be less of a chemical reaction than a physical one and has many of the attributes of a surface contact phenomenon between surfaces of different electrical potential.

III. THE PROCESS OF FERTILIZATION.

The actual process of fusion, with the exception of fertilization by conjugation, furnishes little material for descriptive purposes, two cells come together and fuse, probably with cytolysis of the contiguous cell membranes. In hologamic forms of ciliates (*e. g.*, in *Balantidium coli* according to Brumpt) which are extremely rare,

two individuals come together as in pseudo-conjugation of gregarines; they secrete a common cyst membrane and then fuse completely.

In isogamic and often in anisogamic fertilization, fusion begins as a rule with union of the flagellated ends, if the gametes are motile



FIG. 146.—*Cycloposthium bipalmatum* and *Diplodinium triloricatum*; conjugation. A, *Cycloposthium* with the two migrating pronuclei in the chamber formed by the two peristomial spaces; B, same, the two migrating pronuclei have passed from the peristomial chamber into the gullets; C, *Diplodinium* with migrating pronuclei in their passage from one individual to the other; p, pronuclei. (After Dogiel.)

(*Polystomellina*, gregarines, etc., Fig. 123, p. 235). In *Actinophrys sol* (Fig. 142) according to Bělař, one of the fusing individuals develops a pseudopodium which unites first with the other cell.

With anisogamic fertilization the microgamete is usually motile, the macrogamete is stationary and is sought by the microgamete and the same is true also of oögamie fertilization. In some cases

the macrogamete is smaller than the migrating microgamete (Fig. 144, p. 281). In the *Vorticellidae* the macrogamete remains attached while the microgamete is free-swimming.

In hologamous fertilization by conjugation there is no universal mode of fusion. In the majority of ciliates with adoral zones the fusion area is usually the anterior region of the peristomial furrow, the mouth as a rule being involved (*c. g.*, Fig. 146). In exceptional cases the mouth itself is involved in the protoplasmic bridge between the two conjugants (*Paramecium* sp. *Didinium nasutum*, *Spathidium spathula*). In *Stentor* fusion is lateral. Dogiel (1923, 1925), describes an interesting case of conjugation in *Cycloposthium bipalmatum*. Here the two individuals are united end to end, fusion occurring at the borders of the peristomes, leaving the membranelles of the adoral zone intact in a common conjugation cavity (Fig. 146). The wandering pronuclei are provided with tails and, spermatozoa-like, break through the anterior wall and into the conjugation cavity from which each enters the other conjugant by way of the mouth.

A. Meiotic Phenomena.—The meiotic phenomena in many Protozoa are apparently started by stimuli resulting from contact and partial fusion and may be divided into three types: (*a*) Conjugant meiosis, or maturation processes occurring only after union of the participating cells; (*b*) gametic meiosis (Wilson), or types in which the maturation processes are antecedent to union; and (*c*) zygotic meiosis (Wilson) characteristic of forms in which meiotic divisions occur in the zygote subsequent to the fusion of the nuclei. The first of these is illustrated by conjugating Infusoria; the second by the great majority of types in which fertilization is accomplished by permanent fusion of gametes; and the third by a few known cases among the Sporozoa.

(*a*) **Conjugant Meiosis.**—In mature ciliates the protoplasmic organization is such that the stimulus received on contact is apparently all that is needed to start up the nuclear activities associated with the phenomena of chromosome reduction and preparation of the pronuclei. These activities furthermore, have to do almost entirely with the micronuclei. Macronuclei take no part in the process of fertilization but are important in the subsequent reorganization.

With one or two exceptions (*Trachelocerca phocnicopterus*, *Spirostomum ambiguum*, etc.) all of the free-living ciliates thus far described agree in the general course of their maturation phenomena. Maupas (1889), the first to make a comparative study of different ciliates during conjugation, described eight successive phases of the process which are still applicable to practically all ciliates. Of these, Phase A is characterized by the swelling and early changes of the micronucleus; Phase B is the period of the

first meiotic or maturation division; Phase C, the period of the second meiotic division; Phase D, the third nuclear division resulting in the formation of the pronuclei; Phase E, the period of interchange and union of pronuclei; Phase F, the period of the first metagametic nuclear division; Phase G, of the second metagametic nuclear division, and Phase H, the period between the second metagametic nuclear division and the first division of the reorganized cell.

The first four of these phases have to do with the phenomena of maturation, the last four with the process of reorganization of the individual. In *Trachelocerca phoenicopterus* this succession of stages according to Lebedew (1908) is entirely absent and fertilization follows quite a different course. Also in *Euplotes charon* and *Euplotes patella* according to Maupas there is a slight variation in the usual sequence in that an anomalous, additional or preliminary division of the micronucleus takes place in each conjugant prior to the first of the two maturation divisions. In the Peritrichida also a similar preliminary division occurs but in these cases it is limited to the microgamete, the macrogamete following the usual history (*Vorticella monilata*, *V. nebulifera* Maupas; *Carchesium polypinum* Maupas, and Popoff, 1908; *Ophrydium versatile* Kaltenbach, 1915; and *Opercularia coarctata* Enriques, 1907). In the Ophryoscolecidae according to Dogiel (1925) similar progamous nuclear divisions are followed by division of the cells resulting in much smaller conjugating individuals.

If more than one micronucleus is normally present in the ciliate the first meiotic division usually takes place in all of them and the second division may occur in all, or one or more of the products of the first division may be absorbed in the cell. Some multiple micronuclei have been described in conjugating forms of *Paramecium aurelia* (Hertwig, 1889), *Onychodromus grandis* (Maupas, 1889), *Stylonychia pustulata* (Maupas, 1889; Prowazek, 1899) and *Oxytricha fallax* (Gregory, 1923) each individual having 2 micronuclei. Two or 3 micronuclei are present in conjugating *Didinium nasutum* (Prandtl, 1906); 2 to 4 in *Uroleptus mobilis* (Calkins, 1919); 4 or 5 in *Blepharisma undulans* (Calkins, 1912) and 16 to 18 in *Bursaria truncatella* (Prowazek, 1899).

1. *Phase A. The Prophase Stages of the First Meiotic Division.*—In many ciliates in which the history of maturation has been followed there is very little to distinguish the first meiotic mitosis from the usual vegetative divisions beyond a slight swelling of the micronucleus, fragmentation of its homogeneous chromatin and formation of its chromosomes. This appears to be the case in *Loxophyllum meleagris* (Maupas, 1889), *Spirostomum teres* (Maupas, 1889), *Euplotes patella* (Maupas, 1889), *Colpidium colpoda* (Hoyer, 1899), and in *Blepharisma undulans* (Calkins, 1912). In the case of *Colpidium colpoda* Hoyer (1899) described a typical tissue-cell

spireme but this is so exceptional among ciliates that it cannot be accepted without confirmation.

In the majority of ciliates this first meiotic mitosis is markedly different from somatic mitoses. In different species of *Paramecium* (*caudatum*, *aurelia* and *bursaria*) a typical prophase stage occurs in the form of a crescent derived from the homogeneous micronucleus which first draws out in the form of a long cylinder (Fig. 57, p. 103). In *Chilodon uncinatus* the micronucleus draws out into a long comma-shaped band and in *Cryptochilum nigricans* (Maupas, 1889), *Vorticella monilata* and *Vorticella nebulifera* (Maupas) and in *Opercularia coarctata* (Enriques, 1907) a similar chromatin rod extends in some cases the entire length of the cell.

Still another type of prophase, the "candelabra" (Collin, 1909) or "parachute" nucleus (Calkins, 1919) is found in *Orychodromus grandis* (Maupas), *Bursaria truncatella* (Prowazek, 1899), *Didinium nasutum* (Prandtl, 1906), *Anoplophrya branchiarum* (Collin, 1909), *Oxytricha fallax* (Gregory, 1923), *Uroleptus mobilis* and *halseyi* (Fig. 32, p. 64), *Euplotes*, Turner (1930), *Conchophthirus* (Kidder, 1933). In these cases the nucleus swells to two or three times the usual diameter with the compact chromatin at one pole (Figs. 32, 162). In *Uroleptus mobilis* there is an endobasal body within the nucleus; this divides, one-half passing to the periphery of the nucleus at the pole opposite the chromatin mass while the other half remains with the chromatin (Fig. 32, p. 64). The distal centrosome is the focal point of the spindle fibers which spread out from it to the fragmenting chromatin mass and forms one pole of the mitotic spindle.

In the transformation of the crescent type of prophase Maupas, Hertwig and Hamburger all agree that the spindle is formed by the shortening of the long axis of the crescent. Calkins and Cull (1907) and Dehorne (1920), however, find that the division center or achromatinic substance which forms the poles of the spindle migrates from its apical position in the crescent to the center of the convex side, and that this new position marks one pole of the spindle (Fig. 147).

In the parachute type the second pole is formed by the outgrowth from the chromatin mass of a second pole similar to the first, the chromatin granules thus being left in the nuclear plate position or center of the spindle figure (Fig. 32, p. 64).

2. *Phase B. The First Meiotic Division.*—Exact knowledge of the formation of chromosomes and their division is scanty, due in part to the large number of chromosomes and to their small size. Maupas (1889) made no attempt to enumerate the chromosomes; nor did he describe their formation beyond the brief account of the fragmentation of the homogeneous chromatin masses of the micronuclei. Hertwig (1889) believed that there were 8 or 9 chromo-

somes in *Paramecium aurelia*, basing his view not on the chromosomes but on the number of fibers which he could distinguish in the connecting strand between the two daughter nuclei. Later

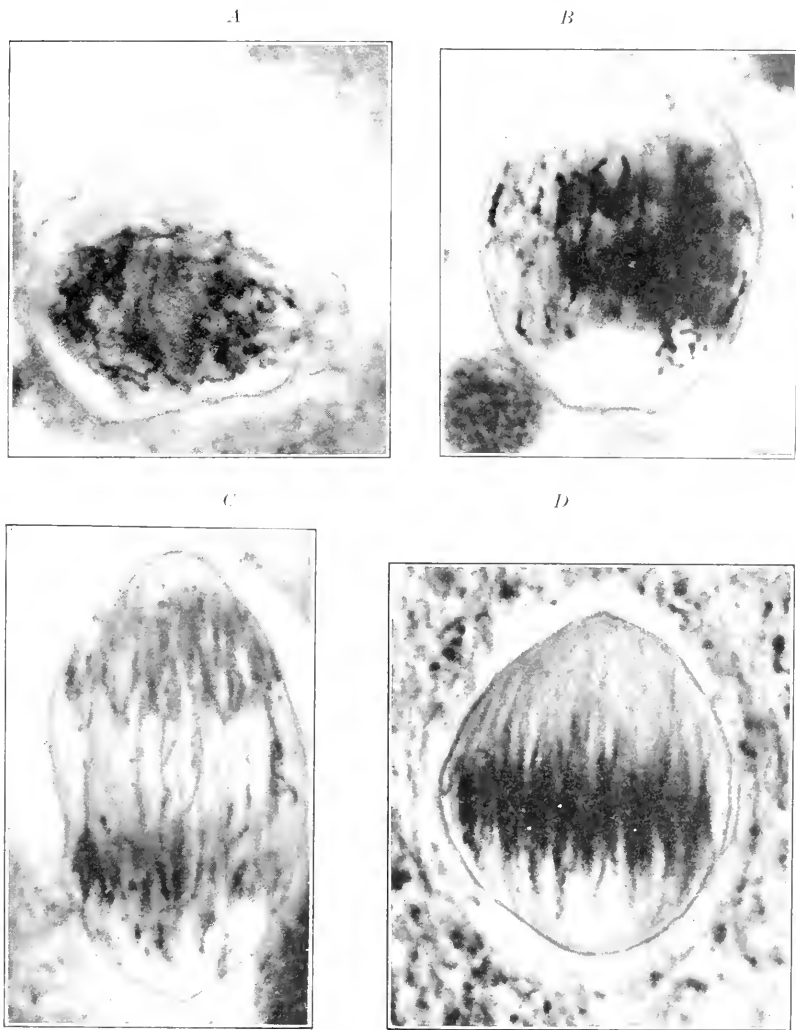


FIG. 147.—*Paramecium caudatum*; A, B, C, stages in the first meiotic division during conjugation; A, shortening of the crescent and formation of pole-plate on upper side; D, prophase of second meiotic division. (After Calkins and Cull.)

observers have found that the number in all species of *Paramecium* is much greater than this, running up to more than one hundred. Dehorne (1920), on the other hand, finds no chromosomes at all,

the chromatin being in the form of a continuous single looped thread which divides by transverse division (Fig. 57, p. 103. Cf. Fig. 147).



FIG. 148.—*Didinium nasutum*, section of conjugating individuals. Second meiotic division of the nuclei (*P*). (Original.)

In more favorable types of ciliates than *Paramecium* the number of chromosomes has been made out with some degree of accuracy. Prandtl (1906) found 16 in *Didinium nasutum* (Fig. 148). Prowazek (1899) was a little in doubt whether there were 12 or 13 in the nuclei of *Bursaria truncatella* (Poljansky, 1928, enumerates more

than 100), but described 6 chromosomes in *Stylonychia pustulata*. Stevens (1910) described 4 chromosomes in *Boveria subcylindrica*, but gave no details of their formation or reduction. Enriques (1908), confirmed by MacDougall (1925) found 4 in *Chilodon*

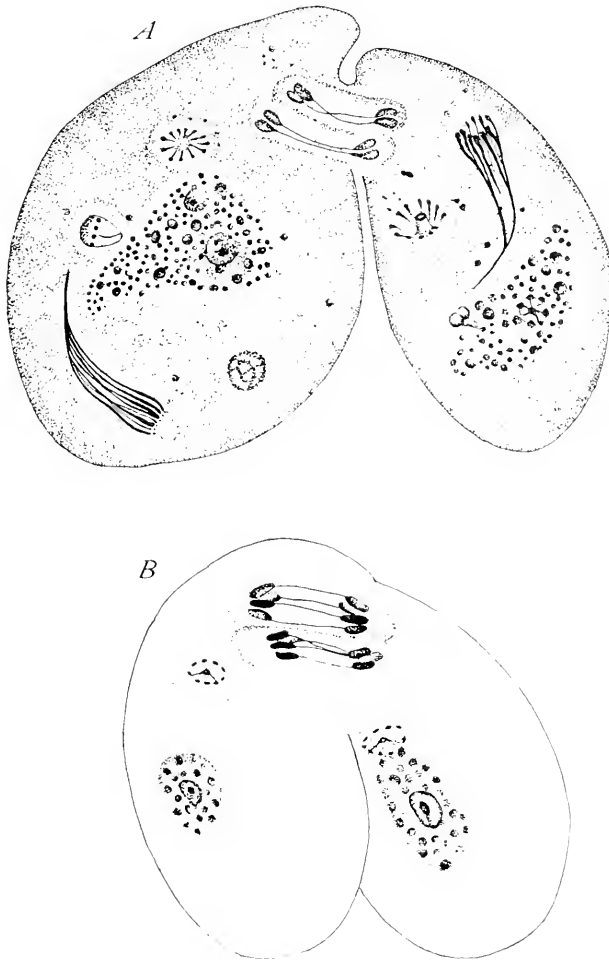


FIG. 149.—*Chilodon uncinatus*. Third division and interchange of nuclei of diploid (A) and tetraploid (B) stock. (After MacDougall.)

uncinatus; Popoff (1908) 16 in *Carchesium polypinum*; Enriques (1907) the same number in *Opercularia coarctata*, and Collin (1909) 6 chromosomes in *Anoplophrya branchiarum*.

Hamburger (1904) is a bit hazy in her account of the origin of

the chromosomes in *Paramecium bursaria*. The late stage in the crescent is regarded by her as a spireme from which the chromosomes are formed as short curved or V-shaped rods. Calkins and Cull (1907) found that the chromosomes of *Paramecium caudatum* are derived from a synezeisis stage which precedes the crescent and that the chromosomes are already divided at the stage which had generally been regarded as the metaphase. According to this account the metaphase stage occurs during the metamorphosis of the crescent into the spindle so that the latter when formed is in the early anaphase stage (Fig. 147).

In other ciliates the chromosomes are formed by the union of chromomeres which are derived by fragmentation of the homogeneous chromatin of the resting micronucleus. The process is completed at the parachute stage and the definitive number is present by the time the second pole of the spindle is completed. In *Uroleptus mobilis* when diffusion of the granules has apparently reached its limit, there are from 16 to 20 chromomeres (48 to 50 in *U. halseyi*)

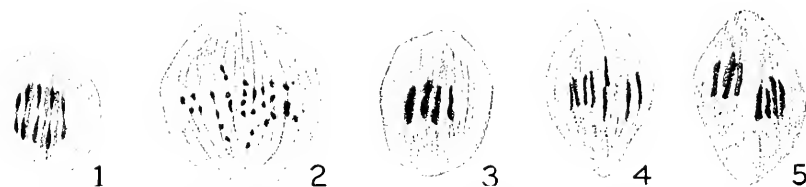


FIG. 150.—*Euplotes patella*, micronuclear chromosomes. 1, In vegetative mitosis; 2, 3 and 4, first, second and third meiotic divisions; 5, first division of the amphinucleus. (After Turner, from University of California Publications in Zoology, 1930.)

(Fig. 32, p. 64). Prandtl's figures show that there are approximately 32 in *Didinium nasutum*. Enriques (1908) and Collin (1909) have described a similar fragmentation of the comma-shaped chromatin rod of *Chilodon uncinatus* and of the homogeneous chromatin mass of *Anoplophrya branchiarum*, the granules of chromatin collecting in the center of the first maturation spindle. In *Didinium*, *Chilodon* and *Anoplophrya* these granules fuse until a definite number of chromosomes result—16 in *Didinium* (Fig. 148), 4 in *Chilodon* (8 in the tetraploid form found by MacDougall, 1925, Fig. 149), and 6 in *Anoplophrya* and 8 in *Euplotes patella* where each is made up of four previously separated chromomeres (Turner, 1930, Fig. 150). In *Uroleptus mobilis* a similar fusion of granules results in 8 chromosomes (Fig. 32, p. 64). *Uroleptus halseyi* differs in many respects from *U. mobilis*. Its micronucleus is larger and lacks an endobasal body. The first pole of the first meiotic spindle is formed by condensation of the karyolympth which draws away from the peripheral chromomeres. The second pole is formed by migration of part of the condensing substance, and between the two poles the nuclear

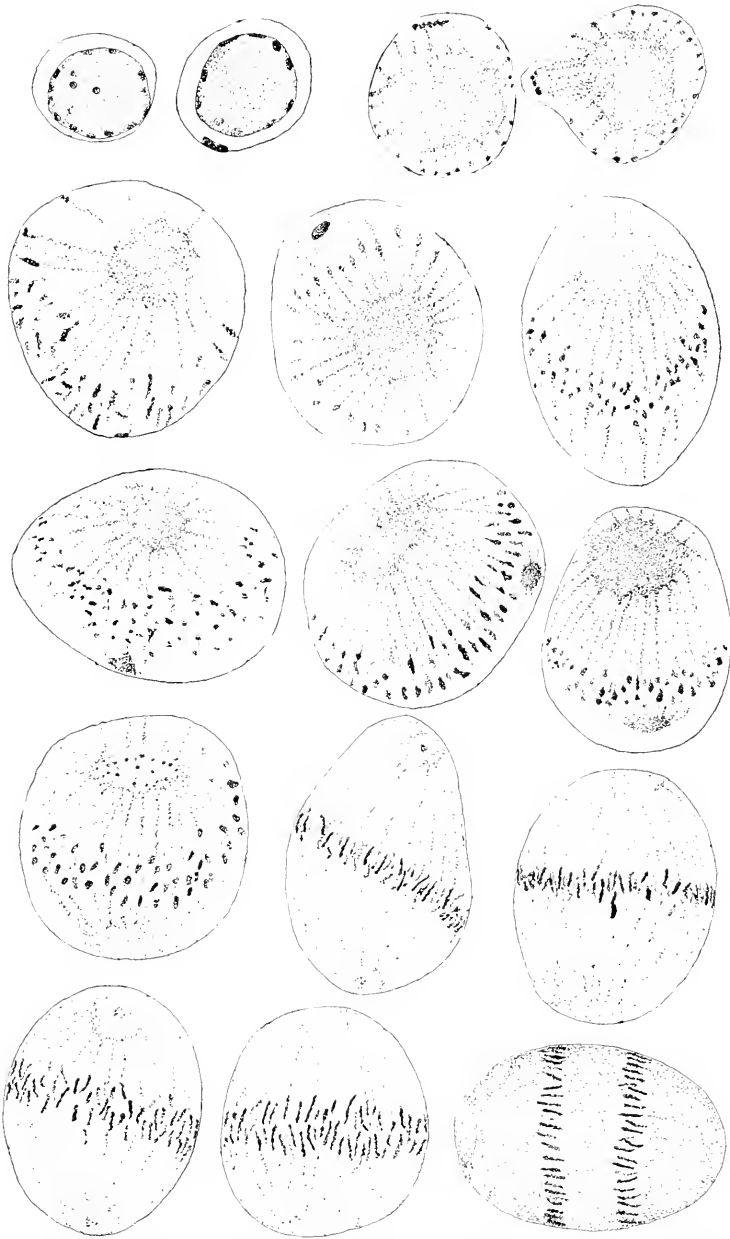


FIG. 151.—*Uroleptus halseyi*. Formation of chromosomes, spindle, and first meiotic division of the micronucleus. $\times 1750$. (After Calkins, Arch. f. Protistenkunde, courtesy of G. Fischer.)

plate is formed with 48 to 50 chromosomes. With the first division these separate into two groups, each with 24 chromosomes (Fig. 151).

3. *Phase C. The Second Meiotic Division.*—Prior to Prandtl's work on *Didinium* there were no conclusive observations on the reduction of chromosomes in ciliates. He found that the 16 chromosomes characteristic of the first maturation division become reduced to 8 with the second division. Since his work appeared there has been a number of authentic observations along the same line. Thus Enriques (1907) found a reduction in number from 16 to 8 chromosomes in *Opercularia coarctata* and the same observer (1908) described a reduction from 4 to 2 in *Chilodon uncinatus* (Fig. 149), reduction occurring at the second division. Other cases of the same type are *Carchesium polypinum* (Popoff, 1908) with reduction from 16 to 8; *Anoplophrya branchiarum* (Collin), from 6 to 3; and *Uroleptus*



FIG. 152.—*Uroleptus mobilis*. The second meiotic division and reduction in number of chromosomes during conjugation. (After Calkins.)

(Calkins, 1919) from 8 to 4 (Fig. 152). In all cases the second meiotic division appears to be unaccompanied by any of the preliminary activities which characterize the first division. In some the nuclei do not return to a resting condition between the two divisions, but in other cases, *e. g.*, *Chilodon* (MacDougall, 1925), the second spindle forms from a resting nucleus.

In ciliates with a multiple number of micronuclei the number participating in the second division appears to bear no constant relation to the number derived from the first division. In cases having but one micronucleus in the vegetative stages the numerical relations are fairly constant, two spindles in the second meiotic division being the rule. There are, however, some exceptions. Thus in *Paramecium bursaria*, according to Handburger (1904), one of the nuclei formed by the first division degenerates without forming a spindle so that only one nucleus undergoes the second division.

Other exceptions are found in *Euplotes patella* in all *Vorticellidae* and *Ophryoscolecidae* examined up to the present time. Here the micronucleus undergoes one or more preliminary mitoses prior to the first meiotic division.

In ciliates with two micronuclei both undergo the first maturation division. According to Prowazek (1899) the 4 resulting nuclei of *Stylonychia pustulata* divide again, thus forming 8 products at the second division. According to Maupas (1889), however, 2 of the first 4 nuclei of *Stylonychia pustulata*, and of *Onychodromus grandis* as well, degenerate so that only 2 second maturation nuclei are formed. Gregory's (1923) observations indicate that a variable number take part in the second division of *Oxytricha fallax*.

In forms with many micronuclei in the vegetative stage there seems to be no general rule as to the number which undergo a second division. Prandtl found a variable number in *Didinium nasutum*, Prowazek a large number in *Bursaria truncatella*, and Calkins a variable number in *Uroleptus mobilis*; while 1 and 4 nuclei are rarely found, 2 or 3 are characteristic.

In summing up the accumulating evidence on meiotic phenomena in the ciliates the conclusion may be drawn that the history in the main is similar to the history of meiosis in Metazoa. Chromosomes of definite number are characteristic of most species and this number is reduced to one-half during one or the other of the two divisions.

4. *Phase D. The Third Division. Pronuclei Formation.*—A third division of the nuclei subsequent to reduction in number of chromosomes is characteristic of all ciliates in which fertilization has been carefully studied. It is extremely difficult to interpret this final division which gives rise to the pronuclei (see *infra* p. 319). In the majority of cases it appears to be a transverse division which, if judged by Metazoa, would make it a second reduction division. One of the products is a wandering pronucleus which migrates, the other is a stationary pronucleus which ultimately fuses with the migratory pronucleus from the other individual. There is some evidence that the migrating pronucleus is equivalent to a spermatozoon (Dogiel, 1925).

The third division spindles are always characteristic and different from the spindles of the meiotic divisions. Not only are they frequently heteropolar, but the late telophase state is characterized by long connecting strands of nuclear substance (Fig. 153). There is no uniformity in regard to the number of nuclei to undergo this third division although only one of the dividing nuclei provides the two functional pronuclei. *Anoplophrya branchiarum*, *Paramacium caudatum*, *Chilodon uncinatus*, *Colpidium colpoda*, *Leucophrys patula*, *Glaucoma scintillans*, *Loxophyllum melcagris*, *Spirostomum teres*, *Bursaria truncatella*, *Blepharisma undulans*, *Boceria*

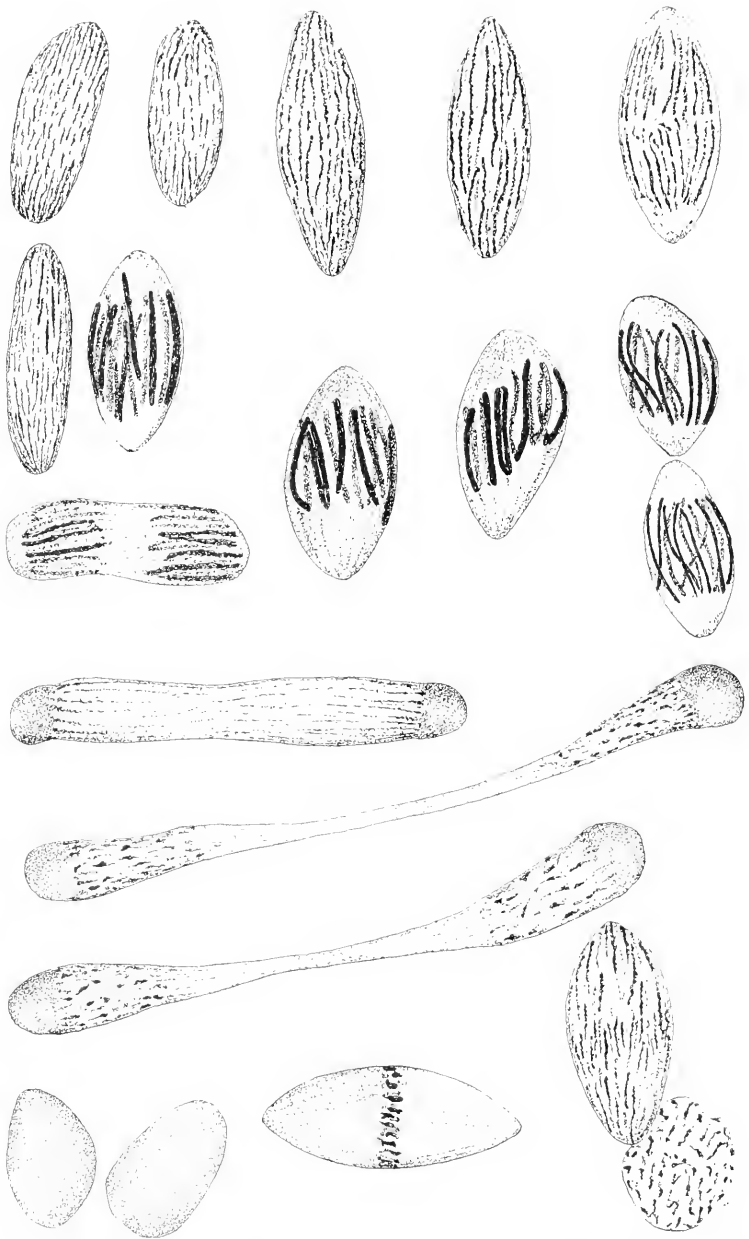


FIG. 153.—*Uroleptus halseyi*. Second and third meiotic divisions of the micronucleus, approach of gametic nuclei, metaphase of first zygotic nuclear division and second zygotic nuclear division. $\times 1750$. (After Calkins, Arch. f. Protistenkunde, courtesy of G. Fischer.)

subcylindrica, *Lionotus fasciola*, and in the *Forficellidae*, only 1 nucleus undergoes this third division. In *Onychodromus grandis*, *Stylonychia pustulata*, and *Euplotes patella*, 2 nuclei; in *Oxytricha fallax* (Gregory), 2 or 3, and in *Uroleptus mobilis*, 2, 3 or 4 nuclei, undergo the third division.

Prandtl (1906) was the first to note a difference in size between the wandering and the stationary pronuclei (*Dilicium nasutum*), Calkins and Cull (1907) described a similar difference in pronuclei of *Paramecium caudatum* and were able to trace this difference back

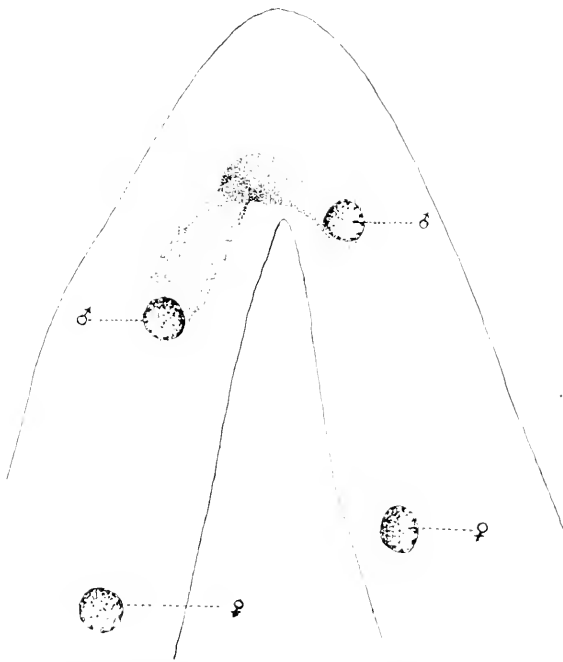


FIG. 154.—*Uroleptus mobilis*, conjugation. The interchange of pronuclei, each preceded by a characteristic "attraction sphere." (After Calkins.)

to a heteropolar third division spindle. In other cases there seems to be no characteristic difference in size between the two pronuclei although other differences may be evident. Thus Maupas noted the presence of a dense aggregate of cytoplasmic granules at the forward pole of the advancing pronucleus of *Euplotes patella* and Prandtl, more pronounced astral radiations about the wandering pronucleus of *Dilicium nasutum*. In *Uroleptus mobilis* such radiations are absent, but a fairly homogeneous condensed "sphere" of cytoplasmic substance precedes the wandering pronucleus in its migration (Fig. 154).

What is the significance of this third division? The answer can be only speculative at the present time. The absence of definite chromosomes in some cases, *e. g.*, *Paramecium*, and the occurrence of heteropolar mitotic figures lend some support to the view that it is a differential division whereby male chromatin, as suggested

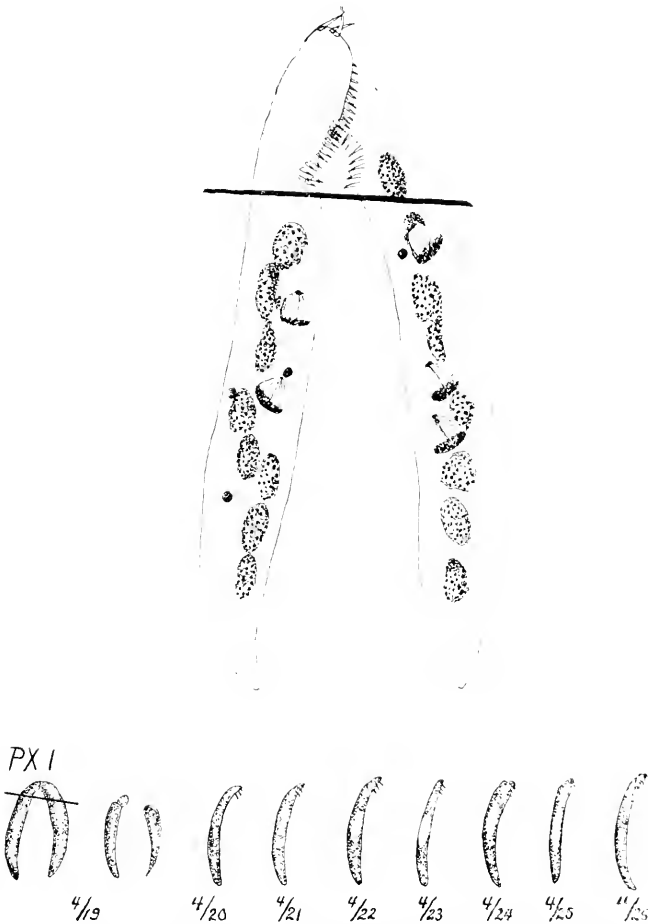


FIG. 155.—*Uroleptus mobilis*, cut during conjugation as indicated. In this case the conjugants were in the prophase stage of the first meiotic division. *PXI*, history of reorganization without fertilization. (After Calkins.)

by Schandinn (1904) is separated from "female" chromatin, the balance between the two being established by union of the wandering and the stationary promeiceli. Such an hypothetical balance would be maintained if there were no interchange of promeiceli and the third division does not take place, a condition realized in what

Woodruff and Erdmann (1914) called endomixis (see p. 317). Experimental evidence leading to definite conclusions has not yet been advanced. Calkins (1921) made an attempt in this direction by cutting conjugating pairs of *Uroleptus mobilis* in such a way that the two migrating pronuclei were removed while the two individuals, now separated, possessed only the stationary pronuclei (Fig. 155). These individuals were then followed in cultures, the process of reorganization was completed, the cells regenerated perfectly, and in successful issues, normal rejuvenescence and a typical life history resulted. The crucial point so far as the present matter is concerned was not determined, viz., from what elements were the new macro- and micronuclei derived? Did the stationary pronucleus in its "unbalanced" condition give rise to the new nuclear elements as it would have done were it an amphinucleus? Was there a fusion prior to the degeneration of other pronuclei of the stationary pronucleus with one of the "male" pronuclei of which there may be as many as four in each conjugant? Or did the stationary pronucleus degenerate, its place being taken by one of the other pairs of pronuclei? Some evidence that the last alternative was the case is afforded by the fact that the conjugating pairs if cut apart at an early period in conjugation may not undergo the third division, some one of the products of the second division acting as an amphinucleus, thus realizing the condition during "endomixis." See also, in this connection, the merotomy experiments of Ilowaisky (1926) on *Stylonychia mytilus* and *Paramecium caudatum* conjugants.

(b) **Gametic Meiosis** (Wilson, 1925).—In the preceding section instances of meiotic divisions subsequent to cell fusion were interpreted as due to stimuli mutually imparted to the conjugating individuals. For this the protoplasm must be in a mature condition, that is, with an organization considerably modified from that of the young or immature organisms. In a later section evidence will be given which indicates that under proper conditions the stage is all set for a similar all or none series of phenomena without, however, the stimulus of contact (see p. 317, endomixis). An analogous condition termed here gametic meiosis if accompanied by subsequent cell fusion of gametes, is characteristic of the majority of Protozoa in which fertilization is accomplished by the fusion of cells. Unfortunately the history of the chromosomes is known in but few cases but there is scarcely a paper on the fertilization of Protozoa that does not describe two rapidly-following divisions of the nuclei prior to fusion, and these are called maturation divisions, and the resulting nuclei "reduction nuclei." In *Actinosphaerium cichhornii* according to Hertwig (1898) the first evidence of the process is encystment of the adult organism and excretion of waste matters contained in the protoplasm. The nuclei are reduced in number to from 5 to 10 per cent of the original number by fusion and absorption

in the protoplasm. The cell then divides into as many daughter cysts as there are nuclei and these Hertwig calls cystospores No. 1, each of which secretes a gelatinous envelope about itself. The nucleus then divides by mitosis followed by division of the cell into two daughter cells which he calls cytospores No. 2. The nuclei of the latter undergo two successive "maturation" divisions resulting in one pronucleus and two "polar bodies" in each (Fig. 156), the latter degenerating and disappearing. The two cytospores of the second order now unite again, reforming cytospores No. 1 and fertilization is completed by fusion of the pronuclei. Bělař quite recently (1922) has given a more complete description of

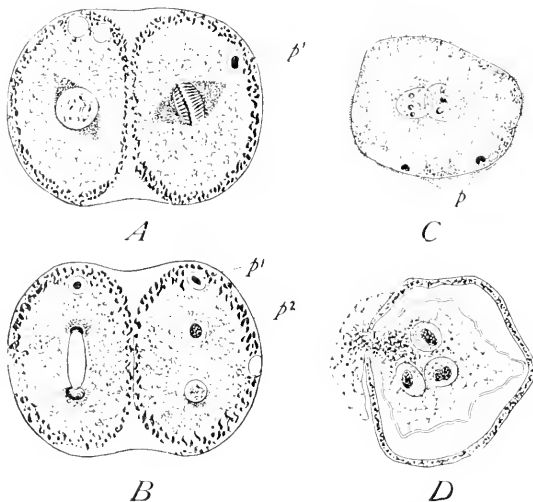


FIG. 156. *Actinosphaerium cichorii*. A, two gametes ("cytospores No. 2") resulting from the division of the same mother-cell; B, both "polar bodies" are formed in the right gamete, the second one forming in the left gamete; C, the cell bodies of the gametes have fused, and the nuclei are fusing; D, young organism leaving cyst; p , p^1 , p^2 , "polar bodies." (After Hertwig.)

the process in the allied form *Actinophrys sol*. The individuals draw in their pseudopodia, ordinary vegetative division of the nucleus follows, and the cell divides into two. By this division which Bělař terms the "progamous" division, the two gametes are formed and after each of them has undergone two meiotic divisions of the nuclei they reemite to form the zygote. One of them anticipates the other in these divisions and develops a pseudopodial process which the other lacks. By this process the first fusion of the two cells takes place. The original cell thus is a gametout and the fusing gametes are sister cells, one of which shows an incipient sex difference in its precocious activity and by its pseudopodium-like process. (Fig. 142, p. 278.) There are 44

chromosomes in the vegetative mitoses of *Actinophrys sol* and after the progamous division the gametic nuclei swell, chromosomes arrange themselves in pairs (parasynapsis) oriented toward one pole of the nucleus. These double chromosomes shorten and ultimately form the nuclear plate of the first meiotic spindle (Fig. 157). Here the two parts of the double chromosomes are separated and pass to the resulting nuclei, each of which thus has 22 single chromosomes. A second meiotic division results in the longitudinal splitting of these 22 chromosomes so that the pronuclei and the two "polar bodies" in each gamete have 22. One of the products of each division degenerates and is absorbed in the cytoplasm, and these are compared with the polar bodies in Metazoa. The two gametes then fuse, their nuclei fuse and the zygote becomes encysted (Fig. 142). In this case the chromosome cycle is remarkably similar to that of

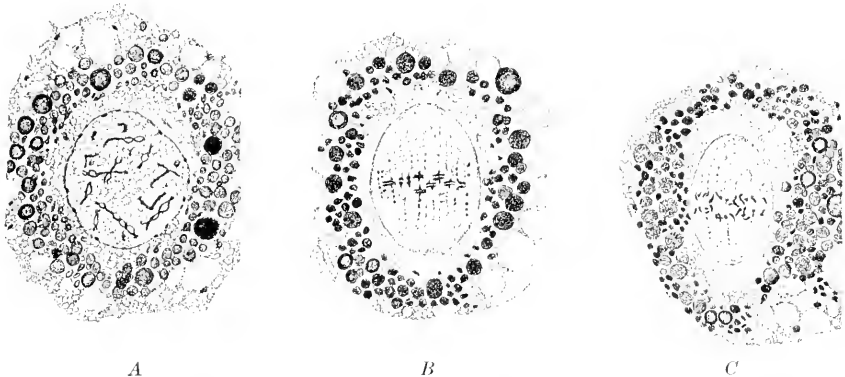


FIG. 157.—*Actinophrys sol*. A, contraction of the double chromosomes of strepsimine stage; B, metaphase of reduction division; C, anaphase of equational division. $\times 1900$. (After Bělaf, *Archiv f. Protistenkunde*, 1926, courtesy of G. Fischer.)

chromosomes of the metazoan egg and sperm in their maturation divisions.

Analogous processes may take place in other types of Protozoa in which fusion of gametes occurs, but the chromosome history is known in but few cases. In Gregarinida there are several progamous divisions of the gamonts, the last of which, according to Mulsow's (1911) observations of *Monocystis rostrata*, being a reducing division whereby the chromosomes are reduced in number from 8 to 4 (Fig. 55, p. 101). Mulsow's interpretation is confirmed for *Monocystis* by Calkins and Bowling (1926).

(c) **Zygotic Meiosis** (Wilson).—Reduction in number of chromosomes subsequent to nuclear fusion of gametes occurs in rare instances but the phenomenon may be more widely spread than is at present admitted. Two well authenticated cases are the coccidian

Aggregata eberthi and the gregarine *Diplocystis schneideri*. Dobell (1915) describes 6 chromosomes in the vegetative divisions of *Aggregata eberthi*, and Jameson (1915 and 1920) describes 3 in *Diplocystis schneideri* (Fig. 56, p. 102, and Fig. 158). These numbers remain constant in both organisms during gametogenesis, the mature gametes have the same numbers while the diploid numbers 12 and 6 are present only in the zygotes (Figs. 56 and 158). With the first division of the zygotes the two sets of chromosomes unite in homologous pairs; in *Aggregata* 1 pair consists of long chromosomes, 1 pair is very short and 4 pairs are intermediate in length (Fig. 56). The nuclei resulting from this first metagametic division have 6 chromosomes each in *Aggregata* and 3 each in *Diplocystis*, and these haploid numbers are retained throughout the vegetative cycles.

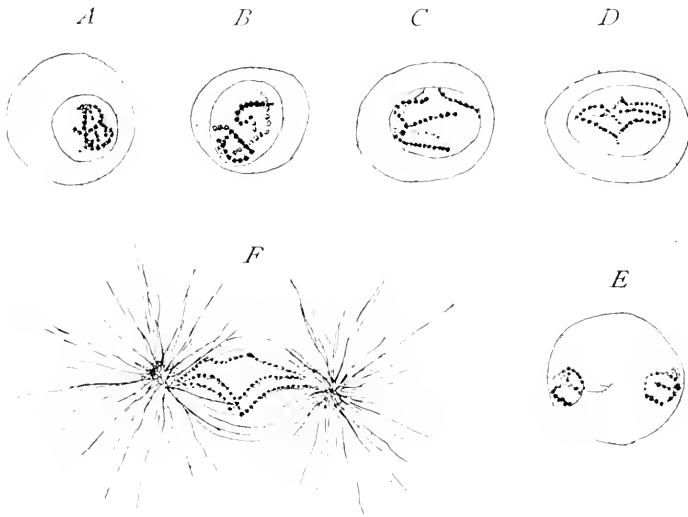


FIG. 158.—*Diplocystis schneideri*. Zygotic meiosis. A to E, nucleus of the zygote forming 6 chromosomes (the diploid number), and the first metagametic division; F, anaphase of the sixth progamous division preparatory to gamete formation, with 3 longitudinally split chromosomes, the haploid number. (After Jameson.)

The generalization made by Dobell and Jameson to the effect that this method of reduction is probably universal among the Telosporidia is hardly justified by these two cases. Few species indeed have been studied with respect to the reduction of chromosome number and only one—*Monocystis rostrata*—by Mulsow (1911), with sufficient care as to cytological detail to be admitted, and here, as stated above, reduction occurs with the final progamous division of the nuclei. Dobell and Jameson would explain this divergent case as due to confusion by Mulsow of stages of two different gregarines, one with 8 the other with 4 chromosomes, but

Mulsoy's contention is proved by finding final progamous spindles in the anaphase stage with 4 chromosomes in each daughter plate (haploid) while other progamous spindles are present in the same section with 8 chromosomes in each daughter plate (diploid) (Calkins and Bowling, 1926). Evidence in support of Dobell and Jameson's generalization is furnished by the fact of the frequent occurrence of an odd number of chromosomes in nuclei of different gregarines. Thus 5 chromosomes were found by Shellack (1907) in *Echinomera hispida* and the same odd number by Léger and Duboscq (1909) in *Nina gracilis*; while 3 were found by Shellack in *Monocystis ozata* (1912). Such odd numbers are not difficult to interpret if reduction takes place at the first metagametic division but they lead to questionable hypotheses of "odd chromosomes" (Léger) "accessory chromosomes," etc., if reduction is interpreted as taking place prior to fertilization.

B. Disorganization and Reorganization.—(a) **Phenomena of Disorganization.**—While the meiotic processes are probably universal accompaniments of fertilization they do not comprise all of the phenomena taking place at this period. Evidences of disorganization are apparent in the cell quite independent of the gametic nuclei. Metagametic activities involving reorganization of the protoplasm are equally characteristic of the fertilized cell and lead to the production of young organisms with full potential of vitality. Disorganization and reorganization, although probably closely related, are different in character and will be discussed separately.

The destruction of the old macronucleus in Infusoria is one of the most significant of the phenomena attending conjugation (Fig. 139, p. 273). Here is an organ of the cell which is generally regarded as intimately connected with metabolic activities of the organism; which has functioned throughout vegetative life of the race and has divided with each division of the cell. Yet at conjugation the macronucleus degenerates through hypertrophy and fragmentation and the fragments are ultimately absorbed in the protoplasm. The process is fundamentally the same in all ciliates differing only in details.

If the organization of a ciliate is dependent upon the specificity of the proteins, carbohydrates, fats, salts and water which enter into its make up, then this large bulk of nucleo-proteins distributed to all parts of the cytoplasm must bring about a markedly different matrix with which the new amphinucleus and its products are to react. Zweibaum (1922) concluded that products of metabolism during vegetative activity gradually poison the nuclear substances so that both synthetic and oxidizing activities are weakened, but at conjugation and with fragmentation of the macronucleus the contained ferments are freed from their toxic bonds, and activity is fully restored. The intake of oxygen is much greater after con-

jugation than before, a fact which Zweibaum (1921) interprets as due to reorganization and the freeing of oxidases by nuclear disorganization. To this mass of nucleo-proteins is also added three-quarters (*e. g.*, *Paramecium*) to fifteen-sixteenths (*Uroleptus*) of the substance of the old micronuclei, which is likewise absorbed in the cytoplasm.

Not only is the old nuclear material broken down and distributed but, in some instances at least, the formed metaplastids of the cell are similarly destroyed and absorbed. This is well illustrated by the disappearance of the old pharyngeal basket and some of the cilia in *Chilodon uncinatus* (MacDougall, Fig. 112, p. 222). This is perhaps relatively unimportant at conjugation since the same thing happens at each division of the cell during vegetative life, but it is evidence in support of the view that stabile substances of the organism, substances that have accumulated with continued vegetative life are reduced to labile substances at this significant period of the life history.

In a similar manner the many nuclei of *Actinosphaerium cichhornii* (300 or more) according to Hertwig (1898) are fused or absorbed prior to fertilization. As there must be a limit to the number that fuse (if any?) the great majority of nuclei must be absorbed in the protoplasm, for only a few (up to 20) become nuclei of gamonts (see p. 307).

In gregarines also there is a similar fragmentation of some of the nuclei leading to collections of chromidia which appear to function in the formation of sporoducts (see p. 239). In Mycetozoa and Neosporidia also some of the nuclei are destroyed in connection with the formation of accessory structures of the fruiting bodies (elaters, sporoducts, spore capsules, etc.).

The conclusion is forced upon us that this period of fertilization is marked by far-reaching changes in organization. Some of these, as in ciliates, have a prospective value for the young organisms while others are differentiations serving a useful purpose for the limited period of fertilization in organisms whose individual metabolic activities are approaching the end, and these are evidence of extreme specialization.

(b) **Metagamic Activities and Reorganization.**—Under this heading we include all changes which take place in the organism immediately after formation of the amphinucleus. In ciliates the fragmentation and absorption of the old macronucleus may continue for several days after union of the gametic nuclei but the further activities of the amphinucleus appear to be independent of the other happenings in the cytoplasm. These activities have to do primarily with the differentiation of the characteristic cell structures of the new organism. Thus in *Chilodon* and other Chlamydodontidae a new oral basket is formed and some if not all of the cilia are renewed;

whether or not new cirri, membranelles, and undulating membranes are formed and the old ones absorbed, has not been fully determined by observation but this appears to be the case in *Uroleptus mobilis*.

The most important of the changes at this period have to do with the formation of the new macro- and micronuclei. The inaccurate statement is often made to the effect that the new macronucleus is formed by the metamorphosis of a micronucleus. This is strictly true only in cases of parthenogenesis. In fertilization both macro- and micronucleus are formed from products of the amphinucleus,

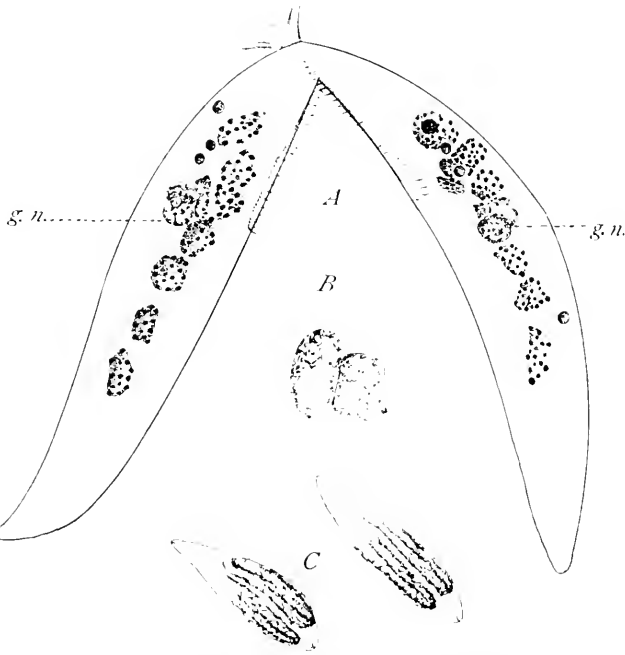


FIG. 159.—*Uroleptus mobilis*; conjugation at the stage of nuclear fusion: *g. n.*, gametic nuclei about to fuse; *B*, same enlarged; *C*, elongation of amphinucleus shortly after fusion. (After Calkins.)

and both types of nuclei are formed by metamorphosis of such products. In the majority of cases the first metagametic division of the amphinucleus results in two equivalent nuclei. In *Uroleptus mobilis* this division occurs very soon after fusion and before complete mixture of the two pronuclei is established (Fig. 159). This is shown by the occasional finding of nuclei in which 4 of the 8 chromosomes are in the anaphase stage while the other 4 are in the metaphase (Fig. 160). The two products of this division have different fates. One of them divides again to form two nuclei which lose their vesicular character and condense into minute and

homogeneous bodies, the micronuclei. The other one forms a heteropolar spindle and divides into two unequal products the larger of which is vesicular and persists as the new macronucleus, the smaller one is spheroidal and compact and ultimately disappears

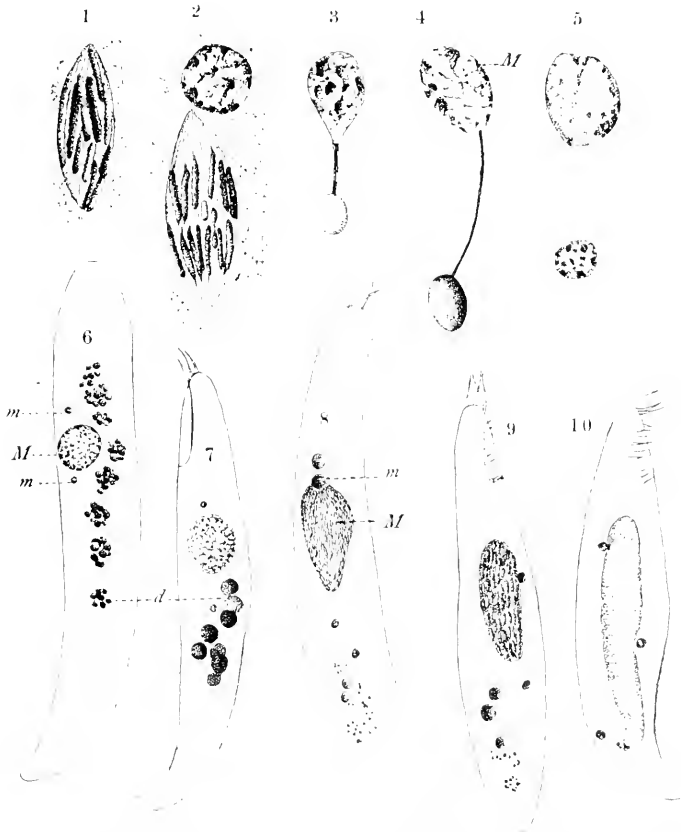


FIG. 160.—Origin of macronucleus after conjugation in *Erobolus mobilis*. (1) first metagametic mitosis of the amphinucleus; (2) one of the progeny of this division dividing again; (3), (4), (5) telophase stages of second division of the amphinucleus resulting in a new macronucleus (above), and a degenerating nucleus (below); (6 to 10), stages in differentiation of the young macronucleus and disintegration and absorption of the old macronucleus; in (10) two new micronuclei are in mitosis preparatory to the first division of the ex-conjugant. (M) new macronuclei; (m) new micronuclei; (d) degenerating old macronuclei. (After Calkins.)

by absorption (Fig. 160, 4). The young macronucleus sometimes called the "placenta" becomes finely granular and loses its staining capacity which is not regained for a period of from three to five or more days. During this period the young macronucleus appears like a vacuole in a center of a cell and is distinctly visible in the

living cell. It is small at first but grows in size from day to day while nucleic acid is formed and deposited in continually growing chromomeres (Calkins, 1930; see p. 84), until finally the placenta occupies fully two-thirds of the cell. It then condenses into a compact homogeneous ellipsoidal nucleus, invisible in the living cell, and now stains intensely with chromatin dyes (Fig. 160, 10). It is now ready for the first macronuclear division and divides twice prior to division of the cell. It is perhaps significant that a similar dense ellipsoidal nucleus is formed by fusion of the 8 macronuclei prior to cell division in vegetative life (see p. 220).

An essentially similar history of the amphinucleus occurs in *Colpidium colpoda* (Hoyer, 1899), *Stylonychia pustulata* (Maupas, 1889) and *Lionotus fasciola* (Prowazek, 1909). In *Paramecium caudatum* the amphinucleus divides twice without differentiation and all 4 products divide a third time, 4 of the resulting 8 nuclei become micronuclei and 4 become macronuclei (Calkins and Cull, 1907). Here there is no degeneration, but in *Paramecium putrinum* according to Doflein (1916) and in *Paramecium bursaria* (Hamburger, 1904) 3 of the 8 nuclei degenerate. Three divisions of the amphinucleus are also characteristic of *Cryptochilum nigricans* (Maupas, 1889), *Carchesium polypinum* (Popoff, 1908), *Vorticella monilata* and *Vorticella nebulifera* (Maupas, 1889) and *Ophrydium versatile* (Kaltenbach, 1915). In these, 7 of the 8 resulting nuclei form macronuclei while the eighth forms the micronucleus. All 7 fuse to form 1 macronucleus in *Cryptochilum* (Maupas) but in the others each forms a macronucleus the 7 being separated by successive cell divisions until finally each cell has 1 (Popoff, Maupas, Kaltenbach).

In *Didinium nasutum* (Prandtl, 1906), *Paramecium bursaria* (Hamburger, 1904), *Glaucoma scintillans*, *Leucophrys patula*, *Spirostomum teres* and *Stylonychia pustulata* (Maupas, 1889) differentiation occurs with the second division; 2 of the 4 nuclei become macronuclei and 2 micronuclei while none degenerates. A very exceptional history occurs in *Bursaria truncatella* according to Prowazek (1899). Here no differentiation occurs until 16 nuclei are formed; 2 to 5 of these become macronuclei; 3 or more become micronuclei and the remainder degenerate. This history, however, is not confirmed by Poljansky (1928) in a more critical examination of this phase of *Bursaria*.

In Sporozoa metagamic activities take quite a different form. The majority of gregarines become gamonts which form many gametes (in *Ophryocystis* only two), which copulate within the gametocyst (Fig. 120, p. 231). The amphinucleus of each zygote divides, usually three times, to form eight products, each of which becomes the nucleus of a sporozoite. In *Diploecystis schneideri* the first of these divisions results in the reduction in number of

chromosomes to one-half (Jameson, 1923; see p. 310). In the Coccidia the number of metagamic divisions is still further increased. Here the zygote as well as the amphinucleus divides to form from two to many sporozoite-forming centers—the sporoblasts—each of which becomes enclosed in a special sporoblast capsule (sporocyst) where it divides, usually only once, to form sporozoites (see p. 530). In *Aggregata eberthi* as in *Diplocystis* the first division of the zygote results in halving the number of chromosomes (Dobell, 1916). The Hemosporidia differ in that capsule-bearing sporoblasts are not formed. Here the zygote grows to large size and the amphinucleus divides repeatedly until myriads of sporozoites are formed. In these types of Protozoa, therefore, metagamic activities involve actual reproduction, and reproduction here is a sequel to fertilization.

Other groups of Protozoa differ widely in their metagamic activities and some types give unmistakable evidence of ontogenetic development. Thus zygotes of Foraminifera grow directly into the more or less complex asexual generation (microspheric). Here the amphinucleus divides repeatedly while the cell divisions are suppressed.

Other changes of a metagamic nature have to do with the clearing up of accumulated substances in the cytoplasm. Zweibaum (1922) finds that relatively large droplets of neutral fat which are characteristic of vegetative phases of *Paramecium* are broken down prior to conjugation while smaller droplets of another type accumulate. Among these he was able to detect a larger amount of cholesterol ester than normal and a great quantity of what he interpreted as fatty acids. After conjugation these small drops disappear and neutral fats reappear. A similar accumulation of fat-like droplets and "lipoplasts" is described by Bělař (1922) in *Actinophrys sol* as characteristic of the copulating gametes and of the zygote, but the accumulation breaks down and disappears with germination of the latter. Macrogametes of Coccidia have an analogous store of cytoplasmic substances of the nature of lecithin which also disappears during metagamic activities.

There is some evidence, therefore, that specific products of metabolism accumulate in cells of Protozoa prior to fertilization and that these are utilized as are yolk substances of metazoön eggs in the early metagamic activities. Their disappearance after fertilization indicates that in this respect also, the general make up of the cytoplasm is reorganized.

IV. PARTHENOGENESIS.

Parthenogenesis may be briefly defined as the development of an organism from an egg cell (or its equivalent, *e. g.*, a ciliate) which has not been fertilized. The phenomenon occurs spontaneously in a few animal groups and may be induced artificially in

eggs from animals of widely different phyla which usually undergo fertilization before development.

The chief biological interest of parthenogenesis centers in the nuclear phenomena. Under ordinary conditions of fertilization two polar bodies are formed by the maturing egg and with their formation the number of chromosomes is reduced to one-half so that egg pronucleus and polar body nuclei are haploid. It follows, therefore, that in artificial parthenogenesis all tissue cells of the body are haploid. The same phenomenon occurs, naturally, in the development of the drone honey bee, or of the male rotifer and may be referred to hereafter as Type 1. In the great majority of parthenogenetic eggs, however, the second polar body is not formed and the nucleus remains diploid as for example in parthenogenetic aphids or female rotifers; this may be designated Type 2. A third possibility, in theory, would be cases where two polar bodies are formed which, with the pronucleus, are haploid but the egg becomes diploid by later fusion of the pronucleus with one of the polar body nuclei. This which may be called Type 3 has not been established with certainty in any metazoön but was suggested as a possibility by Boveri (1887) and described by Brauer (1893) as one type of parthenogenesis in the eggs of *Artemia*.

In Protozoa many cases of so-called parthenogenesis have been described some of which fall in line with one or another of the three types in Metazoa as outlined above. These phenomena may be grouped under two headings—so-called endomixis of Woodruff and Erdmann (1914) and autogamy, a widely used term in connection with Protozoa.

A. Endomixis.—Under this term Woodruff and Erdmann (1914) described “a complete periodic nuclear reorganization without cell fusion in a pedigreed race of *Paramecium*.” At regular intervals of approximately thirty days they found that the old macronucleus of *Paramecium aurelia* gives rise to buds or fragments which are absorbed in the cytoplasm. There appears to be some difference in the details of macronucleus fragmentation between individuals in 1914 and more recent individuals. Thus Woodruff and Spencer (1922) find that ribbon or skein formation prior to fragmentation and characteristic of conjugation, which was very rare in 1914, had become much more common in 1921. Each of the two micronuclei divides twice, forming 8 products some of which form new micronuclei, some new macronuclei. The possible combinations of nuclei and their relations are shown in Fig. 161. Later, Erdmann and Woodruff (1916) demonstrated a similar periodic reorganization at intervals of approximately sixty days in *Paramecium caudatum*. In this case the single micronucleus divides three times, forming 8 nuclei, 4 of which become macronuclei, 2 possibly degenerate and 2 persist as new micronuclei.

Reorganization process

Conjugation

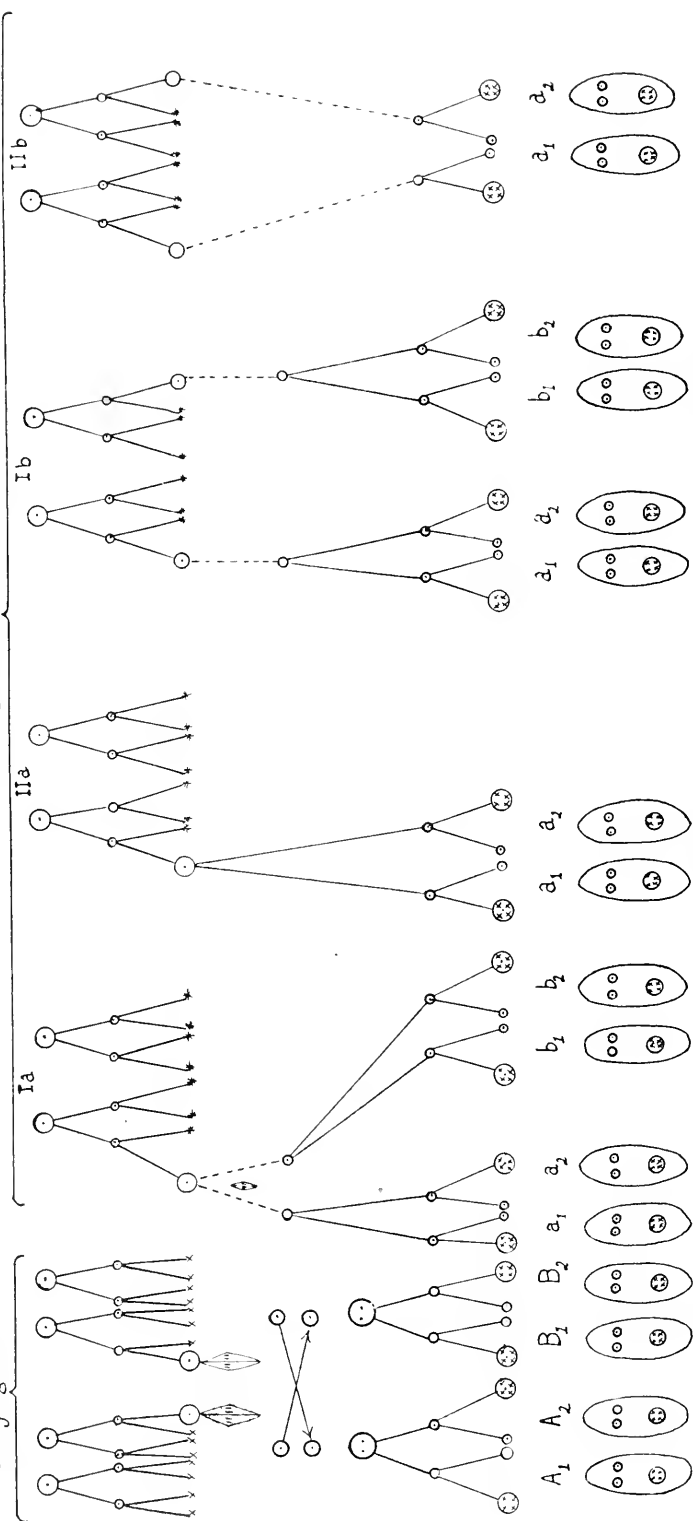


FIG. 161.—Diagram of reorganization in *Paramoecium aurilia* after conjugation and during endomixis. (After Woodruff and Erdmann.)

In *Paramecium*, therefore, the first two divisions of the micronuclei in endomixis correspond to the reducing divisions in conjugation, the third division as absent in *aurelia* but present in *candatum*. Ivancic (1928) described a similar nuclear history during the processes of encystment of *Chilodon uncinatus*. If reduction occurs with the first two divisions the four products in endomixis are equivalent to haploid nuclei so far as the chromosomes are concerned, and correspond, therefore, to the first type of parthenogenesis above. But they are likewise equivalent to the fertilization nucleus and develop with the diploid number of chromosomes. This number unfortunately, is too large in *Paramecium* to permit of accurate counting, while in ciliates with a small number of chromosomes, endomixis takes place during encystment where cytological details have not been made out in any case. Fermor (1912) indeed described the union of the two macronuclei and of the two micronuclei in *Stylonychia pustulata* during encystment, but the account of the phenomenon is incomplete and on its face implies the fusion of diploid nuclei. This is so improbable from the chromosome standpoint that the result cannot be accepted without confirmation. Later work by Howaisky (1926) failed entirely to confirm Fermor's interpretation of the happenings during encystment of *Stylonychia*.

As indicated above (p. 303) the difficulty over haploid and diploid chromosome number reaches an extreme in connection with the third division of the ciliate nucleus. If reduction in number occurs during the first two meiotic divisions then the pronuclei are formed by a third division of an haploid number of chromosomes. If this division is transverse as appears to be the case with *Paramecium*, this third division might also be a reducing division, and the amphinucleus coming from the union of such nuclei would be haploid. If the third division, however, is equational the pronuclei would still have the haploid number and their fusion would result in a diploid amphinucleus. The latter appears to be the correct solution. Gregory (1923) for example describes 24 dumb-bell-shaped chromosomes in the nuclear plate of the first meiotic division of *Oxytricha fallax*. This number is reduced to 12 dumb-bell-shaped chromosomes with this first division and each dumb-bell divides longitudinally. The equational halves are separated at the second division and 12 dumb-bells form the equatorial plate of the third division (Fig. 162). The two halves of the dumb-bell are finally separated with this third division, 12 single chromosomes passing to each pole. The pronuclei thus have 12 single chromosomes and the amphinucleus formed by their union has 24. An equivalent process occurs in *Uroleptus halseyi* where there are 48 chromosomes in the first division reduced to 24. These 24 are reduced to 12 in the second division and these 12 are divided transversely in the third division (Figs. 151, 153). The interpretation here

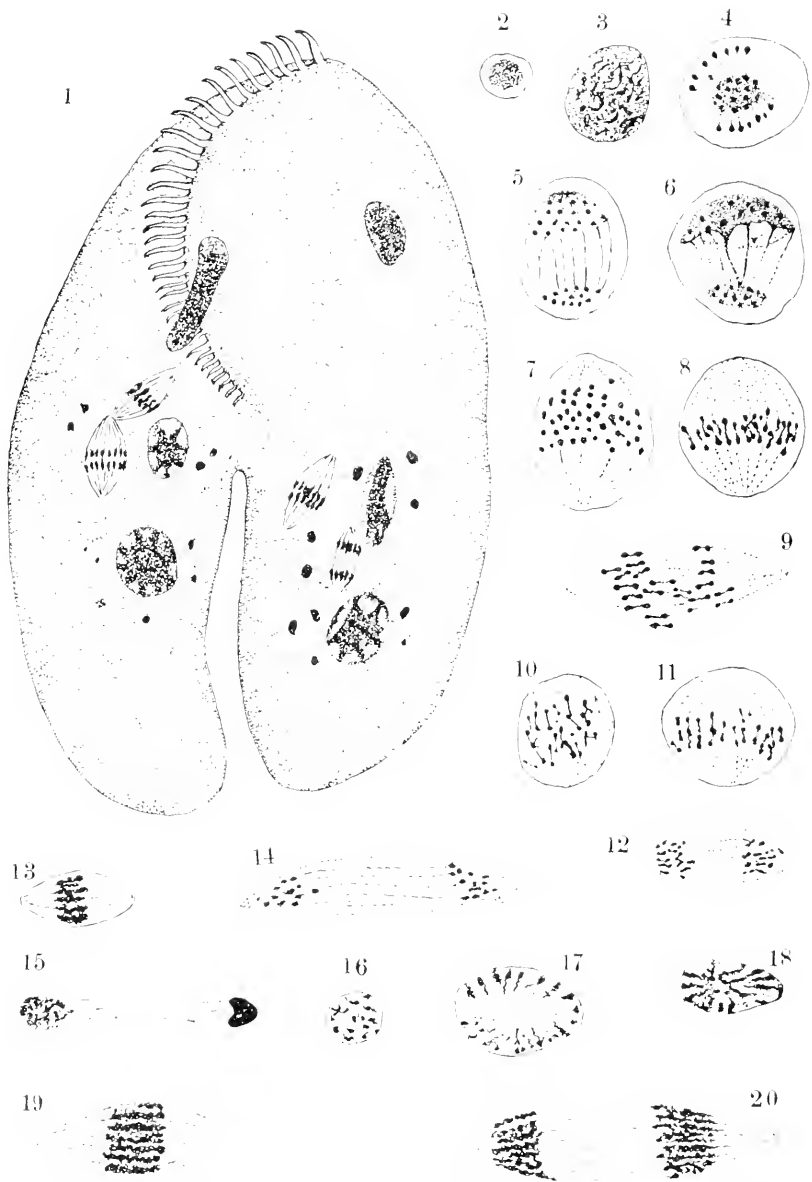


FIG. 162.—*Oxytricha fallax*; conjugation and meiosis. 2 to 9, formation and division of the first meiotic nuclear spindle and separation of the twenty-four dumb-bells into two groups of twelve dumb-bells each; 10 to 12, the second meiotic division; 13 to 15, the third division; 16, one of the pronuclei; 17 to 20, the first zygotic division. (After Gregory.)

depends upon the origin of the 24 or 48 chromosomes of the first division. In *Oxytricha* the meiotic process begins with a spireme which fragments into granules, approximately 48 in number. Association of these granules, 2 by 2, results in 24 dumb-bells. If the number of chromosomes were 48 this would be synapsis in the usual sense. The reduced number, however, is 12 and only 24 chromosomes make up the amphinucleus. If the granules are homologous and in pairs, and if like unites with like to form the dumb-bells, then division of the 24 chromosomes of the first nuclear plate in meiosis would be equivalent to equational division. The latter interpretation satisfies the conditions in other ciliates (*e. g.*, *Chilodon*, *Uroleptus*, *Didinium*, etc.), and the anomalous condition in ciliates generally may be cleared up by the assumption of two equational and one reducing division per chromosome at meiosis, as against one equational and one reducing division in Metazoa. With all forms, furthermore, reduction occurs during the first two meiotic divisions. The difficulties, however, cannot be cleared up by *a priori* reasoning in attempts to homologize protozoan and metazoan meiosis. In *Uroleptus halseyi* all three meiotic divisions during conjugation are *transverse divisions* and these chromosomes find their place in the theory of the gene only on the assumption that each chromosome represents one gene and one gene only (Calkins, 1930).

A further difficulty arises with parthenogenesis. Woodruff and Erdmann regard the first two divisions of the nucleus at endomixis as equivalent to the first two divisions in conjugation. If this is true the chromosomes are presumably reduced in number by either the first or the second division and the reorganization nucleus would be haploid from which the normal number of chromosomes in endomictic animals would have to be reestablished by division of each of the chromosomes present. In the case of *Oxytricha fallax* cited above, barring fusion of nuclei during endomixis, no evidence for which has been advanced in any ciliate, the functional nucleus would have 12 dumb-bell-shaped chromosomes. If the chromosomes remain double a race of haploid individuals would be formed. At the next endomictic period these would again be halved, and so on. This, however, is unbelievable. If on the other hand the parts of the dumb-bell should separate, then the normal diploid number would be restored with two sets of homologous chromosomes and the 48 chromosomes would be formed by the further division of the 24, and this would be intelligible on the above assumption of a single gene per chromosome.

Still further difficulties are added by the merotomy experiments with conjugating *Uroleptus mobilis*. A pair in conjugation at the period of pronuclei interchange is cut across the angle as shown in Fig. 155. The angular apex thus cut off and one of the arms are

fixed and stained to determine the stage of maturation. The other arm is cultivated. Since other pronuclei usually degenerate, it is evident that only one pronucleus is present in the piece cultivated, and this one contains the haploid number of chromosomes. The possibility remains open, however, that this pronucleus may unite with a sister pronucleus formed by sister nuclei, and which do not degenerate, but for this there is no evidence. In this case it would be parthenogenesis of the third type above. When such cutting experiments are successful the resultant organisms regenerate perfectly and undergo typical life histories and each individual has the normal number of chromosomes.

The most probable interpretation of such merotomy experiments appears to be that the diploid number of chromosomes is restored by chromosome division.

The conclusion follows that so far as chromosomes are concerned, endomixis and amphimixis after prolonged in-breeding as in *Uroleptus* are similar in results. The cellular processes of reorganization are identical in both and Woodruff is quite right in stating that amphimixis is unnecessary for continued life of a ciliate. In respect to vitality, endomixis and amphimixis are equivalent and so long as one or the other occurs continued vitality is possible. Furthermore it may be argued that if an equivalent reorganization is accomplished in any other way then neither endomixis nor amphimixis by conjugation is necessary. Evidence of this third possibility is furnished by observations on *Actinophrys sol* (Bělař, 1922) and by the animal flagellates. If this is a correct interpretation then there is a possibility of harmonizing the many conflicting results and views advanced in relation to the much discussed problem of indefinitely continued vitality.

B. Autogamy.—Autogamy or self-fertilization in Protozoa is a logical sequence of endogamy. If a gamont of *Actinophrys sol* should not divide to form gametes which later fuse (see above, p. 308), and if the gamont's nucleus should divide and the two products should undergo meiosis, and the two pronuclei should then unite, all in the same one cell, then the process would be called autogamy. Or if pronuclei from the same individual ciliate should unite, it would be autogamy. In short autogamy is the realization of Type 3 of parthenogenesis above.

The phenomena which have been described and interpreted as autogamy, particularly as they occur in parasitic forms, are rather cautiously interpreted today and many careful observers, perhaps too careful, are inclined to regard the earlier descriptions of autogamy as dealing with degeneration phenomena rather than with normal vital activities. One illustration, that of *Sappinia diploidea*, appears to be well established. The organism has two nuclei which lie closely together (Fig. 163). Both nuclei divide at cell division

(Fig. 163, *B*). Two such amoebae become enclosed in a common cyst but do not fuse. According to Hartmann and Nägler, the two amoebae are products of division of one amoeba, the apposed nuclei of each organism then fuse into one. This fusion is followed by two reduction divisions of the fused nuclei, three of the products degenerating. Two amoebae then fuse again and their nuclei come to lie side by side. The question of autogamy obviously depends upon the origin of the two amoebae in the common cyst. If they

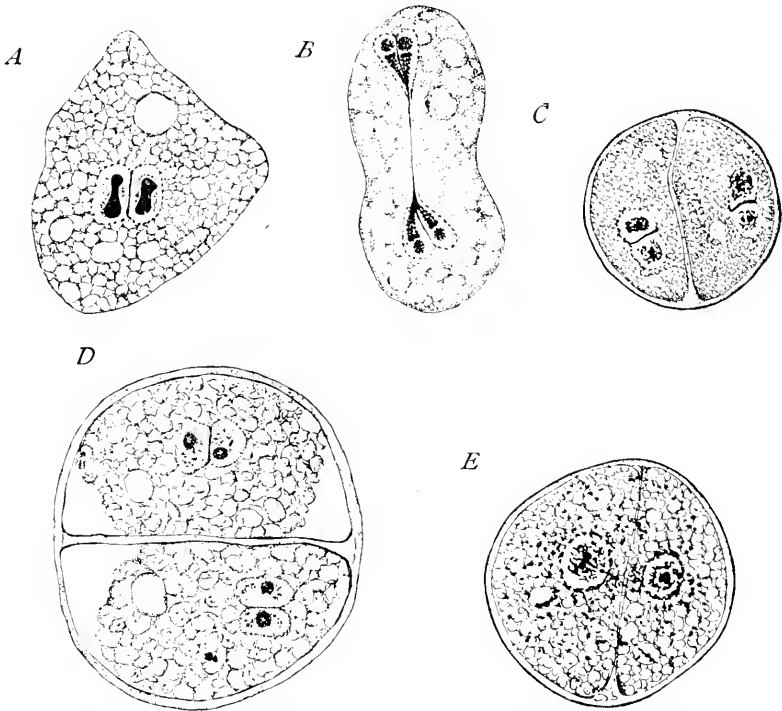


FIG. 163.—*Sappinia diploidea*. The ordinary vegetative individual has two nuclei which divide independently at cell division. With encystment these nuclei form spindles (*B*) and the cells divide (*C*, *D*); the two pairs of nuclei then unite, forming two fusion nuclei after which the cell bodies reunite, thus forming the vegetative binucleated cell. (After Hartmann and Nägler.)

do not come from the same parent cell, the phenomenon is one of delayed exogamy.

Autogamy appears to be characteristic of the Neosporidia among the Sporozoa and the processes are fairly uniform in Myxosporidia, Microsporidia and Actinomyxida. Multinucleate cells are typical of the nutritive or vegetative stage and in some cases the nuclei are dimorphic. Spores are formed endogenously and during the continued vegetative activity of the organism. The process was well

described by Schröder (1907) for *Sphaeromyxa sabrazesi*, a parasite of the sea horse, where the multinucleate ameboid body of the parasite contains two kinds of nuclei distinguishable by size and structure. Within the protoplasmic body small areas become differentiated from the surrounding cytoplasm. These areas, characteristic of the Myxosporidia, each contain 2 nuclei, 1 of each kind (Fig. 164, K-Q). With the development of the pansporoblast, each nucleus divides in such order that 7 daughter nuclei finally result from each, the 14 nuclei behaving as follows: 2 are destined to degenerate as "reduction nuclei;" 4 become the centers of capsule and shell formation; 4 become centers of polar capsule formation; and 4 remain as germinal nuclei. The protoplasm of the pansporoblast divides into two halves (M), the sporoblasts, and each contains 6 of the nuclei, while the 2 degenerating nuclei remain outside. The 6 nuclei are thus differentiated into somatic and germinal nuclei 4 in each case going into somatic differentiations of the spores (shells, polar capsules and threads) and 2, presumably 1 of each of the original two kinds, remain as pronuclei (N, O, P).

Many different observers have noted this binucleated stage of the young spore, and the problem of fertilization in Myxosporidia appears to be bound up with their further fate. Schröder believes that they unite later and so complete the fertilization, a belief which he was able to prove in a later publication (1910). Keyselitz (1908), working on *Myxobolus pfeifferi*, likewise believed in the union of an analogous pair of nuclei during either the final stage of development of the spore or in the young animal immediately after leaving the spore case (Fig. 164, A-I). Davis (1916) observed the union of such nuclei in *Sphaerophora dimorpha* but was somewhat skeptical of his own observations, but Erdmann (1911 and 1917) confirmed Schröder in actually observing the fusion. Awerinzew (1909) on the other hand, working with *Ceratomyxa drepanopsettae*, believed that fusion or fertilization does not occur in the spore stage but after the initial development of the young animal (see also Kudo, 1924). When the latter has reached the stage with 4 nuclei, 2 of the nuclei become trophic while the other 2 become germinal giving rise by division to "microgametes" and macrogametes which fuse after "reduction." Mavor (1916) working with an allied species (*Ceratomyxa acadensis*) found uninucleate young forms which, upon the first division of the nucleus, give rise to dimorphic nuclei as described by Awerinzew. The fusion of "gametes" which Awerinzew described was confirmed in part by Keyselitz (1908) in connection with *Myxobolus pfeifferi*. Here the pansporoblasts which Keyselitz names the "propagation" cells, arise in the protoplasm of the adult organisms in the same manner as in other Myxosporidia, but the nuclei, and with them the cell body of the germinal area, divide (Fig. 164, A, B, C). The prop-

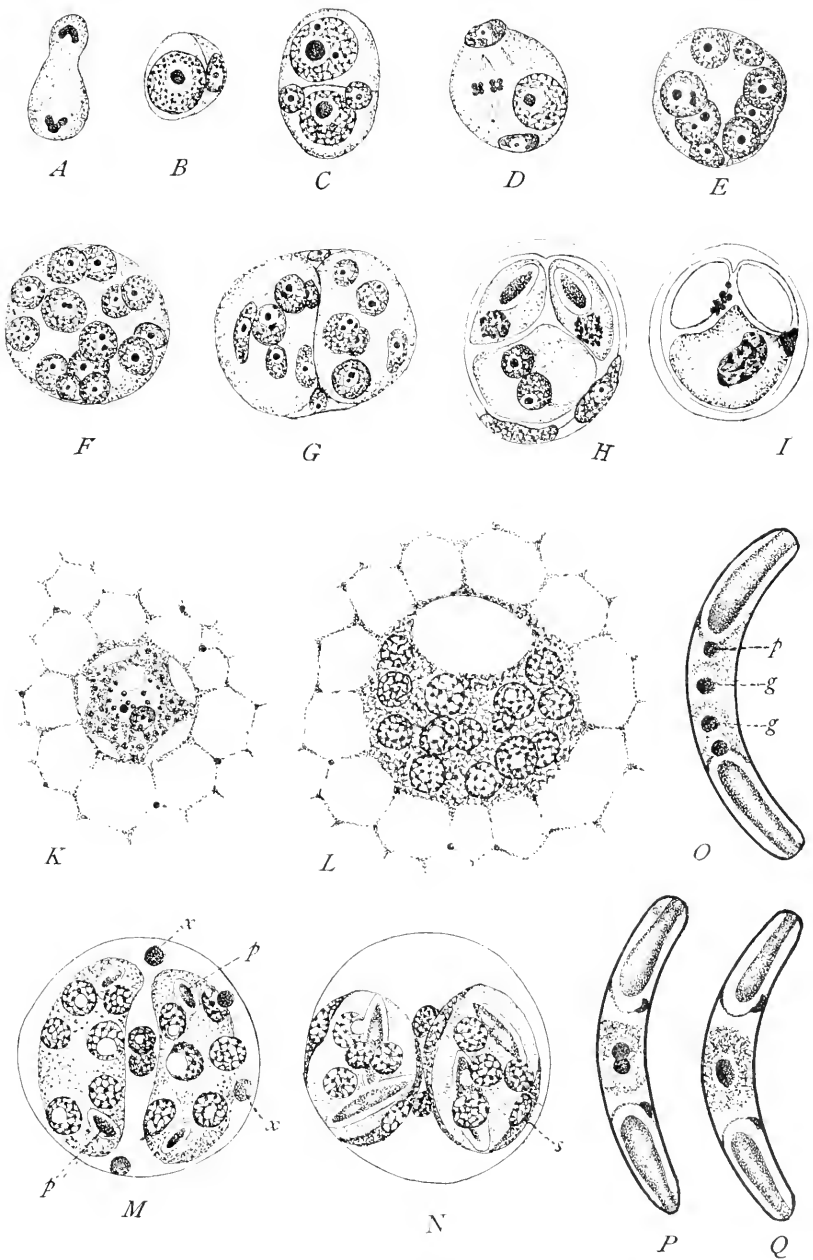


FIG. 164.—*Myxobolus pfeifferi* (A to I) and *Sphaeromyxa sabrazesi* (K to Q). See text, (After Keysseltz and Schröder.)

agative cells later unite 2 by 2 and are at first separated by a thin cell wall, which later disappears. Within this united mass the nuclei divide until there are 14 as in *Sphaeromyxa*. Such cases of fusion are interpreted by Erdmann (1917) as plastogamous in character.

A more complicated history is furnished by Naville (1931) for *Chloromyxum leydigi*. Here the initial organism has a large number of nuclei which divide by mitosis, each nucleus with 4 chromosomes. These are succeeded by heteropolar mitoses with 2 chromosomes at each pole. Two types of nuclei are thus formed, large and small, each with 2 nuclei. Upon internal bud formation a large nucleus unites with a small one. After fusion the fusion nucleus divides several times, each time with 4 chromosomes. Later each undergoes a reducing division and nuclei with 2 chromosomes again result. Each of these haploids divides to form a group of 8 nuclei in the sporogenous plasmodium and spores are formed as in other species, each spore having 2 haploid nuclei which unite later.

These observations indicate that fertilization in Myxosporidia belong in the group of autogamous phenomena. In the closely related Microsporidia there is considerable difference of opinion in connection with the time and place of fertilization if it occurs at all. Stempell (1902, 1904, 1909) and Fantham and Porter (1912) give evidence to indicate that union of nuclei occurs as in Myxosporidia and after the spore leaves its capsule. Mercier (1909), Swarczewsky (1914) and others believe that the formation of heterogametes occurs prior to sporulation as described by Awerinzew for *Ceratomyxa*; Debaisieux (1913, 1915, 1916) also believes in a process of autogamy prior to sporulation in *Glugea danilewskyi*, *G. mulleri*, *G. anomala*, and in microsporidian parasites of Simulium larvae.

Similarly a process of autogamy occurs prior to sporulation in Actinomyxida. Here, according to the observations of Caullery and Mesnil (1905) on *Sphaeractinomyxon*, the youngest stages are found as intestinal parasites of the tubificid worm Clitellio, and are either uninucleated or binucleated. The observers were inclined to believe that the uninucleated stage comes first and that it represents, possibly, a sporozoite. Whatever may be the origin of the binucleated form, the 2 nuclei divide and 2 of the 4 resulting nuclei become somatic nuclei connected with the formation of the cyst wall. The remaining nuclei and cell body now divide until there are 16 independent cells. These unite 2 by 2, fertilization thus occurring endogamously, and 8 spores are finally formed.

In many of these cases so-called reduction nuclei have been described as indicating processes comparable with chromosome reduction in meiosis. Up to the present time, however, while well-marked chromosomes of definite number have been described by George-

witsch (1915), by Davis (1916) and by Naville (1931), there is little evidence of reduction in number either before or after nuclear fusion, with the exception of Naville's account, and this is difficult to harmonize with meiotic phenomena in either protozoa or metazoa. Erdmann (1917) has shown that so-called reduction nuclei inside the spore are masses of chromatin or perhaps glycogen, which serve a purpose in the formation of the spore membrane. The extremely minute size of the nuclei and the technical difficulties make the general problem very difficult to solve in Cnidosporidia.

From the foregoing review it is apparent that the changes of a cumulative character are taking place during the vegetative activities in all types of organization. Such changes are manifested by structural or functional peculiarities at different stages, the most marked of which are at periods of maturity and old age. Some of these are peculiar to certain types only, *e. g.*, the old age structural differentiations of Mycetozoa and Sporozoa. Others, particularly those occurring at maturity, are more universal but differ in degree in different cases, the least evident being those of hologametes and conjugating Infusoria, and the most evident are those in which complete anisogamy occurs. One widely spread effect of such differentiation is the phenomenon of meiosis or reduction in the number of chromosomes. This also occurs at various periods, furnishing a basis for the categories of conjugant meiosis, gametic meiosis and zygotic meiosis.

Whatever may be the interpretation of the phenomenon, the fact is obvious that all products of fertilization are labile, active organisms quite different in character from the conjugants, hologametes, or gametes which participated in their production. Apparently the same protoplasm, however, is continuous from the old to the young, and during transition certain processes, here described as disorganization and reorganization, have taken place. These processes, as I believe, are responsible for the renewal of vitality and for the inauguration of a new life cycle in a new organism, evidence for which is given in the following chapter.

CHAPTER IX.

EFFECTS OF REORGANIZATION AND THE ORIGIN OF VARIATIONS IN THE PROTOZOA.

IN the preceding chapters we have developed the ideas that life is organization plus its activity; that vitality is the sum total of actions, reactions and interactions between and amongst the aggregate of substances which make up protoplasm; that minute differences in the aggregate of substances constitute differences in organization; that no two organizations are identical; that with continued metabolism the protoplasm of a given individual undergoes changes in organization which are gradual but progressive; that such changes may be manifested by structural differentiations and by physiological activities which are characteristic of certain periods in the life cycle; and that progressive differentiation leads to a condition or protoplasmic stability such that metabolic activities weaken or cease altogether.

We have no desire to belittle or ignore the fact that observations are not all in accord with the conclusions outlined above or to underestimate the significance of data which apparently do not agree with them. We are attempting however, to formulate a conception of organization and vitality which will embrace as large a field of observational results as possible and to give a rational interpretation of them. An important part of such an interpretation is concerned with the effects of fertilization and parthenogenesis which are briefly considered in the present chapter.

I. EFFECTS OF REORGANIZATION ON VITALITY.

If our fundamental thesis that continued metabolism leads to functional weakening and ultimate cessation of vitality is correct it follows that for continued life some reconstructive or reorganizing operation is necessary. The phenomena attending cell division, together with experimental evidence (see Chapter VI), indicate that such reorganization may occur with each division of the cell, and that vitality of the protoplasm immediately after division is normally unhampered by accumulated products of activity in the form of metaplastids or of substances which are becoming inert. The deep-seated changes in organization which accompany fertilization and parthenogenesis have a similar but even more profound effect, for the protoplasm is entirely made over and new cell organs are

present for activity in a renewed cytoplasmic body, the aggregate resulting in a new organization and new vitality.

"Conjugation is a physiological necessity for maintenance of the race" (Hartmann, 1921; p. 114). This indeed is one of the oldest views as to the effect of conjugation of the ciliates. It is unfortunate perhaps that the phenomena involved became labeled with fanciful terms signifying renewal of youth (*Verjüngung* of Bütschli, 1876; *Rejuvenescence* of Maupas, 1889), terms which many hard-headed biologists find it difficult to accept. It might or might not have made some difference if the phenomena had been interpreted as a series of reactions whereby protoplasmic impedimenta are removed leaving a renovated organism and a possibility of unhampered vitality. It is in this sense that the term *rejuvenescence* is used in these pages.

Another interpretation of the phenomena, however, was early given in connection with theoretical biology. The union of two individuals in conjugation, or in fertilization generally, involves the fusion of two organizations represented either by nuclei alone as in conjugation, or by nuclei and cell bodies as in merogamy. The term *amphimixis* (Weismann) was applied to this phenomenon and its significance was interpreted as a means of inaugurating variations which would turn out to be useful or not in the grilling process of natural selection.

Of the two interpretations the former appears to be the more comprehensive and fundamental since it deals with vitality and applies not only to phenomena of fertilization but to effects of parthenogenesis as well, and may be still further extended to include the effects of periodic reproduction by cell division. The general truth of the latter interpretation is undeniable and has been repeatedly confirmed in experimental zoology, but we avoid the stigma of teleology by assuming that *amphimixis* arose in connection with the satisfying of some fundamental protoplasmic need. In other words and on this supposition, gametes were developed not as a means of ensuring *amphimixis* but as a result of vital activities and changes in organization which rendered them unable to continue metabolic activities without fusion.

It would seem that the fundamental truth of this generalization requires no argument insofar as it concerns merogamy. The fertilized egg cell is a new organism with a new potential of vitality having the possibility of development with differentiations leading to the adult organism. It is the beginning of a new life cycle for which the stimulus to development is furnished by the sperm cell. The facts of parthenogenesis, however, show that this potential is in the substance of the egg itself and that it, without participation of the sperm cell, may likewise be the beginning of a life cycle. The egg cell furthermore does not have the same organization as

did the primordial germ cell, or endothelial cell, from which it came. Reorganization of the protoplasm of that endothelial cell has taken place in its metamorphosis to an egg cell and is brought about by the often-described process of oogenesis and maturation. In this phenomenon of endothelial cell metamorphosis we find the homologue in Metazoa of the reorganization processes of the Protozoa.

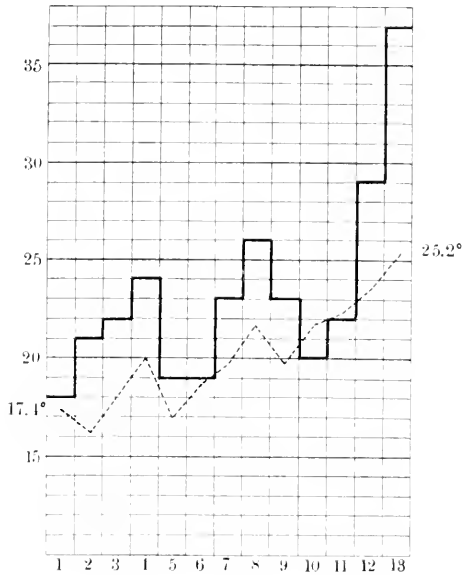
The nearest approach to the metazoön egg and spermatozoön condition amongst animal Protozoa is found in the group Coccidioromorpha amongst Sporozoa. Here, no less than in Metazoa, the fertilized egg is the beginning of a new life cycle or, by metagametic divisions, gives rise to sporozoites, each of which is the beginning of an independent life cycle with its characteristic phases and differentiations. Few biologists would question the application to Sporozoa of the term life cycle, and yet no single individual spermatozoön has ever been followed through the sequence of changes from fertilization to fertilization. This cyclical history of Sporozoa is forgotten by those who speak of a life cycle in Protozoa as a myth. They have in mind only the ciliated Infusoria and the phenomena of conjugation; indeed the controversy over the effects of fertilization in Protozoa has been limited almost exclusively to the Infusoria.

Actual experiments to test the effects of conjugation on vitality of the Infusoria have been few in number, the majority of investigators stopping with experiments to determine the need of conjugation, *i. e.*, whether or not vitality as measured by the division-rate actually undergoes a diminution to a point where death ensues if fertilization fails (see Chapter VII). Jennings (1921) has pointed out that Maupas himself never claimed that the power to reproduce is restored by conjugation, although his experiments did lead him to the conclusion that ciliates undergo senile degeneration and natural death. This inconsistency on Maupas' part requires some explanation here for it is usually overlooked. His general conclusion is carried in the statement: "In regard to Infusoria my culture experiments have demonstrated that these Protozoa do not escape the general law of senescence" (1888, p. 273). From this conclusion we would naturally infer that senescence means a weakening of the general physiological processes including the power to reproduce by division. But Maupas apparently had no such conception of senescence for he adds: "The power of multiplication follows no such diminishing and parallel course. It is maintained almost intact even a long time after the other functions, and the entire organism, are shown to be greatly reduced by senile degeneration" (1888, p. 273).

The inconsistencies in Maupas' conclusions have been pointed out in another place (Calkins, 1923); it is sufficient here to state that exact data in the form of daily records of divisions were kept

by Maupas for only three series of individuals, and data for only one series (*Stylonychia pustulata*) were published in full. The graph shown in Fig. 165 was constructed from these published data and it certainly appears to bear out his conclusion concerning

Stylonychia pustulata



Stylonychia mytilus

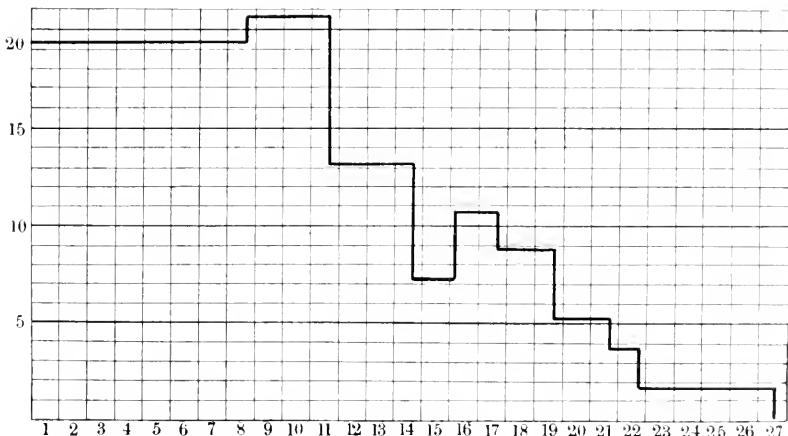


FIG. 165.—Vitality graphs of *Stylonychia pustulata* and *S. mytilus* from records by Maupas.

multiplication. For another series, however (*Stylonychia mytilus*), data were given for a different purpose and from these the graph shown in Fig. 165 (below) was constructed. From this graph it is apparent that his conclusions regarding multiplication and vitality do not agree with his records. Maupas' experimental evidence in connection with vitality after conjugation thus counts for very little either for or against rejuvenescence.

A much more carefully planned and executed series of experiments to test the effect of conjugation on the division-rate were carried out on *Paramecium* by Jennings (1913) and later by his students (Stocking, 1915; Raffel, 1930, *et al.*). He found: (1) That ex-conjugants in only a few exceptional cases have a higher division-rate than do non-conjugants of the same strain; (2) that conjugation causes a decrease in division-rate of the great majority of ex-conjugants; (3) that conjugation causes a high mortality among ex-conjugants; (4) that it causes a marked increase of weak, sickly, and abnormal individuals. From these results it would appear that conjugation is a highly unprofitable habit of the Infusoria which, if freely indulged in by *Paramecium*, would soon lead to the extermination of the race. The annual crop of *Paramecium*, however, remains about the same and we are forced to interpret Jennings' results as due more probably to the conditions under which the experiments were carried on than to the effects of conjugation (see *infra* p. 350, and Calkins, 1923).

The question of increased vitality after conjugation receives a definitely affirmative answer with Woodruff and Spencer's experiments with *Spathidium spathula* (1924). Conjugation tests furnished material from pure lines for conjugation and ex-conjugants were isolated and followed out in isolation cultures. The daily division-rates for parent and offspring series were compared with great exactness. Ninety-four different ex-conjugant series were thus available for comparison with their respective parental series. Of these the parent series died in 15 cases during the first fifteen days of life of the ex-conjugants but the latter "all actually divided more rapidly than their respective parents" (p. 187) during the periods in which the parents were alive. In 67 cases both parents and offspring continued to live and divide for more than fifteen days, the offspring in all cases dividing more frequently than the parents. Eighty-two cases therefore out of 94 ex-conjugant series showed a definitely marked increase in vitality as measured by the division-rate, as a result of conjugation: "it is evident that conjugation directly induces an immediate acceleration of the reproductive activity" (1924, p. 188). The same conclusion is reached for the full life history of ex-conjugants in comparison with the remaining life of the parental series after conjugations have occurred. "Since conjugation is the sole variable involved

in ex-conjugant and parental cultures it is evident that conjugation directly induces not only an immediate acceleration of reproduction but also an acceleration which persists at least as long as the life of the parental cultures. These results are in opposition to all results which indicate that conjugation is devoid of a profound physiological stimulation of the metabolic activities of the cell expressed in reproduction" (*loc. cit.*, p. 189). Thus in *Spathidium spathula* not only are the division-rates of ex-conjugants higher than those of the parental strains but the ex-conjugants actually outlive the parent protoplasm, hence the authors further conclude: "Conjugation typically has a high survival value in the life of the organism" (p. 196).

It is significant that Woodruff and Spencer studiously avoid use of the term "rejuvenescence" in their work. They speak of an increased division-rate of ex-conjugants and of the "survival value" of conjugation but not of renewal of vitality. As these are the two essential factors which characterize the phenomena of rejuvenescence we are justified in including Woodruff among the proponents of rejuvenescence. The two factors were discussed in an earlier analysis of rejuvenescence (Calkins, 1920) in which it was pointed out that the division-rate expresses the "intensity" of vitality and the length of life in division days the "endurance;" the latter is evidently the same as Woodruff and Spencer's "survival value."

The experimental work on *Spathidium spathula* was a confirmation of the work on *Uroleptus mobilis* which was begun in 1917. A single ex-conjugant was the progenitor of all the material that has formed the subject of the investigation. The method employed throughout was the usual isolation culture method (see p. 248). In the following account of the experiments the term "series" always means an ex-conjugant with the progeny formed from it by division; the progeny being represented by five pure lines which are continued by isolation cultures until vitality is exhausted and death ensues. Conjugation tests at regular intervals provide material for filial series. Up to January 1, 1925, 125 different series had been studied; 116 of them had followed the usual history and had died out and 9 series were under culture. The last of these 9 series represents the F 29 generation of successive conjugations since the original ex-conjugant was isolated. Abundant statistical data were accumulated during these seven years and these furnish valuable evidence in favor of the theory of rejuvenescence.

The analysis of this evidence has been the subject of many papers by numerous writers (Calkins, Woodruff, Jennings, Robertson, *et al.*) from which the general conclusions may be drawn that renewal of vitality follows conjugation, and that the extent of renewed vitality as well as the continued vitality depend upon the age of the parental protoplasm at the time of conjugation. The

following synoptic table shows not only these facts but also that for *Uroleptus mobilis* vitality may be maintained at an optimum by conjugations during youthful periods of consecutive series (see also Fig. 166). Experimental data show that parthenogenesis (endomixis) also brings about a similar restoration to an optimum vitality.

1. **Renewal of Vitality as a Result of Conjugation.**—In Chapter VII it was shown that the life cycle of an ex-conjugant of *Uroleptus mobilis* begins with high vitality; this gradually weakens during a period of from nine to twelve months and ends with death of the

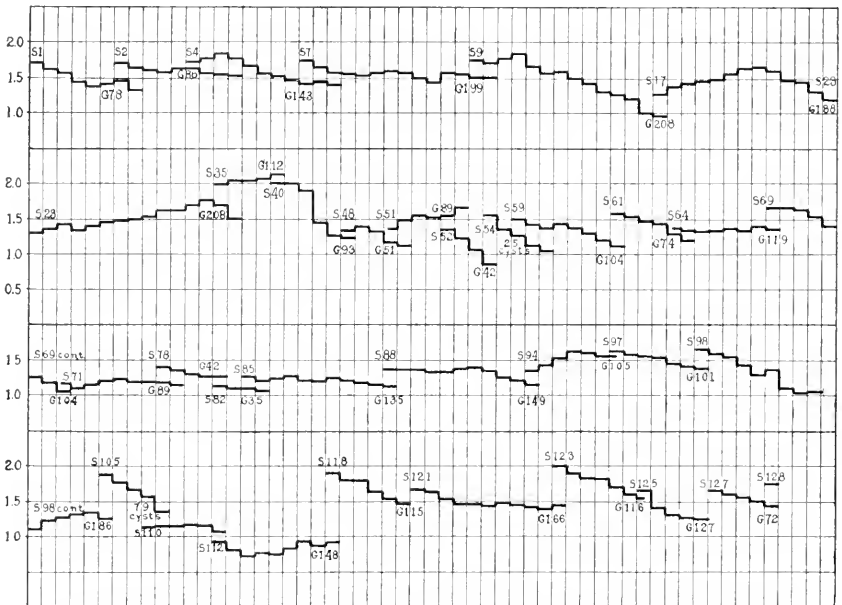


FIG. 166.—Condensed vitality graphs showing the descent of *Uroleptus mobilis* from November, 1917 to 1926. S = series; G = generation age of parents.

last individual representing that protoplasm if reorganization by fertilization or parthenogenesis has been prevented. A full pedigree of a late series (128) is illustrated by the graphs shown in Fig. 166. Conjugation between the progeny of an ex-conjugant occurs whenever a conjugation test is made after the series is mature (see p. 271). An ex-conjugant from such a mating has a higher vitality as expressed by the division-rate than the individuals of the parent series which had not conjugated. The test for this is shown by a comparison of the division-rate of the parent protoplasm which has not conjugated with the division-rate of the protoplasm that had conjugated, both protoplasts running simul-

taneously and under identical conditions in isolation cultures. If such conjugations occur early in the life history of the parent series both parent and offspring run simultaneously for some months; if late in the life history of the parent the offspring series outlives the parent, in some cases for many months. An arbitrary test of the difference in vitality of parent and offspring is furnished by a comparison of the division-rate of the ex-conjugant for its first sixty days of life with the division-rate of the parent during the same calendar sixty days. The difference between the two rates indicates the difference in intensity of vitality between parent and offspring. In the accompanying synoptic table data are listed for all series from 1 to 120, including series number, relative vitality (column 2), number of generations attained (column 3), number of division days (column 4), parent series (column 5), age of parent series at time of conjugation (column 6); number of divisions of parent subsequent to conjugation (column 7); intensity of vitality of parent and offspring and differences between these intensities (columns 8, 9 and 10). The division-rates represent the numbers of divisions which any individual of a series would undergo in ten days.

The last column of the table on pages 336, 337 and 338 gives an emphatic affirmative to the question: Does conjugation effect a renewal of vitality?

2. Intensity of Vitality and Extent of Renewal.--An important matter which is usually overlooked in experiments of this nature is the intensity of vitality of the parent protoplasm at the time of offspring-forming conjugations. The metabolic activity, growth and reproduction, of an organism are not unlimited, each species having its limit of vitality. As more water cannot be forced into a jug that is already filled, so it is impossible, under constant temperature conditions, to increase vitality in protoplasm that is already functioning to its full capacity. In *Uroleptus*, however, conjugations do not occur when the protoplasm is at its maximum of vitality and the difference in intensity of vitality between parent and offspring depends upon the age of the former at the time of conjugation. With offspring from young parents the parental vitality is relatively high and the difference in intensity for the first sixty days of life of the offspring between parent and offspring is frequently so small as to fall within the limits of fluctuating variations or of experimental error. This was the case for example in Series 2, 4, 64, 71, 78, 79, 85, 96, 97, 102, 104, and 111 where the difference in intensity is less than two divisions in ten days. Reference to column 6 of the table shows that all of these series came from young parents. Such slight differences afford little positive evidence of rejuvenescence and failure to take into account the age of parents explains a number of discordant results in the literature of this subject. With advancing age of the parent protoplasm the differ-

SYNOPTIC TABLE OF SERIES OF UROLEPTUS MOBILIS.

Series, 1.	Offspring.			Parents.			Intensity.		
	Relative virality, per cent, 2.	Age.		Series, 5.	Age, 6.	Divisions after offspring, 7.	First 60 days after off- spring.		Difference, 10.
		Divisions, 3	Days of division, 4				offspring, 8.	Parents, 9.	
1	90.9	313	267	17.3	15.5	+ 1.7
2	97.7	349	294	1	75	235	17.2	14.0	+ 3.2
3	73.6	271	215	1	137	176	17.2	16.0	+ 1.2
4	94.1	317	256	2	86	263	17.5
5	81.2	291	253	Cyst	17.5
6	78.6	268	245	16.8	9.1	+ 7.7
7	89.0	322	238	4	143	174	17.1	4.4	+ 12.7
8	69.0	253	197	1	311	2	17.9	1.0	+ 16.9
9	74.5	273	210	7	199	123	17.3	15.0	+ 2.3
10*	68.5	250	196	Cyst	18.7
11	71.3	257	188	8	188	65	18.4	8.9	+ 9.5
12	79.5	268	197	10	108	...	18.2	14.8	+ 3.6
13	95.0	327	287	9	116	137	15.6	12.0	+ 3.6
14	5.4	23	35	7	316	6	3.4	0.6	+ 2.8
15	48.1	180	185	8	245	8	13.9	0.8	+ 13.1
16	76.3	285	236	9	208	65	12.3	6.9	+ 5.0
17	85.9	301	255	9	208	65	12.7	6.9	+ 5.8
18	88.1	317	265	13	120	207	15.5	12.4	+ 3.1
19	110.4	397	598	11	225	32	10.4	1.0	+ 9.4
20	71.8	281	286	13	135	192	11.8
21	81.3	313	376	13	158	169	14.3	14.2	+ 0.1
22	64.5	264	291	13	158	169	16.7	14.2	+ 2.5
23	89.3	328	225	9	208	65	13.1	5.6	+ 7.5
24	57.8	226	168	18	270	47	15.0	3.8	+ 11.2
25	52.7	191	165	22	183	99	14.0	6.6	+ 7.4
26	42.7	163	125	22	205	59	15.2	7.7	+ 7.5
27	46.3	181	123	22	205	59	15.5	5.4	+ 10.1
28	52.9	202	163	18	270	47	15.6	3.8	+ 11.8
29	51.5	192	136	18	270	47	14.5	3.8	+ 10.7
30	31.8	120	101	18	270	47	15.0	3.8	+ 11.2
31	51.3	202	140	18	270	47	16.0	3.8	+ 12.2
32	44.0	151	148	13	291	36	12.4	4.0	+ 8.4
33	43.6	137	120	32	64	...	14.4
34	10	33	80
35	94.6	370	311	23	208	120	19.7	15.2	+ 4.5
36	78.2	302	318	23	248	80	21.3	8.5	+ 12.8
37	42.6	177	103	34	147	...	19.6	6.4	+ 13.2

38	55	192	296	35	92	278	206	186	210
39	48	187	295	28	176	246	198	6	167
40	87	302	270	35	112	258	200	9	131
41	81	286	219	19	235	...	203	8	35
42	50	187	134	37	170	107	185	6	21
43	90	316	268	19	293	...	186	0	26
44	39	147	199	19	332	...	122	1	0
45	105	394	357	39	394	93	136	9	17
46	90	320	330	40	90	212	152	1	28
47	5	20	42	24	224	2	23	2	1
48	79	5	193	41	93	193	136	0	0
49	65	0	238	40	140	162	138	6	0
50	79	0	319	41	130	117	131	7	3
51	70	4	267	48	51	223	110	1	21
52	29	0	105	51	89	178	111	1	21
53	60	0	211	45	142	252	136	7	3
54	54	0	210	42	63	153	126	6	39
55	71	0	262	50	160	159	150	8	65
56	61	3	193	53	121	93	105	5	5
57	60	0	219	49	226	12	117	8	16
58	41	5	167	53	157	57	3	8	109
59*	82	7	250	53	51	165	143	7	33
60	71	3	270	59	51	...	119	6	80
61	97	3	319	59	104	...	165	1	21
62	96	4	316	60	97	173	159	1	4
63	31	1	123	56	170	23	136	6	0
64	91	1	346	61	71	245	137	2	0
65	92	7	351	61	110	209	137	0	117
66	69	1	233	57	181	245	145	2	14
67*	36	3	131	63	106	...	134	0	4
68*	30	9	138	63	143	...	153	6	0
69	99	1	350	64	119	227	135	6	0
70	95	0	355	66	141	192	176	8	8
71	83	6	311	69	116	231	150	1	8
72	81	8	312	64	219	127	146	6	10
73	76	8	286	61	225	121	108	2	36
74	104	7	3
75
76
77	89	1	331	70	96	254	131	8	...
78	75	0	288	71	89	225	138	2	33
79	65	9	291	72	145	197	132	0	6
80	65	0	256	73	105	183	104	0	2
81	51	8	206	74	132	11	124	0	24
82	54	1	218	75	142	246	112	6	0
83	68	1	239	76	140	202	114	6	13
84	73	6	261	78	86	185	118	0	3
85	90	4	319	82	35	155	112	2	0
							121	2	1

Series discarded after fifty days; same as 71.
 Failed to reorganize.

Discarded after fifty days; same as 77.

SYNOPTIC TABLE OF SERIES OF UROLEPTUS MOBILIS—Continued.

Series, 1.	Offspring.		Parents.		Intensity.		Difference, 10.	Series given away. Series lost by accident.	
	Relative virality, per cent.	Age.	Series, 5.	Age, 6.	First 60 days after off- spring.				
					Divisions, 3.	Days of division, 4.			offspring, 8.
86	
87*	87.2	305	236	85	135	184	13.6	10.6	+ 3.6
88	103.6	367	405	87	68	...	12.0	13.6	- 1.6
89	90	356	290	87	88	...	15.3	12.6	+ 2.7
91	76.9	310	274	89	278	89	15.4	11.5	+ 3.9
92	87.0	329	222	91	52	258	16.0	13.7	+ 2.3
93	93.6	330	256	91	70	240	15.8	13.7	+ 2.1
94	92.2	335	313	88	149	136	13.6	10.8	+ 2.8
95	92.4	350	297	90	168	188	17.2	13.2	+ 4.0
96	83.6	348	301	93	123	227	15.0	14.6	+ 0.4
97	79.1	299	285	94	165	230	16.2	14.3	+ 1.9
98	86.0	308	245	97	101	198	16.0	13.8	+ 2.6
99	79.5	299	219	95	171	137	18.7	13.8	+ 4.9
100
101	66.0	299	162	98	92	216	13.6	10.6	+ 3.6
102	92.7	348	259	99	93	206	15.0	13.2	+ 1.8
103	67.2	248	267	99	205	197	15.7	11.7	+ 4.0
104	70.3	266	236	102	111	237	19.0	19.0	0.0
105	76.6	289	239	98	186	122	18.7	12.5	+ 6.2
106	44.3	181	193	105	116	173	12.9	11.6	+ 1.3
107	15.4	56	55	102	247	101	7.7	8.1	- 0.4
108
109	49.3	201	162	105	185	104	10.9	7.5	+ 3.4
110*	63.5	236	267	105	105	104	11.3	6.4	+ 4.9
111	74.5	313	256	110	72	164	10.8	9.6	+ 1.2
112	77.6	316	256	110	72	164	9.1	9.6	- 0.5
113	6.2	35	34	110	125	111	4.4	7.6	- 3.2
114*	53.6	194	224	111	174	97	8.3	9.4	- 1.1
115	81.3	311	221	109	174	27	10.5	2.8	+ 7.7
116	87.1	341	278	111	125	188	12.8	12.2	+ 0.6
117	86.3	312	292	115	48	236	13.4	10.4	+ 3.0
118	88.1	332	287	115	148	168	19.3	15.2	+ 4.1
119	50.8	188	134	114	75	119	17.6	13.1	+ 4.5
120	117	130	182	15.2	8.6	+ 6.6

Died in twenty days; reversion from series 89.

Series failed to reorganize.

ence in intensity between parent and offspring becomes more pronounced. The young ex-conjugant returns to the full capacity of the species while the parent protoplasm shows the vitality characteristic of its age. The difference between them is now beyond the range of fluctuating variations or of experimental error and furnishes unmistakable evidence of rejuvenescence. Series 7, 11, 24, 27, 28, 29, 30, 31, 36, 57, and 63, which exceed their parents in rate of division by from 8 to 10 divisions per ten days, illustrate this point, and reference to column 6 shows that these series came from parents well along in age. With extremely old parents finally the difference in intensity between parents and offspring reaches its maximum and if parents have less than 35 divisions subsequent to their age at the time of conjugation (column 7), the offspring have an intensity of from 11 to 16 divisions per ten days more than the parent protoplasm (Series 8, 15, 39, 63).

3. Relative Vitality of Different Series and Effect of Parents' Age on Vitality of Offspring.—Do ex-conjugants from old parents have as much vitality as do ex-conjugants from young parents? That is, is the organization of offspring affected by the depleted vitality of the parent? Except in extreme cases these questions cannot be answered by comparison of the intensities of vitality of the two series. For example a series living two hundred days and dividing 300 times would have an average intensity of vitality indicated by 15 divisions in ten days; another series living only fifty days and dividing only 75 times likewise has an intensity of 15 divisions per ten days. It would be far from exact to say that the two series have the same vitality; here the time factor or endurance is not taken into account. Hence to compare vitalities of two different series both intensity and endurance must be represented. The method adopted (Calkins, 1920) rests on the principle of reference to a common, ideal life cycle represented by a numerical constant. The number of generations by division and the days of life of a series have a definite relation expressed by a percentage of such an ideal constant. Such percentages indicate the relative vitality of the different series and are listed in column 2 of the table.

With these percentages expressing relative vitality it is possible to compare different series in respect to the effect of age of parents on the vitality of offspring. There is unmistakable evidence contained in the table that offspring from old parents in the great majority of cases have a much lower relative vitality than do the parental series, or series from young parents. This is best illustrated by instances where two or more offspring series are taken off at different periods in the life history of the same parent. Such a sequence is illustrated by Series 2, 3, 6 and 8, all of which came from Series 1, and with a difference of 28.7 per cent in relative vitality between the first (Series 2) and the last (Series 8) offspring. Another

striking illustration is shown by Series 7 and its two offspring, Series 9 and 14; Series 9 came from Series 7 when the latter had lived more than half of its life and its relative vitality was about 15 per cent lower than its parent. Series 14 came from the same parent when the latter had only 6 more divisions in its life history and the effect of its old age is shown by the relative vitality of 5.4 per cent of its offspring, Series 14. It is quite evident that the protoplasmic organization of the parent is not the same at the beginning and at the end of its life and that the effect of the change is indicated by the organization and activities of its offspring. Some interesting and perhaps significant surprises have turned up, however, from such old age conjugations and it is possible that mutations may arise at such times. Thus Series 19 came from parents that were 225 generations old and with only 32 more generations to live. The expectation would be a low relative vitality for this old age offspring, but on the contrary it had a relative vitality of 110.4 per cent, the highest on record.

In our experience it has been impossible to restore an extremely weak series to a vigorous condition by conjugation; all such attempts result in still weaker series. It is possible, however, to restore comparatively weak series to full strength, a result which Woodruff and Spencer also obtained with *Spathidium spathula*. This is well shown by Series 60 and 62, in which the relative vitality is raised from 70.3 to 96.4, or by Series 66 and 70, in which it is raised from 69.1 to 95.0, etc.

4. **Rejuvenescence After Parthenogenesis (Endomixis).**—Woodruff's long culture of *Paramecium aurelia* furnishes an excellent illustration of continued vitality through reorganization by parthenogenesis. The fluctuations or waves in his graph (Woodruff, 1921) indicate a series of depressions followed by increased vitality; reorganization occurs during the periods of depression. Different culture media have no effect in changing the frequency of endomixis in time but may cause an increase or decrease in the number of interendomictic generations by divisions (Woodruff, 1917). According to Jollos (1916) external factors may call out parthenogenesis in *Paramecium* at any stage in the life history, and according to Young (1917) sudden sharp changes of medium may bring on endomixis prematurely, but the sequence always lapses to the regular routine and usually by the next period. If endomixis does not occur the race invariably dies. "This indicates strongly, if it does not prove that a periodic occurrence of the definitive endomictic phenomena is a *sine qua non* for the continued life of the race" (Woodruff, 1917, p. 462).

With *Uroleptus mobilis* the evidence for rejuvenescence through parthenogenesis is of the same kind as that from conjugations. Reorganization without fertilization takes place during encystment and the cysts are formed early in the life history of a series (see

p. 268). On emerging from its cyst the organism is treated as though it were an ex-conjugant and the first five individuals are maintained as five pure lines of the series. Such series are indicated in the table, p. 336, by an asterisk. The vitality of the first sixty days of a cyst series is compared with that of the parent series for the sixty days following encystment and the results are practically the same as with ex-conjugants. In some cases the cysts are kept dried for a period of weeks or months but this has no effect upon the vitality of the organism when it emerges. In all cases the evidence of rejuvenescence is the same as for ex-conjugants from young series.

The general results of these experiments with *Uroleptus mobilis* leave little ground for reasonable doubt of the rejuvenating effect of conjugation. The view of Woodruff and Spencer (1924) that loss of vitality and death here are due to conditions of the milieu seems rather far-fetched when we consider that series after series with the similar sequence of renewed, waning, and exhausted vitality pass by in apparently endless succession, and all in the same milieu so far as it is possible to make it the same, from the beginning of the experiments to the end. It is quite a different question whether or not conditions of the medium can be so altered as to bring about the same results as conjugation. The explanation must be looked for in the protoplasmic happenings at the period of conjugation or of endomixis (see Chapter VIII). In both cases these result in a rearrangement of the chromatin and cytoplasm which according to Erdmann (1921) gives rise to new sets of autocatalyzers and new cytoplasmic matrices for their activation.

The general and philosophical aspects of the phenomena described above, particularly those pertaining to the so-called physical immortality of the ciliates, are important or not according to the individual point of view. To my mind the phenomena in these forms lead to the conclusion that Protozoa and Metazoa are fundamentally alike in respect to protoplasmic continuity and protoplasmic death, the difference between them is bound up with our definitions of the "individual." So far as immortality of Protozoa is concerned, Hertwig's (1914) conclusions appear to sum up the situation: "However these investigations may turn out, one may say this now, that the doctrine of the immortality of the Protozoa in the form established by Weismann at a time when we did not know anything of the fertilization processes of the Protozoa, cannot be retained. The beautiful investigations of Erdmann and Woodruff do not detract from my conception based on former work and repeated here, but furnish a new affirmation that death in many-celled animals is the result of peculiarities which are present in everything that is alive, and that the life process contains within itself the germ of death and that the harm connected with it (death)

may be postponed in Protozoa by reorganization processes. In many-celled animals, however, these cannot be applied, the more the life of the single cell depends on the total organization." (Hertwig, 1914, p. 580.)

II. HEREDITY AND VARIATIONS IN PROTOZOA.

Owing to the relative simplicity of the organisms with which we are dealing there are few structural characteristics that can be used in a study of variations. Variations in size are often noted but

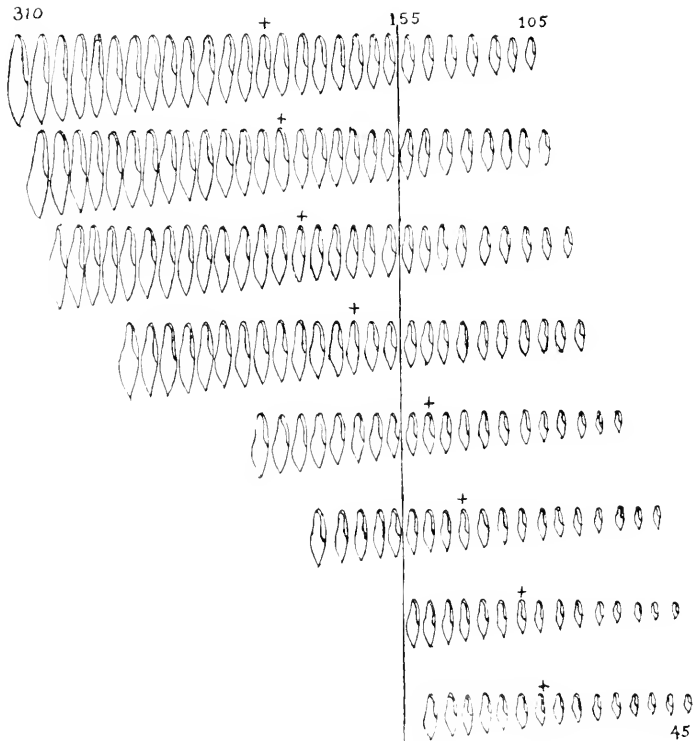


FIG. 167.—Size variations in eight families of *Paramecium*. (After Jennings.)

these in themselves do not furnish reliable data, a *Dileptus gigas* for example may be 250 microns in length or only 25 microns (Fig. 6, p. 27) according to the food it gets. Similar differences due to temporary conditions are evident in all organisms that are studied for a sufficient length of time. In a mixed population, however, size differences may indicate fixed variations as was clearly shown by Jennings (1909) for *Paramecium* (Fig. 167).

It is difficult to distinguish between fluctuating or cyclical variations and germinal variations and the distinction cannot be realized where the germinal history is unknown. The difficulty is increased by the fact that comparatively few life histories of Protozoa are known. Many variations that have been recorded may be cyclical in nature and repeated in all life histories of individuals of the species. These correspond to differentiations in ontogeny of Metazoa and have been more fully discussed in Chapter VII. The fact that such variations breed true by cell division is to be expected for the organism could not do otherwise. The test comes with amphimixis or parthenogenesis.

A. Uniparental Inheritance.—It is quite possible that changes in the genotype or organization of Protozoa may occur and remain permanently, and such changes may be due to environmental or to internal causes. Changes due to environmental causes, to be permanent, would have to so affect the germinal make-up that reversions would not occur. Thus individuals formed by reversions from the double *Uroleptus* described in Chapter VII (p. 245) never regenerated the double organism but lived as single individuals of *Uroleptus mobilis* (Series 91 of table, p. 338). Here the organization was unchanged although the new double type of organism lived for four hundred and five days and divided 367 times.

Variations due to environmental changes should be retained as long as such changes are maintained. Thus Zuelzer obtained a very different type of organism by transferring *Amoeba verrucosa* from fresh to salt water. The variation lasted as long as the organisms were kept in salt water but reverted to the original form on transference to fresh water again. Jennings (1921) cites a number of cases of bacteria in which the organization appeared to be permanently changed by a temporary change of drastic character in the medium. Similar results have been obtained with Protozoa where adaptations or responses of the organism to solutions of gradually increasing concentrations or to slowly increasing temperature changes have apparently become permanent, or at least endure for many generations by division. Among the first, and the more extensive of such experiments, were those of Dallinger and Drysdale (1873) in connection with the life histories of different flagellates. Dallinger (1907) in particular, working with remarkable patience and perseverance for seven years was able to accustom three species of flagellates which are described as *Tetramitus rostratus*, *Monas dallingeri*, and *Dallingeria drysdali* to temperatures which are fatal to these organisms under normal conditions of 60° F. At the beginning of the experiment all individuals were killed by a sudden change to 78° F., but by accustoming them to slowly increasing temperatures acting for long periods they became adapted to this condition. Such adapted individuals were then

subjected to further increases in temperature, the change from one degree of heat to another often requiring months of patient waiting. Finally he obtained individuals which continued to live vigorously in a temperature of 158° F. Here was a change in organization or an adaptation to changed conditions which persisted as long as the conditions were maintained and until an accident brought the experiment to an end.

Similar but less extensive experiments have been carried on with other Protozoa. Within the last few years Middleton (1918) and Jollos (1913, 1923) have tested the effect of increased temperatures on ciliates. Middleton (1918) separated progeny of an individual of *Stylonychia pustulata* into two groups, one of which was kept for some thirty days at a relatively high temperature (about 30° C.) the other at a low temperature (10° C.). The set at 30° C. divided more rapidly than those at 10° C. They were then transferred to a common intermediate temperature in which the previously warmed individuals continued to divide more actively than the cooled set.

Experiments of this type and others to be described below show that changes in organization can undoubtedly be produced in Protozoa. If such changes are permanent they may be interpreted as mutations; if not permanent they have little more value than the fluctuating variations which accompany changes of metabolism. The great majority of changes which have been described are certainly not mutations but illustrate the flexibility of protozoan organizations and broaden the limits within which fluctuating variations are known to occur. Such variations ultimately revert to type and although they may last for many generations by division, they have no permanent effect upon the organization. Jollos (1913) terms them "enduring modifications" (Dauermodifikationen). Other frequently-cited illustrations of this type of variations have to do with the effects of minute doses of poison on the organization. Some races of *Trypanosoma* for example, may become adapted and immune to weak doses of arsenic—the so-called poison-fast, arsenic-fast, atoxyl-fast races first described by Ehrlich. Bignami (1910) thus interprets malaria relapses as due to quinine-fast organisms. Such modified types retain their immunity for long periods and through many successive generations of transplants but they apparently belong to this type of enduring modifications. Gonder (1912) has shown that poison-fast races of *Trypanosoma lewisi* lose their acquired immunity by passing through the rat flea. Also races of *Trypanosoma* without parabasal bodies (Blepharoplastlose) first obtained by Werbitzski (1910) by injecting pyronin into the host's blood, would live for many generations of transplants without this kinetic element, but the parabasal body ultimately reappears. Here too in all probability should be included

the so-called mutations in *Radiolaria* described by Haecker (1909) the observations in this case being somewhat casual and not followed up experimentally so that the matter of permanency is in doubt.

The extensive experiments on *Paramecium* made by Jollos (1913, 1923) offer many illustrations of change in organization and subsequent return to normal, sometimes after many vegetative divisions, sometimes after endomixis, and again only after conjugation. The effects of arsenic acid calcium compounds, and extreme temperatures, were lasting through one or more periods of endomixis and conjugation, but such effects were ultimately lost. A significant fact, however, is the difference in effect produced by treatment with arsenic or heat at critical periods. If treated during vegetative life the results were as described above, *i. e.*, temporary changes or enduring modifications. If treated during the later phases of conjugation, that is, during the period of reorganization of the ex-conjugant (Jollos calls it the "sensitive" period) then the effects were found to be permanent in a very small percentage of cases. Such changes are evidence of a change in the organization itself, or in the genotype, and were found to be lasting for generations by conjugation. Jollos is apparently right in speaking of such cases as mutations.

In this connection also we should include the numerous attempts to perpetuate abnormalities in Protozoa. Popoff (1909) by centrifuging *Stentor* when about to divide, produced individuals in which the original beaded nucleus was unequally distributed, one individual receiving 16 beads, the other only 3. Both individuals reorganized perfectly after fission, but the one with 3 beads was about one-quarter the size of the individual with 16 beads. The two types were persistent and divided normally for a short time, the progeny of the smaller form regenerating the normal number of beads. The cultures were then lost so that the further history is unknown. In another case a dividing *Stentor* was suddenly cooled so that the division processes ceased. The individual was then placed under conditions of normal temperature, conditions where it reorganized into a single but very large individual. From it a race of giant *Stentors* was obtained by reproduction, the individuals breeding true for a period of about six weeks. An analogous experiment by Chatton (1921) was made on the ciliate *Glaucoma scintillans*, by treating individuals in the early phase of division with a dilute solution of sodium bromide (16 to 1000) for ten minutes. The division processes were hastened by the change in osmosis and when nearly divided the individuals were restored to their normal medium where the division planes were lost and the two nearly divided halves were again resolved into one. In this manner Chatton obtained individuals with two mouths, several micronuclei and only one macronucleus each. On reproduction some of the

offspring were similarly distomous, while some, as with the *Uroleptus mobilis* double individual, reverted to the single type. The double individuals were maintained in culture for a period of five months (sic) when they were abandoned, Chatton believing that they might be continued indefinitely by division. Analogous double individuals were obtained by Dawson (1920) by the fusion back to back of amiconucleate individuals of *Oxytricha hymenostoma*. The double individuals reproduced double individuals for 102 generations by division. Dawson's monsters ultimately died. The permanence of Chatton's *Glaucoma scintillans* may well be questioned and it is unfortunate that he discarded the race after only five months of culture. The double *Uroleptus* at the age of five months was more vigorous than at the outset, but like all other series of *Uroleptus* it ultimately died. It lived and reproduced, however, for more than fourteen months (see p. 244).

Similarly with mutilations. The mutilated portions are passively handed down to progeny by division, but the organization is not affected and in the course of a few divisions the normal type is regenerated. This was demonstrated by Jennings (1908) and confirmed by Calkins and by Peebles (1911, 1912) in cutting off the anterior or posterior end of *Paramecium*, leaving a truncated individual which did not regenerate but divided to form a perfect individual from the posterior end and a truncated individual from the anterior end (Fig. 108, p. 216); after a few divisions both anterior and posterior individuals were perfectly normal. Abnormal projections such as spines or clefts in the cortex, etc., are likewise passively transmitted to descendants by division for a limited time, but no permanent change in organization is brought about.

In general the upshot of all experiments with poisons, heat, abnormalities, etc., is failure to modify the organization of Protozoa in any permanent manner. The experiments of Jollos of treating *Paramecium* at the time of reorganization are, however, possible exceptions.

Modifications of the organization which arise from within the organism itself, on the other hand, may be permanent. Such modifications are possible through the sifting out of germinal characteristics in the course of continued metabolic activity and division. Some are manifested by morphological characters which afford a basis for selection on the part of the investigator. Experiments to this end have been carried out mainly by Jennings and his associates. The underlying principle in such selection work is that a single individual from a "wild" population is the result of a great number of hereditary characteristics stored up in the past through amphimixis and united now in the organization of the single individual. Such an individual, if cultivated under uniform conditions, gives rise to progeny showing diversities in structure or function

which are probably ancestral characters. The extreme individuals showing such diversity are selected and bred independently.

Jennings has clearly shown that such differences are characteristic of all the pure lines he has studied and his findings have been confirmed by Root (1918) for *Centropyxis aculeata*; by Hegner (1919) for *Arcella dentata*; and by Reynolds (1923) for *Arcella polypora*. While the fundamental character (genotype) of a race is maintained there are minor differences in organization which may or may not be manifested by structural peculiarities. This is strikingly shown in Jennings' studies on *Difflugia corona* (1916), a favorable form since the characteristics of the shell can be measured or counted and the structure does not change after it is once formed. In such a study Jennings says the method of evolution by slow and gradual change rather than by sudden jumps or mutations becomes visible. "We begin to exercise selection within the single family. On the one hand we select all the long-spined individuals and place them together; on the other hand we select all the short-spined ones and place them together. In the long-spined group we continue to save for generation after generation only the individuals that are long-spined; in the short-spined group only the offspring with short spines. In the same way we select other sets for numerous spines and for few spines; for large shells and for small shells; for many teeth and for fewer teeth.

"And now as we keep this up for generation after generation we find that the correspondence between parent and progeny becomes more and more marked. We find that our single family is breaking up into many different groups which differ from one another hereditarily. We get finally what appears to be two diverse races—one with long spines, the other with short spines—the difference continuing for generation after generation. A third set has constantly large shells, while others consistently produce small shells. We also get stocks hereditarily different for numbers of spines; and for numbers of teeth. Our single stock, derived by fission from a single parent, has gradually diversified itself into many stocks that are hereditarily different. If this is what we mean by evolution, we have seen evolution occur" (Jennings, 1921, pp. 75-78).

In a similar manner Root (1918) and Hegner (1918) studied uniparental inheritance in *Centropyxis aculeata* and in *Arcella dentata* and obtained results of the same nature. External agents (lack of food, salts, temperature, etc.) may bring about similar variations in size of shell, numbers of spines, etc., which persist as long as the conditions are maintained (Hegner, 1919). From this it appears that external conditions may inhibit the expression of germinal factors, but not permanently.

The interpretation as given by Jennings of these clear-cut results appears to be fundamentally sound and its significance is not less-

ened by the chromidia problems which are associated with all of these testate rhizopoda. If, as generally believed, the chromidia give rise to germ nuclei, there is some chance of this hereditarily important chromatin being unequally distributed at cell division, for the mass of chromidia is not halved with the same precision as is the chromatin of the nucleus or nuclei. Whether or not chromidia are responsible the interesting fact remains that demonstrable variations in organization occur with continued reproduction. It remains to be determined, however, whether the variations will still breed true after endogamous fertilization and reorganization, or will revert to the form of the original wild individual; then only will the matter of permanency of the changed organization be settled. Jollos (1924), exercising selection in *Arcella vulgaris*, *Arcella discoidea*, and *Arcella polyppora* obtained abnormalities in parents and offspring which he interpreted as due to environmental conditions, especially to the accumulations of metabolic waste. With cultivation under better conditions of the medium such abnormalities gradually disappeared with reversion to the normal.

Further evidence of the sorting out of mixed characteristics was given by Calkins and Gregory (1913). The first 4 of the individuals formed by an ex-conjugant of *Paramecium caudatum* were individually isolated and the history of their progeny was followed out in 32 pure lines, 8 from each of the original 4 individuals. The history of these 4 strains in one experiment is condensed in Fig. 168. Pure lines that died are indicated by X and the 4 sets of 8 lines each came from the 4 individuals A, B, C, and D. Physiological differences in the progeny of these 4 are indicated by the division-rates and by the ability to conjugate, the progeny of A for example giving epidemics of conjugation at each test while similar tests gave no conjugations in the progeny of B, C, and D until nine months of culture, and then in very small numbers. Similar variations in size were characteristic of the different quadrants. It is possible that such results are due to the segregation of germinal materials during three metagamic divisions of the amphinucleus, each of the original four cells receiving a different combination of macro- and micro-nuclei.

In general, all results that are based upon physiological differences must be cautiously interpreted. Thus with *Uroleptus mobilis* individuals from the progeny of single ex-conjugants may be selected at appropriate periods to show marked differences in division-rates. One such individual may reproduce at the rate of 17 divisions in ten days; another individual from the same line will reproduce at the rate of 8 divisions in ten days, and a third may divide at the rate of only 2 divisions. One might erroneously argue that these individuals represent the sifting out of an hereditary complex and the argument would apparently be supported by

results of conjugation between individuals of each set. In the first set the high division-rate would appear to be inherited; in the third set the low division-rate in most cases would appear to be inherited but such series invariably die. The real test is shown by conjugation

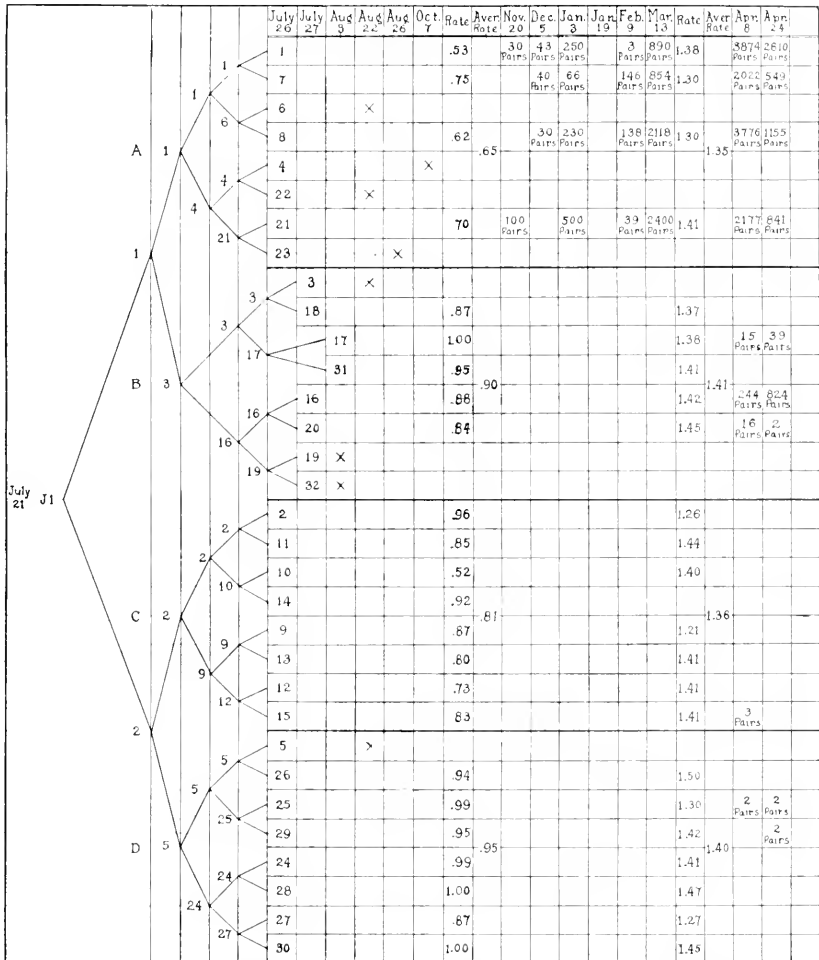


FIG. 168.—Variations in the progeny of a single ex-conjugant of *Paramoecium caudatum*. (After Calkins and Gregory.)

in the second set which results in optimum division-rates. In such sets of progeny, as shown above, the differences in vitality of the offspring through conjugation are due to differences in vitality of the parent. With low vitality of offspring from old parents it might

be argued that here is an example of the inheritance of an acquired characteristic, whereas it is merely a matter of general vitality.

B. Biparental Inheritance.—Through amphimixis there is a possibility of introducing changes in the organization of a species from within. The new amphinucleus is a new creation and its interaction with the cytoplasm must differ from previous interactions. The cytoplasm is also different in cases of merogamy and in cases of conjugation. In merogamy there is a fusion of cell bodies as well as of nuclei; in conjugation the old macronucleus, a product of the old amphinucleus, is distributed throughout the cytoplasm and absorbed. As a result of the interactions of new nucleus and new cytoplasm, new structures and new activities or changed activities may ensue.

While *a priori* such origination of variations in Protozoa is a logical consequence, as a matter of fact it has been rarely observed in Protozoa. Here genotypes as well as fixed and congenital variations usually vary little from the fluctuating variations of a species. The remarkable fixity of the genotype is indicated by the world-wide distribution of the common species, and is clearly demonstrated by long-continued cultures of any given species. Vitality also is remarkably constant as illustrated by Woodruff's long culture of *Paramecium aurelia*, or by cultures of *Uroleptus mobilis* in which the average relative vitality of the first 12 series representing the F to F₄ generations by conjugations was 83 per cent and the relative vitality of a recent set of series representing the F₁₈ to the F₂₂ generation was 85.6 per cent. Here, although there was an interval of six years between the two sets compared, the vitality remained practically the same.

Despite this constancy there is some unmistakable evidence of variations in the Protozoa. There is also considerable evidence that has been misinterpreted as mutations. Among the latter, abnormalities in reorganization may be responsible for apparent mutations. Thus a bi-micronucleated, short race of *Paramecium caudatum* was obtained as a result of conjugation of two normal individuals (Calkins, 1906). Its two micronuclei, shortened body and rounded posterior end were characteristic of *Paramecium aurelia* and the latter was erroneously interpreted as a mutation of *Paramecium caudatum*. The *aurelia* characters persisted for 45 generations by division when they were lost, and reversion to the *caudatum* type occurred, presumably during a period of endomixis. In like manner we may account for the amiconucleate races of many ciliates (Hance, Moody, Dawson, Woodruff, etc.), the amiconucleate condition persisting for many generations, but ultimately ending in death, since failure to conjugate is characteristic of such races. These are evidently not cases of mutation but temporary abnormalities resulting from imperfect reorganization.

An exceptional case of mutation is that of *Chilodon uncinatus* described by MacDougall (1925).

A single individual of *Chilodon uncinatus* was isolated by MacDougall (1925) in December. Its progeny were maintained in pure line cultures until lost in June. In May, larger forms appeared in the cultures and these increased until they outnumbered the smaller forms, few of which could then be found. Cytological examination showed that the larger form was morphologically identical with the smaller form, with the exception of the micronuclei in which the chromosomes were eight in number as against four in the smaller form. MacDougall worked out the meiotic divisions for both types and found a similar history in both (Fig. 149, p. 299) and correctly interprets the tetraploid form as a mutant from the ordinary diploid type.

The entire matter of heredity in Protozoa, together with rejuvenescence and related problems have been fully treated in a remarkably frank and impartial manner by Jennings (1929) in his excellent monograph on the *Genetics of the Protozoa*, to which the reader is referred.

The Protozoa, finally, cannot be regarded as simple organisms which may be permanently changed in structure or function at will. Each type has a remarkable tenacity of life which we believe is organization and its activity, and which may be temporarily modified by environmental changes, but in which permanent changes are rare, and when they occur must come apparently from within. Organization, on the one hand, is continuous and has been handed down from the indefinite past to the species which we know today. Vitality, on the other hand, may be discontinuous and variable and is manifested by the sum of activities which take place in the organization at any time. Death is not of necessity the cessation of vitality but the disintegration of the organization after which vitality is impossible.

CHAPTER X.

GENERAL ECOLOGY, COMMENSALISM AND PARASITISM.

As stated in the introductory chapter (p. 25), Protozoa may be found wherever there is moisture. The general distribution is also outlined, particularly with regard to deep sea forms. It is pointed out, furthermore, that fresh water forms, both genera and species, are for the most part cosmopolitan, so that a piece of research begun in New York may be continued on similar forms in Siam, China or Australia. Similar or identical species may be found in both fresh and salt water or brackish water. There are, however, a certain number of ecological centers which permit of a rough classification into: (1) Water-dwelling forms; (2) semi-terrestrial forms; (3) soil-dwelling forms; (4) sapropelic forms; (5) coprozoic forms; and (6) parasitic forms.

1. **Water-dwelling Protozoa.**—Without too much exaggeration this caption might well be applied to all Protozoa; here, however, it is limited to those Protozoa which live in ordinary exposed waters, where certain ecological conditions lend themselves to the vital needs of some types and are fatal to others. These needs have to do in the main with food requirements and oxygen pressure and a rough classification, without taxonomic value, was suggested by Kolkwitz in 1908. This was based upon the requirements of water-dwelling forms in respect to the amounts and conditions of organic matter present. Habitat groups were proposed under the terms katharobic, oligosaprobic, mesosaprobic and polysaprobic. Katharobic types are rare, for their environment is fresh water springs, running rivers and streams which are free for the most part of organic matter but rich in oxygen.

Oligosaprobic types are those which are able to live in waters with little organic matter but rich in mineral matters. The chief types here are the chlorophyll-bearing Protista, but some types of Protozoa also are able to live (*Amoeba proteus*, *Laurencia odor*, *Trachelius* sp., *Frontonia* sp., *Ophrydium versatile*, etc.).

Mesosaprobic types are numerically greater than those of other habitat groups, for in this environment active oxidation is going on and organic matter is decomposing. In addition to many algal forms we find here flagellates such as *Bodo*, *Tetramitus*, *Anthophysa* and *Peranema*, and many common ciliates including *Paramecium*, *Coleps*, *Spirostomum*, *Colpoda*, *Chilodon*, *Stentor*, *Stylonychia*, *Euploes*, *Vorticella*, etc. Heliozoa are represented by *Actinophrys* and *Actinosphaerium*.

Polysaprobic types finally live in waters with little free oxygen but with sulphuretted hydrogen, carbonic acid and other products of putrefaction advertized by their foul odors. In this group are all of the open sewage Protozoa (see p. 357), as well as some of the sapropelic fauna (Lauterborn) which live in a medium quite free from oxygen at the bottom of ponds where ill-smelling gases (methane, carbonic acid, sulphuretted hydrogen, etc.) accumulate. These are anaërobic forms some of which, characterized by fantastic shapes, are unable to live under aërobic conditions (see p. 356).

2. **Semi-terrestrial Protozoa.**—Semi-terrestrial protozoa may be found in moss, sphagnum, etc. (many types of testate rhizopods and a few flagellates and ciliates).

Not only food and oxygen but relative alkalinity and acidity are also determining factors in the life of given types. *Acanthocystis aculeata*, for example, lives well with a hydrogen-ion concentration (pH) of 8.1 but dies in a less alkaline medium with pH 7.4 (Stern, 1924). Other forms may live in a distinctly acid medium and some may live in waters having a wide range of pH. In standing waters with decomposed matter at the bottom, the pH at different levels is variable which accounts in part for the sequence of forms in a hay infusion (Woodruff, 1912; Bresslau, 1926, *et al.*) and the segregation of specific types at different levels.

With the varying conditions to which Protozoa are adapted and under which they live and thrive, it is probable that some types are more readily adaptable to a parasitic mode of life than others. Anaërobic forms, for example, are already partially adapted.

3. **Soil-dwelling Protozoa.**—It is to be expected that an occasional water-dwelling form of Protozoa should be found in the soil, particularly where moisture abounds. It is also possible that coprozoic forms under suitable conditions in the soil might have a more or less extended life. The so-called soil Protozoa, therefore, might well include representative genera and species of both water-dwelling and coprozoic forms.

Modern studies of representative soils from all over the world, including all types, have demonstrated, however, that the great numbers of Protozoa found cannot be accounted for on any such casual basis. In this connection Sandon (1927) states that soil forms constitute a fairly well-defined group, with characteristic functional needs and cannot well be regarded as an accidental collection.¹

Sandon's conclusion is supported by the facts of distribution which he adduces. These apply to all groups of Protozoa of which he

¹ Sandon, H.: The Composition and Distribution of the Protozoan Fauna of the Soil, London, 1927, p. 63.

describes no less than 250 species capable of living in the soil. With arable soils the maximum numbers are found, as a rule, at a depth of 4 to 5 inches while the sub-soil is generally free from them. Such soil-dwelling types are able to live under partial anaërobie conditions and are usually bacteria-eating holozoic forms. As such they become important factors in all economic matters concerning soil productiveness.

Of the flagellate group Sandon describes seven species which seem to be limited to this habitat (*Allantion trachyphloen*, Sandon; *Allas diplophysa*, Sandon; *Colponema symmetrica*, Sandon; *Phalansterium solitarium*, Sandon; *Sainouron mikroteron*, Sandon; *Tetranitus spiralis*, Goodey; *Anisonema minus*, Sandon; and *Cercobodo vibrans*, Sandon). An eighth species, *Parapolytoma satura*, Jameson, is questionably limited to the soil.

The relative frequency of animal flagellates found in 146 soils from different parts of the world is shown in the table on p. 355, condensed from Sandon's Chart II.

It is safe to say that the first 10 of these are characteristic of flagellates of the soil and may be found practically anywhere, particularly in arable and garden soils. The last 10 may well be regarded as chance specimens and without significance in soil biology. The twenty-eight intermediate species may or may not be present, depending upon environmental conditions of food (bacterial), moisture, relative acidity, etc., the great majority being species which are also found as water-dwelling or as coprozoic forms. Furthermore, methods of examination are not sufficiently perfected to determine whether a given form actually lives in the soil or is dormant there and has developed in the artificial culture medium subsequently used. It is quite possible that certain cysts never develop in earth, and it is also quite possible that active forms in the soil are killed by drying or by the conditions of culture.

In addition to the list of flagellates described by Sandon other workers have described different species from the soil, bringing the total number of soil flagellates up to about 75 species. This estimate, however, includes several forms which should be regarded as rhizopods (particularly the Bistadiidae and flagellated swimmers of the Mycetozoa) and plant flagellates. Amongst additional animal flagellates we should include *Codosiga botrytis*, Ehr. (Goodey, 1911, rare); *Salpingoeca convallaria*, Stein, and *S. ampullacea*, Braun (Wolff, 1912); *Bodo terricolus* (Martin, 1912); *Pleuromonas jaculans*, Perty (Fellers and Allison, 1920; Fantham, 1922-1924; Wolff, 1912); *Phyllomonas contorta*, Klebs (Wolff, 1912); *Ilexamitus inflatus*, Duj. (Fellers and Allison, 1920); *Monas guttula*, Ehr. (several observers); *Monas vivipara*, Ehr. (several observers); *Astasia* sp. (Fellers and Allison, 1920); *Peranema trichophorum* (various observers); *Urceolus*

cyclostomum, Stein (Fantham and Paterson, 1923-1924); and *Heteronema acus*, Stein (Fellers and Allison, 1920).

FLAGELLATES OF THE SOIL FROM 28 STATIONS IN ALL PARTS OF THE WORLD, IN THE ORDER OF FREQUENCY, CONDENSED FROM SANDON, 1927.

	Per cent.
1. <i>Heteromita globosa</i> , Stein	found in 143 soils, or 98.0
2. <i>Cercomonas species</i>	found in 99 soils, or 67.8
3. <i>Oikomonas termo</i> , Ehrbg.	found in 93 soils, or 64.3
4. <i>Allantion mikroteron</i> , Sandon	found in 72 soils, or 50.0
5. <i>Phalansterium solitarium</i> , Sandon	found in 56 soils, or 38.3
6. <i>Tetramitus spiralis</i> , Goodey	found in 37 soils, or 25.3
7. <i>Spongomonas sp.</i>	found in 34 soils, or 23.1
8. <i>Sainouron mikroteron</i> , Sandon	found in 32 soils, or 22.0
9. <i>Cercomonas crassicauda</i> , Alexeieff	found in 31 soils, or 21.0
10. <i>Cerobodo vibrans</i> , Sandon	found in 27 soils, or 19.0
11. <i>Helkesimastix foecicola</i> , W. and Lap.	found in 23 soils, or 16.0
12. <i>Allas diplophysa</i> , Sandon	found in 21 soils, or 14.0
13. <i>Anisonema minus</i> , Sandon	found in 17 soils, or 12.0
14. <i>Proleptomonas foecicola</i> , Woodeock	found in 15 soils, or 10.0
15. <i>Spiromonas angusta</i> , Dujardin	found in 13 soils, or 9.0
16. <i>Scytomonas pusilla</i> , Stein	found in 12 soils, or 8.0
17. <i>Actinomonas mirabilis</i> , Kent	found in 11 soils, or 8.0
18. <i>Mastigella sp.</i>	found in 10 soils, or 7.0
19. <i>Heteromita sp.</i>	found in 8 soils, or 6.0
20. <i>Cerobodo agilis</i> , Moroff	found in 6 soils, or 4.0
21. <i>Tetramitus rostratus</i> , Perty	found in 6 soils, or 4.0
22. <i>Monas sp.</i>	found in 6 soils, or 4.0
23. <i>Petalomonas angusta</i> , Klebs	found in 6 soils, or 4.0
24. <i>Entosiphon sulcatum</i> , Dujardin	found in 6 soils, or 4.0
25. <i>Monosiga ovata</i> , Kent	found in 5 soils, or 3.0
26. <i>Phyllomitus amylophagus</i> , Klebs	found in 5 soils, or 3.0
27. <i>Phyllomitus undulans</i> , Stein	found in 5 soils, or 3.0
28. <i>Petalomonas sp.</i>	found in 5 soils, or 3.0
29. <i>Bodo celer</i> , Klebs	found in 4 soils, or 3.0
30. <i>Bodo saltans</i> , Ehr.	found in 4 soils, or 3.0
31. <i>Colponema symmetrica</i> , Sandon	found in 4 soils, or 3.0
32. <i>Heteromita obovata</i> , Lemmermann	found in 4 soils, or 3.0
33. <i>Cephalothamnion cycloum</i> , Stein	found in 4 soils, or 3.0
34. <i>Polytoma sp.</i>	Less than 3.0
35. <i>Mastigamoeba limax</i> , Moroff	Less than 3.0
36. <i>Heteromita ovata</i> , Dujardin	Less than 3.0
37. <i>Menoidium sp.</i>	Less than 3.0
38. <i>Chlorogonium sp.</i>	Less than 3.0
39. <i>Bodo caudatus</i> , Dujardin	Less than 1.0
40. <i>Bodo edax</i> , Klebs	Less than 1.0
41. <i>Heteromita compressa</i> , Lemmermann	Less than 1.0
42. <i>Phyllomitus sp.</i>	Less than 1.0
43. <i>Cladomonas fruticosa</i> , Stein	Less than 1.0
44. <i>Tetramitus pyriformis</i> , Klebs	Less than 1.0
45. <i>Spiromema multiciliata</i> , Klebs	Less than 1.0
46. <i>Chilomonas sp.</i>	Less than 1.0
47. <i>Cryptomonas</i>	Less than 1.0
48. <i>Petalomonas medicanellata</i> , Stein	Less than 1.0

Rhizopods and ciliates are represented by fewer genera and species than are flagellates. Of the 250 species of Protozoa living in the soil Sandon enumerates only 48 species of rhizopods and 35 species of ciliates; nor are they so widely distributed among the 146 soils from all parts of the world. *Limax* amebae were registered from 49.5 per cent of all sample soils examined; *Hartmannella hyalina*, from 42 per cent; *Nuclearia*, from 27 per cent; *Trinema enchelys*, from 22 per cent; *Trinema lineare*, 19 per cent; and *Nägleria gruberi*, from 17.5 per cent. Of ciliates, *Colpoda cucullus*, *Colpoda steinii* and *Cyrtidium glaucoma* were present in 56, 47 and 23 per cent respectively.

Few generalizations, however, can be made regarding soil-dwelling forms as distinct from other Protozoa, and there is but little evidence that morphological adaptations follow such a mode of life. This phase of Protozoölogy, however, is still young and further study will undoubtedly lead to important deductions as well as to practical results.

4. **The Sapropelic Flagellates.**—Under the term "sapropelic fauna" Lauterborn (1901) included Protozoa which are able to live in media partly or wholly free from oxygen. Some of the soil flagellates, particularly those living deep in the soil, are partially anaërobie, and might well be included here. The majority of sapropelic flagellates, however, live in sulphurous waters and in sewage, especially in the deeper zones of sewage filtration tanks where oxygen is entirely absent (polysaprobic forms, Kolkwitz, 1908).

The sapropelic fauna, according to Lauterborn, includes those forms which live and multiply in the slime on the bottom of fresh ponds or salt water pools and ditches. This slime consists, for the most part, of plant débris and animal excrement and remains, while inorganic mineral matters are reduced to a minimum. Necessary conditions leading to the accumulation of the necessary ingredients for building up this environment are: (1) A rich growth of vegetation in the surface water; (2) standing water free from currents; (3) protection against intense sunlight. In still waters dead plants and animals from the surface settle on the bottom where the protein materials decompose rapidly, giving rise to foul-smelling gaseous products such as sulphuretted hydrogen, marsh gas, carbonic acid and the like. If direct sunlight is present there is an active production of oxygen by green plants, and with the aid of aërobie bacteria progressive oxidation causes the splitting up of organic matters until stabile inorganic combinations result. Under such conditions a slime suitable for sapropelic forms does not accumulate, hence for a proper medium oxygen must be absent.

With the proper anaërobie conditions a fairly characteristic sapropelic fauna develops. Many types are intermediate and may live as semi-anaërobie forms, but others are obligatory anaërobies

and die in the presence of oxygen. Amongst the animal flagellates, Lauterborn (1916) includes as sapropelic forms: *Mastigamoeba trichophora*, Lauterborn; *Trepomonas agilis*, Duj.; *Hexamitus inflatus*, Duj. (also reported from sewage); *Rhynchomonas nasuta*, Stokes; *Pteridomonas pulex*, Penard; *Physomonas socialis*, Kent; *Heteronema acus*, Ehr.; *H. spirale*, Klebs, and *Menoidium pellucidum*, Perty. Of rhizopod types he enumerates *Pelomyxa palustris* and *Pamphagus armatus* as characteristic forms, while amongst the ciliates he finds several types which are found nowhere else (*Dactylohlanyx pisciformis*, *Discomorpha pectinata*, *Legendrea bellerophon*, *Coenomorpha melusula*, *Saprodinium dentatum* and *Pelodinium reniforme*).

With these sapropelic forms should be added the anaërobie organisms which live in sewage; a list of such forms found in Imhof tanks by Lackey (1925) includes the following animal flagellates:

COMMON FORMS.	RARE FORMS.
<i>Mastigophora:</i>	
Bodo caudatus ¹	Anthophysa vegetans
Bodo mutabilis	Bodo angustus
Bodo ovatus	Distigma proteus
Cercomonas crassicauda ¹	Eutosiphon sulcatus ¹
Cercomonas longicauda	Heteronema sp.
Cercomonas ovatus	Menoidium incurvum
Clautriavia parva	Peranema trichophorum
Dinomonas vorax	Petalomonas carinata
Hexamitus inflatus ²	Petalomonas medicanellata ¹
Mastigamoeba longifilum	Platythea micropora
Mastigamoeba reptans	Salpingoeca Marssonii
Mastigella simplex	
Monas amoebina	
Monas minima	
Notosolenus orbicularis	
Oicomonas socialis	
Pleuromonas jaculans	
Tetramitus decissus	
Trepomonas agilis ²	
<i>Rhizopods:</i>	
Euglypha alveolata	Amoeba proteus
Hartmannella hyalina ¹	Chlamydoxys stercorea
Dimastigamoeba gruberi ¹	Trinema lineare ¹
Vahlkampfia guttula	Vahlkampfia albida
Vahlkampfia limax	
<i>Infusoria:</i>	
Holophrya sp.	Aspidisca costata
Metopus sigmoides	Colpoda inflata Chilonon sp.
Saprodinium putrinum	Cinetochilum margaritaceum
Trimyema compressa	Cyclidium glaucoma
	Glaucoma scintillans

5. **The Coprozoic Protozoa.**—Coprozoic Protozoa are forms which pass through the digestive tracts of animals while encysted. Mixed with water, dung containing such cysts forms a nutrient medium

¹ Also reported as soil-dwelling.

² Also reported in sapropelic fauna.

in which ex-cystment occurs and the freed organisms live and multiply for a limited period. When their world dries up many of the active organisms have encysted. Such cysts may be carried with dust into food substances of man and other animals, and through the agency of such contaminated food they are carried, while remaining encysted, into the intestine where they do not develop but which ultimately will provide a nutrient medium for their development. In artificial cultures made up with feces of different animals many such coprozoic Protozoa may be found, and it is obvious that unwary observers may mistake them for parasitic forms of the intestine.

At the present time at least, it is hardly feasible to speak of a definite coprozoic fauna since many of the cysts which pass through an intestine may contain organisms capable of living in stagnant waters, or as parasites in the intestines of different types of animals. There are several forms of flagellates, however, which develop from cysts in dung and which in the unencysted condition are not known as parasites. Amongst such coprozoic flagellates perhaps the most common type is *Bodo caudatus*, Duj., which, as would be expected, is also common in sewage; *Rhynchomonas nasuta*, Stokes, is coprozoic in cockroaches (Parisi), but seems to be widely distributed in fresh (Stokes, Bělař) and in salt water (Griessmann). *Cercomonas longicauda*, Duj., from human feces also occurs in sewage.

PARASITIC PROTOZOA.

By virtue of protoplasmic irritability there is a constant reaction of the organization to environmental stimuli (see Chapter V). The reaction may be manifested by morphological or physiological changes which we interpret as adaptations. If the stimuli are too drastic the protoplasmic response is too vigorous and disintegration results. A given stimulus or set of stimuli may result in controlled reactions by one type of organization, while similar stimuli may be fatal to other types. This principle is well illustrated by the protozoan parasites where complete adaptation to the environmental stimuli within a given host has resulted in organizations which disintegrate upon exposure to the different stimuli of a free-living existence, and, *vice versa*, free-living forms are killed by the drastic change to the conditions of an animal host. Great numbers of species of Protozoa have become adapted to the specific environments of different animal hosts and no type of animal is free from the possibility of protozoan infection.

We can imagine a series of progressive adaptations whereby free-living types may respond favorably to conditions of a partial anaërobic medium (many such facultative aërobic forms are known). Further adaptation to complete anaërobiosis is shown by the sapro-

pelic and sewage-dwelling fauna. Such forms are partially prepared for survival in the digestive tracts of animals, and these chances are enhanced if their protoplasmic responses to stimuli provide a resistance to the digestive fluids of the animal gut. We know of no case amongst Protozoa where such resistance has been demonstrated as a response to stimuli from the digestive tract, and must go as far afield as the nematode worms for evidence. Here it has been demonstrated that extracts from *Ascaris lumbricoides* contain anti-ferments which neutralize the digestive ferments of the host (Weinland, 1902 and 1908).

Ectoparasitic Protozoa.—An ectoparasitic mode of life in most cases is not sufficiently different from a free-living condition to call for special morphological changes. Attached forms on algae or detritus of different kinds may find an equally good anchorage on shells of molluscs, carapace and appendages of arthropods, gill structures of diverse types of fresh and salt water animals. Such forms have the advantage of moving from place to place with their hosts or of utilizing the food-bearing currents passing over their gills. There is some evidence of adaptation to particular hosts even in these ectoparasitic forms. Thus one can usually find *Zoöthamnium affine* and *Lagenophrys nassa* on the legs of *Gammarus pulex* and *Spirochona gemmipara* and *Dendrocometes paradoxus* on the gill lamellae while other species of the same genera are usually found on *Asellus*. In some cases special adaptations for such a mode of life have been developed. Thus the suctorian *Trichophrya salparum* adheres like a saddle to a gill bar of *Salpa* (Fig. 100, p. 192) or the vorticellid ciliate *Ellobiophrya donacis* (Chatton and Lwoff, 1929, 1923) in which the usual adhesion disc (as in Scyphidia) is drawn out in two arms which encircle a gill filament of the lamelli-branch *Donax vittatus* (Fig. 104, p. 202). More frequently an attaching organ ("scopula," Fauré-Fremiet, 1910) is provided with spines or hooks as in *Trichodina* species or *Cyclochaeta* on *Hydra*. A specific thigmotactic reaction appears to keep *Kerona pediculus* on the ectodermal surface of *Hydra fusca*.

Such forms, however, can scarcely be called parasites for they apparently cause no ill-effects on the host. Schröder's term "Planktonepibionten," or simply epibionts, appears to be more appropriate. Ectoparasites in a strict sense are rare and appear to be limited to fish hosts where the flagellate *Costia necatrix* grows to such numbers that vitality, especially of young fish, is greatly impaired. Of the ciliates, *Chilodon cyprini* furnishes a similar case, while *Icthyophthirius multifiliis*, by boring into the skin of fish, becomes a more deeply-lying parasite and the cause of distributed ulcerations.

Endoparasitic Protozoa.—In this group adaptations which are often highly complex are mostly physiological and are directed toward the preservation of the individual against the antagonistic

reactions of the host, as well as toward the perpetuation of the species. Many of them are obligatory parasites of specific animal types and must find their appropriate environment to live. The first question that arises is: How do they get into the body? As a matter of fact the host, for example, the human body, is fairly well protected and gateways to the insides are limited practically to the mouth, nasal passages and the skin. The most obvious of these is the mouth leading into the digestive tract, and infection may follow the intake of contaminated food and drink. By far the greatest number of protozoan parasites are introduced by this contaminative method. Minute germs and cysts may be taken in with air currents through the nose and throat, but this method is mainly limited to bacterial infections and if we exclude the questionable Chlamydozoa, protozoan infection by this method is practically unknown.

While probably the majority of endoparasitic Protozoa are harmless, others are pathogenic and in each group with the probable exception of the ciliates we find gradations between the two, while with the Hypermastigida and termites we find a perfect symbiosis (p. 199).

The skin is a most effective barrier against infection and so long as it is kept in good condition infection by this means is reduced to a minimum. Abrasions, hang-nails, casual cuts, etc., however, are portals of entry and bacteria, spirochetes or small flagellates may gain access to the blood through such injuries. Or the skin may be punctured by biting bugs, arachnids, flies, mosquitoes, leeches and the like, and disease germs may be transmitted in this way. Scratching the skin at points of irritation, thereby providing entrance for possible parasites deposited with feces by ticks, mites or other ectoparasites, is another means of inoculative infection. Only rarely do Protozoa have invasive power of sufficient strength to penetrate the unbroken skin and then only in the more delicate coverings; such a disease is the so-called horse syphilis caused by *Trypanosoma equiperdum*.

Obviously the most important of these modes of infection is that by contaminated food and water taken into the digestive tract through the mouth. Once adapted to the conditions of the gut, intestinal parasites are prepared for further explorations and adaptations which may lead to parasitism in various organs of the host. According to their seat of parasitism, internal parasites may be grouped as entozoic (gut-dwelling), celozoic (lumen-dwelling), hematozoic (blood-dwelling), cytozoic (intracellular), histozoic (tissue-dwelling), karyozoic (intranuclear), etc.

In connection with the life history of trypanosomes Minchin (1908) developed the thesis that hematozoic forms originate from entozoic parasites in the same host. Support for this point of view,

even if not acceptable for trypanosomes, is certainly given by the life histories of several diverse types of the protozoan parasites. Amongst flagellates, for example, the genus *Trichomonas* is one of the most widely distributed types in man. While there is some question of the identity of species, representatives of the genus have been recorded from the human mouth (many observers), from the toe-nails (Wenyon), from sputum (many observers), from the pleural cavity, from the vagina (many observers), in urine (many observers), etc. Wenyon (1920) demonstrated the passage of forms from the intestine into the surrounding tissue, and Pentimalli (1923) found them in the blood. Similarly the widely distributed entozoic genus *Giardia* and other flagellates—*Eutrichomastix*, *Octomitus*, etc., are frequently present in great numbers in the blood (Reichenow). Even more striking instances of adaptation from entozoic to hematzoic mode of life are shown by coccidimorpha amongst the Sporozoa. Here in *Shellackia*, *Lankesterella*, *Hepatozoön*, etc., infection is contaminative and blood parasitism is developed in varying degrees. In all of these, infection is by the contaminative method, the sporozoites of *Shellackia* and *Hepatozoön* develop and reproduce like typical coccidia in epithelial cells of the gut. In *Hepatozoön* (Miller) the gametocytes enter the blood where they are engulfed by phagocytes. These are eaten by the mite *Lelaps echidninus*, fertilization takes place in the gut and sporozoites are formed in the body tissues of the mite—the latter when eaten by a rat enter epithelial cells and repeat the cycle. In *Shellackia* there is a similar history, but macrogametes penetrate the gut wall of the host—a lizard—and are fertilized in the deeper tissues where sporozoites are formed. These make their way into the blood where they enter red blood cells or leukocytes. Here they remain dormant until eaten by a mite and the mite eaten by a lizard. In the lizard the cycle is repeated. *Lankesterella*, a blood parasite of the frog, is a typical hematzoön. Here the initial sporozoite stage is a gut parasite of the frog, eaten with infected leeches (*Hemiclepsis marginata*). Unlike the other forms mentioned, no development occurs in the frog's gut but the sporozoites penetrate the gut wall and enter the blood where, as intracorpuseular parasites, they grow and reproduce as hematzoa.

While the above cases illustrate the change from an entozoic to a hematzoic mode of life in the same individual they do not cover the whole story of the blood parasites. It is perfectly possible for a gut parasite of one animal to become a blood parasite in an entirely different type of animal. This indeed was regarded by Leger (1904) as the mode of origin of mammalian trypanosomes. Developing as entozoic parasites of insects they were inoculated when the insect began to feed on mammalian blood and, finding a suitable medium for growth and reproduction, they multiplied until

each infected individual became a source of contamination for other insects of the same type. The cycle thus established by adaptation to the different kinds of host would continue indefinitely. However this may be with trypanosomes, and it seems to be the most probable hypothesis, there is little doubt about it in the case of malaria parasites. Here the original host was the mosquito in which fertilization and development take place in the gut and gut wall while the sporozoites are liberated in the body cavity. In all of these cases the protective cysts which all strictly gut parasites form and which safeguard the germs against an unfavorable external environment are quite unnecessary. The second host replaces the cyst.

Effects of Protozoan Parasites on the Host.—Pernicious effects of parasites depend largely upon the site of parasitism, cytozoic forms, for example, being far more destructive than celozoic, coccidia more often fatal than gregarines or Cnidosporidia or intestinal flagellates. In general the more recent the association of host and parasite the more serious are the effects upon the host, but with physiological adaptive responses on the part of both host and parasite, a balance is ultimately established which leads to commensalism or even to symbiosis (as in the association of termites and hypermastigida). South African cattle are little if at all affected by *Trypanosoma brucei*, but European cattle succumb. Domestic cattle and the wild animals of Africa thus become carriers of the disease.

Functional derangement of the host may be brought about in different ways some of which may be due to occlusion or massing of parasites in bloodvessels, ducts or lymphatics, thus shutting off the blood supply and food of vital organs. Thrombus formation in capillaries of the brain or of other vital organs, due to massing of parasites, makes tropical or pernicious malaria the most dreaded of malarial diseases. The characteristic lethargy and accompanying symptoms of African sleeping sickness are due to lack of nourishment and atrophy of nerve cells in the base of the brain, caused by the occlusion of smaller bloodvessels by accumulations of parasites and lymphocytes in the perivascular spaces. Or impairment of function may be due to the destruction of large numbers of secreting cells—the coccidian *Cyclospora karyolytica*, for example, destroys so many secreting cells of the intestine that the disease in ground moles is fatal in 100 per cent of cases (Schaudinn). Secondary organic complications may be due to the overactivity of vital organs—thus in malaria so much hemoglobin is liberated that the liver cannot take care of it all and the excess is passed on to the kidneys, resulting in hemoglobinuria and functional impairment of the excretory organs.

Secretions by parasites in many cases cause cytolysis of tissue

cells and so lead to ulcers and to abscess formation, as in amebic dysentery. The disintegrating proteins of such cells produce toxins which by autointoxication impair the vitality of the host. Less frequently there are specific toxins which poison the host but in only a few cases have such products been determined—sarcocystine from the sporozoan *Sarcocystis* is the one toxin which has been extracted (Laveran and Mesnil, 1899).

Evidences of toxin action, both by direct poisoning and by serological reactions of the host have been demonstrated in many cases, rarely indicating direct secretion by the parasite (*Sarcocystis* by Kasperek, 1895) but more often indicating a toxic compound (endotoxin) which is liberated with death of the parasite or formed as a chemical product from the breaking-down of substances composing the body of the parasite (*Endamoeba dysenteriae*, *Leishmania*, *Trypanosoma*, malaria organisms). In connection with host reactions a great deal of work of serological nature has been done especially in reference to the detection of protozoan infections. Precipitation, agglutination, lysis and complement-fixation tests have been developed to a high degree and are of the greatest importance in detecting even mild infections (see Taliaferro, 1930, p. 411, for serological methods). Craig (1926) has demonstrated two types of toxin from *Endamoeba dysenteriae*: one, a hemolysin capable of dissolving human red blood corpuscles; the other, a cytolyisin capable of breaking down the epithelial cells of the intestinal mucous membrane of man and cats. Noguchi (1924) by serological methods demonstrated the difference between morphologically identical species of *Leishmania* (*L. douorani*, *L. tropica* and *L. braziliense*). Parasiticidal reactions of the host to trypanosomes and malaria organisms have been shown by Taliaferro (1926) and for trypanosomes the parasite destroying agent was found, by experiments *in vitro*, to be a lysin by Schilling (1902), Lingard (1904), Franke (1905) and Rodet and Vallet (1906), and by Massaglia (1911–1912); also by experiments *in vivo* by Diesing (1905), Klein and Möllers (1906) and Johnson (1929). Similar reactions of the host cause a diminution in rate of reproduction of the parasites, or even its cessation (Taliaferro, 1924; Coventry, 1925).

It is evident from the few references given above to a vast field of protozoan research that definite and often specific changes occur in the blood of individuals infected with different kinds of protozoan parasites. If the results of such changes, in the form of parasiticidal lysins, agglutinins, etc., are retained in the blood, they would be effective against reinfection. Active immunity thus established by infection in a host may be of longer or shorter duration, but for the most part it lasts only for a short time. It appears to be a potent protection in some types of Leishmaniasis, particularly that produced by *Leishmania tropica*, where a localized ulceration confers

a general immunity. Advantage is said to be taken of this fact by parents in countries bordering the Mediterranean who expose children by inoculation of parasites of Oriental sore on arms or legs and so prevent further infection with possible disfiguring scars on more conspicuously exposed surfaces. Absolute immunity conferred by a single infection of *Theileria parva*, the cause of East Coast fever of cattle, is another example; another case of relative immunity is furnished by a single infection of rats with *Trypanosoma lewisi*; further infections are harmless, although the parasites may not be killed. In the majority of cases, however, the immunity reactions have no permanent value. Here as with bacterial infections the blood may contain natural substances which are inimical to specific parasites. Such individuals are said to be naturally immune. In other individuals a gradual immunity is built up by repeated infections—as in adult natives of a malarial country who have been subject to repeated infections from childhood. Many efforts also have been made to immunize by use of attenuated strains but with dubious results. Some degree of success with attenuated *Trypanosoma brucei* has been obtained (Ponselle, 1923) and with *Plasmodium praecox* of bird malaria (Et. and Ed. Sargent, 1921).

Passive immunity, of transient nature, is established in many types of protozoan disease by inoculation of blood serum from actively or normally immunized individuals. Such serums may act as alexins to stimulate phagocytosis (*c. g.*, Laveran and Mesnil, 1901, with *Trypanosoma lewisi*) or to bring about agglomerations and agglutinations resulting in swelling and disintegration (Trypanosomes and Leishmanias).

Parasitic Flagellates.—The importance of the parasitic flagellates of man centers mainly in the family Trypanosomidae. There is strong evidence to show that these forms, originally, were parasites of the digestive tract of invertebrates, mainly insects, which by contaminative or inoculative methods transmitted their parasites to vertebrates, especially to mammals, where they became adapted to conditions in organ cells and in the blood. Reinfection of the invertebrates follows from their blood-sucking habits and vertebrate and invertebrate thus become mutual carriers of infection which is often pathogenic to the former, but by mutual adaptation apparently harmless to the latter.

Invertebrate forms which are known to harbor intestinal flagellates and some of which have been proved to be, or suspected of being, transmitting agents of vertebrate parasitic flagellates are insects, arachnids and leeches. Of these the insects are by far the most important, Wenyon listing no less than 254 species containing intestinal flagellates, while arachnids are limited to 5 species and leeches to 11. Excluding insects which do not feed on vertebrates,

the number of possible transmitting agents is considerably lessened. There is, obviously, always a possibility of vertebrate infection, either by contamination or by inoculation, from insects which feed on vertebrates, but the transmission is always difficult to prove, and the fact that pathogenic flagellates live and multiply in the digestive tract of insects is no proof that the insect transmits them to mammals, although the inference is highly plausible. So it is or has been with the transmission of pathogenic *Leishmanias* by bed-bugs, flies and fleas or of *Trypanosoma* by biting flies and bugs. In some cases the transmission has been demonstrated without question of doubt and these will be considered in the following pages.

The family Trypanosomidae includes 7 genera which apparently are genetically related and reveal an interesting series in progressive parasitism. These are *Leptomonas*, *Crithidia*, *Leishmania*, *Herpetomonas*, *Endotrypanum*, *Trypanosoma* and *Schizotrypanum* of invertebrates and vertebrates, and *Phytomonas* of invertebrates and plants. These all have the same general type of structure and represent the simplest forms of flagellates (Fig. 169). In all cases the body in motile stages is elongate and ellipsoidal; the nucleus is single and of the usual endosome-bearing type; the kinetic elements are more variable, but there is always a blepharoplast usually connected by fibrils with a parabasal body. Rhizostyles, arising from the blepharoplast are sometimes present but not invariably, even in the same species. Axoplasts, analogous to axostyles, have been reported for one species of *Herpetomonas* (*H. drosophilae*, Chatton and Leger, 1911). The flagellum is of the usual type with axoneme or axial filament originating from the blepharoplast and periplastic sheath. In some forms (*Herpetomonas*, *Leptomonas*, *Crithidia*, *Leishmania* and *Phytomonas*) the kinetic complex (kinetoplast) is anterior to the nucleus; in the fully-developed forms of *Trypanosoma*, it is posterior (Fig. 169, *D*). In all cases a rhizoplast, or endoplasmic portion of the axial filament is present. In forms with the anteriorly placed blepharoplast this is relatively short, but where the blepharoplast is posterior to the nucleus it may be almost as long as the cell as in *Crithidia* forms and the trypanosome form of *Herpetomonas muscarum* (Fig. 169, *B*), here it runs along the margin of the cell restrained by the periplast. In *Trypanosoma* the axial strand becomes the margin of a delicate periplastic ledge to form an undulating membrane which vibrates with the activity of the free axial filament of the flagellum.

Other structures of the cell are less constant and of less importance — cytoplasmic granules of the nature of volutin (see p. 72) are sometimes very abundant; mitochondria and Golgi bodies have received scant attention and play no part in taxonomic or parasitic discussions.

These parasites have no mouth, food-taking being osmotic or saprozoic. They live, normally, in the dissolved food substances of the gut or in the blood but may grow and multiply in the semi-fluid protoplasm of different types of tissue cells. For the most

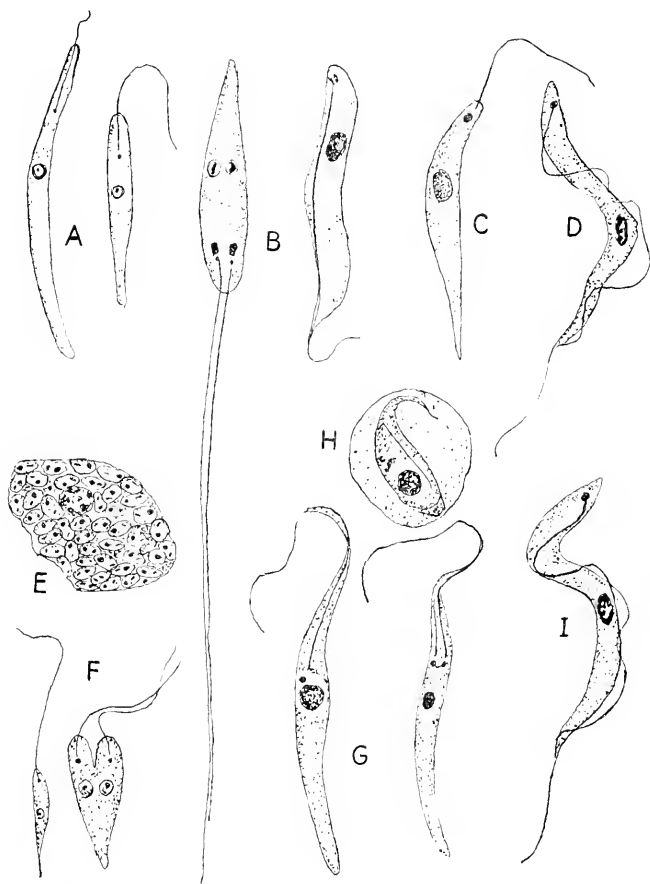


FIG. 169.—Trypanosomidae. A, *Leptomonas ctinocephalis*; B, *Herpetomonas muscarum*, at left individual in division, at right trypanosoma form. C, *Phytomonas dacidi*; D, *Trypanosoma gambiense*; E, macrophage with intracellular phase of *Leishmania donovani*; F, *Leishmania donovani*, flagellated and division stages; G, *Crithidia gerridis* from water bugs; H, *Endotrypanum schaudinni* in blood of sloth; I, *Trypanosoma rhodsiense*. \times ca 2000. (After Weynon, Protozoölogy, 1926; courtesy of Baillière, Tindall & Cox.)

part they grow readily in culture media which must be kept free from bacteria. Novy and MacNeal (1904) were the first to cultivate *Trypanosoma* in the condensation fluid of solid blood agar, their method being somewhat simplified by Nicolle and now gener-

ally used under the designation NNN agar medium. In this medium the same strains of *Leishmania* have been (1925) maintained for upward of fourteen years with hundreds of sub-cultures (Nicolle, 1925).

In a nutrient medium—digestive tract, blood, cell or artificial medium—the normal, fully-developed flagellates reproduce by longitudinal division. Blepharoplast and parabasal body are the first to divide, then the nucleus in which the endobasal body initiates division (Fig. 169, *B* and *F*). In some cases the old flagellum is retained by one of the daughter cells, a new flagellum growing out from the blepharoplast in the other cell. Multiple division of kinetic elements and nuclei, without accompanying cell division—so-called somatella formation—is characteristic of some types, particularly during intracellular stages, *e.g.*, *Trypanosoma lewisi* (Fig. 122, p. 234).

Reproduction by division is not confined to the fully-developed flagellates but may occur in any phase. Thus the “crithidia forms” or haptomonads of *Trypanosoma* may divide while attached to host cells as do the *Leishmania* forms within cells. “*Leptomonas* forms” (nectomonads) likewise divide.

The genus *Leptomonas* is the simplest of this family of parasitic flagellates. It is represented by many species which are widely distributed amongst insects and by one species in nematode worms (*L. bütschlii*, Kent, in *Triloba gracilis*). Encystment occurs in the digestive tract, the cysts passing out with the feces and infection is contaminative. Only one host—invertebrate—is known.

Structural changes are simple, from the fully-developed nectomonad with kinetic complex anterior to the nucleus, and long flagellum, it becomes progressively shorter and loses its flagellum. In this condition it may become attached to epithelial cells of the gut and Malpighian tubes (haptomonads) or it may become still smaller, develop a protecting covering and pass out with the feces.

Crithidia is a second genus of the family with only one host (invertebrate) and causing infection by contamination through the agency of cysts. It also is widely distributed amongst the insects and particularly in Diptera. Structurally it is similar to *Leptomonas* with the kinetic complex anterior to the nucleus. The endoplasmic portion of the axial filament, however, passes to the margin of the body and continues along that margin until it leaves the body at the anterior end, thus giving the impression of a rudimentary undulating membrane (Fig. 170). As in *Leptomonas* the swimming nectomonad becomes progressively shorter, attaches by the flagellar end to epithelial cells where it may reproduce by longitudinal division. Large areas of the exposed surface of epithelial cells may be covered in this manner thus hampering the functional activity of these cells (Fig. 170, *F*).

Leishmania shows an interesting and important step in progressive parasitism leading to serious, often fatal, diseases of man and

other vertebrated animals. Like the two preceding genera it has two significant phases: a nectomonad, *Leptomonas*-like, swimming phase in the invertebrate gut and in the vertebrate blood, and a quiescent phase equivalent to the haptomonads of *Leptomonas* and *Crithidia*. Unlike these haptomonads, however, the quiescent phase is not passed as cellozoic forms on the outer surfaces of cells but as cytozoic forms within the cells, not only of the gut, but of practically all types of cells throughout the body. This leads to cell hypertrophy and disintegration with a corresponding upset of function.

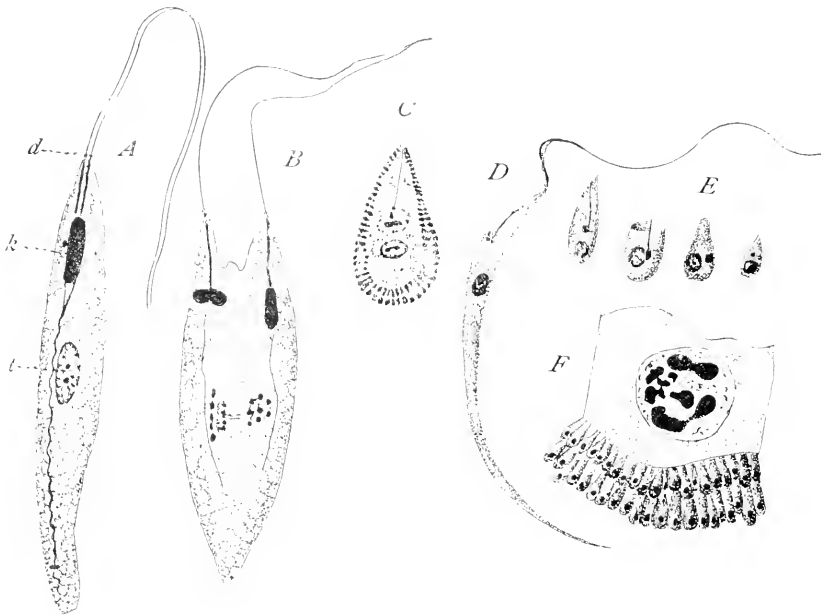


FIG. 170.—Protozoans. A, B, *Herpetomonas musca-domestica*; C, resting stage of same; D, *Crithidia subulata*, nectomonad; E, resting forms of same; F, haptomonads of same attached to epithelial cells; (d) basal bodies; (k) parabasal body; (l) nucleus. (From Calkins after Prowazek and Leger.)

The fully-developed organism is of the *Leptomonas* type (Fig. 169, F; 170, D). This stage occurs in the digestive tract of invertebrate hosts and in the blood of vertebrates, also in cultures. As cytozoic parasites they appear primarily in macrophages and other blood elements, and in cells of the liver and spleen, where they multiply by division, a single cell often containing 100 or more (Fig. 169, E).

Early reports of the parasite interpreted them as spores of peculiar organisms (macrophages) in the blood (Cunningham, 1885) or as *Sporozoa furnuculosa* (Firth, 1891). Their correct interpretation

was given in 1903, following a remarkable series of clear-cut observations which appeared in rapid succession. On May 30, 1903, Leishman published some observations, which he had made a couple of years before on peculiar intracellular bodies found in cases of dum dum fever. These he interpreted as evidence of trypanosomiasis in India. On July 11th, Donovan observed peculiar bodies in the peripheral blood of cases of kala azar. Preparations were sent to Laveran and Mesnil who regarded the "Leishman bodies" as similar to parasites (*Babesia*) of mammalian erythrocytes and on November 3rd named the organism *Piroplasma donovani*. On November 14th and 28th Ross published his conclusions that the "Leishman-Donovan bodies" are not Trypanosomes (Leishman) but a new type of organism which he named *Leishmania*. The correct name of the peculiar organism of dum dum fever or kala azar was thus established as *Leishmania donovani*. The series of observations was not yet complete, however, for in December, 1903, Wright published the results of his study of a case of tropical ulcer which was treated in a Boston hospital, and he named the organism *Helcosoma tropica*. Its resemblance to the Leishman-Donovan bodies was soon recognized, but skeptics refused to admit that the "Leishman-Donovan-Wright bodies" are organisms and held that they might be the results but not the causes of these diseases. All such doubts were dispelled, however, in 1904 when Rogers cultivated *in vitro* material taken from infected blood and spleen cells and demonstrated the transformation of the disputed "bodies" into actively moving flagellated parasites.

Further discoveries followed. Nicolle, in 1908, found a similar organism in cases of infantile ulcer which he named *Leishmania infantum*, and Vianna (1911) discovered the cause of a South American disease known as espundia, which he named *Leishmania braziliensis*.

Clinically there appear to be two types of human leishmaniasis—visceral and cutaneous. The former is characteristic of dum dum fever, also called kala azar (black sickness), the latter of infantile ulcer, tropical ulcer and Brazilian leishmaniasis. Structurally the several species are indistinguishable, but serologically *L. donovani* and *L. infantum* are apparently the same, both differing from *L. tropica* and *L. braziliensis*. In regard to the specificity of the last two there is considerable difference of opinion. Reichenow-Doflein accepts them as independent species while Wenyon considers the evidence inconclusive. *L. tropica* is the cause of localized cutaneous diseases which are widely distributed geographically and known as Oriental sore, Delhi sore, Aleppo boil, Bagdad sore, tropical ulcer, Nile ulcer, etc. *L. braziliensis* causes a similar localized initial cutaneous sore, which heals, but some time later, it may be months, the parasites reappear in the mucous membrane of mouth, nose

and throat, and cause a shocking disease resembling the effects of syphilis except that only soft parts are eaten away.

Infantile ulcer is also a cutaneous disease and differs from kala azar, which is distinctly a visceral disease, yet serologically the organisms involved are one species only. *L. donovani* antiserum will agglutinate not only *L. donovani* but *L. infantum* as well, while it will not affect *L. tropica* or *L. braziliensis*.

The parasites of kala azar occur in all possible parts of the infected human organism as intracellular forms (Fig. 169, *E*). These are small ($2\ \mu$ to $4\ \mu$), round, oval or pyriform bodies, each with a relatively large, dense nucleus and a round, ellipsoidal or rod-like body—the blepharoplast—in the cytoplasm. Division stages, $4\ \mu$ to $5\ \mu$ in diameter, and with double nucleus and blepharoplast, are frequent, showing active multiplication in this non-flagellated stage. They are most numerous in the spleen, liver and bone-marrow but are also plentiful in lymph glands, mesenteries, endothelial cells of bloodvessels, gut wall and skin, but are comparatively rare in the circulating blood where they may be found in macrophages and other cells derived from the endothelial vascular walls. Typical symptoms are irregular fever, anemia, reduced vitality, enormous enlargement of the spleen and frequently of the liver also. Acute cases if untreated usually end in death in a few months, and chronic cases in a year or more.

Diseases due to *L. tropica* are much less severe and do not involve the entire human organism, the sores, up to 1 inch in diameter, healing spontaneously within a few months, leaving a characteristic scar. They are usually on exposed portions of the body, *e. g.*, hands, wrists, legs and face, and one infection usually confers immunity (see p. 363).

South American leishmaniasis is more severe and the clinical symptoms are different, involving not only an initial cutaneous sore, but later infections of the mucous membrane of mouth, nose and throat. The skin lesions are deeper and more persistent than with *L. tropica* and multiple lesions are more frequent; Torres (1920), for example, reported one South American case in which 248 distinct sores occurred on various parts of the body (quoted from Wenyon, p. 426).

Formerly the majority of cases of leishmaniasis ended fatally; today the great majority recover. This is due to treatment with tartar emetic (or the corresponding sodium salt) which was first used with success by Vianno in South American leishmaniasis and later in the same year for cases of kala azar by Di Cristina and Caronia in 1913. Other compounds of antimony have proved useful in combatting resistant forms of *Leishmania* in spleen, bone-marrow, etc. (see Wenyon, p. 423).

The transmission of *Leishmania* is far from established. Experi-

ments have shown indeed that *L. donovani* lives and multiplies in the digestive tracts of various kinds of blood-sucking arthropods—mosquitoes, sand flies, fleas and bed-bugs, but no experiments involving transmission to man have been successful. With *L. tropica* the evidence is more positive and numerous successful experiments in producing skin ulcers from leptomonas forms in sand flies of the genus *Phlebotomus* have led to the general belief that this type of insect at least is capable of transmitting not only *L. tropica* but *L. braziliensis* as well.

The genus *Herpetomonas*, while not a parasite of vertebrates, is interesting in having a stage in which it resembles a trypanosome. In this stage the axial filament, as a rhizoplast, runs along the margin of the cell, without however raising the periplast to form an undulating membrane (Fig. 169, B).

Trypanosoma, in its fully-developed phase, differs from related forms of protomonads in having an undulating membrane, the margin of which is formed by the axial filament of the flagellum which ends in a free whip or terminates at the anterior end (Fig. 169, D, I). The axial filament arises posterior to the nucleus. Near it is a conspicuous granule, homologized by Kofoid and his school as a parabasal body. This, by use of the Feulgen nucleal reaction, has been shown to contain thymonucleic acid (see p. 118). The combination of blepharoplast and parabasal is termed the kinetoplast by Wenyon. The nucleus is usually spherical, with the usual protomonad endosome lying in a clear space within a nuclear membrane. The cytoplasm is usually clear and homogeneous but contains volutin granules, as a rule, and a small vacuole frequently lies near the kinetoplast. Reproduction is always by longitudinal division which begins with the kinetoplast. The cell divides first at the flagellar end, the posterior end with the kinetoplast dividing last.

These few structural characters afford very little basis for division of the genus into species, while the numerous changes which the same species may undergo in the course of its life history make it still more difficult. Size is some help, the largest forms occurring in cold-blooded vertebrates. Other characters are relative length of flagellum, distance from kinetoplast to posterior end, rounded or pointed posterior end, position of the nucleus, etc. The tendency is to name a trypanosome according to the host in which it is found provided there are no specific structural characters by which it can be identified—such methods may swell the synonyms but they are relatively harmless until the full life history is worked out in each case.

The following list of species,¹ while not complete, gives some idea of the distribution of trypanosomes and of the enormous literature on the subject:

¹ Compiled from Wenyon, *Protozoology*; Biological Abstracts; Zoölogical Record, and miscellaneous sources.

TRYPANOSOMA IN MAMMALS.

- Trypanosoma aconsys*, in spiny mouse, Wenyon, 1909.
Trypanosoma acouchii, in agouti, Brimont, 1909.
Trypanosoma akodonti, in vole mouse, Carini and Maciel, 1915.
Trypanosoma annamense, in dog, Blin, 1902; ox, Schein, 1907; horse, Blanchard, 1888; mule, same.
Trypanosoma arvicanthi, in *A. barbarus*, striped mouse, Delanoč, 1915.
Trypanosoma asini, in donkey, Dschunkowsky and Luhs, 1909.
Trypanosoma auricularis, in *L. zebra*, striped mouse, Wenyon, 1909.
Trypanosoma bandicotti, in *Nesokia gigantea*, Lingard, 1904.
Trypanosoma berberum, in horse, Sergeant, *et al.*, 1912.
Trypanosoma brucei, in buffalo, Bruce, 1913; *Felis* (cat), Bruce, 1895; gnu (wildebeeste), Bruce, 1897; *Cephalophus grimms* (duiker), Bruce, 1913; reed-buck, Bruce, 1903; water-buck, Bruce, 1913; dog, Bruce, 1915; donkey, Bruce, 1905; goat, Bruce, 1915; horse, Bruce, *et al.*, 1895; hyena, Bruce, 1895; mule, Bruce, *et al.*, 1895; oribi, Bruce, 1913; ox, Bruce, 1895; wart-hog, Bruce, 1913; pig, Macfie, 1916; stein-buck, Bruce, 1903; koodoo, Bruce, 1895; eland, Tante, 1913; bush-buck, Bruce, 1899; Speke's antelope, Duke, 1921; mpala, Kinghorn and Yorke, 1912; hartebeest, Bruce, 1913.
Trypanosoma camelensis, in camel, Yakimoff, Schokker and Koselkine, 1917.
Trypanosoma caprae, in reed-buck, Bruce, 1913; water-buck, same; goat, Kleine, 1910; oribi, Bruce, 1913; sheep, Fehlandt, 1911; koodoo, Bruce, 1914; eland, Bruce, 1913; bush-buck, same; mpala, Bruce, 1914.
Trypanosoma cazalboni (= *T. vivax?*), in reed-buck, Rodhain, *et al.*, 1913; puku, same; dog, same; donkey, Bouffard, 1907; goat, Bonet, 1908; roan antelope, Rodhain, *et al.*, 1913; ox, Cazalbon, 1904; sheep, Bonet, 1908; koodoo, Rodhain, *et al.*, 1913; bush-buck, same.
Trypanosoma cephalophi, duiker, Bruce, 1912.
Trypanosoma citelli, in *Citellus richardsoni* (ground squirrel), Watson, 1912.
Trypanosoma clerei, in *Midas midas* (yellow-banded marmoset), Leger and Pettit, 1909.
Trypanosoma congolense, in buffalo, Bruce, 1913; camel, Broden, 1906; duiker, Kinghorn and Yorke, 1912; reed-buck, Bruce, 1913; water-buck, same; dog, Martin, *et al.*, 1908; donkey, Broden, 1904; goat, Martin, *et al.*, 1909; puku, same; roan antelope, Kinghorn and Yorke, 1912; horse, Bruce, 1914; hyena, Bruce, 1913; mule, Hornby, 1919; ox, Broden, 1906; wart-hog, Bruce, 1913; pig, Bruce, 1914; eland, same; bush-buck, Kinghorn and Yorke, 1912; mpala, Bruce, 1914.
Trypanosoma cricetuli, in *Cricetulus griseus*, Patton and Hindle, 1926.
Trypanosoma crociduræ, in *Crocidura rursula*, shrew, Brumpt, 1932.
Trypanosoma cruzi = *Schizotrypanum cruzi*, in *Chrysothrix sciurus*, Chagas, 1909; armadillo, Chagas, 1912; Torres, 1915.
Trypanosoma dendromysi, in *Dendromys* sp., Rodhain, 1915.
Trypanosoma denisi, in spiny-tailed flying squirrel, Rodhain, *et al.*, 1912.
Trypanosoma dimorphon, in dog, Martin, 1906; donkey, Martin, 1906; goat, same; horse, Dutton and Todd, 1903; mule (= *T. congolense?*), Martin, 1906; ox, same; pig, same; sheep, same; bush-buck, Dutton, *et al.*, 1907.
Trypanosoma duttoni, in *Rattus muris*, Thiroux, 1905.
Trypanosoma eburneense, in *Rattus chocha*, Delanoč, 1915.
Trypanosoma elephantis (= *T. brucei?*), in elephant, Bruce, 1909.
Trypanosoma equinum, in donkey, Vital, 1907; horse, Voges, 1901; capybara, Lutz, 1907.

- Trypanosoma equiperdum*, in donkey, Schneider and Bouffard, 1899; horse, Rouget, 1896.
- Trypanosoma evansi*, in dog, Lingard, 1894; donkey, Evans, 1880; elephant, same; buffalo, Lingard, 1899; camel, Evans, 1880.
- Trypanosoma evatomys*, in *Evatomys saturatus*, Hadwen, 1912.
- Trypanosoma gambiense*, in *Cercopithecus pygerythrus*, Bruce, 1911; *Cercop.* sp., Koch, 1909; goat, Klein and Eckard, 1913; ox, Bruce, 1911; sheep, Klein and Eckard, 1913; Speke's antelope, Duke, 1912; Man, Dutton, 1902.
- Trypanosoma grosi*, in *Mus sylvaticus* (*Apodermus syl.*), Laveran and Pettit, 1909.
- Trypanosoma heybergi*, in *Nycteres hispidus*, Rodhain, 1932.
- Trypanosoma hippicum*, in horse, Darling, 1910; mule, same.
- Trypanosoma indicum*, in palm squirrel, Lühe, 1906.
- Trypanosoma ingens*, in duiker, Bruce, 1912; reed-buck, Bruce, 1909; water-buck, Bruce, 1914; puku, Rodhain, *et al.*, 1913; oribi, Bruce, 1913; ox, Bruce, 1909; bush-buck, same; Speke's antelope, Duke, 1912.
- Trypanosoma korssaki*, in striped field mouse, Yakimoff, *et al.*, 1910.
- Trypanosoma legeri*, in *Tamandua tridactyla*, Mesnil and Brimont, 1910.
- Trypanosoma lesouardi*, in spider monkey, Leger and Porry, 1918.
- Trypanosoma lewisi*, var. *primatum*, in monkey, Reichenow, 1917; gorilla, same; chimpanzee, same; brown rat, Lewis, 1879; *Rattus macleari*, Durham, 1908; *R. maurus*, Martin, *et al.*, 1909; potts, Reichenow, 1917; gerbil, Fantham, 1926.
- Trypanosoma marocanum*, in horse, Sergeant, *et al.*, 1915.
- Trypanosoma megadermae*, in *Lavia frons* (African bat), Wenyon, 1909.
- Trypanosoma melophagium*, in sheep, Woodcock, 1910.
- Trypanosoma microti*, in *Microtus arvalis* (field vole), Laveran and Pettit, 1909.
- Trypanosoma minasense*, *Hapale penicillata*, Dios Zuccarini and Werngren, 1925; marmoset, Chagas, 1908-1909.
- Trypanosoma montgomeryi*, in dog, Kinghorn and Yorke, 1912; ox, Montgomery and Kinghorn, 1909.
- Trypanosoma morinorum*, in bat, *Hipposides tridens*, Leger and Baury, 1923.
- Trypanosoma morocanum*, in dog, Delanoë, 1920.
- Trypanosoma multifforme*, in bush-buck, Kinghorn and Yorke, 1912.
- Trypanosoma musculi*, in *Mus musculi*, Pricoli, 1906.
- Trypanosoma myoxi*, in dormouse, Blanchard, 1903.
- Trypanosoma nabiasi*, in rabbit (Europe), Railliet, 1895.
- Trypanosoma nicolleorum*, in long-eared bat, Sergeant, 1905.
- Trypanosoma ninae*, in camel, Yakimov, 1922.
- Trypanosoma otospermophili*, in ground squirrel, Wellman and Wherry, 1910.
- Trypanosoma pecaui* (= *T. brucei*?), in dog, Bonet, 1908; donkey, Cazalbou, 1910; goat, Pecaud, 1909; horse, Cazalbou, 1909; mule, Bouffard, 1908; ox, Cazalbou, 1910; sheep, Pecaud, 1909; camel, Balfour, 1909; pig, Bonet, 1908.
- Trypanosoma peromysci*, in American field mouse, Watson, 1912.
- Trypanosoma pestani*, in *Meles meles* (badger), Bettencourt and France, 1905.
- Trypanosoma petrodromi*, in elephant shrew, Bruce, 1915.
- Trypanosoma phyllostomae*, in So. American bat, Cartaya, 1910.
- Trypanosoma prowazeki*, in ouakari monkey, Gonder and B. Gossler, 1908.
- Trypanosoma rabinowitschi*, in common hamster, *Cricetus cricetus*, Brumpt, 1906.
- Trypanosoma rhesii* in *Macacus rhesus*, Terry, 1911.

- Trypanosoma rhodesiense* (= *T. brucei*?), Stephens and Fantham, 1910.
Trypanosoma simiae, in wart-hog, Bruce, 1913.
Trypanosoma soricis, in shrew (Canada), Hadwen, 1912.
Trypanosoma species, in *Steatomys pratensis* (fat mouse), Plimmer, 1912.
Trypanosoma species, in bush-buck, Dutton, *et al.*, 1906.
Trypanosoma species, in chevrotain, Dodd, 1912.
Trypanosoma species, in striped bat, Iturbe and Gonzalez, 1916.
Trypanosoma species, in howling monkey, Brimont, 1909.
Trypanosoma species, in hartebeest, Montgomery and Kinghorn, 1908.
Trypanosoma species, in hippopotamus, Kleine and Tante, 1911.
Trypanosoma species, in sable antelope, Week, 1914.
Trypanosoma species, in lion, Week, 1914; monkey, Mathis and Leger, 1911.
Trypanosoma species, in *Cercopithecus schmidtii*, Dutton, Todd and Tobey, 1906.
Trypanosoma species, in serval, Week, 1914.
Trypanosoma species, in reed-buck, Kleine and Fischer, 1911; water-buck, same.
Trypanosoma species, in little hamster, *Cricetulus migratorius*, Finkelstein, 1907.
Trypanosoma species, in *Choloepus didactylus* (two-toed sloth), Mesnil and Brimont, 1908; also *Endotrypanum schaudinni* (Mesnil and Brimont, 1908).
Trypanosoma species, in gnu (*Connochaetes gnu* = wildebeeste), Week, 1914.
Trypanosoma species, in guinea-pig (*Cavia porcellus*), Kunstler, 1883.
Trypanosoma species, in *Spermophilus evessmanni*, Laveran, 1911.
Trypanosoma sudanense, in donkey, Roger and Greffulke, 1905; in horse, Chauvrat, 1892.
Trypanosoma talpae, in mole, Franca, 1911.
Trypanosoma theileri, in duiker, Rodhain, Pons, *et al.*, 1912; reed-buck, Kleine and Fischer, 1911; roan antelope, Rodhain, *et al.*, 1913; ox, Theiler, 1902; bush-buck, Dutton, *et al.*, 1906.
Trypanosoma togolense, in donkey, Schilling, 1901; horse, same; ox, same.
Trypanosoma tragelaphi, in Speke's antelope, Duke, 1912.
Trypanosoma uniforme, in buffalo, Duke, 1913; water-buck, same; ox, Bruce, 1911; bush-buck, Duke, 1912; Speke's antelope, Duke, 1912 and 1923.
Trypanosoma venezuelense, in dog, Rangel, 1905; donkey, Tejera, 1920; capybara, Tejera, 1920; mule, same; howler monkey, same.
Trypanosoma vespertilionis, *Miniopterus schreibersi* (bat), Battaglia, 1904; Dionisi, 1899; long-eared bat, Bettencourt and Franca, 1905; Sergent, 1905.
Trypanosoma vivax, in buffalo, Duke, 1913; duiker, Kinghorn and Yorke, 1912; reed-buck, Connal, 1917; water-buck, Kleine and Fischer, 1911; donkey, Hornby, 1919; goat, Ziemann, 1905; roan antelope, Duke, 1923; horse, Yorke and Blacklock, 1911; mule, Hornby, 1919; ox, Ziemann, 1905; sheep, same; bush-buck, Bruce, 1911; Speke's antelope, Duke, 1912.
Trypanosoma xeri, in Ethiopian ground squirrel, Leger and Baurý, 1922.

TRYPANOSOMES IN BIRDS.

- Trypanosoma anellobiae*, in honey-sucker, Johnston, 1910; crow, oriole and fly-catcher, Cleland and Johnston, 1911.
Trypanosoma ardeae, var. *major*, in Florida heron, Leger, 1918; goliath heron, Rodhain, *et al.*, 1913.
Trypanosoma asturinae, in hawk, Stephens and Christophers, 1908.
Trypanosoma avium, in roller, Danilewsky, 1885; tawny owl, same; hang-nest, Novy and MacNeal, 1905.

- Trypanosoma bicanistis*, in hornbill, Stephens and Christophers, 1908.
Trypanosoma bouffardi, in weaver bird, Leger and Blanchard, 1911.
Trypanosoma braunae, in Indian little owl, Stephens and Christophers, 1908.
Trypanosoma brimonti, in bulbul, Mathis and Leger, 1910.
Trypanosoma calmettei, in domestic fowl, Mathis and Leger, 1909.
Trypanosoma caprimulji, in nightjar, Kerandel, 1909.
Trypanosoma catharisti, in black vulture, Mesnil, 1912.
Trypanosoma chouqueti, in tiger bittern, Mathis and Leger, 1911.
Trypanosoma columbae, in pigeon, Stephens and Christophers, 1908.
Trypanosoma confusum, in hang-nest, Luhe, 1906; jay, robin and hang-nest, Luhe, 1906; honey-sucker, Cleland and Johnston, 1911.
Trypanosoma corvi, in jackdaw, Stephens and Christophers, 1908.
Trypanosoma cotyli, in sand martin, Franchini, 1923.
Trypanosoma cypseli, in swift, Franchini, 1923.
Trypanosoma dabbenei, in *Chamaeza brevicauda*, Mazza, *et al.*, 1927.
Trypanosoma curystomi, in roller, Kerandel, 1909, 1912.
Trypanosoma franchinii, in *Nyphocolaptes major*, Mazza and Fiora, 1930.
Trypanosoma francolini, in francolin, Kerandel, 1912.
Trypanosoma fringillinarum, in chaffinch, Woodcock, 1910; finch, same.
Trypanosoma gallinarum, in domestic fowl, Bruce and Coles, 1911.
Trypanosoma guyauense, in hawk, Mesnil, 1912.
Trypanosoma hannai, in rock pigeon, Pittaluga, 1904; pigeon, Mello and Braz de Sa, 1916.
Trypanosoma johnstoni, in weaver finch, Dutton and Todd, 1903.
Trypanosoma lagonostictae, in weaver finch, Murallaz, 1914.
Trypanosoma langeroni, in *Cerebeis spavesius*, Mazza and Fiora, 1930.
Trypanosoma laverani, in American goldfinch, Novy and MacNeal, 1905; rock sparrow, Leger, 1913.
Trypanosoma lithricis, in babbler, Laveran and Marullaz, 1914.
Trypanosoma loxiae, in crossbill, Nöller, 1920.
Trypanosoma mathisi, in martin, Sergeant, 1904, 1907.
Trypanosoma mayae, in house sparrow, Maya and David, 1912.
Trypanosoma mesnili, in American buzzard, Novy and MacNeal, 1905.
Trypanosoma milvi, in kite, Stephens and Christophers, 1908.
Trypanosoma morai, in *Bubuleus ibis*, da Silva, 1927.
Trypanosoma noctuae, in little owl, Schaudinn, 1904.
Trypanosoma numidae, in guinea fowl, Wenyon, 1909.
Trypanosoma nyctecoracis, in night heron, Stephens and Christophers, 1908.
Trypanosoma paddae, in weaver bird, Laveran and Mesnil, 1904.
Trypanosoma pedrozi, in Selater's currasow, Carini and Botelho, 1914.
Trypanosoma polyplectri, in peacock pheasant, Vassal, 1905.
Trypanosoma pycnonoti, in bulbul, Kerandel, 1912.
Trypanosoma schistochlamydis, in tanager, Splendore, 1910.
Trypanosoma syrni, in tawny owl, Nöller, 1917.
Trypanosoma species, in crested lark, Sergeant, *Ed.*, *et al.*, 1904.
Trypanosoma species, in weaver finch, Fantham, 1919.
Trypanosoma species, in waxwing, Ogawa, 1911.
Trypanosoma species, in bulbul, Zupitza, 1909.
Trypanosoma species, in meadow pipit, Nieschulz, 1921.
Trypanosoma species, in hang-nest, Carini and Maciel, 1916.
Trypanosoma species, in swift, Franchini, 1923.
Trypanosoma species, in green heron, Leger, A. and M., 1914.
Trypanosoma species, in buff-backed heron, Zupitza, 1909.
Trypanosoma species, in heron (*Florida caerula*), de Cerquiera, 1906.
Trypanosoma species, in egret, de Cerquiera, 1906.
Trypanosoma species, in Formosan birds, Ogawa and Uegaki, 1927.

- Trypanosoma species*, in goliath heron, Rodhain, *et al.*, 1913.
Trypanosoma species, in yellow bittern, Mathis and Leger, 1911.
Trypanosoma species, in argus pheasant, Z. S., 1925.
Trypanosoma species, in owl, Mathis and Leger, 1911.
Trypanosoma species, in little owl, Franchini, 1924.
Trypanosoma species, in ant-bird, Carini and Botelho, 1914.
Trypanosoma species, in Siberian eagle owl, Böing, 1925.
Trypanosoma species, in green heron, Rodhain, *et al.*, 1913.
Trypanosoma species, in hornbill, Ringenbach, 1914.
Trypanosoma species, in hornbill, Ross, 1911.
Trypanosoma species, in red-legged partridge, Plimmer, 1912.
Trypanosoma species, in goldfinch, Sergeant, 1910.
Trypanosoma species, in kestrel, Böing, 1925.
Trypanosoma species, in cuckoo, Martin, *et al.*, 1909.
Trypanosoma species, in bower bird, Breinl, 1913.
Trypanosoma species, in golden cuckoo, Zupitza, 1909.
Trypanosoma species, in bird of paradise, Plimmer, 1915.
Trypanosoma species, in sun-bird, Leger, A. and M., 1914.
Trypanosoma species, in marsh harrier, Böing, 1925.
Trypanosoma species, in shama, Plimmer, 1914.
Trypanosoma species, in hawfinch, Bettencourt and França, 1907.
Trypanosoma species, in Japanese hawfinch, Ogawa, 1911.
Trypanosoma species, in woodpecker, Novy and MacNeal, 1905.
Trypanosoma species, in wood-pigeon, Böing, 1925.
Trypanosoma species, in magpie, Plimmer, 1912.
Trypanosoma species, in crow, Mine, 1914.
Trypanosoma species, in house crow, Donovan.
Trypanosoma species, in turaco, Minchin, 1910.
Trypanosoma species, in quail, Franchini, 1924.
Trypanosoma species, in hairy woodpecker, Novy and MacNeal, 1905.
Trypanosoma species, in kite, Bettencourt and Franca, 1907.
Trypanosoma species, in yellow hammer, Petrie, 1905.
Trypanosoma species, in redstart, Nieschulz, 1921.
Trypanosoma species, in robin, Bettencourt and França, 1907.
Trypanosoma species, in weaver finch, Fantham, 1919.
Trypanosoma species, in waxbill, Plimmer, 1912.
Trypanosoma species, in falcon, Breinl, 1913.
Trypanosoma species, in kestrel, Wasielewski, 1908.
Trypanosoma species, in francolin, Plimmer, 1912.
Trypanosoma species, in francolin, Ross, 1911.
Trypanosoma species, in francolin, Todd and Wolbach, 1912.
Trypanosoma species, in goldfinch, Sergeant, 1904.
Trypanosoma species, in linnet, Sergeant, 1904.
Trypanosoma species, in common jay, Bettencourt and França, 1907.
Trypanosoma species, in jay, Ogawa, 1911.
Trypanosoma species, in dove, Maya and David, 1912.
Trypanosoma species, in owl, Leger, A. and M., 1914.
Trypanosoma species, in ant-bird, Carini and Botelho, 1914.
Trypanosoma species, in guinea fowl, Keysselitz and Mayer, 1909.
Trypanosoma species, in kingfisher, Zupitza, 1909.
Trypanosoma species, in kite, Breinl, 1913.
Trypanosoma species, in mocking bird, Novy and MacNeal, 1905.
Trypanosoma species, in swallow, Petrie, 1905.
Trypanosoma species, in shrike, Neave, 1906.
Trypanosoma species, in red-back shrike, Sjöbring, 1899.
Trypanosoma species, in parrot, Plimmer, 1913.

- Trypanosoma species*, in black game, Böing, 1925.
Trypanosoma species, in bee-eater, Zupitza, 1909.
Trypanosoma species, in American song sparrow, Novy and MacNeal, 1905.
Trypanosoma species, in bee eater, Minchin, 1910.
Trypanosoma species, in blackbird, Petrie, 1905.
Trypanosoma species, in wagtail, Bettencourt and França, 1907.
Trypanosoma species, in sunbird, Zupitza, 1909.
Trypanosoma species, in vulture, Neave, 1906.
Trypanosoma species, in house sparrow, Novy and MacNeal, 1905.
Trypanosoma species, in tree sparrow, Mine, 1914.
Trypanosoma species, in sparrow, Sergeant, Ed. and Et., 1904.
Trypanosoma species, in warbler, Bettencourt and França, 1907.
Trypanosoma species, in warbler (willow), Nieschulz, 1921.
Trypanosoma species, in tyrant bird, Carini and Botelho, 1914.
Trypanosoma species, in wood-shrike, Rodhain, *et al.*, 1913.
Trypanosoma species, in gray parrot, Zupitza, 1909.
Trypanosoma species, in fire-crested wren, Bettencourt and França, 1907.
Trypanosoma species, in black redstart, Bettencourt and França, 1907.
Trypanosoma species, in wheat ear, Nieschulz, 1921.
Trypanosoma species, in woodcock, Bettencourt and França, 1907.
Trypanosoma species, in barn owl, Bettencourt and França, 1907.
Trypanosoma species, in tanager, de Cerqueira, 1906.
Trypanosoma species, in stork, Migone, 1916.
Trypanosoma species, in ibis, Migone, 1916.
Trypanosoma species, in harpy, Iturbe and Bonzalez, 1916.
Trypanosoma species, in fruit pigeon, Wellman, 1905.
Trypanosoma species, in wren, Novy and MacNeal, 1905.
Trypanosoma species, in song thrush, Petrie, 1905.
Trypanosoma species, in ring ousel thrush, Nieschulz, 1921.
Trypanosoma species, in hoopu, Bettencourt and França, 1907.
Trypanosoma species, in weaver finch, Leger, A. and M., 1914.
Trypanosoma thiersi, in nightjar, Leger, 1913.
Trypanosoma tinami, in timamut, Mesnil, 1912.
Trypanosoma viduae, in weaver finch, Kerandel, 1909.
Trypanosoma zonotrichae, in finch, Splendore, 1910.

TRYPANOSOMES IN LIZARDS.

- Trypanosoma boueti*, in *Mabuia raddonii*, Martin, 1907.
Trypanosoma chamaelonis, in *Chamaeleon vulg.*, Wenyon, 1909.
Trypanosoma gallaji, in *Psilodactylus caudacinctus*, Bonet, 1909.
Trypanosoma hemidactyli, in *Hemidactylus gl.*, Mackie, *et al.*, 1923.
Trypanosoma leschenaulti, in *Hemidactylus leschen.*, Robertson, 1908.
Trypanosoma mabuiae, in *Mabuia quinquetaeniata*, Wenyon, 1909.
Trypanosoma martini, in *Mabuia maculilabris*, Bonet, 1909.
Trypanosoma pertenuis, in *Hemidactylus tri.*, Robertson, 1908.
Trypanosoma platydactyli, in *Tarentola mauritanica*, Catonillard, 1909;
 = *T. mauritanica*, Chatton and Blanc, 1915.
Trypanosoma rudolphi, in *Mabuia agilis*, Carini, 1913.
Trypanosoma species, in *Acanthosaura*, Mathis and Leger, 1911.
Trypanosoma species, in *Agama col.*, Todd and Wolbach, 1912.
Trypanosoma species, in *Lygosoma taeniolatum*, Johnston and Cleland,
 1910.

TRYPANOSOMES IN SNAKES.

- Trypanosoma brazilii*, in *Helicops modestus*, Brumpt, 1914, 1915.
Trypanosoma clozeli, in *Grayia smythii*, Bonet, 1909; *Tropidonotus ferox*,
 same.

- Trypanosoma erythrolampris*, in *Erythrolampris aeschulapii*, Wenyon, 1909.
Trypanosoma najae, in *Naja nigricollis*, Wenyon, 1909.
Trypanosoma phylodriasi, in Brazilian snake *P. natteri*, Pessoa, 1928.
Trypanosoma primati, in *Hypsichina chinensis*, Mathis and Leger, 1909;
Tropidonotus piscator, same, 1911.
Trypanosoma species, in *Bitis aretans*, Dutton, *et al.*, 1907.
Trypanosoma species, in *Diemenia textilis*, quoted from Cleland and Johnston, 1910.
Trypanosoma species, in *Rhadinaea mersemii*, Brumpt, 1914.

TRYPANOSOMES IN CROCODILES.

- Trypanosoma kochi*, in *Crocodylus niloticus*, Laveran and Mesnil, 1912.
Trypanosoma spermophilii, in *Crocodylus catophractus*, Dutton, *et al.*, 1907.

TRYPANOSOMES IN TURTLES.

- Trypanosoma chelodina*, in *Chelodina longicollis*, Johnson, 1907.
Trypanosoma damoniae, in *Damonia reevesii*, Laveran and Mesnil, 1902.
Trypanosoma leroysi, in *Cinixys homeana*, Combes, 1919.
Trypanosoma pontyi, in *Sternotherus derbianus*, Bonet, 1909.
Trypanosoma vittatae, in *Emyda vittata*, Robertson, 1908.

TRYPANOSOMES IN FROGS, TOADS AND SALAMANDERS.

- Trypanosoma borelli*, in *Hyla rubra*, Marehoux and Salimbeni, 1907; also in fish, species of *Leuciscus*, Keysselitz, 1906.
Trypanosoma diemyctili, in *Nolge viridiscens*, Tobey, 1906.
Trypanosoma hendersoni, in *Rana tigrina*, Patton, 1908.
Trypanosoma hylae, in *Hyla arborea*, Franca, 1908.
Trypanosoma inopinatum, in *Rana esculenta*, Sargent, 1904.
Trypanosoma karyozeukton, in *Bufo regularis*, Dutton and Todd, 1903; in *Rana sp.*, Martin, *et al.*, 1909.
Trypanosoma leptodactyli, in *Leptodactylus ocellatus*, Carini, 1907.
Trypanosoma mega, in *Bufo regularis*, Dutton and Todd, 1903; in *Bufo sp.*, Minchin, 1910.
Trypanosoma nelsprutense in *Rana sp.*, Laveran, 1904.
Trypanosoma nereu-lemaitrei, in *Rana esculenta*, Brumpt, 1928.
Trypanosoma parroti, in *Discoglossus pictus*, Brumpt, 1923, 1928.
Trypanosoma parvum, in *Rana clamata*, Kudo, 1922.
Trypanosoma rotatorium, in *Bufo regularis*, Balfour, 1909; in *Hyla arborea*, Danilewsky, 1885, 1888; in *Hyla lesneurii*, Cleland and Johnston, 1911; in *Leptodactylus ocellatus*, Machado, 1911; in *Rana clamata*, Kudo, 1922; *Rana tigrina*, Patton, 1908.
Trypanosoma sergenti, in *Discoglossus pictus*, Brumpt, 1923.
Trypanosoma species, in *Bufo melanostictus*, Mathis and Leger, 1911.
Trypanosoma species, in *Bufo reticulatus*, Brumpt, 1906.
Trypanosoma species, in *Bufo sp.*, Stevenson, 1911.
Trypanosoma species, in *Bufo vulgaris*, Grassi, 1881, 1883.
Trypanosoma species, in Formosan frogs, Ogawa and Uegaki, 1927.
Trypanosoma species, in *Hyla arborea*, Wedl, 1850.
Trypanosoma species, in *Hyla nasuta*, Baneroff, 1890.
Trypanosoma species, in *Hyla venulosa*, Plimmer, 1912.
Trypanosoma species, in *Limnodynastes ornatus*, Cleland and Johnston, 1911.

- Trypanosoma species*, in *Limnodynastes tasmaniensis*, Cleland and Johnston, 1911.
- Trypanosoma species*, in *Microhyla pulchra*, Mathis and Leger, 1911.
- Trypanosoma species*, in *Rana angolensis*, Laveran, 1904.
- Trypanosoma species*, in *Rana catesbiana*, Hegner, 1920.
- Trypanosoma species*, in *Rana clamata*, Hegner, 1920.
- Trypanosoma species*, in *Rana galamensis*, Dutton, *et al.*, 1907.
- Trypanosoma species*, in *Rana guentheri*, Mathis and Leger, 1911.
- Trypanosoma species*, in *Rana hexadactyla*, Dobell, 1910.
- Trypanosoma species*, in *Rana limnocharis*, Mathis and Leger, 1911.
- Trypanosoma species*, in *Rana mascariensis*, Dutton, *et al.*, 1907.
- Trypanosoma species*, in *Rana oxyrhynchus*, Dutton, *et al.*, 1907.
- Trypanosoma species*, in *Rana rugosa*, Koidzumi, 1911.
- Trypanosoma species*, in *Rana temporaria*, Danilewsky, 1885.
- Trypanosoma species*, in *Rana trinodis*, Dutton and Todd, 1903.
- Trypanosoma species*, in *Rappia marmorata*, Dutton, *et al.*, 1907.
- Trypanosoma species*, in *Rhacophorus leucomystax*, Mathis and Leger, 1911.
- Trypanosoma tritonis*, in *Molge pyrrhogastra*, Ogawa, 1914.
- Trypanosoma tumida*, in *Rana nutti*, Awerinzew, 1918.

TRYPANOSOMES IN FISH.

- Trypanosoma abramidis*, in common bream, Laveran and Mesnil, 1904.
- Trypanosoma acerinae*, in ruff, Brumpt, 1906.
- Trypanosoma aeglefini*, in haddock, Henry, 1913.
- Trypanosoma albopunctatus*, in *Plecostomus* sp., da Fonseca and Vaz, 1928.
- Trypanosoma anguillicola*, in eels, Johnston and Cleland, 1910.
- Trypanosoma bancrofti*, in *Copidoglanis tandanus*, Johnston and Cleland, 1910.
- Trypanosoma barbae*, in *Barbus barbus*, Brumpt, 1906.
- Trypanosoma barbatulae*, in loach, Leger, 1904.
- Trypanosoma bleenniini*, in *Bleinnius cornutus*, Fantham, 1930.
- Trypanosoma bliccae*, in *Blicca bjoerkna*, Nixtau, 1929.
- Trypanosoma bothi*, in *Bothus rhombus*, Lebaillly, 1905.
- Trypanosoma callionymi*, in *Callionymus lyra*, Brumpt and Lebaillly, 1904.
- Trypanosoma capigobii*, in *Gobius nudicep*, Fantham, 1919.
- Trypanosoma carassii*, in *Carassius carassius*, Mitrophanov, 1883.
- Trypanosoma carchariasii*, in *Carcharias* sp., Laveran, 1908.
- Trypanosoma catapraeti*, in pogge, Henry, 1913.
- Trypanosoma chagasi*, in *Plecostomus punctatus*, Horta, 1910.
- Trypanosoma chelostomi*, in *Chelostoma* sp., da Fonseca and Vaz, 1929.
- Trypanosoma clarii*, in *Clarias macrocephalus*, Montel, 1905.
- Trypanosoma cobitis*, in giant loach, Mitrophanov, 1883.
- Trypanosoma cotti*, in *Cottus bubalis*, Brumpt and Lebaillly, 1904.
- Trypanosoma danilewskyi*, in common carp, Laveran and Mesnil, 1904.
- Trypanosoma delagei*, in *Bleinnius pholis*, Brumpt and Lebaillly, 1904.
- Trypanosoma dohrni*, in *Bolea monschr*, Yakimoff, 1911.
- Trypanosoma dorbigugi*, in *Rhinodorus dorbigunii*, da Fonseca and Vaz, 1928.
- Trypanosoma elegans*, in *Gobio gobio*, Brumpt, 1906.
- Trypanosoma ferreirae*, in *Characinus* sp., da Fonseca, *et al.*, 1928.
- Trypanosoma flesii*, in *Flesus vulgaris*, Lebaillly, 1904.
- Trypanosoma francirochai*, in *Otocinclus francirochai*, da Fonseca and Vaz, 1928.
- Trypanosoma giganteum*, in long-nosed skate, Neumann, 1909.
- Trypanosoma gobii*, in rock goby, Brumpt and Lebaillly, 1904.

- Trypanosoma granulosum*, in *Anguilla* sp., França, 1908; in *A. vulgaris*, Laveran and Mesnil, 1902.
- Trypanosoma hypostomi*, in *Plegostomus auroguttatus*, Splendore, 1910.
- Trypanosoma langeroni*, in bullhead, Brumpt, 1906.
- Trypanosoma larai*, in *Prochilodus* sp., da Fonseca, 1929.
- Trypanosoma laternae*, in *Platophryo laterna*, Henry, 1913; in *Arnoglossus*, Lebaillly, 1904.
- Trypanosoma leucisci*, in roach, Coles, 1914; Brumpt, 1906.
- Trypanosoma limandae*, in dab, Brumpt and Lebaillly, 1904.
- Trypanosoma loricariae*, in *Loricaria* sp., da Fonseca and Vaz, 1928.
- Trypanosoma luciopercae*, in *Lucioperca volgensis*, Nixitan, 1929.
- Trypanosoma macrodontis*, in *Macrodon trahira*, Bothelho, 1907.
- Trypanosoma margaritifera*, in *Plecostomus margaritifer*, da Fonseca and Vaz, 1928.
- Trypanosoma murmanensis*, in *Gadus callarias*, Nixitan, 1929.
- Trypanosoma nudigobii*, in *Gobius nudiceps*, Fantham, 1919.
- Trypanosoma pelligrini*, in paradise fish, Mathis and Leger, 1911.
- Trypanosoma percae*, in perch, Brumpt, 1906.
- Trypanosoma phoxini*, in minnow, Brumpt, 1906.
- Trypanosoma piracicaboe*, in *Loricaria piracicaboe*, da Fonseca, 1929.
- Trypanosoma piavae*, in *Characinus* sp., da Fonseca, 1928.
- Trypanosoma platessae*, in plaice, Lebaillly, 1904.
- Trypanosoma plecostomi*, in *Plecostomus* sp., da Fonseca and Vaz, 1928.
- Trypanosoma rajae*, in skate, Coles, 1914; ray, Laveran and Mesnil, 1902.
- Trypanosoma regani*, in *Plecostomus regani*, da Fonseca, 1928.
- Trypanosoma remaki*, in pike, Laveran and Mesnil, 1901; pickerel, Kudo, 1921; *Esox reticulatus*, Kudo, 1921.
- Trypanosoma rhamdiae*, in *Rhamdia queleni*, Botelho, 1907.
- Trypanosoma routei*, in *Monopterus javanensis*, Mathis and Leger, 1911.
- Trypanosoma saecobranchi*, in *Saecobranchus fossilis*, Castellani and Willey, 1905.
- Trypanosoma scardini*, in rudd, Brumpt, 1906.
- Trypanosoma scorpaenae*, in *Scorpaena ustulata*, Neumann, 1909.
- Trypanosoma simondi*, in *Auchenoglanis biscutatus*, Leboeuf and Ringenbach, 1910.
- Trypanosoma scyllii*, in *Scyllium canicula*, Laveran and Mesnil, 1902.
- Trypanosoma solae*, in common sole, Laveran and Mesnil, 1901.
- Trypanosoma species*, in climbing perch, Mathis and Leger, 1911.
- Trypanosoma species*, in *Bagrus bayad*, Neave, 1906.
- Trypanosoma species*, in *Barbus carnaticus*, Lingard, 1903.
- Trypanosoma species*, in *Box salpa*, Fantham, 1919.
- Trypanosoma species*, in *Carassius auratus*, Petrie, 1905.
- Trypanosoma species*, in *Chrysiethys auratus*, Wenyon, 1909.
- Trypanosoma species*, in *Clarias angolensis*, Dutton, *et al.*, 1906.
- Trypanosoma species*, in *Clarias* sp., Zupitza, 1909.
- Trypanosoma species*, in *Dentex argurozona*, Fantham, 1919.
- Trypanosoma species*, in *Etioplus maculatus*, Patton, 1908.
- Trypanosoma species*, in Formosan fish, Ogawa and Uegaki, 1927.
- Trypanosoma species*, in *Gobius giurus*, Castellani and Willey, 1905.
- Trypanosoma species*, in *Labio falcipinnis*, Rodhain, 1907.
- Trypanosoma species*, in *Lichia amia*, Fantham, 1919.
- Trypanosoma species*, in *Lota lota*, Keysseltz, 1906.
- Trypanosoma species*, in *Macrones cavasius*, Castellani and Willey, 1905.
- Trypanosoma species*, in *Macrones seenghala*, Lingard, 1904.
- Trypanosoma species*, in electric eel, Rodhain, 1907.
- Trypanosoma species*, in *Mugil* sp., Neave, 1906.

- Trypanosoma species*, in Polypterus sp., Neave, 1906.
Trypanosoma species, in serpent head, Mathis and Leger, 1911.
Trypanosoma species, in Siluris glanis, Keysselitz, 1906.
Trypanosoma squalii, in Squalus cephalus, Brumpt, 1906.
Trypanosoma strigaticeps, in Plecostomus strigaticeps, da Fonseca and Vaz, 1928.
Trypanosoma synodontis, in Synodontis notatus, Leboeuf and Ringenbach, 1910.
Trypanosoma tincae, in tench, Laveran and Mesnil, 1904.
Trypanosoma toddi, in Clarias anguillaris, Bonet, 1909.
Trypanosoma torpedinis, in torpedo, Sabarex and Muratet, 1908.
Trypanosoma triglae, in tubfish, Neumann, 1909.
Trypanosoma yakimovi, in pipefish, Wladimiroff, 1910.
Trypanosoma zunguroi, in Pseudopimelodus zungaro, da Fonseca and Vaz, 1928.

This formidable list of species of trypanosomes is not complete, but zoologically more than nine-tenths of these are probably synonyms. A useful purpose is served by the mere mention of a species of trypanosome in a new host, and until the life history of each is worked out the synonym may be ignored.

The term Trypanosoma was first used by Gruby (1843) as a generic name for blood parasites which, earlier, were regarded as amebae. Little attention was paid to the genus until mammalian trypanosomes were discovered. Attention was particularly drawn to these by Lewis, studying rats in Bombay as a possible means of distributing the plague, when he found active organisms in the blood. Smears were made and sent to Saville Kent for identification. Still more important was the discovery of a mammalian disease associated with trypanosomes in the following year by Evans, who found peculiar organisms in the blood of horses and mules in India with a disease called surra. Smears were likewise sent to Kent who identified them as the same organism as that found by Lewis, and he included them both in his genus *Herpetomonas*, species *levisi*. The correct interpretation of these as Trypanosomes followed a few years later. A great advance was made by Bruce, in 1893, who demonstrated the agency of tsetse flies (*Glossina morsitans*) in transmitting the disease nagana to cattle, while human trypanosomiasis and its transmission by tsetse flies (*Glossina palpalis*) was fully established by the observations on Gambia fever of Forde (1901), Dutton (*Tryp. gambiensi*) (1902), of Castellani who was the first to see trypanosomes in sleeping sickness; and of Bruce (1903) who showed that Gambia fever is an initial stage of sleeping sickness, and that, like nagana, the trypanosome is transmitted by a tsetse fly. Later discoveries showed the presence of trypanosomes in every group of vertebrates (see list, p. 372), many of them producing fatal diseases, while transmission by various kinds of invertebrate hosts—sand flies, biting bugs, mosquitoes, fleas, lice, mites, ticks and leeches—has been established.

Few stages of the life cycle are found in the vertebrate blood. Here they may reproduce by longitudinal division until the blood teems with them or a balance may be established whereby relatively few forms can be found in the circulating blood. Such hosts become carriers for many different species of trypanosomes, as appears to be the case with African wild animals.

Developmental stages, on the other hand, are well known in the invertebrate hosts, the most complete account being that of Minchin and Thompson (1915) for *Trypanosoma lewisi* of the rat in the rat flea, *Ceratophyllus fasciatus*. Here a most unusual somatella phase occurs in the stomach cells of the flea which is described on page 233.

The young trypanosomes after leaving the stomach cell may enter other stomach cells and repeat the process, or they may pass down the intestine to the rectum where they, like *Crithidia*, become attached to the epithelial cells (Fig. 122, p. 234). From here they may swim off as *Leptomonas* forms or remain and divide as *Crithidia* types. The rectum is, apparently, a site of multiplication, nectomonad and haptomonad stages succeeding one another until finally the metacyclic or transmitting types develop from haptomonads.

It is probable that intracellular stages occur in the invertebrate hosts of other species of *Trypanosoma* but the life history is known in relatively few cases. The method of infection of vertebrate hosts depends largely upon the site of accumulation of the metacyclic forms in the invertebrate host. If in the rectum, as is the case with *Trypanosoma lewisi* in the rat flea, infection of the vertebrate is brought about by the contaminative method, *i. e.*, by ingesting the feces of the invertebrate or eating it whole. If, on the other hand, the metacyclic trypanosomes accumulate in the salivary glands, hypopharynx or other mouth parts of the invertebrate host, infection is inoculative. Duke (1913) suggests that trypanosomes of the latter type might be described as having an anterior station, and Wenyon (1926) attempted a rough classification of the pathogenic trypanosomes into those having an *anterior station* and those having a *posterior station* in the invertebrate host. Among the former a further grouping is made by Wenyon according to the known invertebrate host and the anatomical part in which the trypanosome development occurs. Thus in tsetse flies development in the stomach, proboscis and salivary glands is characteristic of *Trypanosoma brucei* (cause of nagana in cattle and of human sleeping sickness in Rhodesia); *T. gambiense* (cause of human sleeping sickness); development in stomach and proboscis: *T. congolense* of cattle, horses and sheep; *T. simiac* of monkeys; development only in proboscis: *T. vivax* of cattle, sheep and goats; *T. caprac* in cattle, sheep and goats, also *T. uniforme* of the same hosts.

In tabanid flies and other blood-sucking arthropods development in this anterior station is characteristic of *Trypanosoma evansi*, the

cause of surra in horses; *T. hippicum* in mules; *T. venezuelensis* in horses and dogs; *T. equinum* of horses and others. In leeches also with inoculative infection, the trypanosomes accumulate in the mouth region.

Development in the posterior station of invertebrates, with contaminative infection, is characteristic not only of *T. lewisi* in the rat flea but of the majority of small mammalian trypanosomes.

Trypanosoma equiperdum, the cause of dourine in horses, has no invertebrate host, transmission occurring at coitus.

The genus *Schizotrypanum* *chagas* differs from *Trypanosoma* in having an intracellular leishmania phase in tissues of the vertebrate host. It was discovered in the form of crithidia by Chagas in Brazil in 1907, in the posterior gut of the biting bug *Triatoma megista*. When inoculated in a marmoset, they gave rise to typical trypanosomes which Chagas called *Schizotrypanum cruzi*. Later Chagas found them in cats and in children and associated them with a widely-spread disease of unknown etiology now generally known as Chagas' disease. The trypanosomes do not reproduce as free flagellates but may enter nearly any type of cell of the body where, as *Leishmania* forms, they reproduce by active division. Another species, *S. pipistrelli*, was found by Chatton and Courrier (1921) in the bat *Vesperugo pipistrellus*, in which it forms large (up to 200 μ) reproductive cysts in various organs of the bat.

Human trypanosomiasis, known as sleeping sickness in Africa, is essentially a disease of the lymphatics. This, however, is a later stage of the disease which, as Bruce demonstrated, begins as an irregular fever which was known clinically as Gambia fever before its relation to sleeping sickness was discovered. At this time the flagellates are multiplying in the blood and may be detected by direct examination more readily than at other times. Their accumulation leads to antibody formation and the trypanosomes are destroyed in large numbers, the irregular fever being due to the liberation of endotoxins through disintegration of the parasites. Search for living forms of trypanosomes during the febrile period is thus almost invariably negative.

In this early period, which may last from one or two weeks to several years, there is little or no evidence of glandular swelling (Bruce, Kleine, Thiroux, *et al.*), indicating that the trypanosomes have not yet become established in the lymphatic system. The use of medicaments (atoxyl, urotropine, tartar emetic, etc.) at this period is usually successful and a cure results, but when the trypanosomes have become established in the lymphatics they are less easily reached, and once established in the cerebrospinal fluid the disease is incurable (Reichenow-Dofflein). Here the trypanosome multiplication is rapid and at the same time the lymphocytes become markedly increased in number. The peculiar nervous and psychic

symptoms (tremors of tongue and knee, shuffling gait, etc.) which characterize sleeping sickness may be due, as Reichenow (p. 582) believes, to the effect of an endotoxin upon the central nervous system and liberated through destruction of the parasites by lymphocytes. Others, notably Mott, Bruce, Wolbach and Binger, Stargardt, Stevenson, *et al.*, interpret these characteristic symptoms as due to penetration of the brain substance by trypanosomes, their accumulation, with lymphocytes, in the spaces about bloodvessels causing occlusion of the smaller ones with accompanying lack of nourishment followed by atrophy of the brain cells.

The Rhodesian type of trypanosomiasis is not caused by *Trypanosoma gambiense* but by *T. rhodesiense* (Stephens and Fantham), which is closely related to *T. brucei*, the cause of nagana in cattle. Like *brucei*, this human trypanosome is ordinarily transmitted by the tsetse fly, *Glossina morsitans*. The disease is more rapid and more severe than northern sleeping sickness. Trypanosomes may enter the cerebrospinal fluid within a week after infection (Kudicke), and untreated cases are usually fatal within a few months, so that characteristic sleeping sickness symptoms, although they have been observed, are not so pronounced as in the equatorial form of the disease.

While sleeping sickness is essentially a disease of the lymphatics, Chagas' disease or Brazilian trypanosomiasis is, according to Chagas, essentially a disease of the endocrine organs. The parasites (*Schizotrypanum cruzi*) are abundant in the peripheral blood, but unlike *Trypanosoma* they do not reproduce in the blood. They penetrate organ cells and there, like *Leishmania*, they divide and multiply until great groups of them are present in cross-striated muscles of the body, in heart muscle and in the central nervous system. Such groups may develop flagella simultaneously, so that in acute cases the blood may be teeming with flagellates (Reichenow). Children are most susceptible to infection, and the disease is most severe with them; but adults are not immune. In acute forms it is prevalent in very young children, but may assume a chronic type in children up to fifteen years of age, in whom it is associated with retarded development of mind and body. Chagas believes it to be the cause, not only of retarded development, but of functional loss of endocrine glands leading to goiter, cretinism and idiocy.

Other flagellated parasites common in man are found in the intestine for the most part. These are: *Embadomonas*, MacKinmon (1911); *Chilomastix mesnili*, Wenyon (1910); *Tricercomonas intestinalis*, Wenyon and O'Connor (1917); *Trichomonas hominis*, Davaine (1860); *Trichomonas vaginalis*, Donné (1837); *Giardia intestinalis*, Lambl (1859). The etiological significance in each case is doubtful, although the possibility is frequently admitted that some of them may augment disorders of the digestive tract,

but it is also possible that they may find under such conditions a more suitable environment for growth and reproduction. Species of *Bodo* which are amongst the commonest coprozoic flagellates have been observed in the urine (Powell and Kohigar, 1920). The intestinal flagellates, particularly *Embadomonas intestinalis*, *Chilomastix mesnili*, *Tricercomonas intestinalis*, *Trichomonas hominis* and *Giardia intestinalis* are usually present in large numbers in diarrheic stools while only cysts, as a rule, are found in normal stools. This certainly suggests an etiological connection, particularly with *Giardia* infections in which periodic attacks of diarrhea occur with passing of quantities of clear mucus in which the flagellates are abundant.

Parasitic Rhizopods.—While Sarcodina are perhaps less striking in their adaptations than are other groups of Protozoa, they are, nevertheless, more or less specialized in conformity with their habitats and modes of life. The fundamental type is spherical and characteristic of suspended or floating forms (Heliozoa and Radiolaria), but adaptations serving a hydrostatic purpose are numerous, particularly in the great group of Radiolaria. Creeping forms are found in superficial slime of ponds and sea or on stalks and leaves of water plants and are more or less segregated in localities where appropriate food is abundant. Thus *Amoeba vespertilio* may be found in fresh water where diatoms and algæ are abundant; *A. proteus* in waters with decomposing organic matter rich in bacteria, or *Pelomyxa palustris* in still fouler waters. *Amoeba terricola*, many testate rhizopods and related forms are more terrestrial, living in moss or damp earth and sand; here also may be found the majority of Mycetozoa, especially on damp and decaying wood. In short, there are few damp places that are devoid of ameboid types.

The Sarcodina are never as spectacular as the Mastigophora or Sporozoa in their adaptations for parasitism, but many types have become adapted to the semifluid habitats of plant and animal hosts or to the more fluid environments of animal digestive tracts. Coprozoic forms are not uncommon, many types, like coprozoic flagellates, passing through the digestive tract while encysted to develop later in the dejecta (e. g., *Dimastigameba*, *Sappinia* species). Conversely the true parasites are active only in the lumina of the alimentary tract and are able to withstand the rigors of an external life only when protected by cysts. Such cysts, through contaminative infection, germinate in the digestive tract where some types of *Endameba* cause acute or chronic intestinal diseases.

Many amebae are ectoparasitic. One, *Amoeba hydrovena* (Entz, 1912), occurs on hydra (*H. oligactis*); another, *A. paedophora*, Cautlery (1906), on the eggs of a crustacean *Peltogaster curvatus*; *A. mucicola*, Chatton (1909), occurs on the gills of marine fish. Protista are not exempt—species of *Sphaerita* parasitize Euglenoids, *Volvox*,

Hematococcus as well as parasitic flagellates, particularly *Trichomonas*, and nuclei of amebae; ciliates of various kinds, and other rhizopods are destroyed by *Nucleophaga*. Algae, diatoms, plant and animal flagellates are all subject to infection by species of *Pseudospora*.

The thick cellulose walls of various plant types may be dissolved by amylolytic ferments formed by certain types. In such cases no sharp line can be drawn between parasitism in a strict sense and processes of holozoic nutrition. These are well illustrated by *Vampyrella spirogyrae* which feeds on *Spirogyra* cells; *V. lateritia*, Leidy, on algae of different kinds, and *V. vorax*, Klein, which lives on diatoms.

Serious and economically troublesome diseases of plants are caused by parasites belonging to the Mycetozoa.

Plasmodiophora brassicae, Woronin, is the best known of this group largely because of its economic importance. It attacks the roots of cabbages and other Cruciferae and produces a characteristic tumor disease known as "Club-root," "Hamberries," "Fingers and Toes," "Kohlhernie," etc.

Minute flagellulae are formed from the cysts in an infected garden and these, in some way, penetrate the root cells of the plant and become myxamebae. The nuclei multiply and they grow in the cells of the plant, different individuals fusing to form plasmodial masses which fill the cell. With exhaustion of the cell contents the process of reproduction begins and results in the formation of great masses of uninucleate "spores."

Invertebrates have not been thoroughly investigated for ameboid parasites, and a big field is open here for research. The earliest on record is a parasite of cockroaches to which Leidy, in 1879, gave the name *Endamoeba blattae*. *Endamoeba minchini* was described by MacKinnon (1914) from the intestine of the crane-fly *Tipula sp.*; *Amoeba chironomi*, Porter (1909), from larvae of *Chironomus*; *Endamoeba belostomi* (Brug, 1922) from the water-bug *Belostoma sp.* of Java and *E. disparata*, *E. simulans* and *E. asbulosa*, Kirby (1927), from termites. A species from the gut of the oyster (*Falkampfia patuxent*, Hogue) was described by Hogue (1921). Endamoebae from other insects include: *E. apis*, Fantham and Porter (1911), in the honey bee; *E. mesnili*, Keilin (1917), in larvae of *Trichocera sp.*; *E. thompsoni* in *Blatta orientalis*.

In entomostraca (*Daphnia* species) a curious sporulating ameboid parasite was discovered by Chatton (1925) and named by him *Pansporella perplexa*. Binucleated spores escape from thin-walled cysts in the gut of *Daphnia* and give rise to uninucleate amebae, whether by division or by fusion of nuclei was not determined. These grow without dividing and finally encyst in which form they are passed out of the intestine. A series of nuclear divisions occur

in the encysted ameba followed by division of the body into binucleated spores which repeat the cycle upon ingestion by *Daphnia*. This history is so unusual for amebae that Chatton placed it in a new family, the *Sporoamoebidae*.

Vertebrates, particularly mammals, have been more extensively studied for parasitic amebae than have invertebrate animals. *Amoeba froschi* was found by Hartmann (1907) in frogs' feces, and *Valkampfia* (Epstein and Lovasky, 1914) from the frog intestine, and *A. lacertae*, Hartmann, and *A. dobelli*, Hartmann, from the intestinal contents of lizards. Other species described from reptiles are: *Endamoeba testudinis*, Hartmann, in the land turtle *Testudo graeca*; *E. barreti*, Hegner and Taliaferro, in *Chelydon serpentina*; *E. serpentis*, Da Cunha and Fonseca (1917), in the snake *Drimobius bifossatus*; *E. varani*, Lavie (1923), from *Varanus niloticus*. Few amebae have been reported from fish. The genus *Proctamoeba salpae*, named by Alexieff (1911) for an intestinal ameba discovered by Leger and Duboscq (1904) in the marine fish *Box boops*, is undoubtedly an Endameba, so Proctameba is a synonym.

Few parasitic amebae have been reported from birds. Fantham (1912) described *E. lagopodis* from the intestine of the grouse, and *E. anatis* from South African ducks (1924), and Tyzzer (1920) found *E. gallinarum*, Tyzzer, in chickens and turkeys.

Amebae resembling the type of *E. dysenteriae* and *E. coli* have been described from mammals of different kinds. Apart from human intestinal forms they have been reported from the mouse: *E. muris*, Grassi (1879), and *E. decumani*, Kessel (1924); from the rat: *E. rattii*; from rabbits: *E. cuniculi*, Brug (1918); from guinea-pigs: *E. cobayae*, Walker (1908) (*E. caviae*, Chatton, 1918); from swine: *E. debliccki*, Nieschultz (1925), *E. polecki*, Prowazek (1912) (*E. suis*, Hartmann, 1913); from sheep: *E. oris*, Swellengrebel (1914), *E. caprae*, Fantham (1923); from cattle: *E. bovis*, Liebetanz (1915); from horses: *E. intestinalis*, Fantham (1920), and *E. equi*, Fantham (1921). In addition to these, successful inoculations of human dysenteric amebae have been made, particularly in cats and monkeys.

Parasitic amebae in man, naturally, have attracted most attention and have been extensively studied. Tropical dysentery is such a dreaded malady that students over the entire world have contributed until today there are few important gaps in the pathological history of the disease or in our knowledge of the causative agent.

Amebic dysentery has had a long and confusing history in which taxonomic synonyms and etiological misfits have played a conspicuous part. The final chapter has not yet been written, but much of the earlier confusion has been cleared and students of the subject are working with a common understanding. In my opinion

the best account of intestinal amebae is given by Dobell (1919). For a clear comprehension of this modern point of view, I have found it expedient and instructive in teaching to divide the history of amebic dysentery into four arbitrary periods with the understanding that no period is clearly marked but all grade into one another in a slow, often backward, but nevertheless sure development. I would designate these periods: (1) Early taxonomic observations; (2) early etiological observations; (3) taxonomic chaos; and (4) modern point of view.

1. *Early Taxonomic Observations.*—With our present knowledge of the intestinal protozoan fauna of man it is difficult to decide whether so-called amebae of the earlier observers were really rhizopods or more or less abnormal forms of intestinal flagellates. The so-called "amebae" mentioned by Lambl (1860), who is usually credited with the discovery of human intestinal amebae, are regarded by Dobell as degenerating individuals of *Trichomonas*, while the value of his observations is further lessened by the fact which has been frequently pointed out, that he also observed the free-living forms, *Difflugia* and *Arcella*, in the same intestinal material. Ten years later (1870) Lewis, in India, whose investigations had already yielded a new mammalian trypanosome, and Cunningham (1871), working on cholera, discovered an intestinal ameba they believed to be non-pathogenic and which may well have been some harmless species of *Endameba*, possibly *coli*.

The first authentic association of an ameba and dysentery was described by Lösch (1875) in Russia. Upon autopsy of an individual who had a well-developed hospital case of dysentery but died of pneumonia, Lösch found an abscess of the liver containing amebae. Mainly negative results followed attempts to infect dogs with material from fresh stools of the victim, and Lösch concluded that with only 1 dog showing dysentery symptoms while 3 were negative his ameba, which he named *A. coli*, was a harmless commensal living in the human intestine. There is little doubt in the minds of modern students that he was really dealing with the active agent of amebic dysentery, in which case, as Dobell, Wenyon, Doflein-Reichenow and others have pointed out, the taxonomic specific name of the dysentery ameba should be *coli*. Lösch's dictum, however, that his *Amoeba coli* was a harmless commensal has influenced all subsequent investigators until the name *coli* is so intimately associated with what has turned out to be a really harmless ameba that it would involve needless confusion if an attempt were made to apply rigorously the rules of scientific nomenclature.

While the specific name *coli* thus got off to a poor start, the generic name *Ameba* for endoparasitic forms was destined to have a short life. Leidy (1879), who was working on his classical monograph on

the "Fresh Water Rhizopods of North America," discovered a parasitic ameba in the intestine of *Blatta orientalis*, which he first named *Amoeba blattar*. Recognizing the impropriety of grouping the relatively huge fresh water and free-living amebae, such as *A. proteus* and the minute intestinal ameba of the cockroach in the same genus, he changed the generic name of *Amoeba blattar* to *Endamoeba blattar*. Sixteen years later Casagrandi and Barbagallo (1895) studied an ameba from man which, apparently in ignorance of Leidy's work, they named *Entamoeba coli*, changing it two years later to *Entamoeba hominis*. Now in my opinion this is the exact equivalent of Leidy's *Endameba*, for in this country we use the form "endo" (witness endoplasm, endoderm, endothelium, etc.) in the same sense that Europeans use the form "ento" (entoplasm, entoderm, etc.). *Endameba* and *Entameba* thus are the same, the form depending on the custom of the country where used, and there is little justification for employing them, as Dobell, followed by Wenyon, suggested, to represent two distinct genera. If there is a generic difference between the intestinal amebae of the cockroach and that of man, which is by no means established, then some at least of the human forms should be included under Chatton and Lalung-Bonnaire's name, *Löschia* (1912).

2. *Early Etiological Observations.*—This period marking the beginning of a long controversy over the pathogenicity of intestinal ameba may be arbitrarily fixed between the approximate dates 1880 and 1902. Leidy's generic name was little used until the late '90's; indeed not until after Casagrandi and Barbagallo had introduced the form *Entameba*. At the beginning of this period it was generally believed that the human intestinal forms belong to one species which, following Lösch, was known as *A. coli*. The controversy then was over the question whether or not *A. coli* is pathogenic, and the cause of dysentery. Grassi (1879, 1882, 1883, 1888) found amebae widely distributed in feces of normal individuals as well as in those suffering from diarrhea, and when cysts of the organism are swallowed by humans they give rise to amebae which multiply in the intestine but cause no symptoms of dysentery or other intestinal upset (1888). He was emphatic in concluding that the ameba with which he worked and which he regarded, erroneously, as the same as Lösch's "*A. coli*," is altogether harmless to man. The seed thus planted by Lösch developed into a healthy weed with Grassi, became a permanent plant with Schaudinn (1903) and has never been uprooted. *Endamoeba coli* as a harmless parasite had come to stay.

The pathogenic importance of the so-called *A. coli* was also well supported at this early period. Lösch started it and was supported in the '80's by Kartulis in Egypt (1885, 1886, 1887, 1891), by Koch (1883), Koch and Gaffky (1887) and others. Sections of intestinal

ulcers (Koch) showed ameboid bodies but, according to Dobell, while he evidently regarded these as amebae his observations were not sufficiently definite to justify positive conclusions. The work of Kartulis was more convincing and his evidence, including observations on some 150 cases of intestinal ulcer (1886) with the discovery of amebae in all, together with amebae in liver abscesses, and later (1904) of amebae in abscesses of the brain, went far to establish, clinically, the etiological connection between "*Amoeba coli*" and dysentery.

What is probably the most thorough of the clinical works of this period was the study of Councilman and Lafleur (1891) of the pathology of amebic dysentery and amebic abscess of the liver. The possibility of two types of *Amoeba coli* in the human intestine, one pathogenic, the other harmless, while evident now in the conflicting observations of Grassi and Kartulis, does not seem to have been considered by the earlier workers. It was fully considered, however, by Councilman and Lafleur, who not only suggested the possibility, from the evidence of their work, but went so far as to name the innocuous form *Amoeba coli*, while to the pathogenic form, capable of invading tissues and of causing liver abscesses, they gave the new name *Amoeba dysenteriae*. This classical work on the pathology of dysentery has received but scant attention from later workers, particularly the more influential European parasitologists. There is absolutely no doubt that Councilman and Lafleur recognized, gave adequate descriptions of, and named the cause of amebic dysentery, which today is generally known as *E. histolytica*. It is difficult to see any adequate reason why the specific name *dysenteriae* should have been ignored save that *histolytica* is more euphonic and more descriptive of the havoc made by the ameba. The reasons given by Dobell (1919) seem trivial and unworthy of that astute critic, viz.: that Councilman and Lafleur in spelling failed to capitalize the generic name *Amoeba* and failed to italicize the full name as a zoölogist would have done. Dobell ignores the ending *iae* which alone sets it apart from an ordinary descriptive term. Again Dobell says (*Ibid.*, p. 28): "I regard '*Amoeba dysenteriae*,' Councilman and Lafleur, as ruled out because it is a synonym of '*Amoeba coli*,' Lösch." He accepts *E. histolytica*, however, so this ruling does not seem to be forceful enough to set aside Schaudinn's term which is equally well a synonym of *A. coli*, Lösch. When the subtleties of the legal profession are employed for scientific ends and a matter settled on a *post hoc* technicality which may be applied or not according to the whim of the individual, we are rather close to unfair dealing. It may be too late to remedy the injustice, for the name *Eutamoeba* (or *Eutamoeba*) *histolytica* is now in general use, but it will never have a clear title. It is gratifying to note that Chatton and Kofoid retain the name *E. dysenteriae*.

Nor is the title clear for the generic name *End(Ent)ameba* unless the ameba of the cockroach (*E. blattae*) and the dysentery amebae of the human intestine are continued to be regarded as cogenetic. If, and it may be true, these amebae are generically different, then some other name must be used for the human parasites, for *Endameba* goes with *E. blattae*, Leidy. Dobell and Wenyon and Reichenow (1928) recognize this difficulty but are not sure that these amebae belong to different genera. In case they do, they agree in proposing *Endameba* for *E. blattae* and the form *Entameba* for *E. coli* and the pathogenic species of man. This, however, is a mere subterfuge, for they are only different spellings of the same term. Dobell shows that in case *Endamoeba coli* is shown to be generically different from *E. blattae*, then Chatton and Lalung-Bonnaire's (1912) name *Löschia* would have priority.

Returning from this controversial digression to the host-parasite relations of the intestinal amebae of man, we find that throughout the decade 1890-1900 there was little recognition of two types of amebae—one harmless, the other pathogenic. Quincke and Roos (1893) and Roos (1894) indeed spoke of "harmless" and "pathogenic" forms, the former being non-pathogenic to cats upon infection with amebae per os or per anum. Casagrandi and Barbagallo (1895-1897), who introduced the generic name *Entamoeba coli* in ignorance of Leidy's *Endamoeba*, returned to Grassi's contention that there is only one form of ameba which they termed *E. coli* (1895) but later changed to *E. hominis*.

Schaudinn (1903), also ignorant of Councilman and Laffleur's work, was convinced by work of earlier observers and more so by his own observations and experiments that there are two distinct species of intestinal amebae, one harmless, the other pathogenic. He had an excellent opportunity to rectify the mistake which was then in its infancy of regarding Lösch's *E. coli* as a harmless ameba, but he failed. He accepted Casagrandi and Barbagallo's generic name *Entameba* but regarded their *E. hominis* as the same thing as Lösch's *A. coli*, and such was his great influence at that time that this name *E. coli* was attached, firmly but erroneously, to the common non-pathogenic ameba of man. For the pathogenic species he proposed the name *Entamoeba histolytica*.

With the establishment of two species of *Ameba*—one of which is pathogenic, some of the old difficulties which were engendered by Grassi's and similar work on the one hand, and by that of Kartulis on the other, were cleared up. Throughout this period, however, there were skeptics who could not be convinced that any ameba is an etiological agent in human dysentery, for cases of dysentery in which no evidence of amebae could be found were turning up repeatedly. This difficulty was finally removed by the discovery by Shiga (1898), confirmed by Flexner, of the Shiga-Flexner bacillus

as the cause of bacillary dysentery. Thus by the end of our second period two important points had been established, viz.: the occurrence of two types of amebae in the human intestine, and the occurrence of at least two types of dysentery due to different kinds or organisms.

3. *Period of Taxonomic Chaos.*—It is quite evident from the foregoing that the term taxonomic chaos with propriety might be applied to the entire history of dysentery. It is particularly applicable, however, to the first decade of the present century when, owing to the prestige of Schaudinn, incorrect interpretation of the life history of *Endamoeba dysenteriae* (*histolytica*) resulting from his work stood in the way of progress for more than a decade. In his paper on the reproduction of certain rhizopods Schaudinn (1903) described the life histories of the foraminiferon *Polystomellina crista*, Lam., the testate rhizopods *Centropyxis aculeata*, Ehr., Stein, and *Chlamydothryx stercorica*, Cienkowski, and the parasitic amebae of the human intestine. In connection with the first three he was convinced that chromidia give rise to the nuclei of gametes (see p. 69) and thus play an important rôle as germinal chromatin. It is not surprising, therefore, that he ascribed an important part to what he termed chromidia in the parasitic amebae. In respect to these chromidia the life histories as he interpreted them in *Endamoeba coli* and *E. dysenteriae* (*histolytica*) are complicated.¹ In this account emphasis was laid by Schaudinn on: (1) The structural differences in nuclei of *E. coli* and *E. histolytica*; (2) formation of encysted amebae with 8 nuclei, giving rise to 8 spores, in *E. coli* but absence of all cysts in *E. histolytica*; (3) reproduction by peripheral, chromidia-holding buds in *E. histolytica* but not in *E. coli*; and (4) infection by spores of *E. coli* and by "resistant buds" in *E. histolytica*; (5) pathogenicity of *E. histolytica* and harmlessness of *E. coli*.

In this same year (1903) Huber made observations and experiments which, had they received the attention they merited (see Dobell, 1919), would have saved subsequent confusion. From a case of typical amebic dysentery he observed amebae and their cysts, the former infecting cats when introduced *per anum*, the latter infecting cats per mouth. The cysts were reported as containing 1, 2 and 4 nuclei but never more than 4. In the glamor of Schaudinn's prestige this latter important point was ignored. Viereck (1907) and Hartmann (1907) found them and, since the cysts had 4 nuclei and Schaudinn had stated that *E. histolytica* does not form cysts, they regarded them as a new species of *Endameba*. The former named it *E. tetragena*, the latter *E. africana*. Hartmann recognized *E. africana* as the same as *E. tetragena* which had been published somewhat earlier in the year. The observations were

¹ See Calkins Protozoölogy, 1909, p. 296.

quickly confirmed and, as was to be expected, *E. tetragena* was reported as a much more widely spread dysenteric ameba than *E. histolytica*. A small precystic phase of *E. histolytica* was regarded as a distinct species to which Elmassian (1909) gave the name *E. minuta*. Koidzumi (1909) created a new species, *E. nipponica*, for a variety of amebae, some of which were probably *E. dysenteriae* (*histolytica*). Other synonyms, originally suggested for the most part as new species, were: *Entamoeba schaudinni*, Lesage (1908); *E. hartmanni*, Prowazek (1912); *E. braziliensis*, Aragao (1912); and several others since 1912. At this time (1912) suspicions as to the identity of these suggested species began to appear in the works of Darling (1912), Whitmore (1911) and James (1914) which turned to certainty in the work of Walker (1911) and Walker and Sellards (1913) who demonstrated the identity of *E. histolytica*, *E. tetragena* and *E. minuta* and so brought to an end this particular period of confusion, and, in addition, added many important points concerning the distribution and transmission of the organisms of dysentery.

4. *The Modern Period.*—The general acceptance of the organism now known as *Endamoeba coli* as a harmless commensal, together with the proof that the organism *Endamoeba dysenteriae* (*histolytica*) is pathogenic to man, was the basis for a good start in the modern period.

There is little doubt that *Endamoeba dysenteriae* (*histolytica*) is a dimorphic species which, in one phase, is a tissue-penetrating type which, presumably by secretion of a proteolytic ferment, causes cytotoxicity of tissue cells leading to ulcerations and abscess formation. Such a ferment has been extracted by Craig from cultures. The other type is the *minuta* form which shows a more complete adaptation to the intestinal environment of man. This is the type found in carriers and, were it not for the possibility of its transformation into the pathogenic phase, might well take its place with *E. coli* and other harmless amebae of the intestine. It reproduces by division in the intestine, however, and is regarded as the typical form of the dysentery-causing ameba (Mathis and Mercier, Reichenow, etc.) which under certain conditions may revert to the larger pathogenic form (Kuenen and Swellengrebel, 1913). Dobell, on the other hand, maintains that it is a pre-cystic condition giving rise only to the encysted form with from 1 to 4 nuclei. These cysts are present in the formed stools while living *minuta* forms may be found in fluid stools or after a purgative. The dysenteric forms are not ordinarily found in stools, but may be present in the discharge from ulcers. In artificial culture medium the pathogenic form quickly passes into the *minuta* phase. Successful cultures were made by Cutler (1918), by Boeck and Drbohlav (1925) and with remarkable results by Cleveland and Sanders (1930). The latter were able not only to cultivate the organisms indefinitely and in

amazing numbers, but to bring about encystation and excystation at will while, at any stage, dysentery in kittens could be produced.

The tissue-invading forms of *E. dysenteriae* are usually from $20\ \mu$ to $30\ \mu$ in size but variations above and below these limits may occur. The organism quickly degenerates outside the body and becomes quiet with a thick hyaline ectoplasm, but under normal conditions it shows great activity, moving occasionally like a *limax* type of ameba or more frequently by the formation of large blunt pseudo-

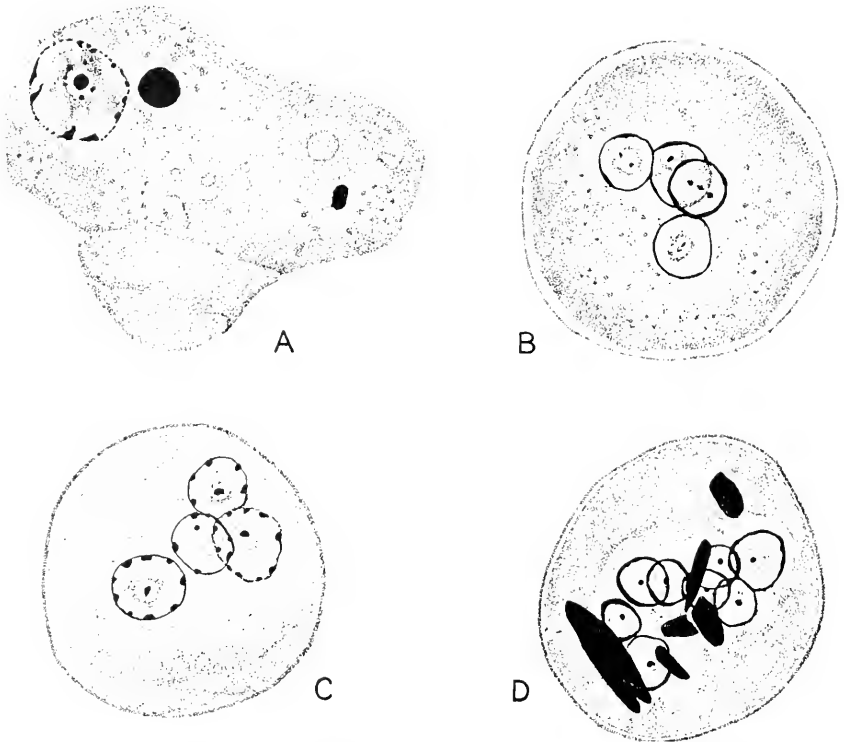


FIG. 171.—*Endamoeba dysenteriae*. A, typical trophic ameba with red blood corpuscle found in dysenteric stools; B and C, encysted individuals about ready for excystation; D, cyst with eight nuclei and chromatoid bodies. (After Cleveland and Sanders, Arch. f. Protistenkunde; courtesy of G. Fischer.)

podia which are suddenly formed and withdrawn with equal speed. The endoplasm is densely granular and in addition to the nucleus contains food vacuoles, "chromatoid" bodies and numerous small granules which stain *intra vitam* with neutral red (Dobell).

The nucleus is difficult to see during life of the organism, owing to the densely granular cytoplasm. In fixed preparations it may be seen to have a delicate membrane studded internally by chromatin granules, and with a minute homogeneous endosome (Fig. 171).

Between the latter and the membrane there is a delicate linin reticulum. Kofoid and Swezy (1924-1925) describe the division of the endosome after migrating to the periphery of the nucleus, and formation of a spindle figure with a centrodesmose on which 6 chromosomes divide.

Nutrition of the tissue invading form is primarily by endosmosis; erythrocytes are frequently found in the food vacuoles sometimes in large numbers, but just as often there are none at all. Bacteria are present only exceptionally.

The *minuta* form is much smaller than the invasive form and there is a greater variation in size—the variations being so consistent that many authors (*e. g.*, Wenyon and O'Connor, 1917; Dobell and Jepps, 1917, 1918) regard them as distinct races. The sizes of the cysts which they form likewise vary. Wenyon (1926) gives the limit of size of the *minuta* amebae from $7\ \mu$ up to the size of the invasive type while the cysts vary in size from 7 to $18\ \mu$.

Reproduction of the ameboid forms by simple division keeps up the number of parasites in the intestine, and may continue indefinitely in carriers. Conditions leading to the formation of *minuta* types and precystic amebae in the intestine are only matters of surmise, but with encystment multiple division into 4 small amebae occurs. The cysts are usually spherical with smooth walls and from $5\ \mu$ to $20\ \mu$ in diameter, and when fully developed contain 4 nuclei (in some rare cases 8 may be present, Wenyon). In addition to nuclei so-called chromatoid bodies are present in the cytoplasm. These are of considerable diagnostic value for they are much more rare in cysts of *E. coli*. They are usually in the form of rods with rounded ends but may be filamentous, or irregularly shaped bodies sometimes 2 or 3 in number, sometimes many. These appear to be absorbed during the external life of the cyst. During the formation of the cyst the glycogen which is present at the commencement of encystment disappears. Cleveland and Sanders have studied ex-cystation. These cysts give rise to an ameba free from chromatoid bodies and with 4 cystic nuclei. So-called "meta-cystic development" (Dobell) results in the formation of 8 young, uninucleate amebae but not always by the same process. The 4 cystic nuclei may all divide after which the cell divides into 8 uninucleate forms. Or one of the 4 cystic nuclei may divide to form 2 meta-cystic nuclei which with cytoplasmic division gives rise to an ameba with 2 meta-cystic nuclei, and a sister cell but larger with 3 cystic nuclei. In other cases 2 or 3 of the 4 cystic nuclei may divide and be cut off with some cytoplasm which ultimately result in uninucleate forms. Hence meta-cystic amebae may be found with any number of nuclei, from 1 to 8. Cleveland and Sanders describe 24 such combinations.

While probable that *E. dysenteriae (histolytica)* may be carried

by the blood to various sites in the body, it is only in rare cases that organs apart from the digestive tract become infected. Brain abscesses are very rare, and in these only the large tissue-invading amebae are present and cysts are not found (Wenyon). Different observers have reported similar amebae in urine, in ducts and tubules of the male reproductive organs, in the lungs and even in the skin. The so-called amebae described by Kofoid and Swezy (1922) from the bone-marrow in cases of arthritis deformans and from degenerating lymphatic glands of Hodgkin's disease, and regarded by them as *End. dysenteriae*, have not been taken seriously by the majority of other students of the Protozoa.

Other Amebae of the Human Intestine.—Amebae of different kinds are characteristic intestinal parasites of all kinds of animals. In man, apart from *E. dysenteriae* (*histolytica*) and its many form changes, they are not pathogenic. Mutual adaptation has made them, for the most part, harmless guests of the alimentary tract, while as stated above, even *End. dysenteriae* in "carriers" is a harmless commensal.

Dientamoeba fragilis was discovered by Jepps and Dobell in 1918. It is a minute ameba (3.5μ to 12μ) and very active with, characteristically, 2 minute nuclei. It is very delicate and apparently quite rare. The binucleate condition, together with the structure of the nuclei and the rare occurrence of cysts (Kofoid, 1923), are definite characters which distinguish it from *Endameba*. Opinions differ as to its pathogenic character, but Dobell (1919) regards it on the whole as a harmless type.

Eudamoeba coli is a common but harmless commensal in the digestive tract and never becomes a tissue-invading form. For the casual observer it may be easily mistaken for *E. dysenteriae* but with abundant material and with different stages of the organism there is now no excuse for such a mistake. It is larger than *E. dysenteriae* (15μ to 30μ), has a more transparent protoplasm so that the nucleus is visible in life and contains ingested food of different kinds but rarely if ever does it ingest red blood corpuscles. The relative scarcity of endoplasmic granules makes the difference between endoplasm and ectoplasm less noticeable than in *E. dysenteriae*. The nucleus is larger and the endosome more conspicuous than in the dysentery causing ameba. The most characteristic feature, however, is the cyst with its 8 nuclei (not infrequently with 16). The pre-cystic forms are somewhat smaller than the active ameba but the cysts are larger than in *E. dysenteriae* (10μ to 30μ), usually 15μ to 20μ .

The so-called *Councilmaniana lafleuri* of Kofoid and Swezy (1921) is now generally regarded by protozoölogists as referring to modified or aberrant types of *Eudamoeba coli*.

Other amebae of the digestive tract are *E. giugivalis* found on

the teeth and in the mouth; *Endolimax nana* (Wenyon and O'Connor, 1917), one of the commonest amebae in man, and *Iodoamoeba butschlii* (Prowazek, 1912).

Parasitic Ciliata.—A considerable volume could be written on the parasitic ciliates. This is attested by the many great monographs on limited groups of this class of Protozoa, *e. g.*, on Opalinidae, Astomida, Oxytrichida, Ophryoscolecida, etc. Highly spectacular life histories, such as those of *Leishmania*, *Trypanosoma* and *Plasmodium*, and economic importance in connection with human affairs are absent here. Absent also are the pathogenic effects of parasites of the *Endameba* type or of biologically significant adaptations to complete symbiosis which characterize the *Hypermastigida*. Nevertheless the parasitic ciliates represent a group which illustrate in high degree the phenomenon of commensalism with morphological differentiations which place them with the most complex types of Protozoa and the most highly organized types of single cells.

Infection in all cases is contaminative and made possible by protective cysts in which the fundamental organizations may remain dormant for years. With the exception of *Balantidium*, pathogenic effects in man are of little importance. By mere numbers, however, especially of ectocommensals, functional activity of the host may be weakened or even suppressed as when gills, eyes and skin are covered with cysts due to *Ichthyophthirius multifiliis*. Entodiniomorpha, on the other hand, as commensals in the forestomach of ruminants are interpreted as approaching the symbiotic relationship of *Hypermastigida* in termites. Dogiel (1928) estimates the number of ciliates in cattle as 50,000 in 1 cc. of rumen contents and Ferber (1928) carries the number in sheep and goats up to 900,000 in 1 cc. The ability of these ciliates to digest cellulose and to build up albumin in their own cell bodies is indirectly advantageous in the nutrition of their hosts through the added supply of their body protein (Dogiel, Ferber, Reichenow-Dofflein).

Morphological evidences of adaptation to an endocommensal mode of life are shown (1) by degenerative changes, and (2) by specializations for protection, adhesion, movement and multiplication. Modifications of a degenerative character are shown by the absence of mouth in Opalinidae and the Astomida in general and the substitution of saprozoic food-getting methods for holozoic methods which are characteristic of the free-living ciliates. Opalinidae are not only mouthless but they also lack the dimorphic nuclei—macronuclei and micronuclei—which are distinctive diagnostic features of the Infusoria. The method of fertilization by copulation of gametes and not by conjugation also distinguishes the Opalinidae from the majority of other ciliates. On these grounds Metcalf (1923) proposed a classification involving the separation of

this group from other ciliates as a sub-class Protociliata, while the remainder of the great group of ciliated Infusoria were grouped as Euciliata. In this he has been followed by Doflein, Reichenow-Doflein, Wenyon and the majority of protozoölogists. Personally, however, I cannot subscribe to this point of view; I am second to no one in recognizing the superlative work of Metcalf on representatives of this group, but I do not agree to the separation of 4 genera of astomatous forms as Prociliata from the number of other astomatous forms, which, together with the hundreds of genera of mouth-bearing forms of ciliates are placed in an equivalent group, the Euciliata. Nor can I regard the so-called Protociliata as primitive. The most generalized forms of free-living ciliates, with which the Opalinidae agree in ciliation, are mouth-bearing forms, and the absence of a mouth in parasites is much more probably a degenerative than a primitive character, and is to be regarded as a special adaptation to the conditions of a limited but highly nutritive environment.

Nor can the absence of dimorphic nuclei pass unquestioned. The cell body of an opalinid is filled with discoidal structures which were interpreted by Tommiges (1927) as representing a distributed macromucleus similar in character to that of *Dileptus gigas* (Fig. 25, p. 52). The same point of view has been vigorously maintained by Konsuloff (1922) but actual proof is still lacking. The history of amiconucleate ciliates shows that dimorphic nuclei are not essential for continued metabolism (see p. 225); here, however, the diversity of chromatin in the opalinid nucleus suggests the correctness of Tommiges' (1927) view that these nuclei possess both germinal and somatic components.

Finally the absence of conjugation and the substitution of gametic fertilization is not unique with the Opalinidae. Here by repeated division without intervening growth, gametes of different size (anisogametes) are formed and these fuse in copulation. The same phenomenon occurs in *Glaucoma (Dallasia) frontata*, a free-living ciliate, the only difference being that the gametes are isogamous and derived from the same parent (Calkins and Bowling, 1928). Here fertilization is pedogamous while in *Ichthyophthirius multifiliis* the process has apparently gone one step farther into autogamy according to Neresheimer (1908) and Büschkiel (1910).

The Opalinidae are parasites of frogs and toads primarily. Some species occur in fish, and one, *Protopalina nyanza*, in a reptile. They are represented, according to Metcalf, by 4 genera which differ in form of the body and the number of nuclei. *Protopalina*, Metcalf, and *Zelleriella*, Metcalf, have each 2 nuclei. *Cepedea*, Metcalf, and *Opalina*, Purkinje, have many nuclei. *Protopalina* and *Cepedea* are nearly circular in cross-section; *Zelleriella* and *Opalina* are flat.

The other representatives of the group Astomina are characteristic parasites of invertebrates, particularly of the annelids, where they may be found in the digestive tract, the coelom, or in the tissues of diverse organs. Cepede (1910) has given the best monograph on the group but his classification based on habitat has been much improved by Cheissin (1930; see Key, p. 489). Adaptations for attachment have been developed in the form of hook-like chitinous organs which are deeply anchored in the body (Fig. 202, p. 492) and of suckers with or without hooks (Steinella; Sieboldiellina from Turbellaria). Chitinous skeletal bars are also widely distributed in the group.

Astomida are also found as parasites in medusae (Kofoidella, Cepede), in copepods (Perezella, Cepede), in amphipods and isopods (Collinia, Cepede) and in the gonads of starfish (Orchitophrya, Cepede).

Mouth-bearing forms of endoparasitic ciliates show great modifications and specializations in structure. Taxonomically they are distributed amongst Holotrichida and Spirotrichida, the latter including Heterotricha, Oligotricha (with Entodiniomorpha, Reichnow). (See Key, p. 508.)

The Holotrichida are subdivided into Gymnostomina, Hypostomina, Trichostomina and Hymenostomina, all of which have parasitic genera and some groups in which parasites have not been recorded. Among the Gymnostomida are the ectocommensial *Ichthyophthirius multifiliis* and the enterozoic forms Butschlia, Schuberg (in ruminants); Bundleia, da Cunha and Muniz; Blepharocolon, Bundle, Blepharoconus, Gassovsky, Didesmis, Fiorentini, and Blepharoprosthium, Bundle (all from the horse); Buissonella, da Cunha and Muniz (from the tapir); and Protohallia, da Cunha and Muniz (from the capybara).

Among the hypostomes we have some destructive ectocommensals: *Chilodon cyprinii*, Moroff, for example, causes severe epidemics amongst carp and goldfish. Less destructive, but biologically most interesting, are the ectoparasites known as Foettingeriidae, where the complicated life histories have been carefully followed by Chatton and Lwoff. They appear to be primarily ectoparasites of crabs, where they appear in the encysted condition on the gills. When the exoskeleton of the crab is shed the ciliates leave their cysts and grow apparently on the secretions of the skin. Ultimately the fully-developed forms leave the old host and divide, *Polyspira* in free-swimming condition, *Gymmodinioides* while encysted.

Foettingeria actiniarum, Clap, lives in the gastral cavity of an actinian; here it divides while encysted and the young forms after emerging from the cyst cannot begin life again in the actinian but become attached to crustacea of different kinds where they

form stalked cysts within which they undergo a metamorphosis. If hosts and cysts are eaten by an actinian the metamorphosed ciliates are liberated and these take up life again in the gastral cavity of the coelenterate.

Of the endoparasitic forms the Pycnothricidae (Nicollellidae of Chatton and Perard) are noteworthy because of the varying positions of the mouth, which is connected with an elongated furrow

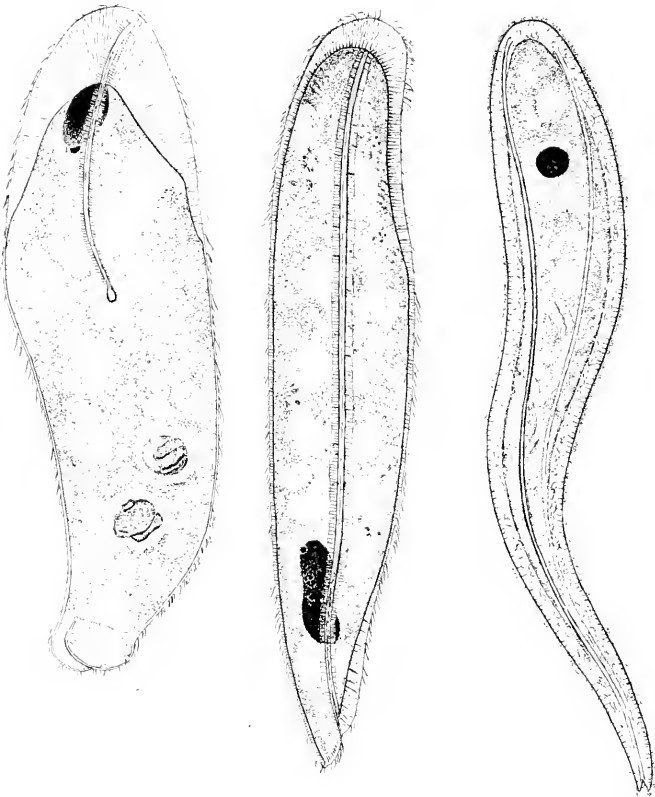


FIG. 172.—Nicollellidae. *Nicollella*, *Collinella*, and *Pycnothrix*. (After Chatton and Perard, Bull. Biol. de la France et de la Belgique, 1921; courtesy of Prof. N. Caullery and Les Presses Universitaires de France.)

running from the mouth to the anterior end. In *Nicollella*, Chatton and Perard, the furrow runs to the mouth which lies at the middle of the ventral surface; in *Collinella*, Chatton and Perard, it runs to the mouth at the posterior end; while in *Pycnothrix*, Schubotz, which is by far the largest of the parasitic ciliates (2 to 3 mm.), it runs down the ventral surface, around the posterior end and back to near the anterior end on the dorsal surface where the

mouth lies (Fig. 172). Here, also, in addition to the mouth opening there are numerous pores opening into the endoplasm throughout the course of the furrow. With this group also we include provisionally *Buxtonella*, Jameson, a parasite of cattle.

Amongst the Trichostomina we have the families Conchoptheriidae which are ectoparasites and endoparasites of molluscs; the Iso-trichidae which are endoparasitic in ruminants; and Cyathodinidae, endoparasites of *Cavia apersa* (see Key, p. 503).

Amongst the Hymenostomina the Ancistridae include ectoparasites and endoparasites in mussels (*Ancistruma*, Strand, and *Boveria*, Stevens) and in holothurians (*Boveria*, Stevens).

These forms are all highly modified ciliates which in some cases have acquired characteristics of the Suctoria. Thus *Hypocoma* possesses a suctorial tentacle which functions as a mouth, and the genera *Pelecypophrya*, Chatton and Lwoff, and *Sphenophrya*, Chatton and Lwoff, have lost all cilia in the fully-developed condition, their earlier presence indicated only by two zones of basal granules of the infra-ciliature. As in Suctoria reproduction occurs by budding from the dorsal surface, the buds being ciliated, not as are the Suctoria, but like the Ancistridae.

Amongst the heterotrichs we find the only ciliated parasites of man represented by species of the genera *Nyctotherus* and *Balantidium*. *Nyctotherus faba*, Schaudinn, is a small form which, according to Reichenow, has been safely identified as an intestinal parasite of man only once (1899) and then in diarrheic stools. *Balantidium* species are more frequently found in the human intestine; here, particularly in *B. coli*, the ciliates may run a normal course in the intestine without causing morbid symptoms, but under conditions of the host which are not understood, they may cause an acute enteritis of the same nature as dysentery. Like *Endameba*, these ciliates may penetrate the gut wall and remain embedded in the deeper tissues.

Balantidium species are widely distributed amongst the lower vertebrates and mammals and *B. coli* is a characteristic parasite of the pig, which is the main source of human infection. A second species, *B. minutum*, was discovered by Schaudinn, together with *Nyctotherus*, in one case; since then it has been observed only sporadically (Pinto in Mexico and Mathewossian in Armenia, according to Reichenow-Dofflein, 1929).

The Oligotrichida are ciliates with greatly reduced ciliation, the adoral zone of membranelles and cirri alone representing the motile organs. In the older systems of classification the Order was divided into three families—Halteriidae, Tintinnidae and Ophryoscolecidae, the last including all of the parasitic forms. These parasites are quite different in organization and complexity from other Oligotrichida, but are of a common type amongst themselves and justify

Reichenow in making them an independent order which he calls the Entodiniomorpha. These are all peculiarly differentiated gut parasites of mammals in which the cilia are reduced and represented only by the adoral zone of membranelles which runs into a deep vestibule at the anterior end of the body (Fig. 2, p. 20). The posterior end is often drawn out into characteristic processes (Fig. 146, p. 293). In *Cycloposthium* and related genera (see Key, p. 515) there are bundles of cirrus-like motile organs in addition to the adoral zone, the various arrangements of these groups of motile organs furnishing the basis for generic distinctions (see Key, p. 513).

THE MORE IMPORTANT SPOROZOAN PARASITES OF MAN.

The Sporozoa comprise a most heterogeneous collection of animal parasites with hosts in every branch of the animal kingdom, and to limit their discussion here to the parasites of man is entirely a matter of expediency.

We follow Doflein in giving a broader interpretation of Leuckart's group Sporozoa than does Wenyon. The latter separates the Cnidosporidia as a distinct Class from the Sporozoa in which he includes only the Gregarinida and the Coccidiomorpha. These two groups are united here as Orders in the Sub-class Telosporidia, Schaudinn, while the Cnidosporidia (Schaudinn's Neosporidia) constitute an equivalent sub-class, but without any obvious relationship to the Telosporidia. With the possible exception of *Sarcocystis* which still has an uncertain taxonomic position, none of the Cnidosporidia are parasites of man.

In a discussion of human sporozoan parasites we are limited, therefore, to the Telosporidia comprising the Gregarinida and the Coccidiomorpha. The former are coelozoic parasites of invertebrates; the latter are parasites of both invertebrates and vertebrates and are much more harmful to their hosts than are the gregarines. This is due to their characteristic cytozoic mode of life which involves the active destruction of tissue cells with corresponding weakening of functions. These are the only forms of Sporozoa which man has to fear and relatively few of them are known to cause human disease.

According to the site of infection the Coccidiomorpha are divided into Coccidia, or tissue-cell-dwelling forms, and Hemosporidia, or blood-cell-dwelling forms. Notwithstanding the difference in habitat and the special adaptations which are characteristic of blood parasites, there is a remarkable uniformity in the life histories of all coccidia and hemosporidia, and a common terminology has been adopted for the different stages. The life cycle of *Eimeria schubergi*, as given by Schaudinn for the parasite of the centipede, *Lithobius forficatus*, although thirty-two years old, is still the clearest and

the most instructive scheme for illustrating the stages in the life history and the adopted terminology.

Eimeria schubergi is a common parasite of the centipede's intestine, infection being brought about by contaminated food. In such food substance the germs of *Eimeria* are protected against drying and other adverse external conditions by cyst membranes, one of which,

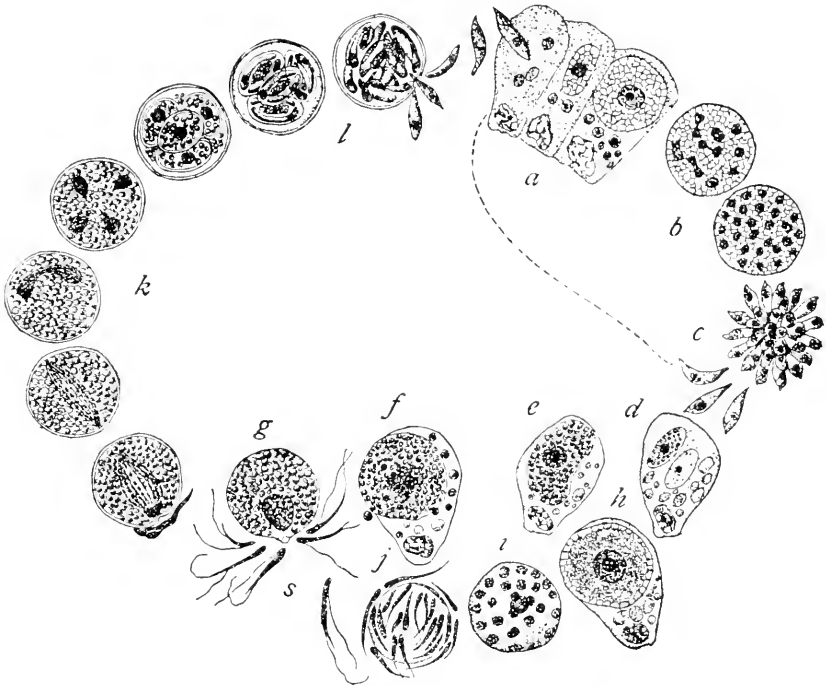


FIG. 173.—*Eimeria Schubergi*. Sporozoites penetrate epithelial cells and grow into adult intracellular parasites (a). When mature, the nucleus divides repeatedly (b), and each of its subdivisions becomes the nucleus of an agamete (c). These enter new epithelial cells and the cycle is repeated many times. After five or six days of incubation, the agametes develop into gamonts; some are large and stored with yolk material (d, e, f), others have nuclei which fragment into chromidia which become the nuclei of microgametes (d, h, i, j). A macrogamete (g) is fertilized by one microgamete (j) and the zygote forms an oocyst (k). This forms four sporoblasts, each with two sporozoites (l). (After Schaudinn.)

the sporocyst membrane, encloses 2 germs termed sporozoites. The second and outer membrane—oocyst—encloses a group of 4 sporocysts (Fig. 173) and 8 sporozoites.

Under the action of digestive fluids the double membrane about the sporozoites are opened and the germs are liberated. They make their way to glandular cells of the intestine and get into the cytoplasm, usually 1 to a cell (Fig. 173, a). In the cytoplasm the sporozoites

zoite grows into an intracellular parasite termed the trophozoite, which fills the greater part of the cell and forces the cell nucleus to one side where it degenerates. The trophozoite grows at the expense of the host cell, and when fully grown its nucleus divides a number of times and the cell body divides into a number of daughter cells. This process is termed schizogony or asexual reproduction and the products are called merozoites. These are liberated into the lumen of the intestine, where they behave exactly like the sporozoites entering epithelial cells which they, also, destroy and grow into adult merozoite-forming trophozoites. The process is repeated a number of times and in this way, by multiple progression, great areas of normal cells are infected and destroyed. Ultimately the merozoites give rise to trophozoites which have a different fate. Some of them grow to full size and as macrogametocytes store up reserve nutriment and become differentiated as macrogametes. Others grow in like manner but instead of storing nutritive substances become free of granules and appear hyaline. The nucleus ultimately begins to divide and its divisions are repeated until many hundreds are present and distributed around the periphery of the cell. These become the nuclei of microgametes which are delicate hair-like cells, each with 2 flagella, distributed over the surface of the mother cell or microgametocyte (Fig. 173, j).

Immediately after fertilization by union of a macrogamete and a microgamete, a fertilization membrane is formed around the zygote. This membrane becomes hardened into the oöcyst or outer protective covering. The zygote is then ready to undergo metagamic divisions, first into 2 and then into 4 cells. Each of these is a sporoblast which secretes a protective membrane about itself—the sporocyst—and then divides into 2 daughter cells, each of which is a sporozoite (Fig. 173, l). Each zygote thus gives rise to 4 sporoblasts with their sporocysts, and to 8 sporozoites, 2 to each sporoblast.

While details vary widely such a general outline of the life history may be applied to all types of Coccidiomorpha. Variations occur in all phases, particularly in the sporogony cycle, where we find wide differences in the number of sporoblasts formed from the zygote, and in the number of sporozoites formed from each sporoblast (see Key, p. 557). In the majority of cases the full life history is completed within one host but in a few cases among Coccidia and in all Hemosporidia two different hosts are necessary, in one, and presumably the original host, only sporogony or sexual phases occur, in the other the usual asexual development and multiplications of the trophozoites. (See p. 406 for adaptations in Hemosporidia).

In general the effects produced by Coccidia are determined by the extent of multiplicative reproduction and the area of devastated cells. The centipede is little affected and so are the great majority

of insects, worms, molluses, crustacea and lower vertebrates. Severity also depends upon single or multiple infections and in the ground mole, according to Schaudinn (1902), infection with *Cyclospora caryolytica* is fatal in all cases. Similarly *Eimeria necatrix*, Johnson, is reported to be fatal to chickens in five days (Tyzzer, 1932). Here the sporozoites are liberated as early as one hour after ingestion and penetrate the gland cells at once; this is followed by rapid growth and multiplication with resulting destruction of great numbers of epithelial cells and death of the host. Blood and mucus containing sporocysts are passed out with the feces which becomes infective material for other birds.

Coccidia have been reported from the greatest variety of animals, both cold-blooded and hot-blooded. In mammals, *Cryptosporidium muris*, Tyzzer (1907), parasitic in the peptic glands is noteworthy because of its minute size and its coelozoic mode of life, no intracellular stages characteristic of the coccidia generally are known. Species of the genus *Eimeria* are parasites in horses, cattle, pigs, sheep, goats, rats, mice, rabbits, cats, skunks, squirrels. They are also found in fowls, geese, ducks, pigeons, pheasants, grouse and in cold-blooded forms, in frogs, newts, salamanders, tortoises, snakes and fish.

The genus *Isospora* (A. Schneider) differs from *Eimeria* chiefly in the third metagametic division, so that only 2 sporoblasts and 2 sporocysts are present in the oöcyst. Each sporoblast gives rise to 2 sporozoites, the oöcyst thus containing 4 instead of 8 sporozoites, as in *Eimeria*. Like the latter genus representative species are found in many different kinds of animals, both vertebrate and invertebrate; here also are the only recognized pathogenic coccidia of man.

It can be easily understood that sporocysts of different kinds of Sporozoa may be eaten with contaminated food. If such cysts are resistant to the digestive fluids of the stomach and intestine they will pass out unchanged with the feces. Such cysts found in the feces may easily be interpreted as the resistant cysts of coccidian parasites of the human intestine. This appears to have been the case with species of the genus *Eimeria* in which *E. clupearum*, Thelohan (1892), and *E. sardinae*, Thelohan (1890), are known intestinal parasites of different fish. The cysts of these species are not infrequently found, although in small numbers, in human feces.

There seems to be little reason for doubt, however, that certain species of *Isospora* are actually pathogenic to man. *Isospora hominis*, Railliet and Lucet (1901), and *I. belli*, Wenyon (1923), are fairly well established in this connection. Wenyon (p. 823) reports an observation by Connal (1922) on a laboratory worker who accidentally swallowed developed oöcysts of *Isospora belli*; six days later abdominal discomfort and diarrhea set in which lasted for

about a month; toward the end of the time oöcysts were found in the feces and these lasted for several days, after which they disappeared and recovery was complete. Similar symptoms and similar cysts have been found by a great number of observers in many different parts of the world. The great wonder is that there are not more cases of coccidian enteritis.

Hemosporidia.—The Hemosporidia are Coccidiomorpha which have become adapted to life in the blood, and with this mode of life the more common contaminative mode of infection is replaced in general by the inoculative method. With this change, new and far-reaching adaptations have been developed which modify to a considerable extent the typical life history of the Coccidiomorpha. One structural change of great importance is the entire loss of protective capsules—oöcyst and sporocyst—which, if present, would make activity in the blood impossible.

Theoretical considerations as to the mode of origin of Hemosporidia and of blood parasites generally have already been considered (see p. 361). Possibility of the origin from the gut of the same host is indicated by some types of Coccidia where infection is contaminative (Hepatozoön, Shellackia and Lankesterella, see Key, p. 566). Here infection of the second host is also contaminative, in these cases through infected blood. With Hemosporidia, fertilization by union of gametes and development to the sporozoite take place in the invertebrate host and the sporozoites are inoculated directly into the blood of vertebrates.

So far as the hematzoic sporozoan parasites of man are concerned the Plasmodiidae and the Piroplasmidae alone are important, the former including the malaria-causing organisms of man and birds.

The cause of malaria, although sporadically seen prior to 1880, was first recognized as a definite organism in that year by A. Laveran, a French medical officer, when he discovered the phenomenon of "flagellation" which we now know is microgamete formation. At that time very little was known about blood-infesting Sporozoa, although ten years before Lankester had observed parasites in frog's blood which were later known as *Lankesterella ranarum*.

The generic name Plasmodium was given by Marchiafava and Celli in 1885. Laveran had named the organism *Oscillaria malariae*, but since the name Oscillaria was pre-occupied, the first-recognized cause of malaria became *Plasmodium malariae*, Laveran. Golgi (1886) showed that there are different types of life history in the blood and suggested the possibility of different species. This was made the basis of Grassi and Feletti's (1890) division of the malaria-causing forms into *Plasmodium vivax*, Grassi and Feletti, *P. malariae* and *Laverania malariae*. These observers believed, with some justification, that the clinical features of pernicious malaria, also

called tropical malaria, combined with the aberrant form of the gametocytes, was sufficient justification for a different generic name. That the point was well taken is shown by the fact that today there are two camps: one, supported by Reichenow-Doflein, maintains the position of Grassi and Feletti, and recognizes the genus *Laverania*; the other, supported by Welch, Schaudinn and Wenyon, cannot see that the shape of the gametocytes with the somewhat more virulent clinical history is sufficiently important to justify a different generic name and, following Welch (1898), the third species was named *Plasmodium falciparum*. A similar difference of opinion concerns the generic name of the organisms causing bird malaria. Some authorities, following Labbé (1894), use the generic term *Proteosoma*; others cannot see that, other conditions being the same, a difference in hosts is of sufficient zoological importance to warrant a different generic name and the bird organisms are also grouped under the generic name *Plasmodium*. Following the example shown in connection with the genera *Trypanosoma* and *Endameba*, it would seem that the weight of authority rests with the advocates of the name *Plasmodium*.

Although Laveran's original discovery attracted much attention, the organism was not immediately accepted by pathologists as the cause of malaria, and it was only after careful work of the Italians, Marchiafava, Celli, Grassi, Feletti and especially of Golgi (1886), that the relationship was established. Golgi, beginning in 1885, correlated the clinical picture of malaria with the developmental stages in the intracorporeal history of the parasite.

The transmission of malaria from individual to individual was another story. R. Pfeiffer (1892) was struck by the resemblance in their life histories, of *Plasmodia* and *Coccidia*, and, not finding sporocysts and oöcysts in the former, suggested that malaria organisms might be transmitted from host to host by some blood-sucking insect. Laveran in the same year and Manson in 1894 independently advanced the same idea, and each suggested the mosquito as the transmitting agent. These suggestions were brilliantly proved, first in France, later in India by Ronald Ross and by Grassi in Italy. Ross (1897), unable to finish his work on human malaria in Paris, continued the work on bird malaria in India. He proved that mosquitoes of the genus *Culex*, and no other kind, are capable of transmitting this type of malaria from bird to bird. Grassi (1900) published a classical monograph on the life history of the organism causing tropical malaria (*P. falciparum*), and with beautiful figures of the developmental stages in the gut of the mosquito. Supplementing Ross's observations on *Culex*, he showed that mosquitoes of the genus *Anopheles* alone have the power to transmit human malaria. Schaudinn (1902) confirmed these findings by working out the life history of *Plasmodium vivax*, the cause

of benign tertian malaria of man. He also added the last link to the chain of evidence by watching the penetration of a human blood corpuscle by a sporozoite fresh from a mosquito's proboscis.

The essential features by which the different types of malaria organisms are distinguished are: (1) Length of time between successive sporulating periods; (2) relative sizes of parasites and human blood corpuscles; (3) effects of the parasites upon human corpuscles; (4) relative numbers of merozoites formed at sporulation; (5) general form of the sporulating organisms; (6) distribution of the melanin; (7) form of gametocytes.

1. The early history of the trophozoite is much the same in all species. After an initial infection sporozoites enter erythrocytes as minute rounded bodies (Plate I) which soon give rise to ring-shape inclusions (signet-ring stage); these are characteristic of all malaria organisms and, except for size, they are all alike (Plate I, Figs. 1, 7, 13). When fully grown the nucleus divides from three to five times, after which the parasite breaks up into as many merozoites as there are nuclei (Plate I, Figs. 4, 10). Upon rupture of the corpuscle the merozoites enter other normal corpuscles and the developmental cycle is repeated.

Invasion of corpuscles and their destruction thus increases by geometrical progression until a stage is reached and enough toxic substances are freed in the blood to give the first definite clinical symptoms of the disease. Such a period of incubation, *i. e.*, from the time of inoculation to the first clinical symptoms, usually lasts from ten to twelve days. A second convulsion (pyrexial attack) occurs after the merozoites liberated at the time of the first attack have grown to full size and again undergo sporulation. The time required for this growth and reproduction differs with different species and furnishes an important diagnostic character for the identification of species. Thus *P. vivax*, the cause of so-called benign tertian malaria, since it is rarely fatal, sporulates at forty-eight-hour intervals (every third day); *P. malariae*, the cause of quartan malaria, on every fourth day or at seventy-two-hour intervals; and *P. falciparum*, at irregular intervals, from quotidian to tertian. Fever charts of clear cases of tertian, quartan and subtertian malaria are thus characteristically different.

2. While the phases of activity of all species of *Plasmodium* are alike, there is a distinct difference in size of the parasites as shown by the proportion of the corpuscle that is occupied. *P. vivax* rarely exceeds three-quarters of the erythrocyte; *P. malariae*, the largest of the *Plasmodium* species of man, may occupy as much as nine-tenths; and *P. falciparum*, the smallest, rarely grows to more than two-thirds the size of the corpuscle.

3. The effects of the parasites upon the infected corpuscles are likewise different; *P. vivax* causes a measurable enlargement (Plate I), while in preparations stained by the Giemsa method

PLATE I



Plasmodium Species.

1, 2, 3 and 4, trophozoite and schizont of *P. malayanense*; 5, female gametocyte of *P. malayanense*; 6, trophozoite of *P. malayanense*; 7, 8, 9 and 10, trophozoites and schizonts; 11, immature gametocyte and 12, male gametocyte of *P. malayanense*; 13, 14, trophozoite and schizonts of *P. falciparum*; 15, gametocyte of *P. falciparum*; 16, trophozoite of *P. falciparum*; 17, gametocytes of *P. falciparum*. (x 2000).

1934—W. S. Knowlton, *Parasitology*, 24: 105. (This copy by permission of the author.)

PLATE II



Plasmodium Falciparum.

Figures A-F Development of the merogony cycle; G-L Development of the macrogamete.

After A. Gupta, Memorias do Instituto Osvaldo Cruz.

infected corpuscles are uniformly stippled with pink spots, the so-called Schüffner dots. The other 2 species cause no enlargement of the corpuscles, on the contrary there is a tendency to reduce them; Schüffner's dots are absent, but irregularly scattered larger dots (so-called Maurer's dots) are frequently present in infections by *P. falciparum*.

4. While the numbers of merozoites formed by the sporulating individual are not always the same but fluctuate about a given mean, this mean or average is quite different in the 3 species. For *Plasmodium vivax* it is 16; for *P. malariae*, about 8; for *P. falciparum*, about 24.

5 and 6. During the growth of the parasite granules of dark substance, known as melanin, malarial pigment, etc., and regarded as products of hemoglobin breakdown, are stored up in the Plasmodium protoplasm. At sporulation this melanin may be distributed irregularly between the merozoites as it is in *P. vivax*, or clumped in the center of the group as in *P. malariae* and *P. falciparum*. In *P. falciparum* the merozoites are irregular as in *P. vivax*, but in *P. malariae* they are grouped rosette-like about the clump of melanin (Fig. 124, p. 238).

7. The gametocytes, finally, afford still another diagnostic morphological character. It is limited, however, for there is not a great difference between those of *vivax* and those of *malariae*. In *P. falciparum* they are distinctly differentiated as crescents, the female crescent with a slightly more definite capsule about it than the male crescent (Plate II). All gametocytes are present in the circulating blood with which they are taken into the stomach of a female Anopheline mosquito.

The evolution of the gametocytes of *P. falciparum* has recently been studied by Aragao (1930), who finds that there is a distinct difference between the male and female gametocytes which may be traced back to the merozoite stages. Merozoites destined to form male gametocytes after entering a corpuscle are spherical, with a distinct nucleus and without the vacuole typical of ring forms (Plate II). The young female gametocyte, upon entering a corpuscle stretches out across the corpuscle in the form of an elongate bar. In all stages of its evolution the chromatin is more definite than in the male gametocyte.

The sexual stages in the life history of Plasmodium, consisting of maturation and fusion of the gametes, development of the zygote and formation of sporozoites, all take place in the body of the mosquito. In these processes there is no important difference in the three species. The gametocytes of the circulating blood in which no further development occurs, under the influence of the changed conditions, are stimulated to undergo their maturation processes whereby the female gametocyte becomes a macrogamete and the male gametocyte gives rise to a small number of

microgametes. After fusion of a macrogamete and a microgamete the zygote becomes a motile vermicle which makes its way to the lining membrane of the gut, penetrates it and comes to rest in the submucosa. Here the amphinucleus divides many times and the cell body increases enormously in size, the delicate fertilization membrane, unlike the resistant oöcyst of the *Coccidia*, enlarging with it. As growth progresses, the sporoplasm breaks up in "islands" which suggest the sporoblasts of *Coccidia*, and about each of them the nuclei are peripherally arranged. The sporozoites are budded out from these islands, each with one of the peripheral nuclei. These are ultimately liberated in the body cavity of the mosquito; make their way to the salivary glands which they penetrate, and come to rest in the lumen from which they finally reach the proboscis.

(For genera of Hemosporidia and other Coccidiomorpha, see Key, p. 566.)

CHAPTER XI.

SPECIAL MORPHOLOGY AND TAXONOMY OF THE MASTIGOPHORA.

THE classification of Protozoa was first put on a modern basis by Bütschli (1882-1888). By this time larval forms of various groups of invertebrates, worms, entomostraca, rotifers, desmids and diatoms, all of which had been included in the Leeuwenhoek group of Animalcula, were properly classified, and the Protozoa were limited to the forms which we know today. For general purposes there has not been much improvement over Bütschli's system whereby the Protozoa were divided into four main groups: (1) The *Sarcodina*, in recognition of Dujardin's pioneer work on the living substance of rhizopods; (2) the *Mastigophora*, a term suggested by Diesing (1865) for Dujardin's group *les flagellés*; (3) the *Infusoria*, a term connoting the original *Infusionsthierc*, and Ledenmüller's term Infusoria, and Dujardin's *les ciliées*; and (4) the *Sporozoa*, a term introduced by Leuckart (1879) for strictly parasitic types of gregarines, and coccidia.

The majority of recent workers have followed Doflein (1901) in dividing the phylum Protozoa into two unequal groups or sub-phyla: (1) The *Plasmodroma*, including Mastigophora, Sarcodina and Sporozoa; and (2) the *Ciliophora*, including Ciliata and Suctorina. The writer fails to see any advantage in the creation of these sub-phyla, although the Infusoria differ from other Protozoa, not only in having dimorphic nuclei and fertilization by conjugation, but also in the possession of the most highly differentiated cortex to be found in the entire group of Protista. The absence of dimorphic nuclei in some groups (Opalinidae), the occurrence of fertilization by copulation of gametes (Glaucoma, Opalinidae) and the interpretation of conjugation as evidence of an ancestral brood of gametes indicate that in these respects the Infusoria fall in line with other Protozoa.

A second change introduced by Doflein (1901) was to divide the Sporozoa into two sub-phyla—*Cnidosporidia* and *Sporozoa*, s.str., the former including *Myxosporidia*, *Microsporidia*, *Sarcosporidia* and *Actinomyxida*; the latter gregarines, coccidia and hemosporidia. This change has much to recommend it and is adopted in the present work. Other, but minor, changes from the classification as given in the first edition of the present work will be found in each of the

sub-phyta treated, while the keys to genera are entirely recast. An important change is the omission here of all groups of chlorophyll-bearing forms. Beginning with Pascher (1914) these were classified as Algae, and they find a much more logical position as branches of the botanical *Stammbaum* than they have in any protozoan relationship. As Protista or as Protophyta they have their unquestioned place, but as Protozoa they are anomalous (see also p. 18). Diesing's term Mastigophora referred primarily to plant flagellates and a new term should be provided for animal flagellates; I suggest the sub-phyllum *Zoömastigophora*.

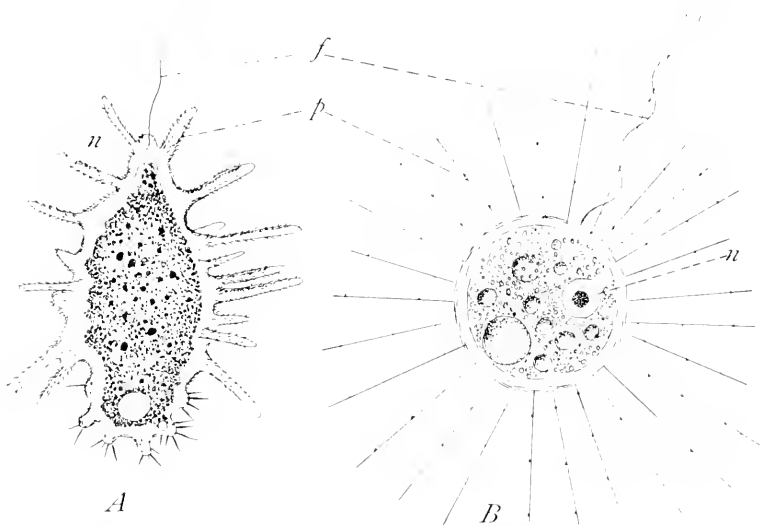


FIG. 174.—Types of Rhizomastigidae. A, *Mastigamoeba aspera*. B, *Actinomonas mirabilis*; f, flagella; p, pseudopodia. (From Calkins after F. E. Schultze and Sav. Kent.)

The only common characteristics of this group of Protozoa are the possession of one or more vibratile motile elements in the form of *flagella*, and reproduction by longitudinal division. In other respects they differ widely in: (a) Complexity of organization, axial relations, symmetry and body form; and (b) distribution and mode of life.

Organization.—Many of the flagellates are simple ellipsoidal monaxonic organisms with a single flagellum at the anterior end (Protonomads); others are ameoboid (Pantastomatida, Fig. 174); some are bilaterally symmetrical (Diplozoic forms); some spherical (*Actinomonas*, etc.) and some are spirally twisted (*Holomastigotidae*, etc.).

While flagella are for the most part all similar in finer structure

(see p. 141), they vary widely in number, size and arrangement on the organism. The most generalized types have 1 flagellum which is directed forward (*Herpetomonas*, *Leishmania*, *Crithidia*, etc.). When 2 are present they may be similar in length and in orientation (*Amphimonadidae*) or of dissimilar length and oriented in the same direction (*Monadidae*) or they may be oriented in different directions (*Bodonidae*, *Embadomonadidae*, *Cercomonas*, etc.). One of the 2 may be adherent to the body (*Cercomonas*), or, retained by the periplast, forms the margin of an undulating membrane (see p. 142) as in *Trypanoplasma*, *Cryptobia*, *Trypanophis*, etc. If 3 flagella are present, 1 is directed anteriorly while 2 are trailing flagella (*Trimastix*, *Dallingeria*, *Macromastix*). When 4 are present, all may be directed anteriorly (*Tetramitus*, *Copromastix*, *Polymastix*); or one may be trailing (*Eutrichomastix*, *Retortamonas*) or retained within a buccal furrow or cytostome, while 3 are directed forward (*Chilomastix*). In some forms the trailing flagellum may be attached to the periplast (*Tricercomitus*) or it forms an undulating membrane (*Trichomitus*, some species of *Trichomonas*). In some forms there are 4 or 5 anterior flagella and an undulating membrane (*Trichomonas*). In another group of forms the single flagellum forms an undulating membrane (*Trypanosoma*, *Myxomonas*). *Myxomonas* (*Dogiel*) may even lose its undulating membrane and turn into an ameboid wood-eating form.

In one group of flagellates (diplozoic forms), including both free-living and parasitic types, the organisms are bilaterally symmetrical. These interesting forms have two sets of flagella placed symmetrically and 1 or 2 nuclei. They are supposed to have arisen by reason of the suppression of cell division after the nucleus and kinetic centers have divided. Similar double forms occur amongst the ciliates where, by treatment with chemicals or ultra-violet rays during division stages, cytoplasmic division is prevented (*Glaucoma*, *Chatton*), or by union during conjugation double individuals result (*Uroleptus*, see p. 245). Free-living diplozoic forms include *Gyromonas*, *Trigonomonas*, *Trepomonas*, *Hexamitus* and *Urophagus* and 2 genera of parasitic forms—*Giardia* and *Octomitus*. The flagella are 4 in number in *Gyromonas*, 6 in *Trigonomonas* and 8 in *Hexamitus*, *Trepomonas*, *Urophagus*, *Octomitus* and *Giardia* (Fig. 17, p. 37).

A multiple number of flagella is quite characteristic of parasitic Mastigophora, particularly parasites of the white ants (Termites). Such polymastigote forms may have a single nucleus (monozoic *Hypermastigidae*) or many nuclei (polyzoic types). The latter, like diplozoic forms above, are supposed to have arisen by multiple division of the nucleus and kinetic complex without accompanying cell division (somatella stage). According to Janicki (1915), each

nucleus is accompanied by a blepharoplast, from which flagella are developed, a parabasal body and an axial thread. Each such group of cellular elements is a karyomastigont (Janicki); in some groups the nucleus is lost but the kinetic complex remains, such enucleate groups being akaryomastigonts. In all cases the axial threads are united to form an axial strand which runs through to the posterior end. *Calonympha*, *Foa*, and *Stephanonympha*, Janicki, are compound individuals of karyomastigonts alone, or of karyomastigonts and akaryomastigonts which are massed at the anterior end of the cell and spirally arranged in *Stephanonympha*

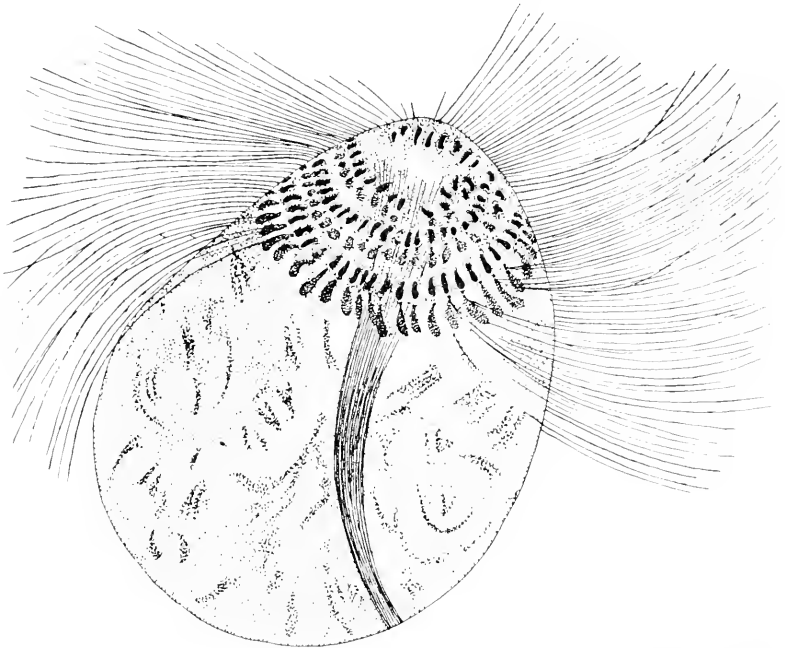


FIG. 175.- *Stephanonympha sylvestri*; with many nuclei, kinetic groups, and flagella. Rhizoplasts unite to form the inner axial strand. (After Janicki.)

(Fig. 175). *Proboscidiella*, Kofoid and Swezy, is likewise multinucleate but differs in having a protrusible proboscis.

A large group of monozoic parasitic forms with from 4 to many flagella leads into the highly complicated hypermastigote flagellates. Parabasal bodies, axostyles and axial strands may be single or multiple in the cell. *Polymastix*, Bütschli, has 4 flagella and an axostyle; *Hexamastix*, Alexieff (1912), has 6; and there are 6 or more also in *Cochlosoma*, Kotlan (1932). *Oxymonas*, Kofoid and Swezy, has 6 flagella and, like *Proboscidiella*, bears a protrusible proboscis. The 2 genera *Pyronympha*, Leidy, and *Dinenympha*,

Leidy, agree in that all of the flagella arise at the anterior end and, like the trailing flagellum of *Tricercomitus*, adhere to the body to form short free whips at the posterior end. The attached flagella in some species form conspicuous spiral ribs down the body, and these in some cases give an appearance of undulating membranes. Axostyles are present which end freely in the endoplasm of *Pyronympha*, but are attached at the posterior end in *Dinenympha* (Fig. 176).

There is little doubt that the *Hypermastigida* are the most highly differentiated of all flagellate types. The differentiations, however, have to do solely with the complications of the kinetic elements or neuromotor system, for the nucleus is invariably single. The group embraces only 1 genus—*Lophomonas* (intestinal parasites of cockroaches) which is not found in termites. Blepharoplasts and basal bodies are numerous while axial threads and so-called parabasal bodies form variously complicated internal structures. In the majority of species the flagella are grouped at the anterior end. Here they form a single group or tuft of flagella in *Lophomonas* (Fig. 105, p. 211); a spirally arranged group of similar tufts (loricula) in *Kofoidea*, Light. In *Joenia*, Grassi, the anterior flagella are separated in two groups, one of which forms an anteriorly-directed tuft, the remainder, like trailing flagella, forming a flagellar mantle about the body. A more or less similar anterior grouping of flagella is characteristic of *Staurojoenia*, Grassi, *Parajoenia*, Janicki, *Joenopsis*, Cutler, *Joenina*, Grassi, *Gymnonympha*, Dobell, and *Leidyonella*, Frenzel. In *Hoplonympha*, Light, they are arranged in two oppositely-directed tufts. They are arranged in longitudinal rows, extending part way down the body in *Microjoenia*, Grassi, and in *Leidyopsis*, Kofoid and Swezy, and in spirally-wound rows from end to end in *Holomastigotoides*, Grassi, *Spirotrichonympha*, Grassi, and *Microspironympha*, Koidzumi. In *Pseudotrichonympha*, Hartmann, a covering of flagella clothes the entire body, the flagella increasing slightly in length toward the posterior end. This dissimilarity of flagella is emphasized in *Trichonympha*, Leidy, where the flagella cover only one-half to two-thirds of the body. The shorter, anterior flagella

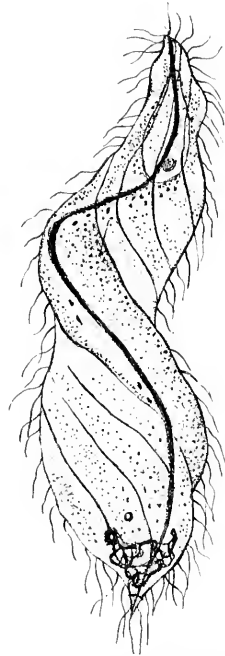


FIG. 176. — *Dinenympha fimbriata*—network of Golgi apparatus at posterior end. (After Brown, Arch. f. Protistenkunde; courtesy of G. Fischer.)

extend anteriorly or laterally while the longer ones extend posteriorly, covering the entire hinder end of the organism.

With these enormously-developed external locomotor organs we would expect to find more or less complicated internal structures for attachment and support. In uniflagellate forms these are relatively simple, the single flagellum originating from and attached to a kinetic element in the nucleus or on its membrane (*Salpingoeca*, J. Clark); or from a kinetic element (blepharoplast) in the cytoplasm (*e. g.*, *Oicomonas*, Kent; *Trypanosoma*, Gruby, etc.). Complications appear with the development of the parabasal body (*Herpetomonas*, *Crithidia*, etc., see p. 111) and with the axostyle while parastyles and epistyles support the undulating membrane and other motile organs. The nucleus is supported by axial threads from the blepharoplasts in *Lophomonas*, by the axostyle in *Trichomonas*, *Joenia* and *Parajoenia* and *Metadevescovina* and by special strands or membranes in *Trichonymphidae*.

Basal bodies are frequently united into apparently solid bars as in the head organ of *Trichonympha* or plates as in *Staurojoenia assimilis*, Kirby, the former acting as a complicated centrosphere (centroblepharoplast, Kofoid) during division (Fig. 54, p. 100). In *Joenopsis polytricha*, Cutler, this becomes a horseshoe-shape structure which bears the basal bodies for the anterior flagella.

With these intestinal forms bacteria are frequently found attached to the body wall and may be mistaken for additional flagella. In some cases they become a part of the organism, forming a fairly complete armature (*e. g.*, *Lophomonas striata*, Bütschli).

The protoplasmic body in all types of flagellates contains the usual cytoplasmic substances. Mitochondria are probably universally present (see p. 73), and volutin (see p. 72) is widely distributed, if not universal. Chromidia are only rarely present (*Rhizomastigidae*).

The presence of Golgi bodies (see p. 77) is not demonstrated in many forms, and some difference of opinion has arisen concerning the chemical identity of certain characteristic structures, particularly the parabasal body. Kofoid (1916) believed it to be of chromatin (nucleic acid) nature, acting as a reservoir of substance to maintain the activity of the kinetic elements. The chromatin make up of the blepharoplast in *Trypanosoma* supports this view. A similar purpose of the parabasal is advocated by Janicki (1915) and by Duboseq and Grassé (1925), but they argue against its chromatin nature and regard it as the homologue of the metazoan Golgi apparatus and the product of the vacuome (Parat). This is based on the fact that the colorable substance is often immediately adjacent to a colorless vesicle. Janicki holds that the parabasals present in large number in the *Polymastigida* and *Hypermastigidae* secrete substances of high potential energy which are used by the

complex motile organs for movement. Duboscq and Grassé homologize the parabasal with the idiosome of spermatozoa which is regarded as a Golgi element, while Grassé (1925) interprets the parabasals in *Trichomonas batrachorum* and *Tetramastix bufonis* as secretory in function, forming minute droplets which break up into smaller elements for distribution in the cytoplasm. Brown (1930), more recently, dissents from this interpretation and finds no evidence in *Dinenympha* or *Pyrsonympha* to support the view that parabasals are homologous with the Golgi apparatus. The latter is present, however, in the form of distributed spherical bodies, which may appear as crescents or rings and which are believed to be secretory in nature. When the granules are present, a Golgi network is absent or much reduced, but a typical network appears at times at the base of the axostyle (Fig. 176).

These diverse points of view leave us very much in the air in regard to the chemical nature and function of the parabasal body so conspicuous in the parasitic flagellates. Their variations in size and shape in the same species certainly indicate their connection with some urgent metabolic need, but for the present at least the nature of this need is enigmatical.

Nuclei are not especially characteristic. In Protomonads it is usually of the centronucleus type—with endosome and frequently with endobasal body (see p. 60). In more complicated flagellates (Polymastigida and Hypermastigida) the endosome becomes greatly reduced or absent altogether and no longer contains the centriole. The latter is either on the nuclear membrane or as a blepharoplast near to it, and, during nuclear activity, it divides with a connecting strand. This strand is homologous with the intranuclear centrodesmose of simpler types, but remains outside the nucleus as a *paradesmose* (Fig. 54, p. 100). Here, therefore, we have evidence of a permanent separation of chromatic and kinetic components of the nucleus, the latter now being permanent cytoplasmic structures. A peculiarity of the chromosomes in some cases is the apparent reduction to one-half the normal number during mitosis (*Giardia microti*, Boeck, 1917; *Trichonympha campanula*, Kofoid and Swezy, Fig. 54, p. 100), although with the possible exception of *Helkesimastix*, Woodcock and Lapage (1915) no fertilization processes are safely established for any type of animal flagellate.

Contractile vacuoles are generally distributed in the free-living forms, where they are invariably simple vesicles. In parasitic forms they are generally absent.

Reproduction of flagellates is typically by simple longitudinal division. In free-living forms the individual in many cases remains connected by stalk-like processes (*Poteriodendron*, Fig. 177), or by dichotomously branched gelatinous tubes (*Cladomonas*) or laterally cemented tubes (*Rhipidodendron*). In some cases they are embed-

ded in masses of jelly (*Spongomonas*, *Phalansterium*). Amongst parasitic forms many species show both simple division and multiple division, during which nuclei and kinetic elements divide two or

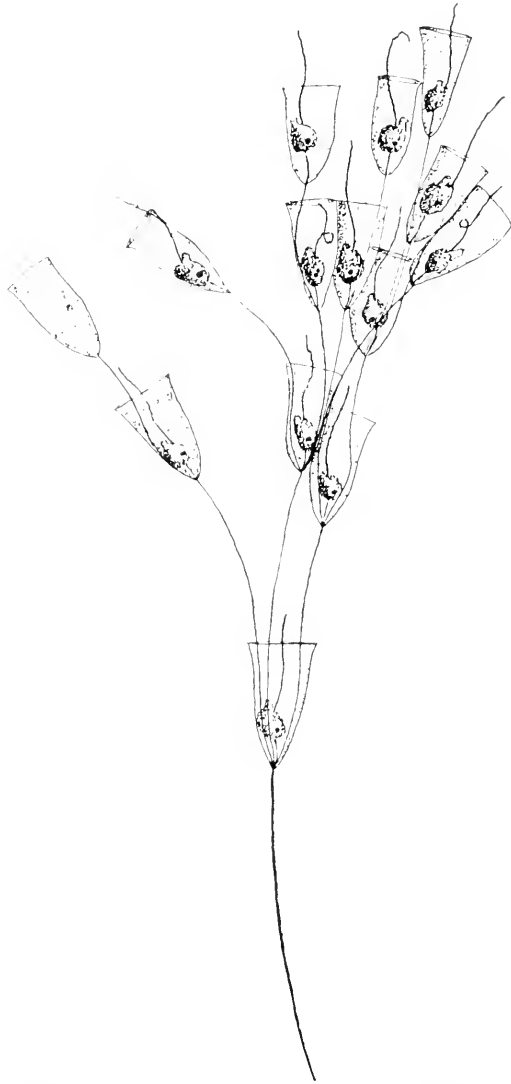


FIG. 177. Arboroid colony of protomonads, *Poteriodendron petiolatum*.

more times without division of the cell body (somatella formation). In rare cases such phenomena are accompanied by the formation of a cyst membrane and the process becomes a typical sporulation (Fig. 122, p. 234).

Adaptations and Mode of Life.—Owing to their remarkable powers of adaptation animal flagellates may be found in practically any place with moisture. They are less abundant in clear drinking waters, where plant flagellates may abound, than in ponds and pools, where decaying vegetation is plentiful; some types of free-living forms have become adapted to the conditions of the soil, others to the putrefactive conditions of dung and feces in general.

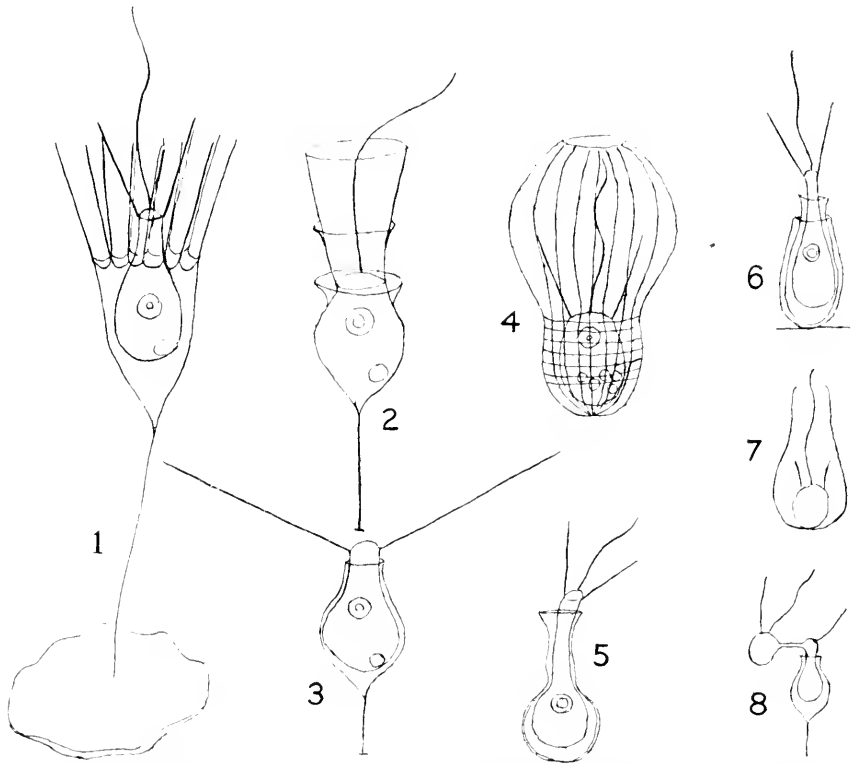


FIG. 178.—Types of choanoflagellates. 1, *Acanthoeca spectabilis*; 2, *Dicrospidella stokesi*, collar with short secondary collar; 3, *Choanocca perplexa*, collar flattened; 4, *Stephanocca ampulla*; 5, *Pachysocca longicollis*; 6, *Diploeca placita*; 7, *Diaphanocca parva*; 8, *Choanocca perplexa* at division, young cell with flagellum leaving sister cell in old house. (After Ellis, Ann. de la Soc. Royale Zoologique de Belgique, 1929; courtesy of M. Forton.)

A favorite haunt for many of these types is in ponds or pools where decomposition is active. Many of them are bottom forms attached to débris or working their way about in the superficial slime. Some are ameba-like (*Rhizomastigidae*) and in addition to their flagella put out pseudopodia from any part of the body. Others are like *Heliozoa* and possess ray-like pseudopodia (*Actino-*

monas). Swimming types have a thickened periplast which may be smooth as in *Phialonema* (Fig. 60, p. 110) or longitudinally and spirally ribbed (*Heteronema*, *Tropidoscyphus*). In one group (Choanoflagellates) a protoplasmic collar surrounds the flagellum (Fig. 178).

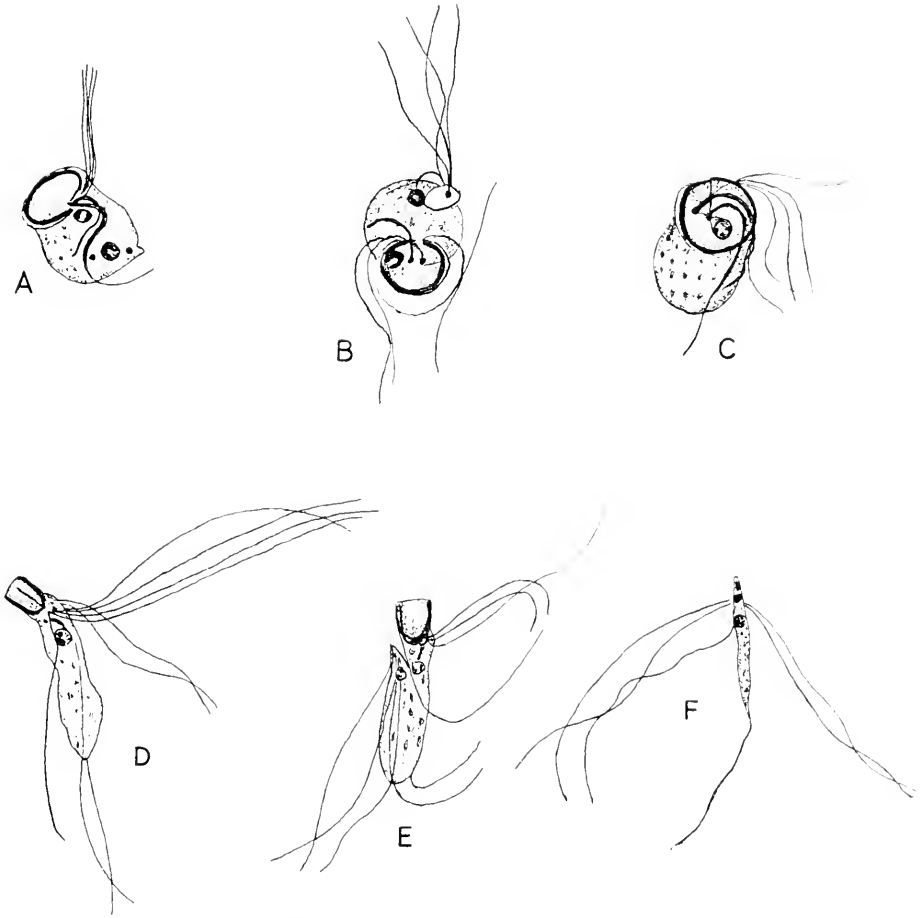


FIG. 179.—Flagellates with suckers, from the ruffed grouse. A, C, normal forms of *Cyathosoma striatum*; B, dividing form of same; D, normal form of *Ptychostoma bonasae*; E, beginning of unequal division of same; F, slender individual, without sucker, resulting from unequal division. $\times 2400$. (After Tyzzer, 1930; courtesy of Am. Jour. Hyg.)

The great majority of animal flagellates, however, have become adapted to the anaërobic conditions accompanying a parasitic mode of life and these flagellates have become a vital factor in the hygienic and economic relations of man, other animals and some plants (see Chapter X, p. 352). Some parasitic forms have developed suckers for attachment (*e. g.*, *Cochlosomidae*, Fig. 179).

SPECIFIC CLASSIFICATION.

1. **The Water-dwelling Flagellates.**—In separating the chlorophyll-bearing flagellates from Protozoa we encounter the difficulty of border-line forms which, except for the absence of chlorophyll, appear to be related to forms with chlorophyll. *Euglena gracilis*, for example, ordinarily has chlorophyll, but upon cultivation in the dark the chlorophyll is lost and the organisms live as saprophytes. Such forms combine, therefore, holophytic and saprophytic modes of food-getting, but it is obvious that they should not be included with animal flagellates. By the same reasoning a number of colorless forms should be retained with their structurally similar colored relations so long as there is no question regarding their homologous structures. Thus the colorless *Chilomonas* is so similar to *Cryptomonas* in structure that it may be regarded as a descendant of a chlorophyll-bearing form which has become permanently adapted to a saprophytic mode of life. So, too, many of the Dinoflagellates have lost their chlorophyll and live as animals do, either by holozoic methods (*Gymnodinium*, *Noctiluca*, etc.) or by parasitic methods (*Oödinium*, *Haplozoön*, etc.). Here the characteristic structures of the Dinoflagellates in swarm spores or adults are so pronounced that the affinities are clearly indicated.

With other colorless flagellates, however, which have been claimed by botanists, the affinities are obscure and there is no more reason for regarding them as recently modified chlorophyll-bearing types than as definitive animals. It may be true that all animal groups should look back to the dim past for their plant ancestors, but this does not mean that modern zoölogy should continue to rest in the lap of botany.

Among such colorless forms which should be transferred to the animal flagellates, *Astasia*, *Menoidium*, *Englenopsis*, *Peranema*, *Urceolus* and *Petalomonas* would be classified as Protomonads; *Distigma*, *Sphenomonas* as Monadidae; *Heteronema*, *Tropidocyphus*, *Anisonema*, *Entosiphon* and *Marsupiogaster* as Bodonidae. These are all free-living holozoic or saprozoic forms living in fresh, salt and brackish waters.

CLASSIFICATION OF THE ANIMAL FLAGELLATES.

Phylum Protozoa, Goldfuss, 1820.

Sub-phylum Zoömastigophora (Animal Flagellata).

CLASS I. PROTOMASTIGOTA.

ORDER 1. *Protomonadida*.

Family 1. Rhizomastigidae.

Family 2. Oicomonadidae.

CLASS I. PROTOMASTIGOTA.

ORDER 1. *Protomonadida*.

- Family 3. Peranemidae.
- Family 4. Trypanosomidae.
- Family 5. Bicoecidae.
- Family 6. Craspedomonadidae.
- Family 7. Amphimonadidae.
- Family 8. Monadidae.
- Family 9. Bodonidae.
- Family 10. Cercomonadidae.
- Family 11. Trimastigidae.

CLASS II. METAMASTIGOTA.

ORDER 1. *Hypermastigida*.

- Family 1. Lophomonadidae, Grassi.
- Family 2. Hoplonymphidae, Light.
- Family 3. Kofoidiidae, Light.
- Family 4. Joeniidae, Grassi.
- Family 5. Staurojoeniidae, Grassi.
- Family 6. Holomastigotidae, Grassi.
- Family 7. Trichonymphidae, Saville Kent.
- Family 8. Cyclonymphidae, Doflein.

ORDER 2. *Polymastigida*.SUB-ORDER 1.—*Monokaryomastigina*.

- Family 1. Callimastigidae.
- Family 2. Dinenymphidae.
- Family 3. Tetramitidae.
- Family 4. Trichomonadidae.
- Family 5. Devescovinidae.
- Family 6. Spironemidae.
- Family 7. Streblomastigidae.
- Family 8. Chilomastigidae.
- Family 9. Cochlosomidae.

SUB-ORDER 2. *Dikaryomastigina*.SUB-ORDER 3. *Polykaryomastigina*.

- Family 1. Oxymonadidae.
- Family 2. Calonymphidae.

CLASS I. **PROTOMASTIGOTA.**ORDER **PROTOMONADIDA.***Key to Families.*

1. Flagellates always with pseudopodia in addition to flagella. 1 Family RHIZOMASTIGIDAE
- Flagellates without habitual pseudopodia 2
2. With one or more posteriorly-directed flagella. 9
- Flagella directed anteriorly. 3

Key to Families.

- 3. With one flagellum 4
 - With two flagella 8
- 4. Non-parasitic forms 5
 - Parasitic flagellates 4 Family TRYPANOSOMIDAE
- 5. With protoplasmic collar about flagellum
 - 6 Family CRASPEDOMONADIDAE
 - Without protoplasmic collar 6
- 6. Body wall distinct 3 Family PERANEMIDAE
 - Body wall indistinct 7
- 7. With proboscis-like process 5 Family BICOECIDAE
 - Without proboscis 2 Family OICOMONADIDAE
- 8. Flagella of similar length 7 Family AMPHIMONADIDAE
 - Flagella of dissimilar length 8 Family MONADIDAE
- 9. With one posteriorly directed flagellum 10
 - With two posteriorly directed flagella
 - 11 Family TRIMASTIGIDAE
- 10. Trailing flagellum leaves body at the anterior end 9 Family BODONIDAE
 - Trailing flagellum attached to full length of body 10 Family CERCOMONADIDAE

PROTOMONADS—Genera.

Family 1. **Rhizomastigidae** Bütschli.

- 1. Flagella and pseudopodia distributed around body
 - Genus (*incertae sedis*)
 - Multicilia* Cienkowsky
 - Flagella originate from anterior end of body 2
- 2. Pseudopodia lobose 3
 - Pseudopodia ray-like 4
- 3. Flagellum arises from nucleus Genus *Mastigamoeba* F. E. Sch.
 - Flagellum independent of nucleus Genus *Mastigella* Frenzel
- 4. Stalked forms 5
 - Body not stalked; ray-like pseudopodia with axial filaments
 - (? Genus *Dimorphiella*) Genus *Dimorpha* Gruber
- 5. Pseudopodia confined to circle about base of flagellum Genus *Pteridomonas* Penard
 - Pseudopodia not limited to flagellum region
 - Genus *Actinomonas* S. Kent

Family 2. **Oicomonadidae** Senn.

- 1. Individuals not cup-dwelling 2
 - Individuals cup-dwelling 6
- 2. Form flattened, leaf-like Genus *Ancyromonas* S. Kent
 - Form spheroidal to ellipsoidal 3
- 3. Not parasitic 4
 - Parasitic—cause of "blackhead" in turkeys
 - Genus *Histomonas* Tyzzer
- 4. Kinetoplast marginal 5
 - Kinetoplast not marginal Genus *Proleptomonas* Woodcock
- 5. Flagellum delicate, short, active Genus *Oicomonas* S. Kent
 - Flagellum heavy, long, sluggish Genus *Rigidomastix* Alexieff
- 6. Cup stalked Genus *Colonoeca* J. Clark
 - Cup without stalk Genus *Platytheca* Stein

Family 3. **Peranemidae** Stein.

1. Body metabolic 2
- Body rigid 3
2. Body with endoplasmic rod ("Stab-organ") Genus *Peranema* Dujardin
- Body without endoplasmic rod Genus *Euglenopsis* Klebs
3. Endoplasm with rod apparatus Genus *Urceolus* Mereschkowsky
- Endoplasm without rod apparatus 4
4. Periplast smooth 5
- Periplast heavy, with 1-7 longitudinal furrows or ridges Genus *Petalomonas* Stein
5. Periplast delicate Genus *Scytomonas* Stein
- Thick and heavy Genus *Thylacomonas* Schewiakoff

Family 4. **Trypanosomidae** Doflein.

1. Undulating membrane absent 2
- Undulating membrane present 6
2. Definitive hosts plants—cysts absent Genus *Phytomonas* Donovan
- Definitive hosts animals 3
3. Vertebrate and invertebrate hosts Genus *Leishmania* Ross
- Invertebrate hosts only 4
4. Protoplasmic body extended around base of flagellum Genus *Crithidia* Leger
- Flagellum without protoplasmic extension 5
5. Kinetoplast always anterior to nucleus Genus *Leptomonas* S. Kent
- Kinetoplast posterior to nucleus in some phases Genus *Herpetomonas* S. Kent
6. Hematozoic forms only in vertebrate Genus *Trypanosoma* Gruby
- Hematozoic and cytozoic forms in vertebrate 7
7. Cytozoic phases in erythrocytes Genus *Endotrypanum* Mesnil et Brimont
- Cytozoic phases in organ cells and tissues Genus *Schizotrypanum* Chagas

Family 5. **Bicoecidae** Stein.

1. Cells with proboscis-like process at flagellum base 2
- Cells with thin periplastic process Genus *Bicoeca* Lauterborn
2. Cells without contractile thread; process sail-like Genus *Histiona* Voigt
- Cells with posterior contractile thread Genus *Poteriodendron* Stein

Family 6. **Craspedomonadidae** Stein.

1. Individuals with one collar 2
- Individuals with two collars 17
2. Individuals without lorica or test 3
- Individuals with lorica 10
3. Individuals solitary 4
- Individuals colonial 5
4. Cells with very short stalks or none Genus *Monosiga* S. Kent
- Cells with very long stalks Genus *Codonomosiga* S. Kent

Family 6. **Craspedomonadidae** Stein.

- 5. Individuals not embedded in jelly 6
- Individuals embedded in jelly 8
- 6. Colonies umbellate, attached Genus *Codonoelulium* Stein
- Colonies free-swimming 7
- 7. Colonies with individuals attached radially
- Colonies band form; individuals side by side Genus *Astrosiga* S. Kent
- Colonies band form; individuals side by side Genus *Desmarella* S. Kent
- 8. Collars not enclosed by jelly 9
- Collars enclosed by jelly Genus *Phalansterium* Cienkowsky
- 9. Individuals irregularly distributed in jelly
- Individuals radially distributed in jelly Genus *Proterospongia* S. Kent
- Individuals radially distributed in jelly Genus *Sphueroica* Lauterborn
- 10. Lorica single 11
- Lorica double Genus *Diploeca* Ellis
- 11. No cirlet of marginal spines 12
- With cirlet of marginal spines Genus *Acanthoeca* Ellis
- 12. Lorica does not enclose collar and flagellum 13
- Lorica encloses collar and flagellum 16
- 13. Individuals attached 14
- Individuals free-swimming Genus *Lagenoeca* S. Kent
- 14. Lorica delicate, thin 15
- Lorica thick with long neck Genus *Pachyoeca* Ellis
- 15. Collar huge, conspicuous, flagellum transient Genus *Choanoeca* Ellis
- Collar small, inconspicuous Genus *Salpingoeca* Clark
- 16. Lorica with definite constriction above collar Genus *Diaphanoeca* Ellis
- Lorica with constriction below collar Genus *Stephanoeca* Ellis
- 17. Individuals without lorica 18
- Individuals with lorica Genus *Diplosigopsis* Francé
- 18. Both collars arise independently 19
- Collars closely attached at base Genus *Dicraspedella* Ellis
- 19. Individuals sessile or with very short stalk
- Individuals with long stalks Genus *Diplosiga* Frenzel
- Individuals with long stalks Genus *Codonosigopsis* Senn

Family 7. **Amphimonadidae**.

- 1. Individuals solitary 2
- Individuals colonial, in jelly 7
- 2. Individuals naked 3
- Individuals in cup Genus *Diplomita* S. Kent
- 3. Body not spirally twisted 4
- Body spirally twisted Genus *Spiromonas* Perty
- 4. Form spherical, ovoid, or spindle-shape
- Form not spherical, ovoid, or spindle-shape 5
- Form ear-shape, ectoparasitic on fish
- Form ear-shape, ectoparasitic on fish Genus *Costia* Leclerque
- Forms diverse—not ectoparasitic 6
- 6. Form horseshoe-shape Genus *Furcilla* Stokes
- Form heart-shape Genus *Streptomonas* Klebs
- 7. Colonies irregular gelatinous masses . Genus *Spongomonas* Stein
- Colonies branched or tubular 8

Family 7. **Amphimonadidae.**

8. Colonies laterally associated tubes—organ-pipe type Genus *Rhipidodendron* Stein
 Colonies branched Genus *Cladomonas* Stein

Family 8. **Monadidae** Stein.

1. Individuals solitary 2
 Colony-forming 5
 2. Naked forms 3
 Cup-dwelling Genus *Stokesiella* Lemmermann
 3. Stalked; slime-covered; radial striations in
 slime Genus *Physomonas* S. Kent
 Free-swimming; not stalked 4
 4. Both flagella active Genus *Monas* Ehr.
 Main flagellum stiff, anteriorly directed
 Genus *Sterromonas* S. Kent
 5. Monads in cups; colony branched Genus *Stylobryon* Fromentel
 Monads not in cups 6
 6. Single cells at ends of branched stems
 Genus *Dendromonas* Stein
 Groups of cells (corbels) at ends of
 branched stems 7
 7. Stalks colorless Genus *Cephalothamnium* Stein
 Stalks colored yellow or brown Genus *Anthophysa* Bory

Family 9. **Bodonidae** Bütschli.

1. Trailer not united with periplast to form
 undulating membrane 2
 Undulating membrane present—parasites 18
 2. One flagellum modified as a proboscis
 Genus *Rhynchomonas* Klebs
 Both flagella active 3
 3. Individual metabolic 4
 Individual rigid 12
 4. With marginal bristles; occasional
 branched pseudopodia Genus *Thaumatomastix*
 Lauterborn
 Without marginal bristles 5
 5. With ventral furrow 6
 No ventral furrow, cytostome apical or
 absent 8
 6. Flagella united at base by membrane
 Genus *Phyllomitus* Stein
 Flagella not united by membrane 7
 7. Trailer short, rarely extending beyond furrow
 (parasitic) Genus *Embadomonas* Mackinnon
 Trailer long, extending through furrow
 beyond posterior end Genus *Colponema* Stein
 8. Trailer used as gliding flagellum Genus *Bodo* (Ehr.) Stein
 Trailer not a glider 9
 9. Trailer used for attachment Genus *Pleuromonas* Perty
 Trailer free 10
 10. Parasitic Genus (*Prowazekella*) *Proteromonas* Kunstler
 In stagnant waters—not parasitic 11
 11. With apical cytostome Genus *Heterouema* Dujardin
 Without cytostome Genus *Dinomonas* S. Kent

Family 9. **Bodonidae** Bütschli.

- 12. Body with keels or ridges..... 13
 Body smooth—no ridges..... 15
- 13. With 1 to 4 ridges..... 14
 With 8 ridges..... Genus *Tropidosecyphus* Stein
- 14. Body flat..... Genus *Sphenomonas* Stein
 Body ellipsoid..... Genus *Notosolenus* Stokes
- 15. Cytostome at end of internal protrusible
 tube..... Genus *Entosiphon* Stein
 Without internal or protrusible tube..... 16
- 16. With ventral furrow to posterior end. Genus *Anisomena* Dujardin
 With pocket-like, deep cytostome..... 17
- 17. Mouth small; cell with rod or "Stab"-
 organ..... Genus *Dinema* Perty
 Mouth large; no "Stab"-organ.... Genus *Marsupiogaster*
 Schewiakoff
- 18. Without transverse bars..... Genus *Cryptobia* Leidy
 With transverse bars..... Genus *Trypanophis* Keysselitz

Family 10. **Cercomonadidae** Kent.

- 1. Without axoneme..... 2
 With axoneme..... Genus *Cercomastix* Lemmermann
- 2. Primary flagellum single..... 3
 Primary flagella two..... Genus *Trimitus* Alexeieff
- 3. Primary flagella very short, inconspicuous
 Genus *Helkesimastix*
 Woodecock and Lapage
- Primary flagella conspicuous..... Genus *Cercomonas* Dujardin

Family 11. **Trimastigidae** Senn.

- 1. Secondary flagella arise from anterior end
 Genus *Macromastix* Stokes
- Secondary flagella arise below anterior end
 Genus *Dallingeria* Kent

CLASS II. **METAMASTIGOTA.**

ORDER I. **HYPERMASTIGIDA** GRASSI.

- 1. Organisms with segmented structure
 Family 8. **CYCLO NYMPHIDAE** Dof.
 Organisms without segmented structure... 2
- 2. Flagella in bundles or tufts at anterior
 end..... 3
 Flagella not limited to anterior bundles... 7
- 3. One bundle of flagella..... 6
 Flagella in more than one anterior bundle... 4
- 4. With two anterior bundles... Family 2. **HOPLO NYMPHIDAE** Light
 With more than two bundles..... 5
- 5. With four bundles..... Family 5. **STAUROJOENIIDAE** Grassi
 With more than four bundles... Family 3. **KOFOIDIIDAE** Light
- 6. Organisms without axostyle... Family 1. **LOPHOMONADIDAE** Grassi
 Organisms with axostyle... Family 4. **JOENIIDAE** Grassi
- 7. All flagella insertion lines spirally wound
 Family 6. **HOLOMASTIGOTIDAE**
 Grassi
- Flagella insertion lines not spirally wound
 Family 7. **TRICIONYMPHIDAE** Kent

Family 1. **Lophomonadidae** Grassi.

Flagella few (5-15)—termite parasite. Genus *Eulophomonas*
Grassi and Foa

Flagella many (?)—cockroach parasite. Genus *Lophomonas* Stein

Family 2. **Hoplonymphidae** Light.

One genus *Hoplonympha* Light

Family 3. **Kofoidiidae** Light.

One genus *Kofoidia* Light

Family 4. **Joeniidae** Grassi.

1. Cell body with transverse furrow. Genus *Joenopsis* Cutler

Cell body without transverse furrow. 2

2. Flagella inserted in longitudinal rows

Genus *Microjoenia* Grassi

Flagella not in longitudinal rows. 3

3. Flagella in one anterior bundle. 4

Flagella arranged in circles or semi-circles. 5

4. Parabasal apparatus a single collar. Genus *Joenia* Grassi

Parabasal apparatus double. Genus *Mesojoenia* Grassi and Foa

5. Flagella in one circle—ring of parabasals

Genus *Torquenympha* Brown

Flagella arranged in semi-circles. 6

6. Flagella in one semi-circle. Genus *Joenina* Grassi

Flagella in two semi-circles; one trailing flagellum. Genus *Parajoenia* Janicki

Family 5. **Staurojoeniidae** Grassi.

One genus with the family characters. Genus *Staurojoenia* Grassi

Family 6. **Holomastigotidae** Grassi.

1. With axostyle. 2

Without axostyle. 3

2. With 4 embedded, but conspicuous, flagellar bands. Genus *Spirotrichonympha*

Grassi and Foa

With many rows of flagella, no flagellar bands. Genus *Holomastigotoides*

Grassi and Foa

3. Periplast with conspicuous spiral folds

Genus *Holomastigotes* Grassi

Periplast without spiral folds. Genus *Spirotrichonymphella*
Grassi

Family 7. **Trichonymphidae** (Kent) Grassi.

1. Flagella arising from anterior two-thirds

of body. Genus *Trichonympha* Leidy

Flagella arising from most of body. Genus *Pseudotriconympha*
Grassi and Foa

Family 8. **Cyclonymphidae** Doflein.

One genus. Genus *Cyclonympha* Dogiel (Fig. 180) (= *Teratonympha* Koidzumi)

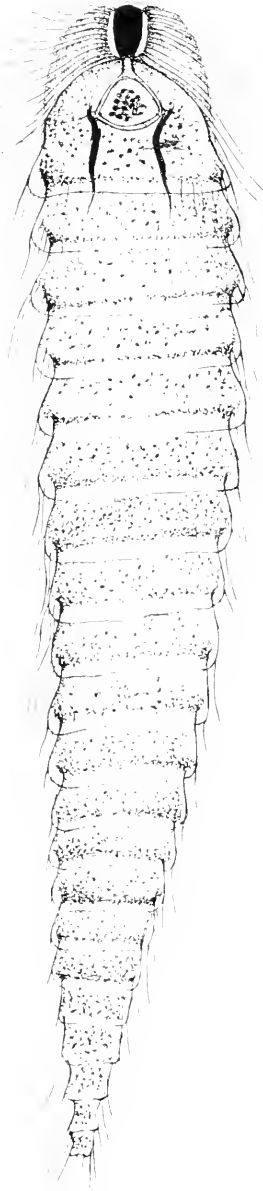


FIG. 180.—*Cytomympha mirabilis*, one of the Hypermastigida. (After Koidzumi.)

ORDER 2. POLYMASTIGIDA.

SUB-ORDER 1. Monokaryomastigina.

KEY TO FAMILIES.

1. Body not spirally wound (see exception in Family 2)..... 2
- Body spirally wound or with spiral stripes..... 8
2. Cytostome, if present, not sucker-like..... 3
- With sucker-like cytostome..... Family 9. COCHLOSOMIDAE
3. Without trailing flagellum..... 4
- With trailing flagellum..... 6
4. Flagella six or more in number..... 5
- Flagella less than six in number..... Family 3. TETRAMITIDAE
5. Flagella grouped..... Family 1. CALLIMASTIGIDAE
- Flagella distributed over body..... Family 2. DINENYMPHIDAE
6. With undulating membrane..... Family 4. TRICHOMONADIDAE
- Without undulating membrane..... 7
7. Without cytostome..... Family 5. DEVESECOVINIDAE
- With definite cytostome..... Family 6. CHILOMASTIGIDAE
8. Flagella four or more in number; anterior..... Family 8. STREBLOMASTIGIDAE
- Flagella twelve or more in number; lateral..... Family 7. SPIRONEMIDAE

KEY TO GENERA.

Family 1. **Callimastigidae** da Fonseca.

- Body spherical..... Genus *Callimastix* Weissenberg
 Body watch-glass shape..... Genus *Selenomonas* Prowazek

Family 2. **Dinenymphidae** Grassi.

1. With axostyle..... 2
- Without axostyle... (Questionable) Genus *Rhynchodinium*
 Da Cunha and Penido
2. Many flagella in spiral rows..... Genus *Dinenympha* Leidy
- Four to eight flagella leaving body at posterior end..... Genus *Pyronympha* Leidy

Family 3. **Tetramitidae** Bütschli.

1. Four flagella in one group..... 2
- Four flagella in two groups..... 4
2. With axial fibril (coprozoic forms)..... Genus *Copromastix* Aragao
- Without axial thread..... 3
3. With cytostome..... Genus *Tetramitus* Perty
- Without cytostome, pseudopodial feeding..... Genus *Collodictyon* Carter
4. Axostyle extends to posterior end..... Genus *Monocercomonas* Grassi
- Axostyle does not run to posterior end;
 body ridged..... Genus *Polymastix* Bütschli

Family 4. **Trichomonadidae** Wenyon(?)

1. With axostyle..... 2
- Without axostyle..... Genus *Trichomitus*
 Kofoid and Swezy

¹ Genus *Hegneria*, Brumpt and Lavier, with 7 flagella; like *Euglena* but colorless and no stigma.

Family 4. **Trichomonadidae** Wenyon(?)

- 2. Flagella less than five 3
 Flagella six in number Genus *Pentatrichomonoides* Kirby
- 3. Parabasal wound around axostyle Genus *Gigantomonas* Dogiel
 Parabasal not wound around axostyle
 Genus *Trichomonas* Donn 

Family 5. **Devescovinidae.**

- 1. With four flagella 2
 With more than four flagella Genus *Metadevescovina* Light
- 2. Without axostyle 3
 With axostyle 4
- 3. Trailing flagellum leaves body at anterior end Genus *Retortomonas* Grassi
 Trailing flagellum leaves body at posterior end Genus *Tricercomonas* Wenyon and O'Connor
- 4. Parabasal wound around axostyle Genus *Devescovina* Foa
 Parabasal rod-like 5
- 5. Parabasal a single rod 6
 Parabasal double; two curved rods Genus *Foaina* Janicki
- 6. Parabasal closely applied to nucleus Genus *Janickiella* Duboseq and Grass 
 Parabasal free from nucleus 7
- 7. Trailing flagellum leaves body near anterior end Genus *Paradevescovina* Kirby
 Trailing flagellum leaves body near posterior end Genus *Tricercomitus* Kirby

Family 6. **Spironemidae.**

- One genus Genus *Spironema* Klebs

Family 7. **Streblomastigidae.**

- One genus Genus *Streblomastix* Kofoid and Swezy

Family 8. **Chilomastigidae.**

- 1. With six flagella Genus *Hexamastix* Alexeieff
 With four flagella 2
- 2. Trailing flagellum in cytostome; no axostyle Genus *Chilomastix* Alexeieff
 Trailing flagellum not in cytostome; with axostyle Genus *Eutrichomastix* Kofoid and Swezy

Family 9. **Cochlosomidae** Tyzzer.

- 1. Truncate anterior end with sucker 2
 Anterior end not truncate; cytostome large
 Genus *Cochlosoma* Kotlan
- 2. Sucker flush with surface of body Genus *Cyathosoma* Tyzzer
 Sucker at end of tube-like prolongation
 Genus *Ptychosoma* Tyzzer

Sub-order 2. **Dikaryomastigina.**

- 1. With more than four flagella 2
 With four flagella (not parasitic) Genus *Gyromonas* S ligo

- | | | |
|---|---------------------------|-----------|
| 2. With eight flagella..... | 3 | |
| With six flagella (not parasitic)..... | Genus <i>Trigonomonas</i> | Klebs |
| 3. Not parasitic..... | 4 | |
| Parasitic..... | 6 | |
| 4. With two posterior cytostomal lobes | | |
| Without cytostomal lobes..... | Genus <i>Trophagus</i> | Klebs |
| Flagella unequal in length..... | 5 | |
| 5. Flagella unequal in length..... | Genus <i>Trepomonas</i> | Dujardin |
| Flagella approximately equal (see also 7) | | |
| | Genus <i>Hexamitus</i> | Dujardin |
| | (in part) | |
| 6. Cytostome present..... | 7 | |
| Cytostome absent..... | Genus <i>Octomitus</i> | Prowazek |
| 7. Cytostome single; anterior..... | Genus <i>Giardia</i> | Kunstler |
| | (= <i>Lamblia</i>) | Blanchard |
| Cytostome double (see also 5)..... | Genus <i>Hexamitus</i> | Dujardin |
| | (in part) | |

SUB-ORDER 3. Polykaryomastigina.

- | | | |
|------------------------|--------|---------------|
| With proboscis..... | Family | OXYMONADIDAE |
| Without proboscis..... | Family | CALONYMPHIDAE |

Family 1. Oxymonadidae Kirby.

- | | | |
|---|-----------------------------|------------------|
| 1. Said to be non-flagellated..... | Genus <i>Kirbyella</i> | Zeliff |
| Flag. anterior..... | 2 | |
| 2. Individual with single nucleus, double | | |
| kinetoplast..... | Genus <i>Oxymonas</i> | Janicki |
| Individual with one to several nuclei | | |
| | Genus <i>Proboscidiella</i> | Kofoid and Swezy |

Family 2. Calonymphidae Grassi.

- | | | |
|--|---------------------------------|---------|
| 1. Nuclei associated with kinetoplasts..... | 2 | |
| Nuclei not associated with kinetoplasts | | |
| | Genus <i>Snyderella</i> | Kirby |
| 2. Nuclei and kinetoplasts associated..... | 3 | |
| Nuclei less numerous than kinetoplasts | | |
| | Genus <i>Calonympha</i> | Foa |
| | (em. Grassi) | |
| 3. Nuclei in one anterior circle..... | Genus <i>Coronympha</i> | Kirby |
| Nuclei otherwise arranged..... | 4 | |
| 4. Each kinetoplast associated with one | | |
| nucleus..... | 5 | |
| Each kinetoplast associated with more than | | |
| one nucleus..... | Genus <i>Diplonympha</i> | |
| 5. Nuclei peripheral, spirally arranged..... | Genus <i>Stephanonympha</i> | Janicki |
| Nuclei central, not spirally arranged..... | Genus <i>Metastephanonympha</i> | |

CHAPTER XII.

SPECIAL MORPHOLOGY AND TAXONOMY OF THE SARCODINA.

THE term Sarcodina was introduced by Bütschli in honor of Dujardin whose studies on the protoplasm of the Foraminifera led him to believe that the living substance of these forms is simpler than that of other living things and justifying his name for it—sarcode. The peculiarity upon which Dujardin based his conclusion constitutes the essential difference between these types and other groups of the Protozoa. A definite cell membrane is usually absent and the body protoplasm in general is more fluid and more tenuous than in other types. In the absence of confining membranes and with the play of internal forces, protoplasmic processes—pseudopodia—are put forth so that the contour of the body may be constantly changing, a phenomenon expressed by the term ameboid movement.

The great majority of Sarcodina are suspended or floating forms (Heliozoa, Radiolaria) and the ground type is homaxonic or spherical, but creeping forms are characteristically flattened, while minor variations of the spherical form lead to the greatest variety of radial ellipsoidal and cylindrical types. They vary in size from a few microns to many millimeters while some forms of fossil Foraminifera are from 1 to 3 inches in diameter.

Unlike organisms in the three other great groups of Protozoa the cortex of the Sarcodina rarely shows much structural differentiation. In the majority of cases it is soft and highly vesicular but shows a marked tendency to form an outer or inner lifeless mantle of chitin. Such lifeless mantles or membranes may be tightly fitting or may be in the nature of tests or houses. They may be of pure chitin as in *Cochliopodium*, *Gromia*, etc., or, more frequently, of chitin impregnated with iron oxides, or still more frequently may serve as a substratum on which foreign particles or plates and scales manufactured by the organism are cemented, as in the majority of testate rhizopods. Or between lamellae of chitin precipitation of calcium carbonate leads to the formation of the limestone shells of the Foraminifera. Skeletons of silica or strontium sulphate of varied patterns and often of exquisite design are characteristic of the Radiolaria, while spicules, rods and plates of silica are widely distributed amongst Heliozoa and Radiolaria.

While many of the Sarcodina are typically uninucleate it may be safely stated that this is exceptional in the group as a whole for the vast majority of Mycetozoa, Foraminifera and Radiolaria are multinucleate. Nuclear dimorphism, however, does not occur and the multinucleate condition is brought about by fusion of cells to form plasmodia as in the Mycetozoa, or by repeated division of nuclei without accompanying division of the cell as in the Foraminifera and Radiolaria.

Contractile vacuoles are typical of fresh water forms and their absence is equally typical of salt water and parasitic forms of Sarcodina. When present they are invariably simple and burst directly to the outside without reservoirs, canals or permanent pores, and they furnish the best evidence for the view that contractile vacuoles here are primarily regulatory in a physical sense, rather than excretory, in function.

The most characteristic feature of the Sarcodina as a group is the ability of the individual cell to throw out protoplasmic processes called pseudopodia, and movements of translation or in food-getting are brought about by the protoplasm in the formation of these processes. It was this ability which led Dujardin in 1841 to distinguish these types as *les rhizopodes* from *les flagellés* and *les ciliées*.

Pseudopodia, however, cannot be described by any one definition. The most casual student of the Protozoa will not fail to recognize a difference between the pseudopodia of *Amoeba proteus* and those of an *Arcella* or *Difflugia*, while the difference is even more marked between these types and the pseudopodia of any foraminiferon, or between these and any heliozoön. These differences are so pronounced that modern students of the Sarcodina beginning with Lang have distinguished no less than four types of pseudopodia under the names of axopodia, myxopodia, filopodia and lobopodia, and there is some evidence that these four types and in the order given represent adaptations of a degenerative nature from an ancestral flagellum-like type of motile organ.

Axopodia are homologous with the flagellum of Mastigophora (p. 145). An axial filament extends from the endoplasm to the tip of the pseudopodium. Many protozoölogists, following Doflein, regard this as essentially a supporting structure, but like the axial filament of a flagellum in many cases it is derived from a kinetic element in the endoplasm and as in the hypermastigote flagellates the axial filaments in many forms form the astral rays of an amphister at division (*v. g.*, *Dimorpha mutans*, Fig. 79, p. 148). In place of the periplastic sheath of the flagellum an axopodium has an investing sheath of cortical plasm in which the protoplasmic granules may be seen streaming back and forth. Many are elastic or mildly vibratile and undoubtedly belong in the category of motile organs since movement of the organism is dependent upon their activity.

Myxopodia are so called because of the tendency to fuse or anastomose when two come in contact. The investing sheath of protoplasm is highly miscible and upon fusion of many pseudopodia a mesh or network, peculiarly characteristic of the Foraminifera, is formed. In this type the axial filament of the axopodia is absent; in its place there is a medullary core of denser substance termed stereoplasmatic axis by Doflein, and interpreted by some as a reminiscence of an earlier axial filament.

Filopodia are homogeneous hyaline pseudopodia possessing in many cases a remarkable elasticity and power of independent movement. It is possible that these pseudopodia do not represent the clear ectoplasm of the *Ameba* type of pseudopodium, but may be homologous with the stereoplasmatic part of a myxopodium, or the highly modified representative of an axial filament.

Lobopodia finally cannot be interpreted properly as motile organs. They are characterized by nothing that can be homologized with structural parts of other types of pseudopodia. They are dependent upon the physical condition of the protoplasm from which they are formed and are present in any type of cell and in any type of animal in which such physical conditions prevail. They are by no means limited to the rhizopods amongst Protozoa but, as shown in Chapter XI, are characteristic of many types of flagellates as well, and they are formed by one type of cell or another in the majority of higher animals.

It is possible of course that the path of evolution has been exactly the reverse of that outlined above and that progressive evolution has resulted in the gradual differentiation of the more complex types of pseudopodia until with Heliozoa we have a prototype of the Mastigophora. Such an hypothesis makes it more difficult, however, to account for such forms as the Bistadiidae or the flagellated phase of different types of Sarcodina.

All types of reproduction are represented; simple division, budding division, unequal division and multiple division (p. 209) and the life histories of different types are so variable that a common or generalized account would be inadequate. In general it is legitimate to say that a two-phase, metagenetic life history is characteristic although certainly not universal. Sexual processes are widely distributed throughout the sub-phylum, but here again these cannot be described as of any common type.

Encystment or resting stages are well known in fresh water forms of Sarcodina, but are absent or have not been described in connection with representatives of the two great groups of marine forms—the Foraminifera and Radiolaria.

Classification of the Sarcodina is fairly well established although minor differences depending upon the individual judgment of relationship in special cases will be found. Division into main groups

is made on the basis of pseudopodia types while minor groups are based upon special structural or functional peculiarities. Thus one great group is characterized by the possession of ray-like pseudopodia with axial filaments and is given here the taxonomic value of Class I, the Actinopoda, and these show the nearest approach to the Holomastigidae amongst the flagellates. A second group—Class II—includes forms with myxopodia, filopodia and lobopodia and is well termed, in recognition of Dujardin, the Rhizopoda. Possible ancestral types for this group may be found in the Rhizomastigidae amongst the Mastigophora.

CLASS I. ACTINOPODA CAULKINS.

These are usually homaxonic or spherical forms living for the most part as suspended or floating organisms. Pseudopodia are typically axopodia but lobose pseudopodia may also be formed, mainly as food-taking organs. The protoplasm is highly alveolar, becoming, in the ectoplasm particularly, vesicular or pseudo-alveolar. A highly differentiated cortex is absent as well as the denser cortical protoplasm which characterizes the Amebidae. In fresh water forms (Heliozoa) one or more contractile vacuoles are present in the vesicular ectoplasm. In the Radiolaria, ectoplasm and endoplasm are sharply separated by a continuous chitinous membrane—the central capsule—within which lie one or many nuclei, while the extracapsular protoplasm is differentiated into zones of more or less specialized ectoplasm.

While several types are naked, the great majority of Actinopoda are provided with spicules, plates, spines or skeletons often of elaborate design and exquisite delicacy. Some forms are covered with a gelatinous mantle in which foreign particles—diatom shells, sand grains, etc.—are embedded. For the most part the spicules and skeletons are composed of silica but in one large group of Radiolaria, the Acantharia, they are horn-like and composed of strontium sulphate. According to Dreyer spicules and skeletons depend upon the vesicular configuration of the protoplasm and upon the quantity of mineral matter precipitated between the alveoli (Fig. 12, p. 33).

In Heliozoa a single vesicular nucleus is the rule, but there may be from 200 to 300 in *Actinosphaerium eichhornii* and several nuclei in *Campylonema nutans*. A multiple number is also characteristic of the Radiolaria, or a single nucleus may become enormously enlarged.

Nutrition is invariably holozoic, living organisms being captured through the agency of lobose pseudopodia (Fig. 97, p. 186). Few observations have been made, however, upon digestive processes or final history of the food (see Chapter V).

Reproduction occurs by division, either binary fission or unequal

division in the form of budding. Multiple division is frequent in Radiolaria where the endoplasm gives rise to a multiple number of flagellated swimmers which may be of similar or dissimilar size (isospores and anisospores). In some cases both kinds are formed within the same central capsule. Whether these are gametes is a matter which, while probable, has not been satisfactorily proved.

Among the Heliozoa sexual processes are fully described only for *Actinosphaerium* and *Actinophrys* in which the peculiar type of pedogamous isogamy is characteristic (see p. 277).

The Actinopoda are divided into two fairly well-defined sub-classes—the Heliozoa of Haeckel and the Radiolaria of Joh. Müller.

SUB-CLASS I. HELIOZOA HAECKEL.

Heliozoa are typically fresh water forms although several species of marine forms are known. They are homaxial and floating in habitat for the most part but stalked and attached forms are occasionally met with (*Wagnerella borealis*, *Clathrulina elegans*, etc.). They are either naked (Aphrothoraca) or covered by a gelatinous mantle without spicules (Chlamydophora), or with spicules (Chalanthoraca) or provided with a definite latticed shell (Desmothoraca).

Pseudopodia are typically radial with central axial filaments which penetrate the endoplasm. Here they end, or rather begin, either in a nucleus (*Actinophrys*, *Camptouema nutans*, etc.), or in a central kinetic granule called the Centralkorn by Grenacher (1869) (*Acanthocystis*, *Sphaerastrum*, *Wagnerella*, etc.). In such cases the nucleus is excentric. In *Camptouema nutans* a single axial filament arises from each of the many nuclei and there are as many pseudopodia as there are nuclei. In *Wagnerella borealis* the nucleus is in the basal plate, while the central granule, with radiating axial filaments, is in an enlargement at the other end of the stalk.

The body protoplasm is alveolar and characterized by two zones which in some cases are clearly differentiated as ectoplasm and endoplasm (e. g., *Actinosphaerium*) but in most genera they are rather indefinite. The ectoplasm is made up of relatively large pseudo-alveoli in *Actinophrys* and *Actinosphaerium* and is very different from the dense ectoplasm of *Amoeba*. The endoplasm is more finely granular and contains one or more nuclei (up to two hundred or more in *Actinosphaerium*). Symbiotic forms are not infrequent in the endoplasm and are regarded as atlagellate forms of algae.

Contractile vacuoles are present in fresh water species but are generally absent in salt water forms. They are developed in the cortex and resemble slightly enlarged ectoplasmic vesicles bursting to the outside.

Nutrition is holozoic, minute lobose pseudopodia being protruded which capture and draw in minute organisms as food. In *Camptonema*, however, the axopodia are able to bend and several of them may be directed toward the capture of living prey.

Reproduction is ordinarily by binary fission or by budding, while incomplete division frequently leads to colony formation as in *Raphidiophrys*. Sexual processes have been described for only a few forms (see Chapter VIII) while flagellated swarm spores, which may turn out to be gametes, are known for *Acanthocystis*, *Clathralina* and *Wagnerella*.

If doubtful forms resembling Heliozoa, but without axial filaments (*e. g.*, *Nuclearia*, *Vampyrella*, etc.), are transferred to the Rhizopoda with which they have most affinities, then the classification of the Heliozoa is simple. The division into orders following Hertwig and Lesser (1874) is based upon the absence or upon the nature of the skeleton elements.

SUB-CLASS II. **RADIOLARIA** HAECKEL.

Broadly stated the Radiolaria are pelagic organisms of the same general type as the Heliozoa but offer many variations from the homaxonic symmetry of the latter. They are exclusively salt water forms, surface-dwelling for the most part, but may be found at great depths of the sea. Pseudo-alveoli are greatly elaborated and form foam-like spheres with radiating axopodia or with soft protoplasmic pseudopodia-like myxopodia, while complex skeletal elements of silica or strontium sulphate afford the greatest variety of structures and designs.

A typical radiolarian may be conceived by imagining a resistant membrane of organic substance, presumably chitin or pseudo-chitin, between the zones of ectoplasm and endoplasm of a heliozoön like *Actinosphaerium*. Such a membrane is present in Radiolaria and is called the "central capsule" (Fig. 181). It separates the intracapsular protoplasm (endoplasm) from the extracapsular protoplasm (ectoplasm). Minute openings, the pylea, through which communication between the two main zones of protoplasm is possible, are uniformly distributed, or arranged in lines and patterns, or limited in number at definite polar positions. These serve as a basis of classification for the main subdivisions of the group according to the scheme early adopted by Hertwig.

The intracapsular protoplasm contains nuclei, fat particles and plastids of one kind or another, and as Verworn showed, it can live independently of the ectoplasm for a time but ultimately regenerates it. The outer or extracapsular plasm is composed of four parts according to Haeckel. The outermost part is a zone of pseudopodia which originate, however, in the more deeply lying fourth zone and

then extend through the gelatinous ectoplasm to the periphery. A second zone—sarcodictyum—is in the form of a meshwork which extends through the third zone of gelatinous material termed the calymma which forms the greater bulk of the ectoplasm. A fourth and most important zone, the sarcomatrix, lies close against the central capsule and is the go-between for the intra- and extra-capsular portions. The sarcomatrix is also the seat of digestion

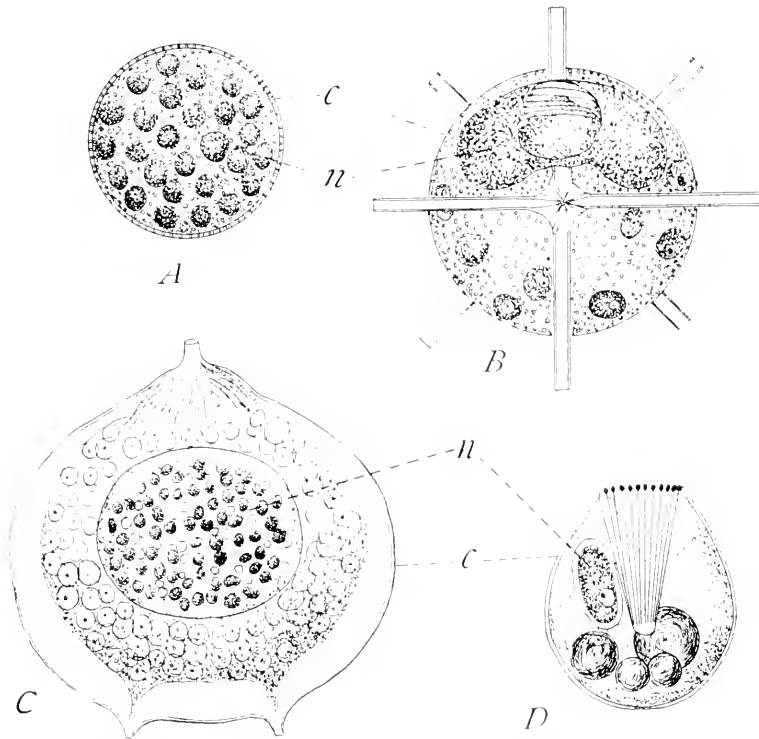


FIG. 181.—Radiolarian central capsules. A, *Thalassolampe*, type of peripylea; B, *Acanthomitra*, type of actipylea; C, *Aulographis*, type of tripylea; D, *Tripterocalpis*, type of monopylea; c, central capsule; n, nucleus. (From Calkins after Haeckel.)

and assimilation, the food coming to it by way of the pseudopodia and the network of the sarcodictyum.

As the means of communication between the central protoplasm and the sarcomatrix is of vital importance to the organism, the arrangement of the apertures in the central capsule offers a good character for the classification of the Radiolaria. Hertwig (1879) who first used this feature, divided the group into four legions as follows: (1) Peripylea, in which the membrane of the capsule is

perforated by pores arranged regularly around the entire surface. (2) Actipylea, in which the pores are said to be arranged in groups or lines over the surface. Schewiakoff (1926), however, in his masterly monograph on the Acantharia, denies the presence of pylea

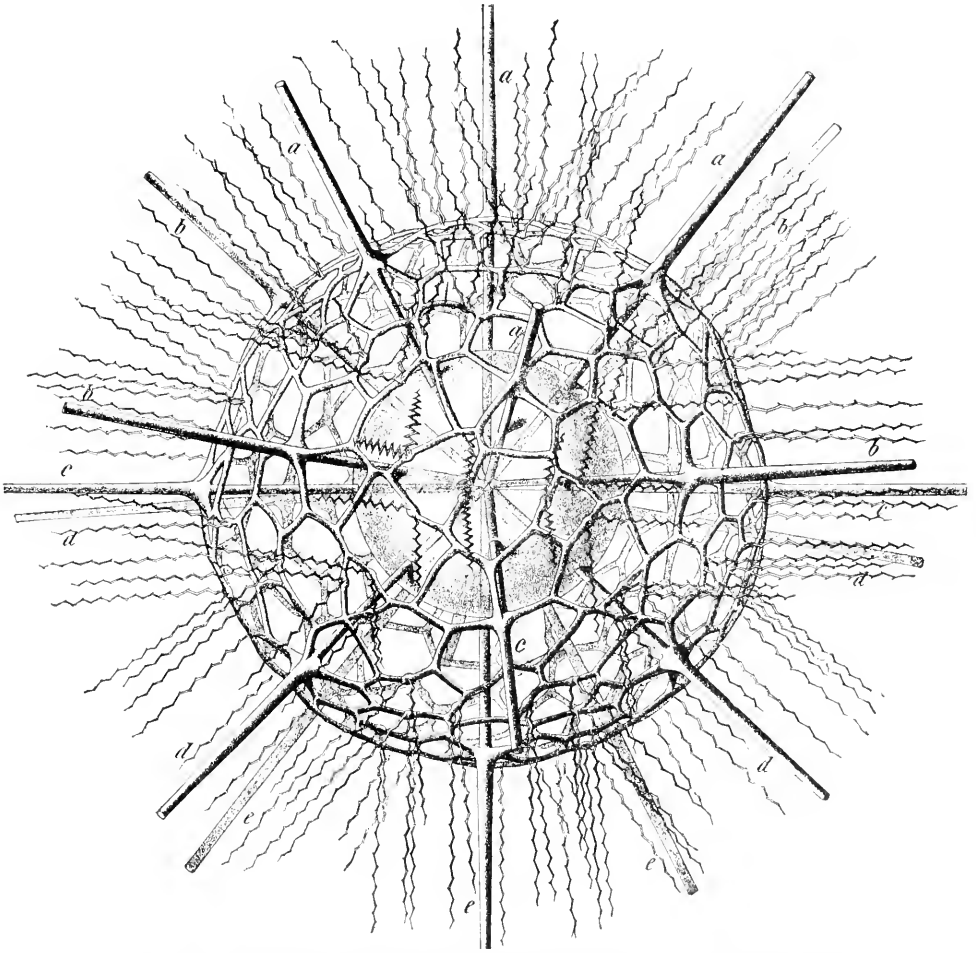


FIG. 182.—*Lichnaspis giltochii*, one of the Actipylea. The spines of strontium sulphate are arranged in accordance with the "Müllerian law" as follows: *a, a, a, a*, northern polar; *b, b, b, b*, northern tropical; *c, c, c, c*, equatorial; *d, d, d, d*, southern tropical; and *e, e, e*, southern polar. (After Haeckel.)

altogether. (3) Monopylea, in which there is only one such group of pores. In these forms the perforated disc may be connected with the center of the central capsule by a conical mass of endoplasm, the podocomus, rich in food particles and granules (Fig. 181).

(4) Canopylea, in which the membrane around the pores is drawn out into funnel-like projections termed astropytes of which one is the primary, the other two secondary. In these forms, furthermore, the central capsule is double. Haeckel found that certain types of skeleton are characteristic of the different types of membrane perforation and gave corresponding names to the four legions of Hertwig, viz.: (1) *Spumellaria*, or practically naked forms. (2) *Acantharia* (Fig. 182), with spicules and bars supposed to be of horn or acanthin, but later shown by Bütschli to be composed of strontium sulphate.—Schewiakoff (1926) separates this group as a distinct sub-class because: (a) Of the chemical make-up and arrangement of the skeletal bars; (b) of the absence of a membranous and perforated central capsule which is replaced here by a more or less thin plasmatic membrane without pylea; (c) of the presence of a clearly-defined hydrostatic apparatus consisting of a gelatinous layer which extends to the ends of the spines and is provided with elastic fibers (myophrisks).—(3) *Nassellaria*, with skeletons and spicules of silica. (4) *Phacodaria* from the presence of a pigmented mass or phecodium around the opening of the primary astropyle.

The Radiolaria are holozoic throughout, and feed upon flagellates, diatoms, small copepods, etc. These are captured through the agency of widespread pseudopodia. Nothing is known about the digestive processes. Symbiotic "yellow cells" (*Zoöxanthellae*) which, with the exception of the Tripylea, are characteristic of the group, may play a part in the nutritive processes.

Reproduction is primarily by binary division which begins with division of the nucleus. This is followed by division of the central capsule and of the extracapsular plasm. In many cases the skeletal structures are also equally divided so that daughter cells must regenerate the missing halves (*e. g.*, *Aulacantha*). Or one daughter cell may leave the parent house and build a new one for itself. Observations, however, are scanty on such phenomena. Repeated divisions of the nuclei and central capsules without accompanying divisions of the extracapsular plasm lead to temporary forms with 2, 4 or 8 central capsules (Thalassicollidae, Tripylea) while this condition is permanent in the huge colony forms (Polycyttaria).

Multiple division leading to the formation of minute bi-flagellated swimmers is not uncommon and has been observed in Peripylea, Actipylea and Tripylea. In some cases only one type—iso-spores—is formed; in other cases what are termed microgametes are formed by one individual, and macrogametes by another, a condition which has led to the conclusion that such anisospores are gametes. This is supported by Hartmann's observation of their fusion. On the other hand, the formation of the two types in one and the same individual throws some doubt on their gamete nature. Chatton (1923) indeed regards them not as belonging to the life history

of the radiolarian, but as swimmers of parasitic dinoflagellates (Merodinium). Such problems remain unsolved until the full development of the swimmers is observed.

We offer no apology for not attempting a special classification of this group or a key to the genera. The enormous number of genera of Radiolaria require monographic treatment which may be found in Haeckel's three volumes of Challenger Reports and in Schewiakoff's monograph of the Acantharia (*Fauna and Flora, Golfes von Neapel*, vol. 37, 1926).

CLASS II. RHIZOPODA VON SIEBOLD.

With the Rhizopoda we find types of derived organization that are not found in the Actinopoda. Myxopodia, filopodia and lobopodia are characteristic, although rarely combined in the same individual. The protoplasm is generally alveolar and may or may not be differentiated into distinct ectoplasm and endoplasm but in general shows less differentiation than in ciliates or flagellates or even in Actinopoda. Protoplasmic inclusions, of the nature of metaplastids, are highly varied while definite plastids are rare. A single chloroplastid of unknown significance, in the form of a blue-green so-called chromatophore, is present in the testate rhizopod *Paulinella* but these are not known elsewhere in the group. Pascher (1929) finds that these "chromatophores" of *Paulinella* are able to live independently of the rhizopod and he regards them as a distinct genus of blue-green algae. Metaplastids such as "chromatoid bodies" are characteristic of the parasitic amebae (Endamebidae), while fat and glycogen-like bodies are widely distributed. These are particularly abundant in the fresh water species *Pelomyxa palustris* Greeff, the highly refringent bodies "Glanzkörper" found here in abundance are interpreted by Stolc and Bott as glycogen-like in composition, by Veley (1905) as albuminous, and by Goldschmidt (1904) as the plastin remains of nuclei which have broken down with the formation of chromidia. The function of these inclusions and of the accompanying bacteria-like organisms (*Cladothrix pelomyxae* Veley) is still a matter of hypothesis. Chromidia, or cytoplasmic chromatin granules, are characteristic and may be permanent or periodic constituents of the cytoplasm (see p. 69).

Living membranes equivalent to the cortical membranes of flagellates, ciliates and gregarines are rarely found here. Transitions toward the chitinous and pseudochitinous tests are present in some forms (e. g., *Cochliopodium bilimbosum*) while the great majority of Rhizopoda have tests of pseudochitin on which mineral substances of quartz, silica or other types are cemented. In Foraminifera, calcium carbonate is precipitated between two such membranes of chitin, resulting in the highly complex and multiform shells of lime stone.

Contractile vacuoles are present in fresh water forms but are generally absent in marine types. They never have the complex canal system such as found in some flagellates and ciliates and are rarely fixed in position. Gas vacuoles are present in some of the testate fresh water forms (*Arcella*).

The majority of Rhizopoda are multinucleate both in fresh water and marine species, the multiple number due mainly to repeated nuclear division aided, in Mycetozoa, by plasmodium formation through fusion. The structure of nuclei is too varied for a general description but the vesicular, endosome type predominates (see p. 50).

Nutrition is holozoic and some progress has been made in working out processes of digestion, digestive ferments, etc. (see Chapter V). Living organisms are captured by pseudopodia or entrapped in the protoplasmic network where they are digested. Cyclosis is invariable and the various protoplasmic granules, digested food substances, etc., are thoroughly mixed.

Reproduction occurs in a variety of ways by division which may be either equal or binary division, budding division, unequal division or budding, and multiple division or sporulation. So-called budding division is the most characteristic and is a form of division apparently limited to the Rhizopoda (see p. 225).

Sexual processes are well developed, microgametes being formed in the majority of cases, which will be reviewed in connection with the several classes.

The classification adopted is an extension of that used by Minchin and includes as primitive forms those questionable Heliozoa-like types which many authors (*v. g.*, Reichenow-Dofflein) include with the Heliozoa.

SUB-CLASS I. **PROTEOMYXA** LANKESTER.

There are but few common characteristics in this group of primitive forms; the most widely spread feature apparently is the usual occurrence of ray-like pseudopodia which recall the appearance of Heliozoa. These have no axial filaments however, and frequently branch or partially anastomose. Flagellated swarm-spore stages are common but the life history is known in few cases. An approach to the Mycetozoa is seen in forms like *Labyrinthula* where the small spindle-shape cells bear long filose pseudopodia which fuse to form a net-like mesh. Most of them are parasites on lower algae and Protozoa.

Family 1. **Labyrinthulidae** Haeckel.—This family is composed of different species of the genus *Labyrinthula* which may be intracellular parasites in diatoms, *Vaucheria*, *Spirogyra*, etc. They frequently become associated in groups or pseudoplasmodia and reproduce by

division. Each individual or aggregate of individuals may encyst to form permanent spore-like resting stages. Flagellated spores are unknown (see Valkanov, 1929).

Family 2. Zoösporidæ Zopf-Delage. — These forms are also endoparasitic in diatoms, algae and various Protozoa, and have filose, Heliozoa-like pseudopodia without axial filaments. They are distinguished by the formation of swarm spores. *Protomonas amyli* Cienkowsky apparently lives only on starch grains. Typical genera: *Pseudospora* Cienkowsky, *Protomonas* Cienkowsky and *Protomyxa* Haeckel.

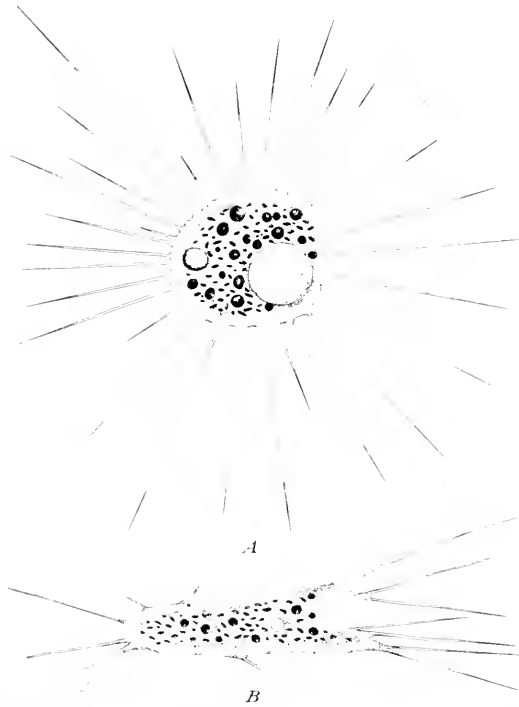


FIG. 183.—*Nuclaria delicatula*, quiescent and moving forms. (From Calkins.)

Family 3. Vampyrellidæ Doflein. — Here also the pseudopodia are very delicate and frequently branch and anastomose and may proceed from all sides of the body or be limited in origin to certain regions. They are frequently parasitic on algae and Protozoa, some forms having the ability to dissolve the cellulose membranes of plant cells, thus making holes through which the protoplast passes into the body of the parasite (*e. g.*, *Vampyrella*; see Lloyd, 1929) or they may enter the plant cells. Products of chlorophyll nutrition frequently form reddish-colored masses (karotin) in their proto-

plasm. Encystment, with cellulose cyst walls, is common. Nuclei are multiple as a rule; reproduction by plasmotomy or by division into uninucleate amebae; flagellated swimmers unknown. Accepted genera: *Nuclearia* Cienkowsky, *Arachnula* Cienkowsky and *Tampanyrella* Cienkowsky (Fig. 183).

SUB-CLASS II. MYCETOZOA DE BARY.

The Mycetozoa were formerly regarded as low types of fungi and under the name of Myxomycetes or "slime molds" were included among the lower plants. The investigations of de Bary, however, revealed the rhizopod affinities, and the relationship with other Sarcodina is now clearly recognized. There is little doubt, however, that Mycetozoa are borderline organisms and their semi-terrestrial habitat leads to modifications and adaptations not met with elsewhere. Many of them are highly complex both as to organization and as to life history and by no stretch of the imagination can they be regarded as simple organisms.

A general idea of the essential characteristics of the Mycetozoa may be gained by following through a typical life history beginning with a recently germinated "spore." This is a small uninucleate ameboid organism known as a "myxameba;" it is active, throwing out pseudopodia and moving energetically about the field. It has a contractile vacuole, and takes in solid food which is digested in a gastric vacuole, or it may live upon dissolved proteins from decomposing organic matter. It may also reproduce by division while in this ameboid condition.

The naked ameboid condition is usually temporary; sooner or later the "myxameba" turns into a "myxoflagellate" by the development of a flagellum. The contractile vacuole is retained and the body, usually ellipsoidal, is highly metabolic and may even give rise to pseudopodia, particularly at the posterior end where the pseudopodia aid in the ingestion of solid food in the form of bacteria, small Protozoa or bits of organic detritus; saprozoic nutrition, however, is also common. Like the "myxamebae" the "myxoflagellates" may reproduce by longitudinal division, in which case the centrioles of the mitotic figure become the basal bodies of the flagella. Myxoflagellates are apparently rather sensitive and show a ready tendency to encyst. Such "microcysts" are temporary and the excysted organism again passes through myxameba and myxoflagellate stages.

According to later investigations of Jahn these myxoflagellates ultimately become gametes; the last division, prior to gamete formation is a chromosome-reducing division, and the haploid gametes fuse to form diploid zygotes. In *Physarum didymoides* the gametes have 8, the zygotes 16 chromosomes.

The zygotes thus formed are very miscible and fusion occurs when two or more come in contact. In this way, and by multiplication of the nuclei by mitosis, and growth, great multinucleated plasmodia arise which may grow to be many inches in diameter and with thousands of nuclei. All observers agree in describing the fascinating spectacle of these sheets of moving protoplasm, a phantasmagoria of living and lifeless granules, nuclei, foreign particles and pigment. The pseudopodia are myxopodia and by their anastomosis great networks of flowing protoplasm form traps for minute organisms utilized as food; some forms, in addition, may be saprozoic in nutrition.

Under conditions which are not entirely known, but some of which are drought and scarcity of food, the entire mass may pass into a resting condition. The fluid protoplasm hardens to form a thick-walled "sclerotium" which is frequently impregnated with calcium salts. The nuclei collect in groups and these become encysted with cellulose walls. Such resting forms may retain life for some years. Ultimately the hardened walls are liquefied and the plasmodium condition is regained, the process requiring hours or days according to the length of time in the dried state.

With maturity of the plasmodium the gametes, or gametocytes, are formed by processes which are quite remarkable for their intricacy and for the complexity of the specialized structures appearing only at the time of fructification. The whole plasmodium may form one "sporangium," but more often the plasmodium breaks up into several "spore"-forming groups or "sporophores," each from a local heaping of the substance of the plasmodium. Part of such a thickening forms an outer investing wall termed the *peridium* which is often further hardened by deposition of lime. Another portion becomes differentiated into a thick network or feltwork, termed the *capillitium*, which is continuous with the outer peridium (Fig. 184). This network is made up of tubes and fibers; some of the latter, termed *elaters*, have a spiral structure and are supposed to function in the distribution of the spores. According to Kränzlin *elaters* arise from the kinetic components of degenerating nuclei.

The formation of the spores varies in details but the essential part of the process is the fragmentation of the residual mass into uninucleate or multinucleate bits of protoplasm. If multinucleate further fragmentation results in uninucleate bits, each of which encysts independently. According to the later observations of Jahn, the supposed fusion of nuclei leading to the uninucleate condition, and interpreted as autogamic fertilization by Prowazek, Kränzlin and earlier, by himself, is only a phase in the degeneration of nuclei many of which are disposed of in this way at this period. Fertilization is exogamic, the gametes being the myxamebae and myxoflagellates which ultimately emerge from the spores.

Liberation of the spores is accomplished in different ways. In some cases a lid is raised off the sporangium; in others the peridium dissolves in spots leaving a fenestrated capsule; in still others the capsule splits longitudinally. The dry, powdery spores are distributed in various ways, air currents playing a conspicuous part, and they finally germinate in the presence of moisture. Myxamebae and myxoflagellates are formed and the cycle is completed.

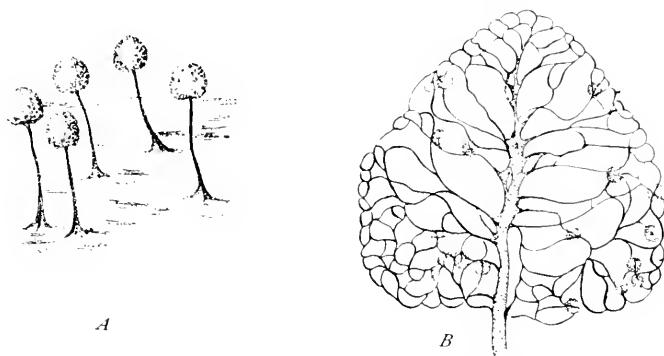


FIG. 184.—Fruiting bodies of *Comatricha nigra*. A, five stalked spore capsules; B, section of capsule with columella, capillitium, and spores. (After MacBride.)

Genera and species of Mycetozoa are distinguished according to the nature of the plasmodia and by the form and organization of the sporangia.

ORDER I. **ACRASIDA** VAN TIEGHEM.

(Pseudoplasmodiidae of Zopf-Delage) Sorophora Lister (in part).

The individual ameboid organisms after a period of creeping by active ameboid movement come together in clusters to form the pseudoplasmodia, the amebae retaining their individuality. Individuals creep up over their fellows and form groups or sori which in some cases are stalked, the stalks being formed by the dried bodies of sacrificial *Amebae*. The sori are formed by other amebae creeping over the stalk and accumulating in a mass at the top. Here each encysts and when a suitable medium is assured the small amebae again creep out, often, however, after a long period of desiccation. Their characteristic habitat is animal dung.

While many competent authorities regard these organisms as remotely related, if at all, to the more complex Mycetozoa, we believe that their affinities are more probably here than with any other group of Protozoa. The three families recognized show different gradations in complexity.

Family 1. Sappiniidae Dangeard.—The single genus—*Sappina* Dangeard—shows the characteristics of the family which differs

from all other Mycetozoa in that not even a pseudoplasmodium is formed, a single ameba going through all the motions of a plasmodium. Stalk and cyst are formed by one individual but the cysts are frequently massed in sporangium-like groups (Fig. 185). The species *S. diploidea*, originally named *Amoeba diploidea* by Hart-

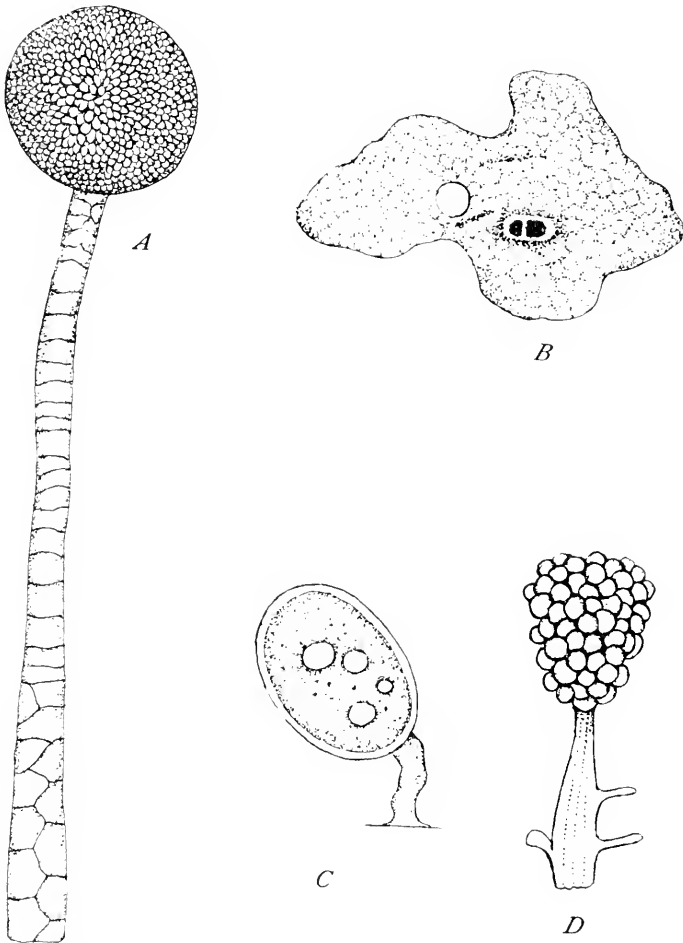


FIG. 185. — *Dictyostelium*, A, and *Sappinia*, B, C, D. (After Doflein.)

mann and Nägler, is much like *limax* types of ameba. Both *S. diploidea* and *S. pedata* Dangeard are binucleated, a condition which arises as the result of the peculiar copulation process shown by *S. diploidea* (see p. 323).

Family 2. Guttulinidae Cienkowsky. — These are small forms which bear stalked or unstalked fruiting bodies covered with

“spores.” The latter have either thin membranes or heavy cellulose walls. The myxamebae foregather in clumps on which the sori originate. Typical genera: *Guttulina* Cienkowsky, *Guttulinopsis* Olive.

Family 3. Dictyostelidae Rostafinsky.—Here the fruiting bodies are borne on simple or branched stalks formed by the hardened bodies of amebae which have migrated from the pseudoplasmodium mass. The polygonal bodies, covered with cellulose membranes, form a sort of tissue over which other amebae migrate to form sori at the top or at the ends of branches (Fig. 185). The myxamebae are characterized by thin, pointed pseudopodia. Typical genera: *Dictyostelium* Brefeldt and *Polyspondylium* Brefeldt.

ORDER II. PHYTOMYXIDA SCHROTER.

(Phytomyxinae Schroter).

Probably as a result of parasitism peridia and capillitia are absent in the representatives of this group. Otherwise they agree with the more complex Euplasmodida. They form true plasmodia and myxoflagellates, but there are no closed sporangia, recalling in this respect the simpler Acrasida. They are parasitic in plant cells and in insects (beetles).

Plasmodiophora brassicae Woronin is the best known of this group largely because of its economic importance. It attacks the roots of cabbages and other Cruciferae and produces a characteristic tumor disease known as “Club-root,” “Hauberries,” “Fingers and Toes,” “Kohlhernie,” etc. (See p. 386.)

Other genera parasitic on plants are *Tetramyxa* Goebel (forming galls on *Ruppia rostellata*) and *Sorosphaera* Schroter (causing tumors in various species of *Veronica*).

The genera *Sporomyxa* Léger and *Mycetosporidium* Léger and Hesse are parasites of beetles (*Scaurus tristis* and *Otiorhynchus usciipes*).

ORDER III. EUPLASMODIDA LISTER.

(Mycetozoa s. str. Myxogastres).

This order includes the great majority of Mycetozoa which in their life histories agree with the description given above (p. 445). Myxamebae and myxoflagellates are invariable, so too are true plasmodia and complex sporangia which with the exception of the family Ceratiomyxidae (Exosporea) are invariably surrounded by a peridium.

The “spores” are usually globular, rarely elliptical, and are often compressed by pressure into polygonal forms. In the majority of cases they are violet in color but colorless, white, yellow, brown and

red sporangia are known. In most cases the "spores" are uninucleate, but forms with two and with four nuclei are known.

In some cases the simultaneously formed sporangia unite to form a common fruiting body in which the individual sporangia may still be distinguished in some types. In other types, however, this independence is lost and one common fruiting body results, with one continuous capillitium. Such fruiting bodies are called aethalia. (See Key for further classification.)

SUB-CLASS III. FORAMINIFERA D'ORBIGNY.

(Reticulosa, Thalamophora.)

This group of the rhizopods includes a large number of bottom-dwelling and marine Sarcodina with anastomosing pseudopodia (myxopodia). A few forms live in fresh water (*Allogromia* species), and some forms are pelagic in the sea (*Globigerina*, etc.). The great majority are provided with tests composed for the most part of calcium carbonate. In some, however, the test is purely organic, consisting of substance of gelatinous or pseudochitinous character (*Allogromia*); or foreign particles of sand, diatom shells and detritus of one kind or another, may be cemented to the pseudochitinous test by gelatinous or chitinous cement. Such tests are usually described as arenaceous, in contrast with the clear lime shells or porcellaneous types. The walls of the shells are either thick and homogeneous or are perforated by minute pores (foramina) through which single pseudopodia are protruded. The cavity of the shells may be a single chamber, septa if present being incomplete (Monothalamous). Or a multitude of chambers may be present, separated by partitions or septa (polythalamous). The latter may be complicated by secondary deposits of lime through which labyrinthine canals and passages give occasion for intricate designs (Fig. 74, p. 138). The surfaces of the shells are usually smooth but in some forms, particularly the floating types of *Globigerina*, spines, ridges, rays, etc., probably assist in floating.

The living substance is usually so fluid that it is rarely quiet and protoplasmic streaming is so characteristic that the Foraminifera have been favorite materials for the study of protoplasm. It is not divided into zones, and the marine forms have no vacuoles. There are numerous foreign bodies as a rule and aggregates of the residue associated with food substances, form masses of fecal material termed "stercome." In many forms living commensals are also present in the form of small yellowish *Cryptomonas*-like forms (*Chrysidella*) which are liberated with sporulation of the host organism.

The living protoplasm fills more or less completely all chambers of the organism. In polythalamous forms protoplasmic strands

passing through pores in the septa maintain all parts of the soft body as a unit mass. In monothalamous and from the last-formed chamber of polythalamous forms, a large mass of protoplasm gives rise to the pseudopodial network which acts as a trap for the capture of diatoms, crustacea, rotifers and other smaller objects used as food. In the perforate types pseudopodia are also protruded through the finer pores (foramina) of the shell.

One large vesicular nucleus is characteristic of both single and many-chambered types. In the latter the nucleus may be confined to the first formed, or inner, chambers, although it may wander throughout the entire organism. In many cases it is replaced by several nuclei, and there is a general tendency throughout the group to form chromidia by multiple division, or fragmentation of the primary nuclei.

Reproduction may or may not be accompanied by fertilization phenomena and throughout the group there is a more or less regular alternation of sexual and asexual processes, accompanied in many cases by morphological evidence of sexual or asexual generation. In its simplest case, asexual reproduction consists of so-called budding division. In *Allogromia*, for example, the protoplasm streams out of the shell mouth and forms a ball of protoplasm of about the same size and shape as the parent organism; on the extruded bud a daughter cell is secreted and after division of the nucleus and migration of one of the daughter nuclei, the bud becomes detached and begins an independent existence. In the polythalamous forms, an initial shell of one chamber contains an organism which grows and buds in a similar manner, but the bud does not become detached. According to the type of budding shell types known as Nodosarian (Fig. 186), Frondicularian and Rotalian, are formed (Fig. 187). A new shell is deposited about the naked bud and thus a second chamber is added to the first, while the protoplasm by

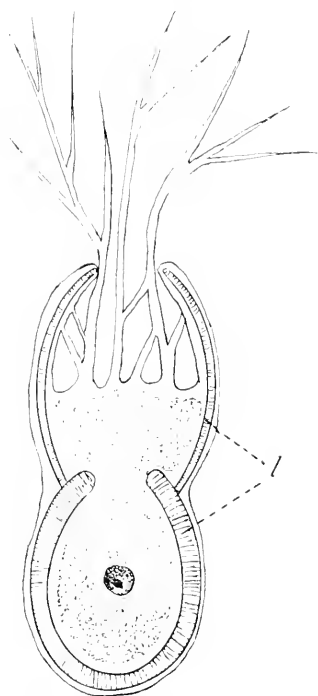


FIG. 186.—Diagram to show the mode of origin of the Nodosarine type of Foraminifera shell.

division of the nucleus, without complete cell division, becomes binucleated or multinucleated. In a similar manner other chambers are added to those already formed until complicated aggregates measuring 3 or more inches in diameter in some cases result (*Nummulites*, etc.). These, however, are to be regarded as single individuals of syncytial nature illustrating growth and differentiation rather than reproduction. With the formation of a brood of reproductive bodies each of which produces a similar multinucleated individual we can speak of asexual reproduction in a strict sense. Thus in *Polystomellina crispa* (Fig. 123, p. 235), after multiplication of the nuclei, the latter give rise by fragmentation to a large number of minute nuclei having the significance of chromidia. The plasm forms islands about each of these minute nuclei, or groups of them, and is then broken up into as many minute cells as there are islands.

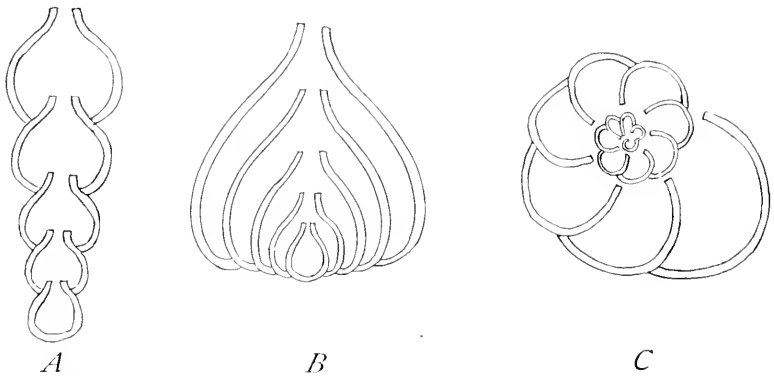


FIG. 187.—Types of polythalamous Foraminifera shells. A, nodosarine type; B, frondicularian type; C, spiral type. (After Carpenter.)

These small cells, in the form of amebulae or amebospores leave the parent shell by way of the foramina or by the mouth opening of the last chamber and after a short period of ameboid movement settle down and secrete the characteristic shell chamber. This initial test (proloculum) is measurably larger than the initial chamber of the organism which formed the amebulae and is called a macrospheric chamber as opposed to the microspheric chamber of the first generation. A new multi-chambered shell is then formed according to the type of structure of the species. When fully grown the protoplasm of this macrospheric generation breaks up into a swarm of small biflagellate flagellispores which leave the parent shell and swim about by means of their flagella. These flagellates are gametes which ultimately unite two by two to form zygotes. The flagella are absorbed and the young zygote secretes the shell material of the first chamber about which other chambers are

formed with growth and budding division until the mature individual again results. Thus there is a typical alternation of generations in the life history of a foraminiferon; the microspheric individual starting from a zygote, with its production of amebulae is an asexual generation while the macrospheric individual starting from an asexual spore is the sexual generation giving rise to gametes. In *Polystomellina* the relative abundance of macrospheric and microspheric shells is 38 to 40 of the former to 1 of the latter (Rhumbler, 1923).

Test dimorphism has led to much confusion in classification, and this difficulty, as pointed out by Cushman (1928), is enhanced by Hofker's (1927) discovery of trimorphic types amongst fossil foraminifera.

Fossil forms are known from the paleozoic to recent times. These have played a conspicuous part in geologic formations and are useful today in economic ways. A complete classification must take such forms into consideration. This is well done in Cushman's system of classification in which 45 families and 411 genera, living and fossil, are keyed and described. As with the Radiolaria it is inexpedient to repeat such keys here and the reader is referred to Cushman's excellent treatise (1928) for family and generic diagnoses.

SUB-CLASS IV. AMOEBAEA.

When rhizopods are mentioned the mental picture in most cases is *Ameba* or some of its close relations amongst the Amoebaea. It is not the largest group of rhizopods but some of the forms included here are amongst the most common types of Protozoa, while their apparent simplicity and enigmatic movement have given them the popular position of the lowest forms of animal life and the phrase "from Ameba to man" is familiar to everyone. They are present in all stagnant, fresh and brackish water; in damp moss or leaves; abundant in the superficial soil, and also abundant as commensals or parasites in all kinds of animals.

In all of the naked forms there is a well-marked differentiation of the protoplasm into endoplasm and ectoplasm. The latter is more dense, the former more fluid and with typical cyclosis. In the shelled types there is frequently a characteristic zonal differentiation.

Pseudopodia are never myxopodia or axopodia. Naked forms have blunt finger-form processes or lobopodia formed by an outflow of ectoplasm and endoplasm. Shelled forms in the majority of types have pseudopodia, composed apparently of ectoplasm only. These have considerable power of movement apart from the usual ameboid type of flowing substance, and may sway or move independently with vigor. In the naked forms pseudopodia may be thrown out from any part of the cell, but in shelled types they are

limited to the region adjacent to the orifice of the shell. In some cases, as in the genus *Cochliopodium*, there is a firm ectoplasm which has many of the features of a chitinous membrane. Pseudopodia pass through it by way of permanent apertures (Fig. 9, p. 31), and when the cell divides the membrane also divides. There are very few of such forms, however, the great majority of shelled forms having a definite chitinous membrane on which foreign particles are attached. In Arcellidae the membrane is clear chitin and in the Euglyphidae the outer elements of the shell are secreted before division and passed out to the daughter individual after the chitin membrane is laid down. The variety of shells is due to the different types of sand crystals, diatoms, detritus of various kinds and even living plant cells.

The nucleus is vesicular and usually single although many types of both naked and shelled forms are binucleated or multinucleated. The entire group is further characterized by the distribution in the cytoplasm of chromidia (see p. 69) which often takes the form of a chromidial network.

With the exception of the parasitic forms, and some of these are also included, the Amoebaea are holozoic in nutrition and proteolytic and amylolytic ferments have been isolated in some cases (see Chapter V).

Notwithstanding the abundance and the wide distribution of these forms of rhizopods there is very little agreement on the part of different observers in regard to the life history. Few Protozoa have been more frequently seen and studied than *Amoeba proteus* and yet little is known accurately about the life cycle. Binary division is characteristic of all the naked forms both free-living and parasitic, and encystment stages are known in all forms. So-called budding division is typical of the testate forms and differs materially from binary fission (see p. 214). Acceptable accounts of sexual processes are limited to the Testacea in which there is a general resemblance to the type of gamete formation characteristic of the Foraminifera (see Chapter VI).

Parasitic forms of the Amoebidae are widely distributed throughout the animal kingdom. They are usually present in the digestive tract but may be ectoparasites as well. The great majority are of the nature of commensals and are harmless, some, however, are pathogenic as *Amoeba mucicola* Chatton, a harmful ectoparasite on the gills of Labridae, or *Eudamoeba dysenteriac*, the cause of dysentery in man (see p. 387).

The organisms included in the Amoebaea fall naturally in one of two groups which have been generally recognized as Amoebida (Gymnamoebida) and Testacea. Following the principle adopted in classifying the Mastigophora where ameboid forms of animal flagellates are retained as Mastigophora only when the flagellum or

flagella are permanent structures of the organism, we include as rhizopods those forms with pseudopodia and temporary flagella; flagella and pseudopodia being more or less interchangeable. These are included here in the family Bistadiidae of Doflein.

ORDER 1. **Amoebida (Gymnamoebida)** EHRENBERG.

Naked forms of Amoebaea, either free-living or parasitic; with one or more nuclei; with contractile vacuole (except in some of the parasitic forms); reproduction by binary fission, multiple division occasional. Encystment widespread.

We recognize four families in this order, viz.: Bistadiidae, Amoebidae, Endamoebidae and Paramoebidae. Separation of the parasitic forms of amebae from free-living forms is hardly justifiable in a natural classification but is tolerated on grounds of expediency.

Family 1. Bistadiidae Doflein.—Organisms characterized by two interchangeable phases—ameboid and flagellated. In the former phase the body is ameboid with lobose pseudopodia. A single nucleus with endobasal body is present; the basal body of the flagellum is formed by division of the endobasal body (Wilson, Puschkarew, *et al.*) and the flagellum grows out from the basal body. (See Fig. 13, p. 34.) Transformation from the ameboid to the flagellated condition involves loss of ameboid movement and change in form to a monaxonic ellipsoidal form. Absorption of the flagellum accompanies transformation again to the ameboid condition. These changes are evidently induced by environmental conditions and, in cultural forms, may be brought about at will. Genera with one, two and three flagella in the flagellate phase are known. Reproduction by division is limited to the ameboid phase, sexual processes unknown. The ameboid phase is represented by small creeping amebae which have been generally included as *Amoeba limax*, and known as "limax" forms. These were separated from the genus *Ameba* by Chatton and Lalung-Bonnaire (1912) under the name *Vahlkampfia*. The forms with a single flagellum in the flagellated stage are retained under the generic name *Vahlkampfia*, although it is by no means assured that all "limax" amebae are thus dimorphic. Forms with two flagella are grouped in the genus *Dimastigamoeba* Alexeieff and forms with three flagella in the genus *Trimastigamoeba* Whitmore. Parasitic forms, regarded by Craig (1906) as a cause of human dysentery and with a flagellated phase with one flagellum, are included in the genus *Craigia*.

Family 2. Amoebidae (authors generally: em. Doflein, em. Calkins).—The usual types of free-living amebae are grouped in this family. Flagella, so far as known, are absent in all stages. Nuclei single, double or multiple; contractile vacuole usually single, present generally in fresh water forms. Reproduction is by simple

division in vegetative forms, by multiple division during quiescent phases. The great majority of forms are aquatic and developmental phases of other types (*e. g.*, mycetozoa) may be easily mistaken for amebae. Others are semi-terrestrial, living in damp earth, moss, etc., where they play a part in keeping down bacteria of the soil (see Goodey).

Family 3. Endamoebidae.—These are parasitic amebae widely distributed throughout the animal kingdom and with characteristic vegetative phases during which the organisms live as harmless commensals or, more rarely, as pathogenic parasites in the host, and with permanent cyst stages by which infection is carried by means of contaminative infection. The genus generally recognized, *Endamoeba*, is represented by a vast number of species with ill-defined diagnostic characters, while many questionable genera are forms about which the taxonomic position is still in dispute (see Chapter X). Nutrition is either holozoic, saprozoic or heterozoic.

Family 4. Paramoebidae.—Forms with single nucleus and peculiar cytoplasmic structure (Nebenkern) variously interpreted as a kinetic element, intracellular parasite, etc. Both free-living and parasitic species. Genus: *Paramoeba*.

ORDER 2. Testacea.

These forms are generally described as amebae with shells; by some they are grouped as a subdivision of the Foraminifera (Doflein). The protoplasmic and test structure, as well as the pseudopodia are so different from Foraminifera that little is gained by this procedure, while the association with naked forms has a long historical backing. They are almost exclusively fresh water forms, although some species are represented in brackish water as well. Many species are semi-terrestrial and abound in moss and similar damp places. The protoplasmic body differs from that of the Amoebidae in having the ectoplasm concentrated at the region of the shell opening, while many forms show a distinct zonal differentiation of the protoplasm. Contractile vacuoles are always present.

Nuclei are either single, double or multiple and are usually accompanied by a zone of chromidia in the form of a dense reticulum from which, according to the observations of numerous observers, the nuclei of gametes are formed (Schaudinn, Zuelzer, Elpatiewsky, *et al.*). It is rather the fashion to doubt this interpretation on the ground that such nuclei are possible parasites, but we shall adhere to it until the critics have a more probable explanation of the nature of the chromidia (p. 69).

Pseudopodia are filopodia which in a few instances have the tendency to branch (Fig. 188). They lack the medullary endoplasm of lobopodia and have a considerable power of independent move-

ment. In *Chlamydothrys* they form a network as in *Allogromia* (Fig. 189).

The tests are simple, one-chambered structures of widely-varied form, frequently ornamented with spines and processes. The basis of all shells is a pseudochitinous membrane which, in some forms, is greatly thickened and constitutes the test; in other cases foreign particles are cemented to the outside of the chitinous membrane (*Difflugia*, *Ceutropyxis*, etc.), and in still other cases silicious plates are precipitated in the endoplasm in the vicinity of the nucleus, and deposited on the chitinous membrane in definite patterns characteristic of different genera (*Euglypha*, *Quadrula*).

Reproduction occurs by longitudinal binary division in forms with a soft chitinous membrane, where membranes divide with the soft

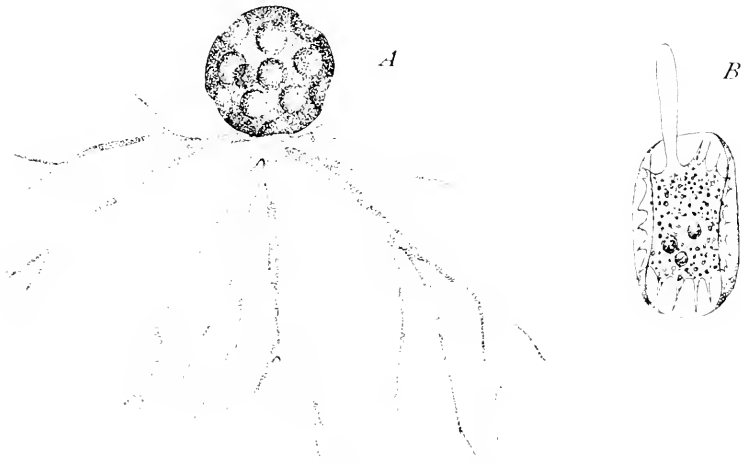


FIG. 188.—A, *Hyalosphenia?* sp. (Original) B, *Pseudochlamys patella* after Clap. and Lachn.

body (*Cochliopodium*); in other cases it occurs by so-called "budding division," whereby the protoplasm swells out of the shell mouth to form a bud which assumes the size and shape of the parent (p. 214). Multiple division also occurs in some types; many nuclei are formed by division; these become the nuclei of small naked amebae which after a short period of free movement and growth secrete the shell characteristic of the species. Fertilization processes have been described for several types (*Ceutropyxis*, *Arcella*, *Trichosphaerium*, *Difflugia*, etc., Fig. 190), the gametes being either amebulae or flagellulae. A typical alternation of generations comparable with that of the Foraminifera was described by Schaudinn for the peculiar genus *Trichosphaerium*. Here asexual processes occur by irregular plasmic divisions (plasmotomy) and by multiple division resulting

in a swarm of minute naked amebae. These develop into an adult form of different type which may likewise undergo plasmotomy leading to the formation of gamonts and gametes. The latter, upon fertilization, give rise to the initial type of organism. In this cycle, the original asexual generation differs from the later sexual generation by the presence of a peculiar type of test consisting of radially-arranged spicules of magnesium carbonate.

The forms included in this Order fall naturally into two families — Arcellidae and Euglyphidae (see Key for genera).

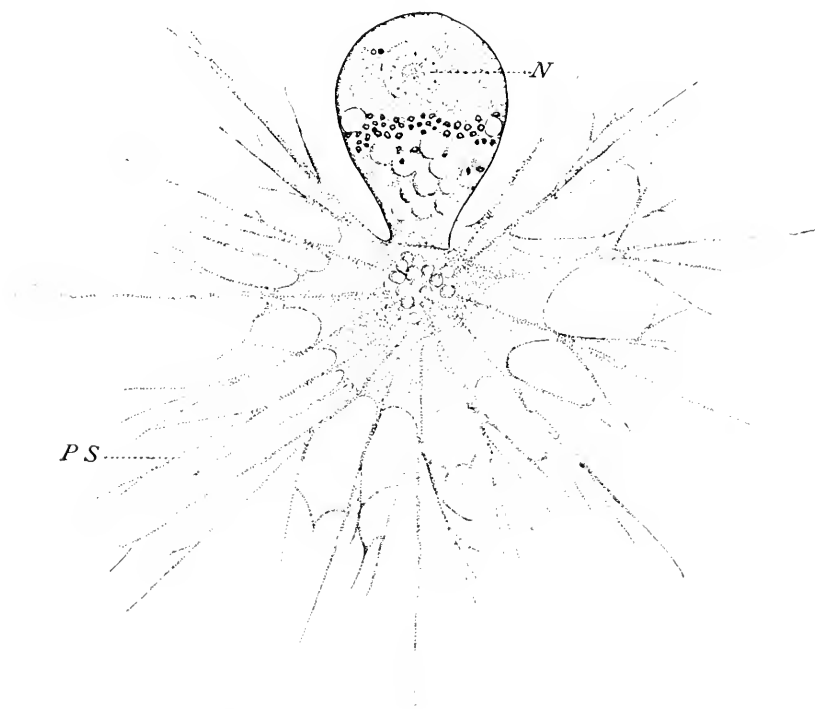


FIG. 189.—*Chlamydomorphys stercorica*. (From Doflein after Schaudinn.)

Family 1. Arcellidae.—Tests transparent or opaque by reason of covering of foreign bodies picked up by the protoplasm and deposited on the outside where they are cemented to the chitinous membrane.

Structure and materials of the shell afford a basis for further classification of the family. They are either pyriform or shaped like a watch-glass; the membrane may be rigid or flexible and the aperture central or asymmetrically placed.

Family 2. Euglyphidae.—In members of this family the test is covered by silicious plates or scales and the pseudopodia are of a filose, branching type. The tests may be either symmetrical or

SUB-CLASS I. **HELIOZOA** HAECKEL.

1. Naked forms; no gelatinous mantle or skeleton..... Order 1. APHROTHORACA
2. Gelatinous mantle present; no spicules or foreign bodies..... Order 2. CHLAMYDOPHORA
3. With isolated or united spicules or plates..... Order 3. CHALAROTHORACA
4. With fenestrated test..... Order 4. DESMOTHORACA

ORDER I. **APHROTHORACA** HERTWIG.

1. Individuals without stalks..... 2
Individuals with stalks..... 6
2. Ectoplasm and endoplasm clearly differentiated—multinuclear..... 3
No clear differentiation between endoplasm and ectoplasm..... 4
3. No central granule in which axial filaments unite..... Genus *Actinosphaerium* Stein
With central granule..... Genus *Gymnosphaera* Sasaki
4. Axial filaments end in nuclei..... 5
Axial filaments end in central granule..... Genus *Ornerella* Dobell
5. All axial filaments end in the single nucleus..... Genus *Actinophrys* Ehrenberg
Multinucleate, each nucleus with one axial filament..... Genus *Camptonema* Schaudinn
6. Stalk hollow..... Genus *Actinolphus* Schultze
Stalk solid..... Genus *Haeckelia* Mereschkowsky

ORDER II. **CHLAMYDOPHORA**.

1. Flattened; with central granule; often colonial..... Genus *Sphaerastrum*
Central granule absent or not observed..... Genus *Astrodisculus*

ORDER III. **CHALAROTHORACA**.

1. Elements of test embedded in outer plasmic zone..... 2
Elements of test not embedded in outer plasmic zone..... 4
2. Spicules chitinous, fine, radially arranged..... Genus *Heterophrys* Archer
Spicules silicious, similar or dissimilar.... 3
3. Spicules loosely embedded; all alike..... Genus *Raphidiophrys* Archer
Spicules of diverse forms and sizes..... Genus *Raphidiocystis* Penard
4. Individuals without stalks..... 5
Individuals with stalks having silicious membrane..... Genus *Wagnerella* Mereschkowsky
5. Test of foreign bodies..... 6
Elements of test made by organism..... 7

6. Test close-fitting about organism... Genus *Lithocolla* Schultze
Test separated from plasm by fluid zone
Genus *Elcorhanis* Greeff
7. Test made up of colorless spherules... Genus *Pompholyxophrys* Archer
Test made up of silicious discs or scales
with or without spines 8
8. Test of tangential scales and radial spines
Genus *Acanthocystis* Carter
Test of tangential, perforated discs; no
spines Genus *Pinaciophora* Greeff

ORDER IV. **DESMOTHORACA.**

1. Capsule spherical, with or without stalk. . 2
Capsule polyhedral, openings small, stalked
Genus *Hedriocystis*
Hertwig and Less.
2. Capsule with stalk, openings large. . Genus *Clathrulina* Cienkowsky
Capsules without stalks, openings small... 3
3. Openings with collars. Genus *Chouanocystis* Penard
Openings without collars. Genus *Elaster* Grimm

SUB-CLASS II. **RADIOLARIA** JOH. MÜLLER.

The great number of genera of Radiolaria make it impossible to give more than a superficial survey of this group. For keys to 4 legions, 21 orders and 36+ families and many hundred genera and species, see monographs by Hertwig, 1879; Haeckel, 1887, and Schewiakoff, 1926. (See p. 442).

CLASS II. **RHIZOPODA** VON SIEB.

1. Naked; Heliozoa-like; radiating pseudo-podia Sub-class 1. PROTEOMYXA
Naked or shelled; pseudopodia not Heliozoa-like 2
2. With myxopodia and plasmodium formation Sub-class 2. MYCETOZOA
No plasmodium formation 3
3. With calcareous shells; marine. Sub-class 3. FORAMINIFERA
Naked or with chitinous tests. Sub-class 4. AMOEBAEA

SUB-CLASS I. **PROTEOMYXA.**

1. Individuals Heliozoa-like; usually solitary. 2
Individuals fuse into thread-like plasmodia
Family 1. LABYRINTHULIDAE
 2. With flagellated swimmers. Family 2. ZOÖSPORIDAE
Without flagellated swimmers. Family 3. VAMPYRELLIDAE
- Family 1. **Labyrinthulidae** Haeck.
Parasitic in algae. Genus *Labyrinthula* Cienkowsky
Free-living in fresh water and earth. . . Genus *Monobia* Schneider
- Family 2. **Zoösporidae** Zopf-Delage.
Intracellular parasites of Algae, Volvox, etc.
Genus *Pseudospora* Cienkowsky
- Starch-eating ameboid forms. Genus *Protomonas* Cienkowsky
Free-living; body red; marine. Genus *Protomyxa* Haeckel

Family 3. **Vampyrellidae** Doflein.

- Form changeable; colorless; ray-like pseudo-
podia Genus *Nuclearia* Cienkowsky
Body branched; naked; pseudopodia delicate
Genus *Arachnula* Cienkowsky
Color reddish; ectoparasitic on Algae. Genus *Vampyrella* Cienkowsky
Color greenish; cysts of cellulose. Genus *Chlamydomyxa* Archer
Body sharply pointed at base of pseudopodia
Genus *Biomyxa* Leidy
Protoplasm of body and pseudopodia yellow
Genus *Rhizoplasma* Verworn
Body yellow; pseudopodia colorless. Genus *Diclitomyxa* Monticelli

SUB-CLASS II. **MYCETOZOA** DE BARY.

- Pseudoplasmodium in some; no peridia nor
capillitia; sporangium a mere mass of
spores. Order 1. ACRASIDA
Parasitic; no peridia nor capillitia. Order 2. PHYTOMYXIDA
Plasmodia; peridia and capillitia. Order 3. EUPLASMODIDA

ORDER I. **Acrasida** VAN TIEGHEM.

Amebae solitary; stalked spore-case

Family 1. SAPPINIIDAE

Amebae grouped; sori from group. Family 2. GUTTULINIDAE

Amebae grouped; stalks of sori hardened

amebae. Family 3. DICTYOSTELIDAE

Family 1. **Sappiniidae** Dangeard.

One genus and species; dung of horse, cow,
dog, etc. Genus *SAPPINIA*

Family 2. **Guttulinidae** Cienk.Cells do not form stalks of sori. Genus *Copromyxa*Short stalks bearing sori. Genus *Guttulina*Family 3. **Dictyostelidae** Rostafinsky.

1. Stalks unbranched. 2

Stalks branched. Genus *Polyspondylium*

2. Spores without definite arrangement

Genus *Dictyostelium*Spores in row like string of beads. Genus *Acrasis*ORDER II. **Phytomyxida**.

1. Tissue parasites of plants. 2

Celozoic parasites of animals. 6

2. Tumor-causing parasites. 3

Tumors not caused. Genus *Ligniera* Maire and Tison

3. Cause of "club root" in cabbage family

Genus *Plasmadiophora* Woronin

Causing gall-like tumors. 4

1. Spores in groups of four. Genus *Tetramyxa* Goebel

Spores massed in balls or plates. 5

5. Spores massed as hollow balls. Genus *Sorosphaera* SchroterSpores in spongy masses. Genus *Sporospora* Brunch

6. Plasmodium forming in intestine of beetle

Genus *Mycetosporidium*

Leger and Hesse

Parasites of fat body and gonads or free in

cavity. Genus *Sporomyxa* Léger

ORDER III. **Euplasmodida** LISTER.

Spores exposed on surface of sporophores

Sub-order 1. **EXOSPOREA**Spores in sporangia Sub-order 2. **MYXOGASTRES**SUB-ORDER 1. **Exospora** ROSTAF.

One genus; spores exposed; no sporangia

Genus *Ceratiomyxa*SUB-ORDER 2. **Myxogastres** FRIES.

(Key to genera adapted from MacBride, 1922.)

Spore mass black or violaceous, rarely ferruginous *Series A*Spore mass never black; usually brown, yellow, etc. *Series B**Series A*With delicate thread-like capillitium; sporangia more or less calcareous . . . Legion 1. **PHYSARALES**With capillitium and columella; rarely calcareous Legion 2. **STEMONITALES***Series B*Capillitium imperfect or none; spores brown, rarely purple Legion 3. **CRIBRARIALES**Capillitium of interwoven plates or tubules; spores pale or ashen Legion 4. **LYCOGALALES**Capillitium of sculptured threads; spores yellow Legion 5. **TRICHALES**Legion 1. **PHYSARALES** MacBr.Fructification often calcareous throughout; capillitium intricate Family 1. **PHYSARIDAE**Lime in peridium only, or also in stipe; capillitium simple Family 2. **DIDYMIIDAE**Family 1. **Physaridae** MacBr. em.1. Fructification an aethalium Genus *Fuligo*

Fructification an aggregate of sporangia 2

2. Peridium calcareous 3

Peridium apparently limeless, at least outside 6

3. Capillitium calcareous throughout . . Genus *Budhamia*

Capillitium largely hyaline 4

4. Sporangia globose; dehiscence irregular

Genus *Physarum*

Sporangia vasiform or tubular 5

5. Dehiscence by lid-covered opening . Genus *Craterium*

Dehiscence irregular; peridium inverted

Genus *Physarella*

6. Sporangia sessile with irregular outlines

Genus *Cienkowskia*Sporangia distinct Genus *Leocarpus*Family 2. **Didymiidae** MacBr. em.1. Fructification an aethalium Genus *Mucilago*

Fructification not an aethalium 2

2. Peridium single 3

Peridium double; outer one gelatinous 4

- Family 2. **Orcadellidae** MacBr. em.
 A single genus Genus *Orcadella*
- Family 3. **Liceidae** MacBr.
 A single genus Genus *Licca*
- Family 4. **Tubiferidae** MacBr. em.
 1. Sporangia stipitate; clustered Genus *Alwisia*
 Sporangia in linear series 2
 2. Spores olivaceous Genus *Lindbladiah*
 Spores umber Genus *Tubifera*
- Family 5. **Reticulariidae** MacBr. em.
 1. Spores brownish or umber 2
 Spores yellowish Genus *Dictydiaethalium*
 2. Sporangia bounded by broad perforated
 plates Genus *Enteridium*
 Sporangia wholly indeterminate Genus *Reticularia*
- Legion 4. LYCOGALALES MacBr.
 One genus only Genus *Lycogala*
- Legion 5. TRICHIALES MacBr.
 1. Capillitium a distinct net; no spiral bands
 Family 1. ARCYRIIDAE
 Capillitium threads fixed or free; no net 2
 2. Capillitium threads free; with spiral bands
 Family 2. TRICHIIDAE
 Capillitium threads attached 3
 3. Threads attached at both ends 4
 Threads attached at one end if at all
 Family 3. PERICHAENIDAE
 4. Threads plain or slightly roughened
 Family 4. DIANEMIDAE
 Threads definitely sculptured Family 5. PROTOTRICHIIDAE
- Family 1. **Arcyriidae** MacBr. em.
 1. Capillitium elastic 2
 Capillitium non-elastic Genus *Lachnobolus*
 2. Capillitium attached at base; no hamate
 branches Genus *Arcyria*
 Capillitium centrally attached, with hamate
 branches Genus *Heterotrichia*
- Family 2. **Trichiidae** MacBr. em.
 1. Capillitium threads long, centrally at-
 tached 2
 Capillitium threads short, free, sometimes
 branched 3
 2. Sculpture spiral Genus *Hemitrichia*
 Sculpture reticulate Genus *Caloneura*
 3. Threads, elaters, marked by spiral bands
 Genus *Trichia*
 Threads with irregular sculpture or none
 Genus *Oligonema*
- Family 3. **Perichaenidae** MacBr. em.
 Sporangia more or less grouped; dehiscence
 irregular Genus *Ophiotheca*
 Sporangia grouped; polygonal; dehiscence by
 lid Genus *Perichaena*

Family 4. **Dianemidae** MacBr. em.

- Capillitium threads attached at one end only
or free.....Genus *Margarita*
- Capillitium threads attached at each end
Genus *Dianema*

Family 5. **Prototrichiidae** MacBr. em.

- A single genus.....Genus *Prototrichia*

SUB-CLASS III. **FORAMINIFERA** D'ORB.

Cushman (1928) has published excellent keys to families and genera of Foraminifera, including 45 families and 411 genera. It is unnecessary to repeat these here.

SUB-CLASS IV. **AMOEBAEA** BÜTSCHLI.

- Naked forms; pseudopodia lobopodia or lam-
ellipodia.....Order 1. AMOEBIDA
- Testate or membraned forms.....Order 2. TESTACEA

ORDER 1. **Amoebida** AUT.

- 1. Diphasic forms, amoeboid and flagellated
stages.....Family 1. BISTADIIDAE
- Monophasic forms, amoeboid only..... 2
- 2. Free-living; water, earth, moss, etc..... 4
- Parasites of cavities and tissues..... 3
- 3. Reproduction by binucleated spores
Family 4. SPORAMOEBIIDAE
- Reproduction by division and by uninucle-
ated spores.....Family 2. ENDAMOEBIIDAE
- 4. Without cytoplasmic "Nebenkern"
Family 3. AMOEBIDAE
- With cytoplasmic "Nebenkern".....Family 5. PARAMOEBIIDAE

Family 1. **Bistadiidae** Doflein.

- Two flagella in flagellated phase.....Genus *Dimastigamoeba* Alexeieff
- One flagellum in flagellated phase.....Genus *Craigia* Calkins
- Three flagella in flagellated phase.....Genus *Trimastigamoeba*
Whitmore

Family 2. **Endamoebidae** Calkins.

- 1. Vegetative forms with one nucleus..... 2
- Vegetative forms with two nuclei... Genus *Dicentamoeba*
Jepps and Dobell
- 2. Encysted stage with huge glycogen mass
Genus *Iodamoeba* Dobell
- Encysted stage without large glycogen
mass..... 3
- 3. Individuals of "limax" type..... Genus *Endolimax* Kuenen and
Swellengrebel
- Individuals of amoeba type..... Genus *Endamoeba* Leidy

Family 3. **Amoebidae** Doflein.

- 1. Actively moving forms with lobose pseudo-
podia..... 2
- Sluggish forms; no definite pseudopodia
Genus *Pelomyxa*
- 2. Large forms, several pseudopodia..... 3
- Small forms moving as one pseudopodium. 4

Family 3. **Amoebidae** Doflein.

- 3. Body discoidal with short conical pseudopodia.....Genus *Dactylosphaerium*
Hertwig and Less.
- Body amoeboid with large lobose pseudopodia.....Genus *Amoeba* Ehr.
- 4. Endosome divides without fragmenting
Genus *Fahlkampfia* Chatton and Lalung-Bonnaire
- Endosome fragments forming typical spindle.....Genus *Hartmannella* Alexeieff

Family 4. **Sporamoebidae** Chatton.

- One genus and species.....Genus *Pansporella* Chatton

Family 5. **Paramoebidae** Doflein.

- Free-living or parasitic, one genus.....Genus *Paramoeba* Schaudinn

ORDER II. **Testacea** M. SCHULTZE.

- 1. Tests simple; membranous, plastic or rigid, 2
Tests rigid, with foreign bodies, plates or scales.....3
- 2. Pseudopodia lobose or simply branched
Family 1. ARCELLIDAE
- Pseudopodia reticulate, forming a network
Family 4. GROMIIDAE
- 3. Chitinous test covered by foreign bodies
Family 2. DIFFLUGIDAE
- Chitinous test with plates made by organism.....Family 3. EUGLYPHIDAE

Family 1. **Arcellidae** Schultze.

- 1. Tests membranous and flexible.....2
Tests membranous; rigid; with or without foreign bodies.....9
- 2. Test like inverted watch-glass; aperture full diameter.....3
Test cup-like or sac-like.....1
- 3. Test with hyaline margin (Fig. 188) Genus *Pseudochlamys*
Clap. and Lachm.
- Tests completely hyaline.....Genus *Pycnidicula* Ehr.
- 4. Tests cup-like.....5
Test bag or sac-like.....7
- 5. Margin of test aperture turned in.....6
Test aperture with diaphragm-like membrane.....Genus *Diplochlamys* Greeff
- 6. Cell body uninucleate (*Rhagostoma*) Genus *Amphizonella* Greeff
Body with more than one nucleus.....Genus *Zonomyxa* Nüsslin
- 7. Crown of test with circular and radial ridges.....Genus *Microcorycia* Cockerell
Crown of test simple; aperture an elastic slit.....8
- 8. Test non-encrusted ovoid sac; aperture linear.....Genus *Capsellina* Penard
Test sac-like, covered with foreign bodies
Genus *Parvulina* Penard

Family 1. **Arcellidae** Schultze.

9. Test rigid; chitinous; without foreign bodies..... 10
 Test more or less plastic; one or more pores
 Genus *Cochliopodium*
 Hertwig and Less.
10. Test symmetrical..... 11
 Test asymmetrical (one species encrusted)
 Genus *Lesquerensia* Schlumberger
11. Tests circular in cross-section..... 12
 Tests ellipsoidal in cross-section... 15
12. Free-living forms..... 13
 Parasitic form (Fig. 189)..... Genus *Chlamydothryx*
 Cienkowski
13. Pseudopodia lobose..... 14
 Pseudopodia short lobose with aciculate tip
 Genus *Diffugiella* Cash
14. Aperture of test with inturned margin
 Genus *Arcella* Ehr.
 Margin not inturned; one lobose pseudopodium..... Genus *Leptochlamys* West
15. Tests yellow or brown; minute; without pits..... 16
 Tests hyaline; transparent; usually with pits..... Genus *Hyalosphenia*
16. Mouth oval placed obliquely to ventral surface..... Genus *Wailesella* de Flandre
 Test mouth slit-like, terminal..... Genus *Cryptodiffugia* Penard

Family 2. **Diffugiidae**.

1. Test circular in cross-section..... 2
 Test ellipsoidal in cross-section (compressed)..... 7
2. Aperture of test circular..... 3
 Aperture of test ellipsoidal, linear, or tri-radiate..... 6
3. Aperture without lobed external collar.... 4
 Aperture with three- or four-lobed external collar..... Genus *Cucurbitella* Penard
4. Aperture excentric in position..... Genus *Centropyxis* Stein
 Aperture central; symmetrical..... 5
5. Test covered with diatom shells; pseudopodia pointed..... Genus *Phryganella* Penard
 Test covered with sand, mud, detritus, etc. (Fig. 190)..... Genus *Diffugia* Leclerc
6. Aperture triangular; inner shell about body
 Genus *Cystidina* Volz
 Aperture ellipsoidal..... Genus *Plagiopyxis* Penard
7. Test with constricted neck and internal shelf..... Genus *Pontigulasia* Rhumbler
 Test without internal shelf..... 8
8. Test with foreign particles on dome only... 9
 Test covered; aperture a long and narrow slit..... Genus *Bullinula* Penard
9. Aperture of test convex..... Genus *Heleopera* Leidy
 Aperture small, ellipsoidal, with thickened margins... Genus *Averintzia* Schouteden

Family 3. **Euglyphidae.**

1. Cells without symbiotic algae 2
 Cells with one or two blue-green symbiotic
 algae Genus *Paulinella* Lauterborn
2. Test curved, retort-shape 3
 Test dome-shape; not curved 5
3. Aperture terminal oblique 4
 Aperture terminal not oblique Genus *Nadinella*
4. Test with regular, small plates; no mem-
 brane Genus *Cyphoderia* Schlumberger
 Test with amorphous plates; aperture with
 membrane Genus *Campascus* Leidy
5. Test circular in cross-section 6
 Test ellipsoidal in cross-section (com-
 pressed) 8
6. Dome with single long spine; plates fine
 Genus *Parceuglypha*
 Dome without spine 7
7. Shell-plates form teeth about aperture
 Genus *Euglypha* Duj.
 Aperture with fringed collarette, no teeth
 Genus *Tracheuglypha* de Flandre
8. Test asymmetrical 9
 Test symmetrical 10
9. Aperture circular; oblique; invaginated
 Genus *Trinema* Duj.
 Aperture oval; oblique; not invaginated
 Genus *Corythion* Tarán
10. Test plates circular or oval 11
 Test plates rectangular Genus *Quadrula* Schultze
11. Test hyaline; transparent; plates numerous 12
 Test brown or colorless; aperture oval
 Genus *Assulina* Ehr.
12. Aperture as in *Difflugia*, circular Genus *Nebula* Leidy
 Aperture linear with undulate border
 Genus *Placocista* Leidy

Family 4. **Gromiidae.**

1. With one test aperture 2
 With two or more test apertures
 Sub-family 3. AMPHISTOMINAE
2. Filose pseudopodia directly
 from plasma Sub-family 1. PSEUDOGROMIINAE
 Reticulate pseudopodia from peduncle
 Sub-family 2. ALLOGROMIINAE

Sub-family 1. *Pseudogromiinae* Wailes.

1. Test of one piece 2
 Test bivalved Genus *Clypeolina* Penard
2. Test smooth; no foreign particles Genus *Lecythium*
 Hertwig and Less.
 Test covered with foreign particles 3
3. Test ovoid; no hair-like cirri Genus *Pseudodifflugia*
 Schlumberger
 Test ovoid; flexible; with hair-like cirri
 Genus *Diaphoropodon* Archer

Sub-family 2. *Allogromiinae* Rhumbler.

1. Test ovoid; plastic; aperture lateral. Genus *Liebeckühnia*
Clap. and Lachm.
Test rigid or plastic; aperture terminal. 2
2. Test oval or pyriform; not encrusted. 3
Test cylindrical; encrusted with foreign
bodies. Genus *Rhychozozomia* Rhumbler
3. Test and organism minute; often colonial
Genus *Microzozomia*
Hertwig and Less.
Test large, oval, solitary. Genus *Allogromia* Rhumbler

Sub-family 3. *Amphistominae* Cash.

1. Test with two apertures. 2
Test with from three to six apertures
Genus *Microcometes*
2. Test minute; hyaline; spheroidal; colored
globule. Genus *Diplophrys*
Test medium; oval; encrusted or not; with
symbionts. Genus *Amphitrema*

CHAPTER XIII.

SPECIAL MORPHOLOGY AND TAXONOMY OF THE INFUSORIA.

SINCE the first discovery of *Vorticella* and allied forms of Protozoa by Leeuwenhoek in 1675, the Infusoria have been among the most favored of living things studied through the microscope. The designation Animalculae, given to include all forms of microscopic life was changed by Ledenmüller to Infusoria in 1760-1763, and the entire phylum of Protozoa were included under this term by the majority of writers down to Bütschli in 1882. Dujardin, 1841, divided the "Infusoires" into rhizopods, flagellates and ciliates, a classification adopted by Bütschli who, however, limited the use of the term Infusoria to Protozoa bearing cilia at some period of the life history. Two classes are universally recognized today, the Ciliata with permanent cilia, and Suctoria with cilia in the embryonic phases only. The classification of the Infusoria approaches more closely to an ideal natural system than is possible at the present time with any other group of Protozoa.

In size the Infusoria vary from minute forms, $12\ \mu$ in length (some species of *Cinetochilum*, *Aspidisca*, etc.), to giant ciliates, up to 3 mm. (*Bursaria*, *Lionotus proceros* (Fig. 44, p. 86), *Spirostomum ambiguum*). Size, however, has little taxonomic value.

The great majority of Infusoria are free-swimming but practically all Suctoria and several minor groups of the Ciliata are attached, while a few are parasitic. The majority of attached forms tend to radial symmetry; free-swimming types show the greatest variety of forms which in many cases may be traced to the effects of mode of life, but the fantastic shapes of sapropelic and of many parasitic types are difficult to reconcile with environmental conditions. The ideal generalized form of Ciliata is a spherical or ellipsoidal organism with the mouth at one end, contractile vacuole near the other, and lines of cilia starting from the mouth and running in longitudinal rows down the body. Shifting of the mouth with distortion of the lines of cilia leads to various modifications of the generalized type which is most closely represented by *Holophrya* or *Prorodon* species (Fig. 191). A ventral surface bearing the mouth is established in the Hypotrichida which includes some of the most highly specialized forms of Protozoa.

Tests, cups or "houses" are found here and there throughout the entire group. Gelatinous secretions forming tubes (*Stichotricha*,

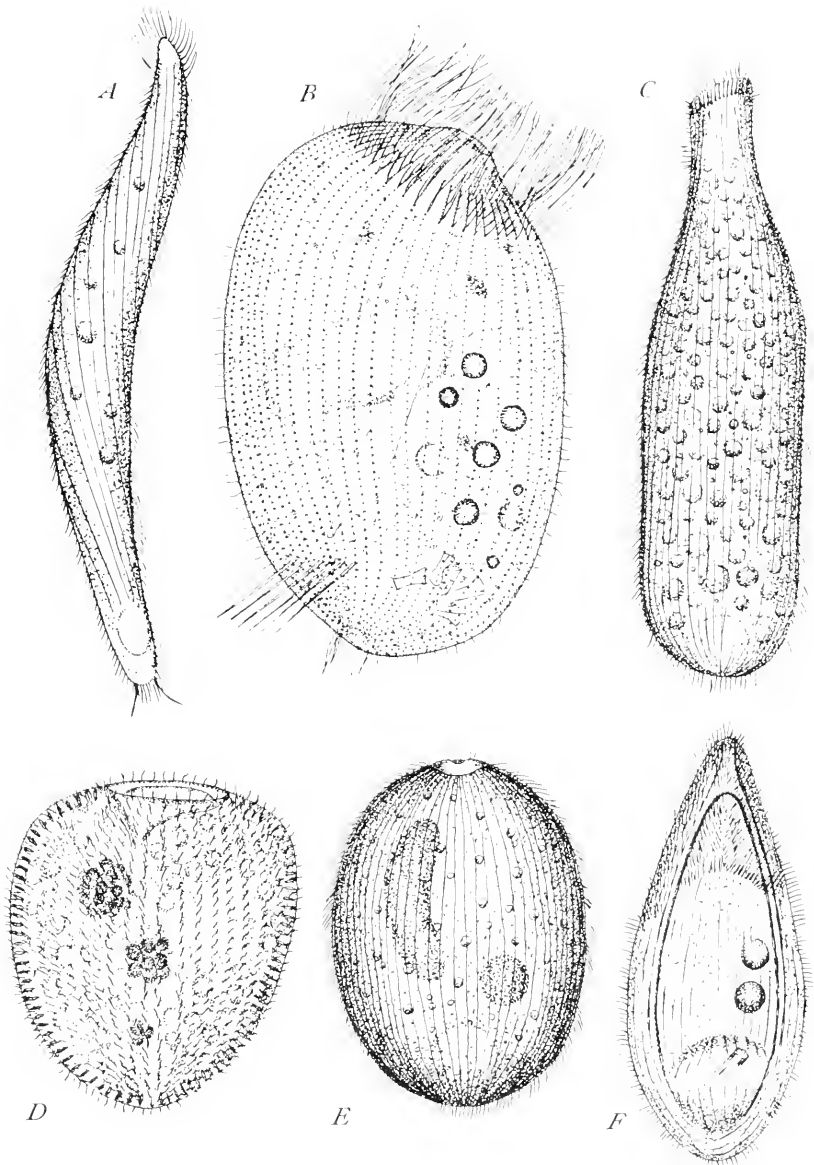


FIG. 191.—Types of Ciliata. A, *Choania teres*, after Calkins; B, *Cyclotrichium oratum*, after Fauré-Fremiet; C, *Enchelys pupa*, after Bütschli; D, *Holophrya gargamellar*, after Fauré-Fremiet; E, *Holophrya discolor*, and F, *Opisthodon mueniensis*, after Bütschli.

Fig. 192; *Stentor*, *Calyptotricha*, etc.) or spheroidal masses (*Ophrydium*) are sometimes found, but cups or "houses" into which the organisms withdraw (*Cothurnia*, *Vaginicola*, *Folliculina*, etc.) or by which they are supported (*Acinetidae*, *Discophryidae*) are more common. Tightly-fitting membranes with sculptured interlocking plates of chitin or pseudochitin are present in *Coleps* and *Tiarina* (Fig. 73, p. 136).

The endoplasm is finely alveolar and much more fluid than the more highly differentiated cortex or ectoplasm. The endoplasm reveals different types of refringent granules during life, some of which have been identified as excretory granules (Prowazek, Nirenstein), others as mitochondria (Fauré-Fremiet, Cowdry) and others as belonging to the Golgi apparatus (Nassonov). In addition to these, reserves of food substances, kinetic elements and metaplastids of different kinds, with the nuclei make up the substance of the endoplasm.

Metaplastids are numerous and widely distributed. Of these trichites, trichocysts and "pharyngeal baskets" are the most characteristic. Trichites are elongate, slender rods usually surrounding the mouth in gymnostomes and are generally interpreted as organs of support or protection. They are not limited to the oral region, however, and in some forms provide a protective cuirass about the posterior region (*Strombidium*). The oral trichites are numerous and closely applied and in some cases form a continuous and smooth tube extending deep in the endoplasm (some *Nassulas*, *Orthodon*, etc.). Trichocysts are shorter and more conspicuous; formed in the endoplasm they assume a radial position in the cortex and may cover the entire surface (*Paramecium*, Fig. 193; *Frontonia*, etc.) or may be limited to certain regions (*Dileptus* proboscis, Fig. 194). In a moving *Actinobolina* they are arranged as in *Paramecium*, but in a quiescent individual each trichocyst is carried out at the end of a long tentacle which this interesting ciliate has the power to protrude for feeding purposes (Fig. 91, p. 163).

The function of the trichocysts is still in dispute (Visscher, 1923). The substance of a trichocyst may be shot out in the form of a long thread which hardens on contact with water. In such forms, repre-



FIG. 192.—*Stichotricha secunda*, a tube-dwelling hypotrichous ciliate. (Original.)

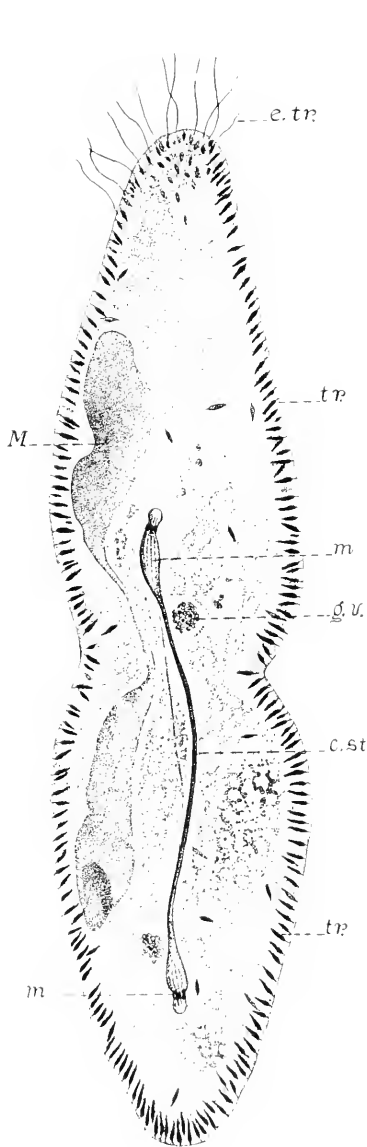


FIG. 193

FIG. 193.—*Paramecium caudatum*. Section of a dividing individual. *c.st.*, connecting strand of dividing micronuclei; *e.tr.*, extruded trichocysts; *g.v.*, gastric vacuole; *M*, dividing macronucleus; *m*, *m*, divided micronuclei; *tr.*, trichocysts. (Original.)



FIG. 194

FIG. 194.—*Dileptus anser*, with beaded macronucleus and twisted proboscis. (Original.)

sented by *Paramecium*, *Frontonia* and other related forms, there appears to be no toxic action connected with the trichocysts, the threads affording protection by the formation of a net-like web about the organism. In other cases, however, there is considerable evidence of toxic action and in such types the long threads are not formed. Visscher (1923) has described such toxic action on the part of the trichocysts of *Dileptus*, and the sudden paralysis of *Halteria grandinella* upon coming in contact with a tentacle of *Actinobolina* is interpreted as due to the toxic action of the minute trichocyst at the extremity of the tentacle (Calkins, Moody). In *Didinium nasutum* there is a zone of rods quite independent of the

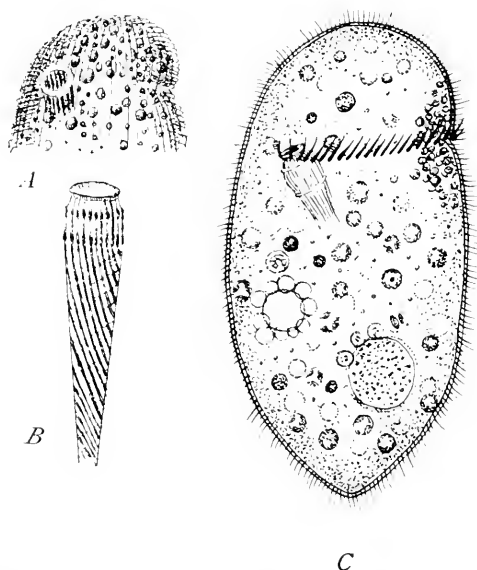


FIG. 195. --*Nassula aurca* (C) and details of basket (A, B), after Bütschli.

pharyngeal trichites and interpreted as trichocysts near the extremity of the seizing organ of this voracious animal (Fig. 98, p. 187). A *Paramecium* jabbed by this proboscis in one of the vigorous darts of *Didinium* is immediately paralyzed and the poisoning is attributed to the trichocyst material. While this interpretation is plausible it cannot be regarded as proved, and it must be admitted that the protoplasm itself may carry the toxic substance. Thus in the Suctoria a ciliate or other small organism is similarly paralyzed upon coming in contact with an outstretched tentacle in which no trichocysts can be demonstrated.

Pharyngeal baskets are characteristic of the Chlamydomontidae where they form conspicuous oral armatures (Fig. 195). The

elements forming the basket are much larger than trichites and are frequently combined in such a manner as to justify the term basket. The rods are usually constant in number in a species and may be united to form a tube at the posterior end of the basket or in some cases may be united throughout. In *Chilodon* the basket is protrusible and serves a useful purpose in food-getting. According to MacDougall (1925) the basket is dissolved in artificial gastric juice (pepsin) indicating a protein composition.

Metaplastic substances frequently appear in the form of pigments which impart a characteristic color to a species. These are probably connected with food metabolism and disappear in the absence of appropriate food materials. Thus the blue pigment "stentorin" of *Stentor coerulesus*, or *Folliculina* or the lavender of *Blepharisma undulans*, the red of *Mesodinium rubrum*, the black spot of *Tillina magna*, etc., are coloring matters of this type. Fats and oils also are frequent inclusions and when brilliantly colored, as in *Nassula aurea*, give a striking and a pleasing picture as the organism rolls through the water.

Symbionts are of frequent occurrence and give to *Paramecium bursaria*, *Stentor viridis*, *Ophrydium versatile* and some *Vorticella* species a bright green color.

Contractile vacuoles are practically universal among ciliates and Suctorina. Held in place in the denser cortex they never move about with cyclosis. They empty to the outside through a covered but thinned orifice in the cortex, the covering being liquefied at systole (Taylor, 1923). The vacuole system often includes canals and reservoirs, reaching a high degree of specialization in some forms, and ciliated excretory canals are said to be present in a few parasitic types (*Pycnothrix*, Schubotz, 1908).

The Infusoria are unique in having an almost universal nuclear apparatus in the form of dimorphic nuclei, macronucleus and micronucleus. Of these the macronucleus is large and usually homogeneous in structure (granular) and is highly variable in shape in different species. In some forms it is multiple and formed by repeated division of an original single nucleus (*Uroleptus*); in other cases attempted division results in a chain of nuclei connected by a common nuclear membrane, giving rise to "beaded" nuclei (*Stentor*, *Spirostomum ambiguum*, *Uronychia transfuga*, etc.). It is frequently rod-shape as in *Diplodinium* (Fig. 2, p. 20), or horse-shoe shape as in *Vorticella*, or very much branched as in *Dendrosoma*, *Ephelota* and other Suctorina (Fig. 196).

Micronuclei are minute and are usually partially embedded in the substance of the macronucleus. There is but little variation in form of the micronucleus in different species, but there is a great variation in the number present. In *Paramecium caudatum* and *P. bursaria* there is but one, while in *P. aurelia* and *P. calkinsi*

there are two, in *P. multimicronucleata* there are many and two are characteristic of the Oxytrichidae, etc. The number of micronuclei runs up to eighty or ninety in *Stentor* and the number is intermediate in several other genera.

Macronuclei are generally regarded as "somatic" nuclei with an important part to play in general metabolism. They disappear by absorption and are replaced by products of micronuclear division at periods of reorganization by "endomixis," or by products of amphinuclei after conjugation. Chromosome formation, with a definite number of chromosomes, has been made out for a number of species

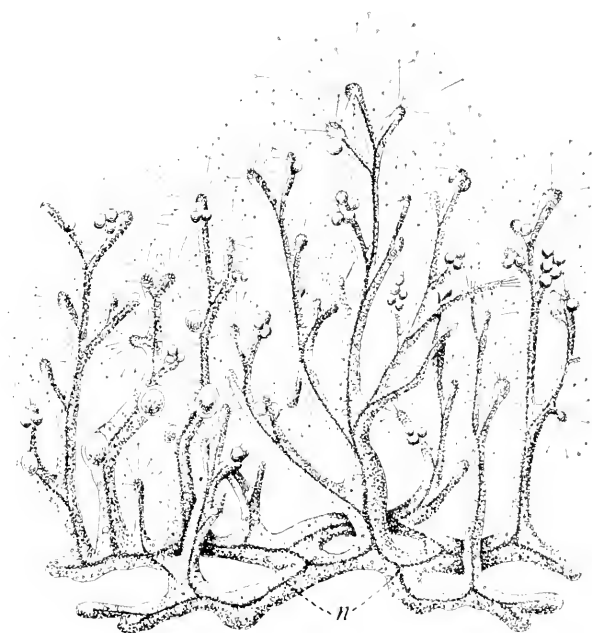


FIG. 496.—*Dendrosoma elegans*; *n*, nucleus. (From Calkins after Kent.)

of ciliates, but no definite chromosomes have been described from macronuclei. Evidence is accumulating to indicate that the micronucleus is the essential element of the cell in conjugation but other evidence is at hand to show that it is not essential for continued vegetative life or for reproduction by cell division. Thus amicro-nucleate races of *Paramecium*, *Didinium*, *Spathidium*, *Oxytricha*, etc., have been maintained for long periods by Woodruff, Dawson and others, while Maupas, Calkins and others have shown that the micronucleus may disappear in long-continued cultures of hypotrichous forms, although the organisms are still able to divide (p. 256). It is evident that different macronuclei represent different

degrees of specialization and that some forms may carry on all processes of asexual activity without a micronucleus and these may represent transition stages to the condition in opalinids in which there is no nuclear dimorphism at all and both sexual and asexual processes are possible with only one type of nucleus. According to McNally this is the condition in *Nassula ornata* or *N. elegans*.

The kinetic elements, including cilia and their derivatives and coordinated systems of intracellular fibrils, represent a neuromotor apparatus even more complex than that of the higher flagellates. In but few cases are there combinations of other types of motile organs with cilia. One such case is described by Penard under the name *Myriaphrys paradoxa*, a form with axopodia and cilia (Fig. 197); another is a combination of cilia with a flagellum.

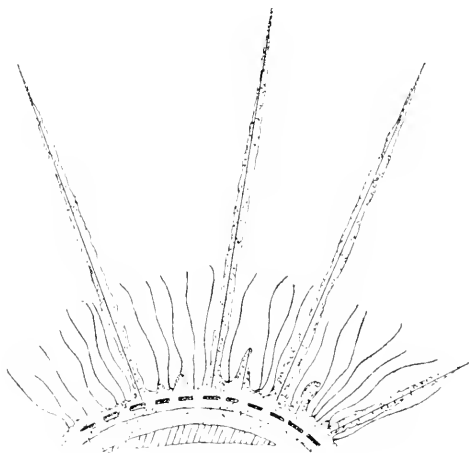


FIG. 197.—*Myriaphrys paradoxa* (?), with cilia and axopodia. (After Penard.)

Monomastix ciliatus described by Schewiakoff. The possibility of the derivation of ciliates from flagellates, in some cases through Heliozoa-like forms, is suggested by such types, but origin of this group involves far too much speculation for serious consideration.

Cilia, by fusion, form locomotor organs of complex nature (see Chapter IV). Undulating membranes, membranelles and cirri are present in the majority of ciliates. A fourth type of combination, membranulae or pectinelles, combines several of the features of flagella. Thus the powerful motile organs of *Didinium* are composed of a few flagella-like, long cilia, while rhizoplasts rim from their basal bodies to the vicinity of the nucleus (Fig. 98, p. 187).

Undulating membranes are limited regionally, to the gullet, margin of the mouth or to a circumscribed area called the peristome. Membranelles are grouped usually in a curved row, the "adoral

zone," around the margin of the peristome, but a dorsal ring of membranelles is present in some parasitic forms (*e. g.*, *Diplodinium*, Fig. 2, p. 20). Here also limited "arches" of apparent membranelles are variously distributed about the body (Fig. 14). Cirri are combinations of cilia of usually the ventral surface, but they may encroach on the dorsal surface (*e. g.*, *Uronychia*); they form groups, as a rule, named according to their position, frontal, ventral, anal and caudal cirri, the number and arrangement forming a basis for diagnosis of genera and species.

At the present time there is need of a more precise characterization of cirri. The Order Hypotrichida, for example, is described by Kahl (1931) as including forms which possess no cilia but only membranelles, membranes and cirri. This distinction upsets the classification, in use for half a century, as given by Stein. Having no cirri, the former hypotrich family Peritromidae is removed to the Heterotrichida while the accepted cilia of the Urostylidae (here included in the Oxytrichidae) are now regarded as simple combinations of cilia, *i. e.*, cirri. Marginal cirri are more complex, frontals and anals still more so, while the great steering and jumping organs of *Uronychia*, *Diophrys*, etc., certainly call for more descriptive terms than cirri. Temporarily the need may be met by use of the expressions: simple cirri, caudal cirri, tactile cirri, frontal, anal and marginal cirri and giant cirri.

The activities of the motile organs are coördinated through a system of longitudinal and transverse fibrils connecting the basal fibrillae coming from the cilia or groups of cilia (p. 152). A coördinating center, termed the motorium, regarded by numerous observers as an artefact (Rees, 1931; Turner, 1933, etc.) has been demonstrated in some forms (*Diplodinium* Sharp, 1914; *Euplores* Yocom, 1918; *Balantidium* MacDonald, 1922; Kidder, 1932, *et al.*).

The "silver line" system, discovered by Klein, is a complex meshwork of granules and fibrils in the cortex arranged in patterns which appear to be characteristic of different species. This, apparently, is a universal coördinating system of the Infusoria (see p. 80).

Myonemes also are widely distributed in the group. In *Steutor* they lie in superficial canals within the cortex and in some cases appear to be conducting as well as contractile elements. In *Epistylis* Schröder has described myonemes running longitudinally from the stalk to the peristome where they terminate in the basal plates of the membranelles (Fig. 70, p. 126); distally they combine to form the contractile strand of the stalk.

A well-defined mouth is present in almost all ciliates (absent in an entire group, only in Astomida). In gymnostomida it is closed save at times of food ingestion; in all other groups it is permanently open. In these latter cases the form of the mouth varies from circular to elliptical, crescentic or triangular openings and in

the majority of cases the mouth leads into a ciliated gullet. Such constant feeders are limited to a bacterial diet and other minute food substances while the gymnostomes, by reason of the distensibility of the oral region are able to take in living organisms even larger than themselves (see p. 186 and Fig. 98).

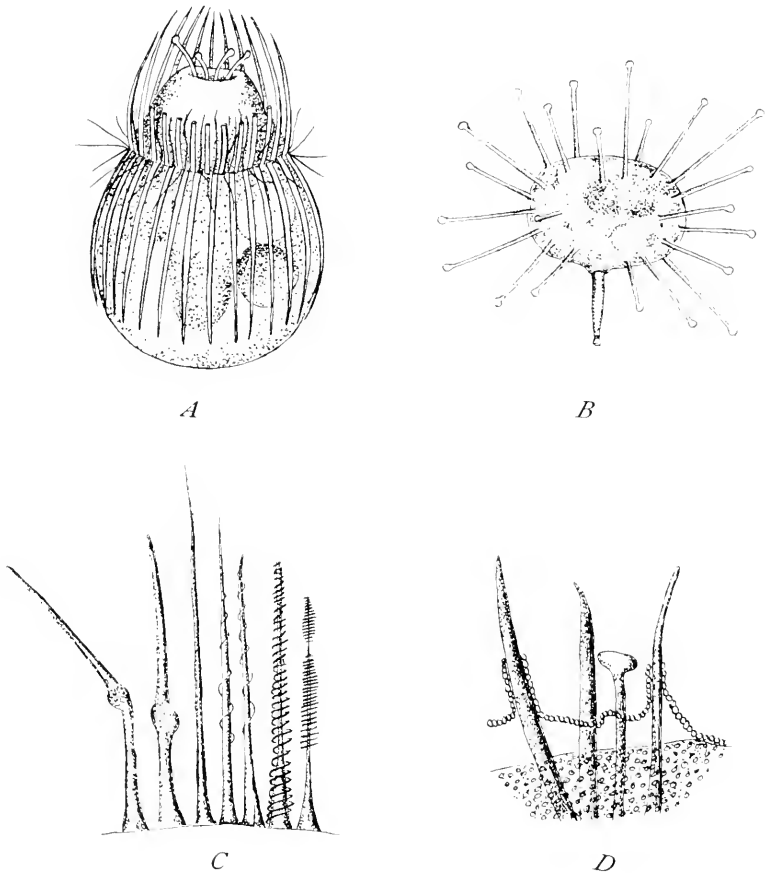


FIG. 98. —Tentacles of Infusoria. A, *Mesodinium pulx*, with four oral tentacles for adhering; B, *Podophrya fura*; C, D, tentacles of Ephelotidae. (A, C, D, from Calkins; B, original.)

In Suctorina, food-taking is of an entirely different type. Mouths are absent but food may be taken in through any one of the many suctorial tentacles. The body wall of a captive organism is cytolized at the point where the tentacle is in contact and the endoplasm of the prey either passes in a stream through the lumen of the tentacle, or the endoplasm of the captor enters the body of the

victim and digests its endoplasm *in situ* (Maupas, 1883). Tentacles for adhesion are also present in *Mesodinium* (Fig. 198).

While the vast majority of Infusoria are holozoic in food-getting, parasitic types may be holozoic or saprozoic (*Astomida*). Proteins are digested by all and carbohydrates in some (*Balantidium* Glaessner, see p. 198).

Adaptations for food-getting, protection during ingestion and other differentiations in the service of nutrition are responsible for most of the cortical structures of the derived organization and these for the most part determine the taxonomic position of genera. Trichocysts, trichites, pharyngeal baskets, etc., have been described (see p. 473). Adhesive discs (Lichnophoridae, Urceolariidae); thigmotactic cilia or special cilia for attachment ("Thigmatricha" of Chatton, Boveriidae, Aucistrumidae, Conchophthiriidae) and suckers for attachment (some *Astomida*, *Mesodinium*, etc.) or for food-getting (Hypocomidae, Suctoria) are widely distributed. The most important, taxonomically, of all of these adaptations are those associated with the ciliate mouth. In the classification adopted here we follow the recent trend (Poche, Kahl, Reichenow-Doflein, *et al.*) in ciliate morphology in which the oral apparatus together with position on the body are primary diagnostic characters. The absence of an adoral zone of membranelles about the mouth (peristome) distinguishes the sub-class Holotricha from other ciliates. The direction of curvature of the adoral zone, and spiral rows of cilia in the sub-class Chonotricha distinguishes the sub-class Peritricha. Here, however, some confusion results from use of the terms left-wound and right-wound. Obviously a left-wound spiral becomes a right-wound spiral if the start is made from the end away from the mouth. Stein, Bütschli and others, until quite recently, viewed the spiral as starting from the mouth and interpreted the adoral zones of *Peritromus*, *Stentor*, *Stylonychia*, *et al.*, as wound to the left, whereas in the *Peritricha* it winds to the right. Kahl, Reichenow-Doflein and other recent writers view the spiral as starting from the end farthest away from the mouth with a corresponding reversal in use of the descriptive terms left and right. Since the stroke of the membranelles and the food currents are toward the mouth, the modern point of view probably has more justification than the older one and is adopted here. It makes a difference furthermore whether the organism is viewed from the ventral or dorsal aspect; for right and left as used above the organism is viewed from the oral side.

The mouth proper may be provided with simple cilia or combinations of cilia, or void of cilia altogether. Those without motile elements are grouped in the order Gymnostomida established by Bütschli. These in turn are distributed in sub-orders according to the position of the mouth. In the sub-order Prostromina the

mouth is at the anterior end of the body and such forms are still regarded as the most generalized types of ciliates. In the sub-order Pleurostomina the mouth is no longer terminal but occurs as an elongated slit (*Amphileptus*, *Lionotus*) or as a circular opening at the base of a more or less pronounced proboscis (*Dileptus*, *Tracheilus*, etc.). In the sub-order Hypostomina the mouth is on the physiologically ventral side as in *Nassula*, *Chilodon*, etc.

The orders Trichostomida and Hymenostomida include forms in which the mouth is provided with cilia or with membranes, free cilia in Trichostomida and undulating membranes in Hymenostomida. There is no great difference between these two orders, and it is frequently difficult to determine whether a particular form belongs to one or the other. Lines of cilia in the gullet, as in *Paramecium*, often give the impression of an undulating membrane.

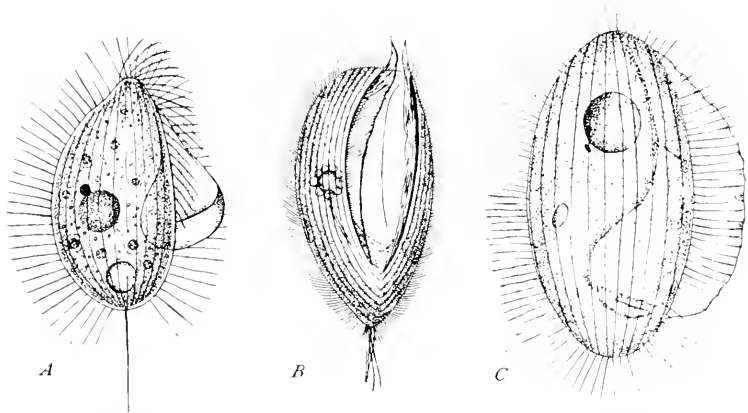


FIG. 199.—Types of ciliates. A, *Cycloidium glaucoma*; B, *Lembadion ballinum*; C, *Pleuronema chrysalis*. (A, C, after Calkins; B, after Bütschli.)

In Hymenostomida the mouth, as a rule, is more complex than in Trichostomida. Undulating membranes surrounding it (peristomial) are frequently enormously developed (*Pleuronemidae*, Fig. 199), forming sail-like traps for food bodies. In other cases the membranes are inside an oral pit or vestibule and such mouth parts are very complicated (Fig. 8, p. 29).

In the sub-class Spirotricha we find the most spectacular types of ciliates; some are huge (*Bursariidae*, *Condylostomidae*, *Stentoridae* of the order *Heterotricha*); some are spirally twisted (*Metopidae*); some highly flexible (*Lichnophoridae*).

Cilia, in addition to the adoral zone of membranelles, cover the body in the majority of *Heterotrichida*—but are greatly reduced or absent in the *Oligotrichida* and *Hypotrichida*, where in the latter

they are replaced in part or entirely by cirri. Here, also, particularly in parasitic forms (Ophryoscolecidae and Cycloposthiidae), the periplast is well developed and the organisms are frequently characterized by fantastic sculpturing (Fig. 146, p. 293).

The sub-class Chonotricha includes a small number of forms with highly exaggerated peristomial structures. These are spirally wound (to the right) in Spirochonidae but are funnel-shape in Chilodochonidae. The characteristic reproduction by budding in these forms suggests a relationship to the Suctoria.

Parasitism in Infusoria, as in other great groups of Protozoa, is widely spread and some of the adaptations to this end merit special consideration. The majority are apparently harmless commensals of digestive tract and body cavity; some, however, are more serious, *Balantidium coli* for example, causing acute enteritis in man and other mammals. Ectoparasitic forms may also be a source of trouble. *Amphileptus branchiarum* gets under the gill mantles of tadpoles and ingests groups of epithelial cells (Wenrich); others form peculiar arms by which they are anchored to gill bars (*Ellobiophrya donavisi* Chatton, Fig. 104, p. 202). In the main, related forms are not strictly parasitic but are attached in gill chambers where a constant supply of food is assured. Special attaching organs, arising from specially modified cilia, are characteristic of holotrichous and of some peritrichous forms. These are best developed in *Trichodina* (common on *Hydra*) where a special attaching organ termed the scopula is characteristic, while the two arms of *Ellobiophrya* mentioned above are interpreted by Chatton as representing a split scopula. Amongst the Holotrichida, ectoparasitism is characteristic of the group which Chatton calls the Thigmotricha (1923). Here a portion of the posterior ciliated region termed the "thigmotactic area" becomes modified as an attaching organ. It is a sucking disc in *Ptychostomum*, a protrusible tentacle in *Hypocomides* and *Hypocoma* which Chatton, correctly, removes from the Suctoria to the Holotrichida. It is rudimentary in *Plagiospira* and not at all evident in *Boreria*. Two types of feeding adaptations are evident in these forms. In one series the peristome and adoral zone become greatly enlarged, forming a helicoid spiral in *Boreria*, *Plagiospira*, *Hemispira* and *Ancistruma*, capable of drawing in food particles from a distance. In another series the oral apparatus becomes rudimentary or lost altogether, food substances being absorbed by osmosis through the general body wall or by tentacles only as in *Hypocoma* and *Hypocomides*.

Lumen-dwelling forms have apparently undergone less degeneration than have ectoparasitic types. In the Astomida such degeneration has been the most extreme. Here mouth and other oral structures are entirely wanting and nutrition is osmotic. In the majority of cases, however, the peristome and mouth are retained

while the cortex is often highly sculptured and fantastic as in the Ophryoscolecidae.

The aberrant Opalinidae are parasitic in Amphibia. Not only are they astomatous, but in certain characters they differ widely from other ciliates so that they have been variously placed in classification. Hartog (1906), for example, placed them with the Hypermastigida of the flagellates. Metcalf (1918, 1923) includes them as Prociliata sharply marked off from the remaining ciliates. In view of the adaptive changes brought about by a parasitic mode of life, it seems more probable that they are degenerate rather than primitive types. There are invariably two or more nuclei but the nuclei are identical with no indication of dimorphism. In the nuclei, however, there are two kinds of chromatin according to Léger and Duboscq (1904) and Metcalf (1909 and 1923). The latter distinguishes these types as "macrochromatin" and "microchromatin," the former in mitosis giving rise to band-form "macrochromosomes," the latter to "microchromosomes" in apparently even numbers (from two to ten). The "macrochromatin" is regarded as functional in vegetative life and, like the macronucleus of other ciliates, gives rise to chromidia (Neresheimer) or otherwise fragments preparatory to absorption in the cell. The "microchromatin" on the other hand is functional during sexual phases. From these considerations it would appear that the dimorphic nuclear conditions of ciliates generally is here represented by each nucleus, but the hypothesis is questionable.

In their sexual phenomena, also, the Opalinidae differ from the majority of other ciliates. Individuals begin to divide rapidly with decreasing size until minute forms result with one, two or more nuclei according to species (Neresheimer, Metcalf). These encyst, the cysts passing out with the feces. Tadpoles ingest the cysts which open in the rectum, giving rise to the same type that had previously encysted. These now multiply, ultimately forming macrogametes and microgametes which fuse on contact. The zygote has one nucleus at first which later gives rise to the binucleated or multinucleated forms, although the exact manner has not been described (Metcalf, 1923).

Reproduction in ciliates generally is typically by binary cross-division and involves a renewal of motile organs, at least this is the case in forms with cirri, and MacDougall (1925) gives evidence to indicate that cilia also are similarly renewed. It thus results that motile organs of both products of cell division are proportionate to the size of the young individuals. Old metaplastids, as pharyngeal baskets, are discarded and new ones are formed in both halves. Nuclear changes during division are quite varied, each species having its own peculiarities of macronuclear condensation and reformation.

Unequal division or budding, while uncommon among ciliates,

is the chief method of reproduction among the Suctoria but also occurs in the Chonotricha (*Spirochona*, etc.). In Suctoria, budding is either external or internal, in the latter case the budding area is invaginated, the margins close over, and a brood chamber is formed from which the embryos escape when formed.

Multiple division or sporulation is also uncommon in the Ciliata, but occurs in some of the more generalized and in some parasitic types. When it occurs it is usually under the protection of a temporary cyst (*Colpoda*, *Ichthyophthirius*).

Sexual processes are practically universal in the group and the main features of the process are similar throughout. In most cases fusion is temporary and pronuclei are exchanged after which the conjugants separate. In some cases, Vorticellidae, fusion is permanent and sexual dimorphism is the rule, in other cases such dimorphism is expressed by the pronuclei, but in most cases there is

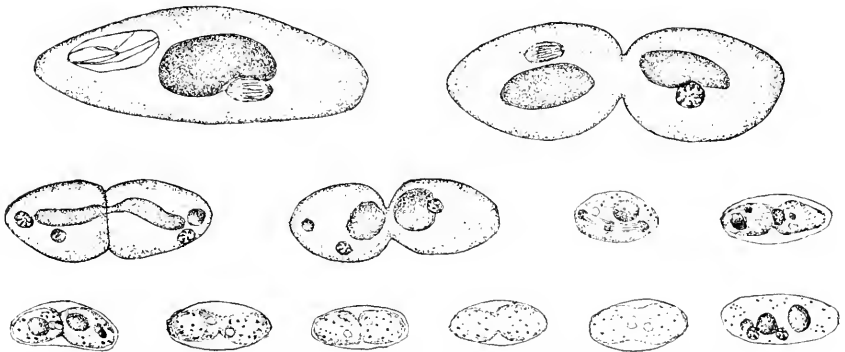


FIG. 200.—*Glaucoma (Dallasia) frontata*. Successive stages leading to the formation of copulating isogametes. (After Calkins and Bowling.)

no sex differentiation whatsoever (see Chapter VIII). In *Trachelocerca phoenicopterus*, *Ichthyophthirius multifiliis*, *Glaucoma (Dallasia) frontata* and in Opalinidae the fertilization phenomena do not follow the usual routine of other ciliates, microgametes being formed and fusion being permanent.

Glaucoma (Dallasia) frontata illustrates a most unusual sexual phenomenon. Here there are two types of fertilization, one by the fusion of gametes, the other by typical conjugation (Figs. 200, 201).

Conjugation always results in physical reorganization of the protoplasm, the old macronucleus is broken up and the fragments are absorbed in the cytoplasm, while a new macronucleus and new micronuclei are differentiated from products of the first or second division of the amphinucleus after fertilization (see Chapter VIII). A similar reorganization takes place at regular intervals of thirty days (*P. aurelia*) or sixty days (*P. caudatum*) according to Woodruff and

Erdmann (1914) who termed the phenomena accompanying this method of reorganization "endomixis" (p. 317). In other types of

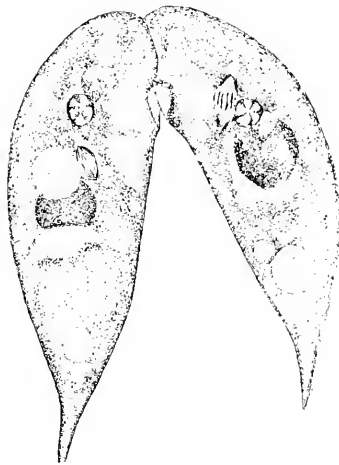


FIG. 201.—*Glaucoma (Dallasia) frontata*. Normal conjugation occurring later in the life history. (After Calkins and Bowling.)

ciliates similar asexual processes of reorganization take place under the protection of a cyst (for significance of reorganization see Chapter IX).

CLASSIFICATION OF THE INFUSORIA.

SUB-PHYLUM. **INFUSORIA** LEDENMÜLLER; EM. BÜTSCHLI.

Class 1. **Ciliata** Perty; em. Bütschli.

Sub-class I. HOLOTRICHA Stein

Order 1. *Astomida* Cepede

- Family 1. *Opalinidae* Stein
- Family 2. *Anoplophryidae* Cepede
- Family 3. *Chromodinidae* Cheissin
- Family 4. *Haptophryidae* Cepede
- Family 5. *Intoshellinidae* Cepede
- Family 6. *Hoptitophryidae* Cheissin

Order 2. *Gymnostomida* Bütschli

Sub-order 1. PROSTOMINA (Prostomata Schewiakoff)

- Family 1. *Holophryidae* Perty
- Family 2. *Actinobolinidae* Kent
- Family 3. *Metacystidae* Kahl
- Family 4. *Didiniidae* Poche
- Family 5. *Colepidae* Kent
- Family 6. *Spathidiidae* Kahl
- Family 7. *Bütschliidae* Poche

Sub-order 2. PLEUROSTOMINA (Tribe Pleurostomata Schewiakoff)

- Family 1. *Amphileptidae* Schouteden
- Family 2. *Tracheliidae* Ehr.
- Family 3. *Lorodidae* Roux.

Class I. **Ciliata** Perty; em. Bütschli.

Sub-class I. HOLOTRICHA Stein

Order 2. *Gymnostomida* Bütschli

Sub-order 3. HYPOSTOMINA Schewiakoff

Family 1. *Nassulidae* SchoutedenFamily 2. *Chlamydotontidae* Claus.Family 3. *Dysteridae* Clap. and Lach.Family 4. *Pycnothricidae* Poche (Nicollellidae Ch. and Pe.)Family 5. *Foettingeriidae* Reichenow-Doflein.Order 3. *Trichostomida* BütschliFamily 1. *Sciadostomidae* KahlFamily 2. *Spirozonidae* KahlFamily 3. *Trichospiridae* KahlFamily 4. *Plagiopyglidae* SchewiakoffFamily 5. *Clathrostomidae* KahlFamily 6. *Colpodidae* PocheFamily 7. *Parameciidae* GrobbenFamily 8. *Marynidae* PocheFamily 9. *Trichopelmidae* KahlFamily 10. *Conchophthiriidae* Reichenow-DofleinFamily 11. *Hypocomidae* BütschliFamily 12. *Boveriidae* PickardFamily 13. *Ancistrumidae* IsselFamily 14. *Isotrichidae* BütschliFamily 15. *Paraisotrichidae* da CunhaFamily 16. *Blepharocoridae* HsiungFamily 17. *Cyathodiniidae* da CunhaOrder 4. *Hymenostomida* HicksonFamily 1. *Frontoniidae* KahlFamily 2. *Ophryoglenidae* KentFamily 3. *Phitasteridae* KahlFamily 4. *Lembidae* KahlFamily 5. *Pleuronemidae* KentFamily 6. *Hemispeiridae* (Hemispeirinae König)

Sub-class II. SPIROTRICHA Bütschli 1889; em. Kahl

Order 1. *Heterotrichida* SteinFamily 1. *Metopidae* KahlFamily 2. *Reichenowellidae* KahlFamily 3. *Spirostomidae* KentFamily 4. *Plagiotomidae* PocheFamily 5. *Condylostomidae* KahlFamily 6. *Stentoridae* Carus (Claus?)Family 7. *Folliculinidae* DonsFamily 8. *Bursariidae* PertyFamily 9. *Peritromidae* SteinFamily 10. *Lichnophoridae* StevensOrder 2. *Oligotrichida* Bütschli 1889Family 1. *Halteriidae* Clap. and Lach.Family 2. *Strombilitidae* KahlFamily 3. *Tintinnidae* Clap. and Lach.Family 4. *Ophryoscotecidae* Claus. (?)Family 5. *Cycloposthiidae* PocheOrder 3. *Ctenostomida* LauterbornFamily 1. *Epalcidae* WetzelFamily 2. *Milestomidae* KahlFamily 3. *Discomorphidae* Poche

ORDER 1. **Astomida.***Key to Families*

1. Without dimorphic nuclei Family 1. OPALINIDAE
 - With dimorphic nuclei 2
 2. Skeleton organs or attaching structures absent 3
 - Skeleton structures or attaching organs present 4
 3. Contractile vacuoles absent or scattered; macronucleus spheroidal, elongate or branched Family 2. ANOPLOPHRYIDAE
 - Contractile vacuole absent; macronucleus fragmented Family 3. CHROMIDINIDAE
 4. Vacuole a dorsal canal Family 4. HAPTOPHRYIDAE
 - Vacuoles in rows or distributed 5
 5. Without supporting elements in cortex Family 5. INTOSHELLINIDAE
 - With supporting elements in cortex Family 6. HOPLITOPHRYIDAE
- Family 1. **Opalinidae** Claus.
1. Form cylindrical; circular in cross-section 2
 - Form flattened; ellipsoidal in cross-section 3
 2. With two similar nuclei Genus *Protoopalina* Metcalf
 - With many similar nuclei Genus *Cepedea* Metcalf
 3. With two similar nuclei Genus *Zelleriella* Metcalf
 - With many similar nuclei Genus *Opalina* Purkinje
- Family 2. **Anoplophryidae** Cepede 1910.
1. Body without anterior sucker 2
 - Body with anterior sucker 8
 2. Without anterior protoplasmic process 3
 - With anterior protoplasmic process 7
 3. Cilia in longitudinal, not spiral, lines 4
 - Cilia in spiral lines Genus *Orchitophrya* Cepede
 4. Contractile vacuoles present 5
 - Contractile vacuoles absent Genus *Metaphrya*
 5. Contractile vacuoles multiple, in rows
 - Genus *Anoplophrya* Dujardin
 - C. V. single, posterior 6
 6. Body pyriform; C. V. sub-terminal Genus *Kofoidella* Cepede
 - Body ellipsoid; C. V. terminal Genus *Perezella* Cepede
 7. C. V. single; macronucleus spheroidal
 - Genus *Herpetophrya* Siedlecki
 - C. V. numerous, in one row; macronucleus elongate Genus *Bütschliella* Awerinzew
 8. Macronucleus spheroidal Genus *Cepedella* Poyarkoff
 - Macronucleus branched Genus *Rhizocaryum* Caul. et Mes.
- Family 3. **Chromidinidae** Cheissin 1930.
One genus only, *Chromidina* (including *Opalinopsis*)
- Family 4. **Haptophryidae** Cepede 1923.
1. Body without sucker; with hook Genus *Lachmanella* Cepede
 - Body with sucker; with or without hooks 2
 2. Body with sucker and two hooks Genus *Steinella* Cepede
 - Highly developed sucker; no hooks or skeleton Genus *Haptophrya* Stein

Family 5. **Intoshellinidae** Cepede 1910.

1. Skeleton elements in form of collar with six spines Genus *Intoshellina* Cepede
2. Skeleton in form of circular disc with teeth
Genus *Monodontophrya*
Vejdowsky

Family 6. **Hoplitophryidae** Cheissin 1930.

1. Spine simple, projecting from anterior end. 2
Spine or skeleton entirely embedded. 3
2. Spine extends beyond anterior end. . Genus *Maupasella* Cepede
Spine ends as an apical point Genus *Protoradiophrya* Rossolimo
3. Spine arrow-like, barbed; none in cortex. . 4
Body with spines in cortex. 5
4. Spine with simple barb; one row of contractile vacuoles. Genus *Mesnillella* Cepede
Spine a tripartite spicule (Fig. 202). . Genus *Hoplitophrya* Stein
5. Body vermiform, not swollen anteriorly
Genus *Radiophrya* Rossolimo
Body vermiform, much swollen anteriorly
with radiating spines Genus *Mrazekiella* Kijenski

ORDER 2. **Gymnostomida.***Key to Sub-orders and Families*

1. Mouth at anterior pole or in immediate vicinity Sub-order 1. PROSTOMINA
Mouth lateral or ventral. 2
2. Mouth lateral; slit-like or round
Sub-order 2. PLEUROSTOMINA
Mouth on anterior half of flat-ventral side Sub-order 3. HYPOSTOMINA

SUB-ORDER 1. **Prostomina (Prostomata** SCHEWIAKOFF).

1. Free-living forms 2
Parasitic forms Family 7. BÜTSCHLIIDAE
2. Mouth region laterally compressed with trichites Family 6. SPATHIDIIDAE
Mouth region not compressed, round cross-section, no neck. 3
3. Mouth opens into receptaculum in anterior part of body (test-dwelling). . . Family 3. METACYSTIDAE
Mouth without receptaculum (not test-dwelling). 4
4. Mouth at tip of apical mound surrounded by circle of motile organs. . . Family 4. DIDINIIDAE
Mouth otherwise. 5
5. Body covered by ectoplasmic, perforated plates. Family 5. COLEPIDAE
Body surface otherwise. 6
6. Body with radially arranged, retractile tentacles (pseudop.). Family 2. ACTINOBOLINIDAE
Body without tentacles Family 1. HOLOPHRYIDAE

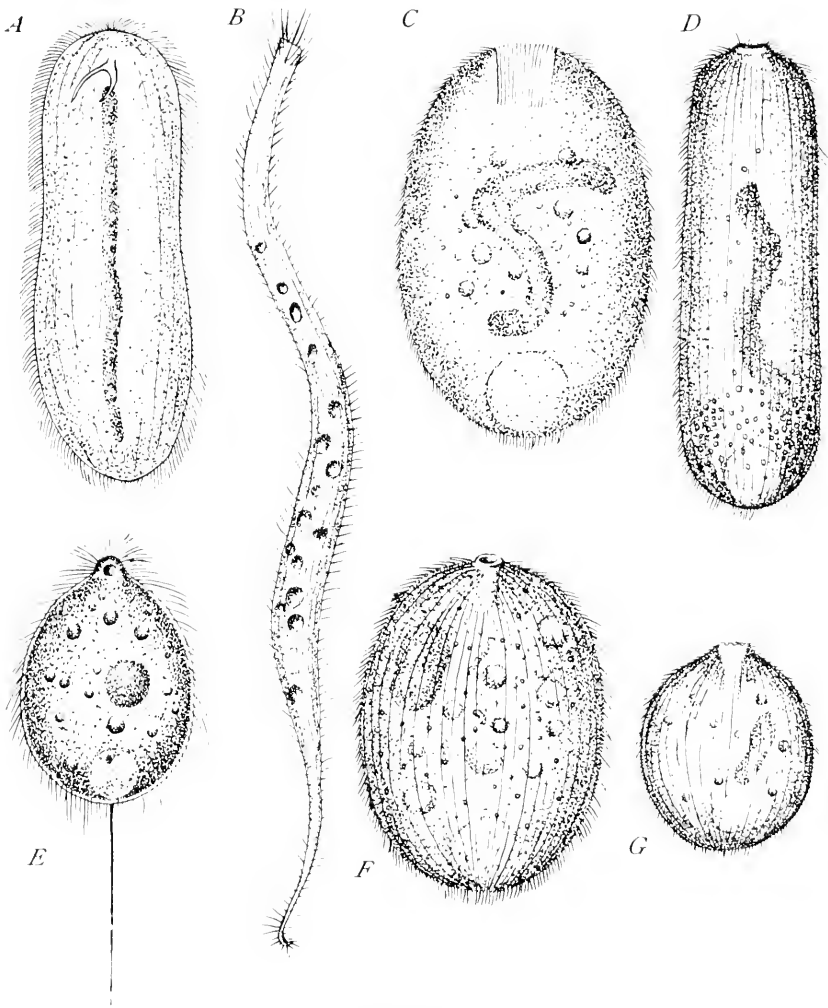


FIG. 202.—Types of Ciliata. A, *Hoplitophrya lumbrici*; B, *Trachlocreca phanicopteris*; C, *Prorodon niceus*; D, *Prorodon faribus*; E, *Urotricha farcata*; F, *Prorodon teres*; G, *Prorodon armatus*. (A, C, D, E, F, G, after Bütschli; B, after Calkins.)

- Family I. **Holophryidae** Perty 1852.
8. Body ovoidal to cylindrical with ventral inclination; with snout-like oral process
 Genus *Lagenophrya* Kahl
 Body otherwise..... 9
 9. Mouth a short slit, directed backwards from pole; no trichocysts..... Genus *Microregma* Kahl
 Mouth not a short, open slit..... 10
 10. Mouth with 3 closely placed rows of small bristles (dorsal brush), no oral papillae. 11
 Mouth without 3 bristles, some with 1; some with widely spread bristles..... 13
 11. Mouth polar; slit-like; usually closed; outer ends of gullet trichocysts not deeply sunk
 Genus *Pseudoprorodon* Blackburn
 Mouth slit-like; gullet surrounded by double trichites which end deep in oral ectoplasm..... 12
 12. Ectoplasm surrounding the mouth flat (Fig. 202)..... Genus *Prorodon* Ehr.
 Ectoplasm surrounding the mouth slightly raised..... Sub-genus *Rhagadostoma* Kahl
 13. Ovoid or short cylindrical; neither elongate nor flask shape; broad truncated anterior end absent..... 14
 Elongate, lance-like cylindrical flask-shape; or worm-like; broad truncated oral end in shorter forms..... 18
 14. Gullet opening surrounded entirely or in part by small papillae..... 15
 Gullet opening not surrounded by papillae. 16
 15. Posterior end with one or several caudal tail cilia, otherwise not ciliated... Genus *Urotricha* C. and L.
 Posterior end ciliated, no caudal cilia
 Genus *Spasmostoma* Kahl
 16. Small; oval; mouth with papillae on right side, running into short ventral groove
 Genus *Plagiocampa* Schew.
 Mouth opening flush with body or slightly raised..... 17
 17. Mouth opening flush with body... Genus *Holophrya* Ehr.
 Mouth opening slightly raised... Sub-genus *Balanophrya* Kahl
 Ectoparasitic on fish..... Sub-genus *Ichthyophthirius* Fouquet
 18. Elongate, lance-like or flask-shape; much flattened, usually with 2 nuclei..... 19
 Not flattened; elongate to worm-like, nuclei diverse..... 20
 19. With long tentacle-like process from terminal mouth..... Genus *Ileoneima* Stokes
 Without tentacle-like process..... Genus *Trachelophyllum* C. and L.
 20. With annular furrow near anterior end, making a head part with spiral rows of cilia (Fig. 85)..... Genus *Lacrymaria* Ehr.
 Without annular furrow and head part... 21

Family 1. **Holophryidae** Perty 1852.

21. Elongate to worm-like species, usually more or less distensible. 22
 Short or flask-shape species; little distensible; ectoplasm without warts. 24
22. Head region narrowed; somewhat contractile, longitudinal or slightly spiral stripes 23
 No spiral stripes; head region not narrowed; no tuft of oral cilia, pellicle warty, exclusively marine worm-like or flask-shape usually very large. Genus *Tracheloverca* Ehr.
23. Without caudal thread; tuft of cilia directed forwards (Fig. 191). Genus *Chaenea* Quenmerstedt
 With two caudal threads. Genus *Urochaenea* Savi
24. Gullet opens to outside with a distinct dome-like process. Genus *Enchelydon* C. and L.
 Gullet mouth round to slit-form; with cross or obliquely truncate anterior end. 25
25. Gullet mouth appears like long cross-cut of anterior end; posterior end with tuft of cilia. Genus *Crobylura* Andre
 Gullet mouth not clean-cut section in appearance; no anal tuft of cilia. Genus *Enchelys* Hill

Family 2. **Actinobolinidae** Kent 1880.

Only 1 genus *Actinobolina* Strand 1926 (*Actinobolus* Stein pre-occupied)

Family 3. **Metacystidae** Kahl 1926.

1. Animals ovoid, without end vesicle; with caudal cilia. Genus *Vasicola* Tatem
 Animals not ovoid. 2
2. Spindle-form; no caudal cilia; closely annulate. Genus *Pelatractus* Kahl
 Cylindrical; usually one, rarely more, caudal cilia, with globular, swollen end vesicle. Genus *Metacystis* Cohn

Family 4. **Didiniidae** Poche 1913.

1. No gullet; special polar area for food-taking (Fig. 191). Genus *Cyelotrichium* Meunier
 Distinct gullet, opening in center on a definite mound. 2
2. Body with one or more circlets of cirri. 3
 Body with circlelet of pectinelles. 4
3. With circlelet of pectinelles about oral mound outside of which is circlelet of cirri
 Genus *Askenasia* Blochmann
 With circlelet of cirri around oral mound
 Genus *Mesodinium* Stein
4. In addition to pectinelles, body uniformly ciliated. Genus *Acropisthium* Perty
 With one to several circlets of pectinelles, otherwise without cilia. Genus *Didinium* Stein

Family 5. **Colepidae** Clap. and Lach. 1858.

1. Body rounded posteriorly; plates separate on pressure. Genus *Coleps* Nitsch
 Body pointed posteriorly, plates firm
 Genus *Tiarina* Bergh

Family 6. **Spathidiidae** Kahl 1930.

1. Mouth region not surrounded by trichocyst-bearing swelling; nor with trichocyst-bearing papillae nor tentacles 2
 - Mouth ventral, unciliated stripes, or papillae or tentacles with trichocysts present 9
 2. Mouth without papilla on its dorsal end; nor surrounded by three tentacle-like arms; occasional processes 3
 - Mouth dorsal, with three arms or with trichocyst-bearing warts 8
 3. Trichocysts of mouth region not heaped in single bundle 4
 - Trichocysts in bundle on dorsal part of mouth region Genus *Cranotheridium* Schew.
 4. Body normally ciliated on both sides 5
 - Body long, worm-like; ciliated on right side only Genus *Homalozoon* Stokes
 5. Mouth area closed in front 6
 - Mouth area open Genus *Enchelydium* Kahl
 6. Small, hyaline, with firm pellicle; with snout-like process Sub-genus *Spathidiella* Kahl
 - Structures otherwise 7
 7. From the mouth area to middle of body an unciliated piece about which the cilia run concentrically Genus *Balantidiodes* Penard
 - Ventral side without unciliated piece; cilia meridional Genus *Spathidium* Dujardin
 8. Mouth area with trichocyst-bearing warts
 - Genus *Spathidiodes* Brodsky
 - Mouth surrounded by 3 trichocyst-bearing arms Genus *Teuthophrys* Chatton and Beauchamp
 9. No tentacles nor warts; mouth area ventral with trichocysts 10
 - Body with trichocyst-bearing warts or tentacles Genus *Legendrea* Fauré-Fremiet
 10. Body ridge runs spirally to posterior right 11
 - Body ridge meridional Genus *Penardiella* Kahl
 11. Anterior end with oblique ventral angle;
 - 2 anterior horns absent Genus *Perispira* Stein
 - Anterior end drawn out into 2 horns . Genus *Diceras* Eberhard
- Family 7. **Bütschliidae** Poche 1913.
1. Entire body uniformly ciliated 2
 - Body not uniformly ciliated 5
 2. Spiral groove from cytostome to posterior end Genus *Paraisotrichopsis* Gassowsky
 - No spiral groove 3
 3. Cilia beat in uniform fashion 4
 - Cilia divided into 3 zones by 2 transverse bands of cilia which beat at different intervals Genus *Blepharozoum* Gassowsky
 4. Cytopharynx at anterior end which is slightly bent Genus *Proralonopsis* Gassowsky
 - Anterior end straight, cytostome large Genus *Holophryoides* Gassowsky

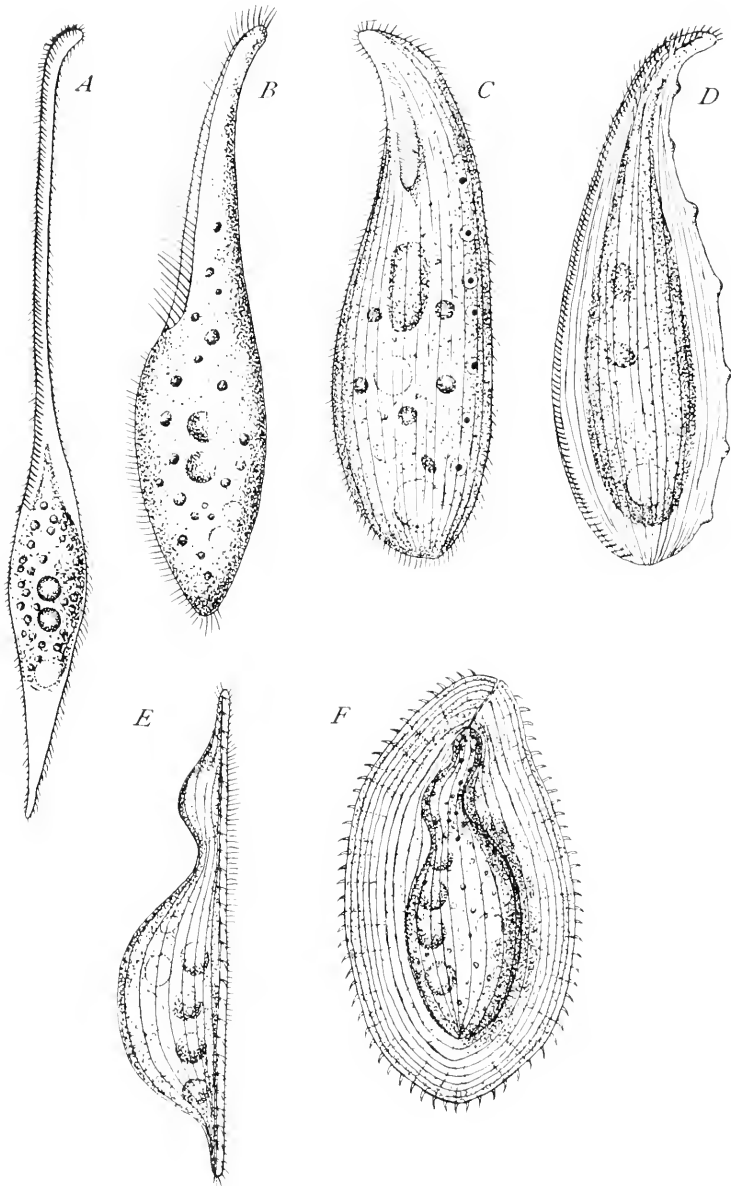


FIG. 203.—Types of Ciliata. A, *Lionotus wrzesniowskiyi*; B, *Lionotus fasciola*; C, *Lorodes castrum*; D, *Lorophyllum mcgagris*; E and F, *Lorophyllum setigera*. (A and C, after Bütschli, the others after Calkins.)

Family 7. **Bütschliidae** Poche 1913.

- 5. Entire body ciliated..... 6
- Parts of body naked..... 7
- 6. Body drawn out into long neck, covered with coarse, long cilia..... Genus *Ampallacula* Hsiung
- Body ovoid, cytostome large and surrounded by longer cilia..... Genus *Bütschlia* Schuberg
- 7. Cilia over less than half the body..... 8
- Cilia over all body except posterior end; a few anal cilia present..... Genus *Blepharosphaera* Bundle
- 8. Body cilia divided into two zones..... 9
- Body cilia divided into 3 zones by 2 transverse naked bands..... Genus *Alloiozoua* Hsiung
- 9. Anterior end drawn into long neck-like process..... 10
- Anterior end blunt or only slightly elevated..... 11
- 10. Macronucleus disk-shaped..... Genus *Polymorpha* Dogiel
- Macronucleus sausage-shaped..... Genus *Blepharoprosthium* Bundle
- 11. Cystostome large at end of short elevation..... 12
- Cystostome small; no elevation; cilia about cytostome and cytopye..... Genus *Bundleia* Cunha and Manix
- 12. Cystostome accompanied by supporting rods..... Genus *Blepharoconus* Gassowsky
- No rod-like structures in gullet.... Genus *Didesmis* Fiorentini

SUB-ORDER 2. **Pleurostomina** (TRIBE PLEUROSATOMATA SCHEWIAKOFF; KAHL).

Family 1. **Amphileptidae** Bütschli; Schouteden.

- 1. Normally ciliated on both surfaces of body..... 2
- Normally ciliated only on right side of body..... 3
- 2. Oral slit does not reach to middle of body; no line of trichocysts..... Genus *Amphileptus* Ehr. em. Bütschli
- Entire ventral surface surrounded by trichocyst-bearing zone..... Genus *Bryophyllum* Kahl
- 3. Ventral surface with flat trichocyst zone; dorsal same or with trichocyst warts (Fig. 203)..... Genus *Loxophyllum* (Dujardin) Wrzesniowsky
- Trichocyst-bearing zones absent..... 4
- 4. Left side entirely without cilia..... Genus *Lionotus* Wrzesniowsky
- Ciliated right side drawn over the dorsal line to the left side so that almost half of the left side is thereby ciliated.... Genus *Acineria* (Dujardin) Maupas

Family 2. **Tracheliidae** Ehr. 1838.

- 1. Anterior end drawn out into a snout or a finger-like process; free-living..... 2
- Ectoparasites on amphipods; anterior body process lancet-like..... Genus *Branchioecetes* Kahl
- 2. With finger-like anterior proboscis; posterior end tail-like (Fig. 194)..... Genus *Dileptus* Dujardin
- Form oval or round; posterior rounded or with barely evident point..... Genus *Trachelius* Schrank

Family 3. **Loxodidae** Roux.

- One genus only (Fig. 203)..... Genus *Loxodes* Ehr.

SUB-ORDER 3. **Hypostomina** SCHEWIAKOFF 1896; EM. KAHL.Family 1. **Nassulidae** Bütschli.

1. Opening of oral basket deep in a vestibule of which the external opening is narrowed by a membrane Genus *Nassula* Ehr.
Opening of oral basket on surface or in a shallow, uncovered depression 2
2. Basket opens in a deep depression with cilia or membrane on anterior edge; small, oval, slightly flattened infusoria, with scattered trichocysts Genus *Cyclogramma* Perty
Basket opens on surface; usually distinctly flattened forms, no trichocysts 3
3. Basket opening, median; body margin with slight or no snout formation Genus *Chilodontopsis* Blochmann
Basket strongly directed to right; right margin of body shows a distinct snout-like process in the mouth region . . . Genus *Orthodon* Gruber

Family 2. **Chlamyodontidae** Claus 1874.

1. The ciliated surface is separated from the unciliated surface by a narrow, hyaline, cross-striped ring Genus *Chlamydon* Ehr.
Cross-striped ring absent 2
2. Ciliated ventral surface limited to a V-shaped median part and overlapped on both sides by non-ciliated part . . . Genus *Phascobodon* Stein
Ciliated surface not thus limited 3
3. Mouth a transverse slit in first quarter of body, with a clapper-like membrane Genus *Gastronauta* Engelm.
Mouth opening circular 4
4. Gullet with distinct basket; a cross row of bristles on the anterior flattened part Genus *Chilodonella* Strand
(= *Chilodon* Ehr.)
Basket indistinct; no cross row of bristles; entire edge of body surrounded by dorsally directed spines Genus *Cryptopharynx* Kahl

Family 3. **Dysteriidae** Clap. and Lach. 1858.

1. Ventral side entirely ciliated; gullet with short stout rods Genus *Hartmannula* Poche
(= *Omychodactylus* Entz)
Ventral surface with unciliated edge on at least one side 2
2. Powerful end spine is continuation of tail end; ciliated area narrowed by unciliated edges on both sides Genus *Scaphidiodon* Stein
Ventral ciliated area limited especially from left 3
3. Ciliated on ventral right side, and in post-oral area where rows shorten from right to left Genus *Trochilioides* Kahl
Postoral cilia formed by extension of pre-oral cilia to right of mouth and parallel to right margin; 1 or 2 adoral cross rows may occur 4

Family 3. **Dysteriidae** Clap. and Lach. 1858.

- 4. Ciliated right area of ventral surface entirely free.....Genus *Trochilia* Dujardin
- Ciliated right area with mouth, in furrow by overgrowth of right ventral surface
Genus *Dysteria*
(= *Cypridium* Kent;
Ervilia Duj.; *Aegyria* C. and L.)

Family 4. **Pycnothricidae** Poche 1913 (= **Nicollellidae** Chatton and Perard).

- 1. Mouth in mid-ventral surface.....Genus *Nicollella* Ch. and Per.
Mouth otherwise placed..... 2
- 2. Mouth at posterior end of body....Genus *Collinella* Ch. and Per.
Mouth dorsal; furrow runs around posterior end..... 3
- 3. Mouth near posterior end.....Genus *Buxtonella* Jameson
Mouth near anterior end.....Genus *Pycnothrix* Schubotz

Family 5. **Foettingeriidae** Chatton and Lwoff 1926.

- 1. Body with pigmented reserve mass..... 2
Body without pigmented reserve mass.... 3
- 2. Chains of buds formed without encystment
Genus *Polyspira* Minkiewicz
Chains of buds formed while encysted
Genus *Gymnodinioides* Min.
- 3. Gastric parasites of Actinians; stalked
cysts in Crustacea.....Genus *Foettingeria* Clap.
No stalked cysts..... 4
- 4. Motile forms in Hydromedusae; cysts in
Copepods.....Genus *Spirophrya*
Clap. and Lach.
Motile forms and cysts in crabs....Genus *Synophrya*
Chat. and Lwoff

ORDER 3. **Trichostomida** BÜTSCHLI 1889.

Key to Families

- 1. Gelatinous test or cup absent 2
Gelatinous test present, animals swim backwards..... Family 8. **MARYNIDAE**
- 2. Small, mostly flattened laterally, with delicate armor-like periplast; cilia sparse, chiefly on right surface in 2-9 broken rows on semicircular or crescentic keel; mouth on compressed ventral surface with obscure membrane-like structures
Family 9. **TRICHOPELMIDAE**
Structures and ciliation different..... 3
- 3. Small to very small ciliates with long caudal cilium, cilia reduced to 3-4 cross spiral rows about anterior half. Family 1. **SCIADOSTOMIDAE**
Ciliation otherwise; no caudal cilia..... 4
- 4. Zone of special cilia extends from mouth to posterior end 5
Spiral zone of special cilia absent..... 6

Key to Families

5. Spiral zone extends from anterior right to posterior left Family 2. SPIROZONIDAE
 Spiral zone extends from anterior left to posterior right Family 3. TRICHOSPIRIDAE
6. Ciliated cross-furrow in anterior half of body runs on ventral surface to mouth Family 4. PLAGIOPYLIDAE
 Ciliated ventral cross-furrow absent 7
7. Mouth in flat oval longitudinal pit with heavy ciliated walls, first quarter Family 5. CLATHROSTOMIDAE
 Mouth deep, funnel-like 8
8. Mouth funnel with strong cilia; mouth about central at base of diagonal peristome Family 7. PARAMECHIDAE
 Peristome from anterior end absent 9
9. Free living, many in moss; oral funnel deep; cilia at top and bottom Family 6. COLPODIDAE
 Ecto- or endocommensals 10
10. Ectocommensals (on invertebrates) 11
 Endocommensals in vertebrates (mammals) 14
11. Attaching organs absent Family 10. CONCHOPHITHIRIIDAE
 Attaching organs present 12
12. Attaching organ tentacular Family 11. HYPOCOMIDAE
 Attaching organs thigmotactic cilia 13
13. Thigmotactic cilia circumoral Family 12. BOVERIIDAE
 Thigmotactic cilia not circumoral Family 13. ANCISTRUMIDAE
14. Entire body covered with cilia 15
 Cilia in certain regions only 16
15. With "concretion" vacuole Family 15. PARASOTRICHIDAE
 Concretion vacuole absent Family 14. ISOTRICHIDAE
16. Mouth occupies entire anterior end; cilia limited to mouth region Family 17. CYATHODINIIDAE
 Mouth not terminal; tufts of cilia above and below mouth and in posterior anal region Family 16. BLEPHAROCORIDAE

Key to Genera

Family 1. **Schiadostomidae** Kahl 1926.

Only one genus—*S. difficile* Kahl

Family 2. **Spirozonidae** Kahl 1926.

Only one genus—*S. caudata* Kahl

Family 3. **Trichospiridae** Kahl 1926.

Only one genus—*Trichospira* Roux

Family 4. **Plagiopylidae** Schewiakoff 1896.

1. Peristome a distinctly ciliated groove or pit 2

Peristome without groove or pit; with crescentic, protruding and stiff lip Genus *Sonderiella* Kahl

2. Gelatinous mantle present Genus *Sonderia* Kahl

Gelatinous mantle absent; peristome furrow near edge of dorsal side, forming distinct notch 3

Family 4. **Plagiopylidae** Schewiakoff 1896.

- 3. Free-living forms.....Genus *Plagiopyla* Stein
- Parasitic in gut of sea-urchin.....Genus *Lechriopyla* Lynch

Family 5. **Clathrostomidae** Kahl 1926.

Only one genus—*Clathrostoma* Penard

Family 6. **Colpodidae** Poche 1913; em. Kahl, 1926

- 1. Mouth a funnel-shaped pit..... 2
- Mouth a long tube, or a narrow diagonal pit..... 4
- 2. Mouth funnel does not include almost half of anterior end..... 3
- Funnel deeply sunk; forms wide opening, partly covered by cilia.....Genus *Bressiana* Kahl
- 3. Mouth opens on the broad side; the right edge is continued horse-shoe-shape around posterior end of mouth and half of left edge; group of posteriorly directed cilia from anterior part of left edge.....Genus *Bryophrya* Kahl
- Mouth opens more towards the left; its left edge bears a cross-striped ciliated area, but no membrane.....Genus *Colpoda* O. F. M.
- 4. Mouth a long, bent, ciliated tube; form like *Colpoda*.....Genus *Tillina* Gruber
- Mouth a flat, diagonal pit; marine form, like *Chilodonella*.....Genus *Woodruffia* Kahl

Family 7. **Parameciidae** Kent 1881; em. Kahl 1931.

Only one genus—*Paramecium* Hill (Fig. 204)

Family 8. **Marynidae** Poche 1913.

- 1. Peristome furrow describes a complete ring about anterior pole; colonial in branching gelatinous tubes.....Genus *Maryna* Gruber
- Peristome furrow confined to ventral surface; in simple cups.....Genus *Myeterothrix* Lauterborn

Family 9. **Trichopelmidae** Kahl 1926.

- 1. Oral funnel supported by delicate rodlets, opening on anterior third or fourth of body..... 2
- Oral funnel without rodlets; mouth opens in middle or near posterior end..... 3
- 2. Peristome extends over into right lateral surface; small membrane in gullet.....Genus *Pseudomicrothorax* Mermod
- Mouth area distinctly set off; gullet opening directed to left; 2 or 3 cirri or membranelle-like structures.....Genus *Trichopelma* Levander
- 3. Mouth opens to left in body center, in membrane-bearing groove.....Genus *Drepanomonas* Fresenius
- Mouth area in little pit near posterior end of left edge.....Genus *Microthorax* Engelmann

Family 10. **Conchophthiriidae** Reichenow-Dofflein 1929.

- 1. Dorsal lobe overhanging mouth.....Genus *Entochipidium* Lynch
- No dorsal lobe..... 2

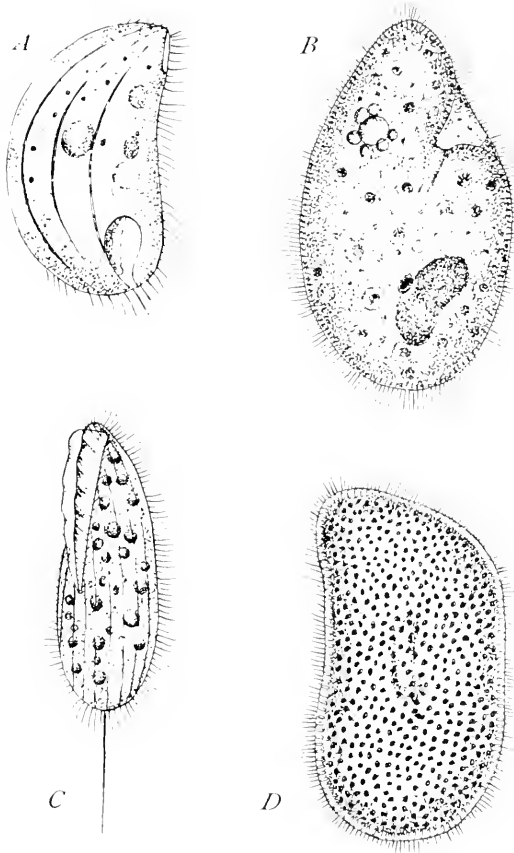


FIG. 204.—Types of Ciliata. A, *Microthorax sulcatus*; B, *Paramecium putrinum*; C, *Lomulus pusillus*; D, *Paramecium bursaria*. (A, B, D, after Bütschli; C, after Calkins.)

- Family 10. **Conchophthiriidae** Reichenow-Doflein 1929.
2. Body much flattened laterally 3
Body slightly flattened; mouth posterior;
oral groove narrow triangle; limpet parasite Genus *Eupoterion* MacLennan
 3. Mouth in anterior third; oral groove S-like;
echinoderm parasite Genus *Cryptochilum* Maupas
Mouth median; oral groove straight . Genus *Conchophthirus* Stein
- Family 11. **Hypocomidae** Bütschli 1889.
1. Adoral cilia apart from attaching tentacle
Genus *Hypocomides*
Cb. and Lwoff
Adoral cilia absent; attaching and food-taking tentacle Genus *Hypocoma* Gruber
- Family 12. **Boveriidae** Pickard 1927.
1. Circum oral spiral begins part way up the body Genus *Plagiospira* Issel
Circum oral cilia around oral end . . . Genus *Boveria* Stevens
- Family 13. **Ancistrumidae** Issel 1903.
One genus—*Ancistruma* Strand (*Ancistrum* Maupas pre-occupied)
- Family 14. **Isotrichidae** Bütschli 1889.
1. Protoplasmic strands from cortex support the macronucleus Genus *Isotricha* Stein
Protoplasmic strands from cortex absent
Genus *Dasytricha* Schuberg
- Family 15. **Paraisotrichidae** da Cunha 1916.
One genus—*Paraisotricha* Fiorentini
- Family 16. **Blepharocoridae** Hsiung 1929.
1. Anal cilia in a single group Genus *Blepharocorys* Bundle
Anal cilia in two groups Genus *Charon* Jameson
- Family 17. **Cyathodiniidae** da Cunha 1916.
One genus—*Cyathodinium* da Cunha

ORDER 4. **Hymenostomida.***Key to Families*

1. With aboral thigmotactic cilia (commensals) Family 6. HEMISPEIRIDAE
Without attaching cilia 2
2. Oral pit not connected with a peristome
Family 1. FRONTONIIDAE
Oral pit at end or at bottom of a peristome. 3
3. Mouth at bottom of sickle-shape, ciliated peristome sunk at right angles to body surface Family 2. OPHRYOGLENIDAE
Mouth at end of peristome running from anterior pole on body surface 4
4. On right edge of peristome a one-layered membrane which forms a pocket surrounding posterior mouth, on left edge a row of cilia or a membrane . . . Family 5. PLEURONEMATIDAE
Peristome otherwise 5

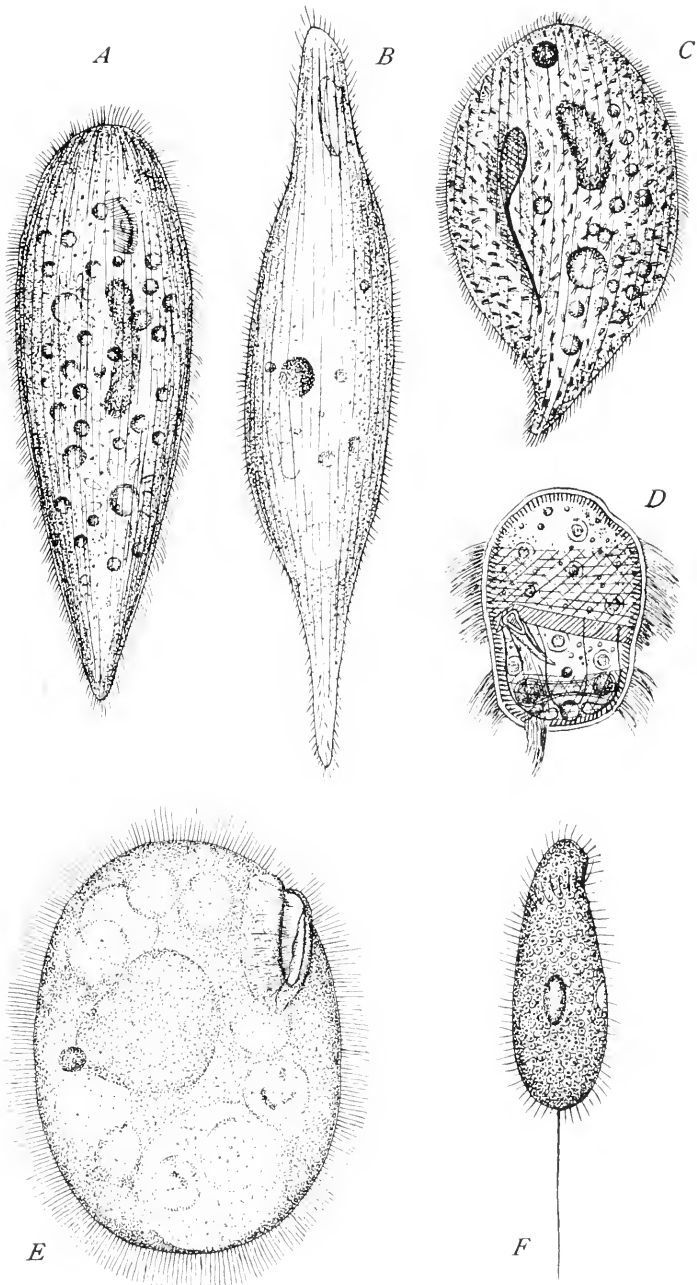


FIG. 205.—Types of Ciliata. A, *Ophryoglena flava*; B, *Glaucocoma frontata*; C, *Frontonia acuminata*; D, *Urocentrum turbo*; E, *Glaucocoma* sp.; F, *Loroccephalus granulatus*. (A, C, D, and F, after Bütschli; B, from Conn after Stokes; E, original.)

Key to Families

- 5. On right edge of peristome are two simple undulating membranes; a distinct pocket is absent..... Family 4. LEMBUDAE
- Peristomial furrows with either a thick area of cilia outside of which is an undulating membrane, or a single thick undulating membrane on the right edge; to right of mouth and posteriorly a pocket with small membrane is sunk below the ectoplasm..... Family 3. PILLASTERIDAE

Family 1. **Frontoniidae** Kahl 1926.

- 1. Except in Lembadion, no long caudal cilia; never a single caudal bristle..... 2
- With one or more caudal cilia, occasionally fused in brush (Urocetrum)..... 22
- 2. Mouth opening pointed anteriorly..... 3
- Mouth opening rounded or truncated anteriorly..... 10
- 3. Mouth at most one-third body length.... 4
- Mouth one-half to four-fifths of body length..... 8
- 4. Mouth truncated posteriorly; undulating membrane on left; partly fused membrane-like cilia on right margin..... 5
- Mouth not truncated posteriorly; but sharp-pointed or rounded..... 7
- 5. Mouth without gullet-like, funnel-form posterior prolongation..... 6
- Mouth with funnel-like gullet into which membrane of left margin continues
Genus *Frontoniella* Wetzel
- 6. Gullet fibrils strong and numerous; posterior to mouth, a distinct line runs to posterior end; form not triangularly narrowed posteriorly..... Genus *Frontonia* Ehr.
- Gullet fibrils delicate, sparse; ventral line indefinite; narrows triangularly at posterior region..... Genus *Disemotostoma* Lauterborn
- 7. Mouth a small, sigmoid cleft; not at anterior pole; with 2 membranes.... Genus *Sigmotomum* Gulati
- Mouth elongate, from anterior pole; 2 membranes; gullet with long fibrils... Genus *Leucophrydium* Roux
- 8. Mouth about one-half body length..... 9
- Mouth three-quarters to four-fifths body length (with long caudal cilia).... Genus *Lembadion* Perty
- 9. Anterior end pointed; body broadly oval
Genus *Leucophrys* Ehr.
- Anterior end rounded, body slender. Genus *Turania* Brodsky
- 10. Mouth oblique (anterior right to posterior left); ectoplasmic lip on right edge.... 11
- No ectoplasmic lip on right margin of mouth..... 13

Family 1. **Frontoniidae** Kahl 1926.

11. Three ciliated structures on inside, left an outer membrane; below it an inner membrane and right, at bottom, a 3-row cilia combination 12
 Only one strong membrane from left edge beats into ectoplasmic lip Genus *Pseudoglaucoma* Kahl
12. Mouth near middle of ventral surface; dorsal ciliated rows bent to right . . . Genus *Glaucoma* Ehr.
 Mouth on right edge of ventral surface; dorsal rows bend sharply to right . Genus *Colpidium* Stein
13. Mouth with 2 or 3 membranes 14
 Mouth with only 1 membrane 16
14. Slimy dwelling; membranes surround mouth at pole, forming pocket behind Genus *Cyrtolophosis* Stokes
 Naked forms; mouth without pocket 15
15. One free membrane on both sides of mouth; anterior rounded Genus *Dichilum* Schewiakoff
 One free membrane on right side, 2 others in oral pit; anterior sharply pointed Genus *Paraglaucoma* Kahl
16. Left and anterior membrane cover mouth cap-like Genus *Stegochilum* Schewiakoff
 Membrane not cap-like 17
17. Membrane inserted inside and continues into posterior funnel 18
 Mouth without funnel continuation 19
18. Form elongate-oval; slightly compressed Genus *Monochilum* Schewiakoff
 Small, flattened, kidney-shape . . . Genus *Chasmatostoma* Engelmann
19. Mouth at or near anterior pole 20
 Mouth distinctly separate from pole 21
20. Mouth a deep pit in the truncated end; in jelly of egg masses Genus *Espejoia* Burger
 Mouth a narrow slit quite near anterior pole Genus *Malacophrys* Kahl
21. Spindle form; delicate groove from pole to small mouth Genus *Bizone* Lepsi
 Mouth without groove; membrane on left side Genus *Aristerostoma* Kahl
22. Cilia arranged in 1 or in 3 girdles 23
 Cilia in longitudinal rows; not arranged in girdle 24
23. Very small (20μ); only 1 median girdle; 1 caudal filament Genus *Urozoma* Schewiakoff
 Caudal cilia many, fused; 2 broad girdles, 1 anterior, 1 posterior to oral girdle (Fig. 205) Genus *Urocentrum* Nitzsch
24. Mouth in center or anterior to center of body 25
 Mouth posterior to center of body 33
25. Body compressed laterally; small furrow from anterior pole with membrane on right side and thick cilia on left . . Genus *Rhinodiscutus* Mansfeld
 Body not laterally flattened 26

Family 1. **Frontoniidae** Kahl 1926.

26. Slightly compressed; mouth depressed;
above middle of ventral surface. 27
Body dorso-ventrally more or less com-
pressed. 29
27. Anterior pole not ciliated; indistinct furrow
from pole to mouth. Genus *Uronema* Dujardin
Anterior pole ciliated, no trace of furrow. 28
28. C. V. terminal; no pocket formed by oral
membrane. Genus *Dexiotrichides* Kahl
C. V. not terminal; oral membranes form
closed pocket. Genus *Uronemopsis* Kahl
29. Body flattened; mouth near right edge;
form ovoid. 30
Body not flattened, form ellipsoidal. 31
30. Mouth with membranous pocket. . . Genus *Saprophilus* Stokes
Mouth small, kidney-shape; a membrane
on left concave edge. Genus *Platymema* Kahl
31. Mouth with anterior and posterior truncate
processes. Genus *Balanonema* Kahl
Mouth without such processes. 32
32. Form plump, worm-like; 4 to 5 caudal cilia;
mouth small, heart-shaped in anterior
fifth of body. Genus *Cardiostoma* Kahl
Not worm-like; anterior pole not ciliated;
cilia in longitudinal or oblique cross
rows; mouth with groove from right
(Fig. 205). Genus *Lorocephalus* Eberlard
33. Broad oval; ventrally flat and ciliated;
dorsal slightly flat or arched without
cilia. Genus *Cinetochilum* Perty
Spindle-shape; not flattened; ciliated on
both sides. 34
34. Very small salt water forms; dancing move-
ment without pause. Genus *Uropedalium* Kahl
Very small moss forms; gliding movement
Genus *Homologastra* Kahl

Family 2. **Ophryoglenidae** Kent 1882; em. Kahl 1931.

Only one genus—*Ophryoglena* Ehr. (Fig. 205)

Family 3. **Philasteridae** Kahl 1931.

1. Peristome with long rows of cilia; always a
tail filament, ectoplasm soft, with tricho-
cysts. 2
Peristome ciliated only on right edge; ecto-
plasmic resistant heavy membrane on
right edge. Genus *Leuboides* Kahl
2. Pocket from end to mouth, with small tri-
angular membrane, nucleus oval. 3
Pocket winds spirally around oral pits;
nucleus elongate. Genus *Helicostoma* Cohn
3. Large marine forms with terminal contrac-
tile vacuoles. Genus *Philaster* Fabre-Dom.
Small; fresh water; contractile vacuole
near center. Genus *Philasterides* Kahl
Here also Genus *Anophrys* Cohn 1866—not confirmed

Family 4. **Lembidae** Kahl 1931.

Only one genus—*Lembus* Colm (Fig. 204)

Family 5. **Pleuronematidae** Kent 1882.

1. Small, test-building, fresh water species
 Genus *Calyptotricha* Phillips
 Not test-building. 2
2. Marine; ectocommensal on Hydraetina
 Genus *Pleurocoples* Wallengren
 Not ectocommensal. 3
3. Undulating membrane without distinct pocket; peristome oblique from anterior right to posterior left Genus *Cteloctema* Stokes
 Undulating membrane pocket distinct; hardly ever oblique. 4
4. Body flat; peristome continues as groove posterior to mouth. Genus *Cristigera* Roux
 Peristome on right side, without groove posterior to mouth. 5
5. Large (70 to 180), striking forms. 6
 Small (up to 50); no semicircular swelling of peristome. Genus *Cyclidium* O. F. Müller
6. Posterior sensory bristles; 1 contractile vacuole; peristome begins at anterior end; semicircular swelling to left at mouth region. Genus *Pleuronema* Dujardin
 Sensory bristles distributed over body, 2 to 3 times longer than cilia; peristome begins on first quarter, without oral swelling; C. V. numerous. Genus *Histiobalantium* Stokes

SUB-CLASS II. **SPIROTRICHA** BÜTSCHLI 1889; EM. KAHL 1931.

Key to Orders

1. Only free cilia present; exceptionally tufts of cirrus-like aggregates. 2
 Ciliation exclusively cirri; dorsal rows of short, delicate, slightly movable bristles
 Order 4. HYPOTRICHIDA
2. Body uniformly ciliated; in flat forms no cilia dorsally; in ectoparasitic Lichnophoridae cilia around edge only of attaching disc; frontal field ciliated
 Order 1. HETEROTRICHIDA
 Cilia much reduced or absent. 3
3. Small, flattened, carapaced forms whose peristome has only 8 membranelles which lie in a ventral hollow. Order 3. CTENOSTOMIDA
 Transverse section circular; cilia much reduced; adoral zone encloses a non-ciliated frontal field. Order 2. OLIGOTRICHIDA

ORDER 1. **Heterotrichida** STEIN.

Key to Families

1. Ciliation complete, with uniform cilia 2
 Ciliation absent or limited to ventral side. . . 9

Key to Families

- 2. Peristome almost free, leading to short and narrow oral funnel which is absent in one family..... 3
 Peristome runs deeply into a funnel-like hollow and is mostly covered. Family 7. BURSARIIDAE
- 3. On right edge of peristomial membranelle zone a narrow zone without cilia; on right of this, in front of mouth, an undulating membrane; between this and membranelle zone a peristomial frontal field difficult to see..... 4
 Frontal field surrounded wholly or in part by spiral adoral zone..... 7
- 4. Adoral zone stretches diagonally to posterior right on ventral side; many forms have an elongated portion which twists spirally around the body.... Family 1. METOPIDAE
 Adoral zone runs for most part in direction of long axis and, just before the mouth opening, bends sharply to right..... 5
- 5. Gullet and undulating membranes absent; mouth usually closed and difficult to find; opens as a slit for food-taking
 Family 2. REICHENOWELLIDAE
 Oral funnel distinct; in typical forms an undulating membrane or a double ciliated furrow before the mouth..... 6
- 6. Parasitic forms..... Family 4. PLAGIOTOMIDAE
 Free-living forms..... Family 3. SPIROSTOMIDAE
- 7. Frontal field not ciliated; one large undulating membrane on its right edge
 Family 5. CONDYLOSTOMIDAE
 Frontal field ciliated; no undulating membrane..... 8
- 8. Frontal field not drawn out in wings; free or in jelly tests..... Family 6. STENTORIDAE
 Frontal field drawn out in 2 wings, in flask-shaped tests..... Family 6. FOLLICULINIDAE
- 9. Free-living, flat, marine forms; ciliated on ventral side only; adoral zone surrounds anterior ventral surface; mouth on left edge near middle of body... Family 9. PERITROMIDAE
 Ectoparasitic marine forms; both ends of body discoid, in middle neck-like
 Family 10. LICHNOPHORIDAE

Family 1. **Metopidae** Kahl 1927.

- 1. Ciliation of body uniform..... 2
 Ciliation reduced; body with "head" region 4
- 2. Ectoplasm soft, yielding..... 3
 Ectoplasm stiff, carapace-like, spirally keeled..... Genus *Tropidotractus* Levander
- 3. Flat oval to ovoid moss forms; C, V, on ventral middle; broad, insunk stripings
 Genus *Bryometopus* Kahl
 Form diverse; free living in water; C, V, terminal; peristome typical..... Genus *Metopus* Clap. and Lach.

Family 1. **Metopidae** Kahl 1927.

4. Cilia of head reduced to lateral zone and dorsal cirri..... 5
Cilia of head reduced to lateral zone and about 8 fused cilia..... Genus *Trochella* Penard
5. One or two rows of dorsal cirri..... Genus *Cænomorpha* Perty
Two single, long cirri..... Genus *Ludio* Penard

Family 2. **Reichenowellidae** Kahl 1931.

1. Elongate, sapropelic, fresh water; C. V. terminal; ciliation meridional..... Genus *Reichenowella* Kahl
Oval; moss dwelling; C. V. numerous; ciliation spiral..... Genus *Balantidioides* Penard

Family 3. **Spirostomidae** S. Kent, 1881.

1. No undulating membrane at mouth..... 2
An undulating membrane on right edge of peristome..... 7
2. Worm-like; contractile..... 3
Not definitely contractile..... 4
3. Usually fresh water; greatly twisted on contraction..... Genus *Spirostomum* Ehr.
Salt water forms; posterior tail-like; very little torsion on contraction..... Genus *Gruberia* Kahl
4. Elongate fresh water forms; 2 rows of cilia in place of undulating membrane..... Genus *Pseudoblepharisma* Kahl
Fresh water forms; not elongate..... 5
5. Oval; with marked ribs; in moss..... Genus *Phacodinium* Prowazek
Small, oval, marine forms; without noticeable ribs..... 6
6. Adoral zone spirally rolled at mouth..... Genus *Spirostomina* Gruber
Adoral zone runs directly to mouth..... Genus *Protoecrucia* da Cunha
7. Peristome-bearing region not narrowed neck-like; no gelatinous membrane.....
Genus *Blepharisma* Perty
Peristome narrowed neck-like; gelatinous membrane; marine forms..... Genus *Parablepharisma* Kahl

Family 4. **Plagiotomidae** Poche 1913.

- Elongate, oval; peristome begins at anterior end; earthworm gut..... Genus *Plagiotoma* Dujardin
- Oval to reniform; peristome beginning sub-terminal; many hosts (Fig. 206). Genus *Nyctotherus* Leidy

Family 5. **Condylostomidae** Kahl 1931.

- Only one genus—*Condylostoma* Bory (Fig. 206)

Family 6. **Stentoridae** Carus 1863.

1. Adoral zone almost completely closed circle; body contractile, "trumpet" animal..... Genus *Stentor* Oken
Adoral zone not complete ring; not contractile..... 2
2. Right peristome edge drawn down ventral surface..... Genus *Climacostomum* Stein
Right peristome edge continuous with right anterior edge of body..... Genus *Fabrea* Henneguy

Family 7. **Folliculinidae** Dons 1912.

1. Neck of test not swollen..... 2
Neck has a basal swelling; tests fastened laterally or on end..... Genus *Parafolliculina* Dons

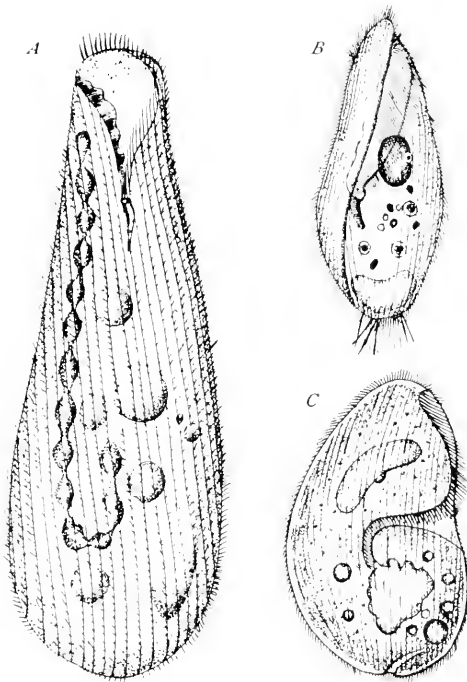


FIG. 206. Types of Ciliata. A, *Condylostoma patens*; B, *Melopus sigmoides*; C, *Nyctoloberus cordiformis*. (A, after Calkins; B, C, after Bütschli.)

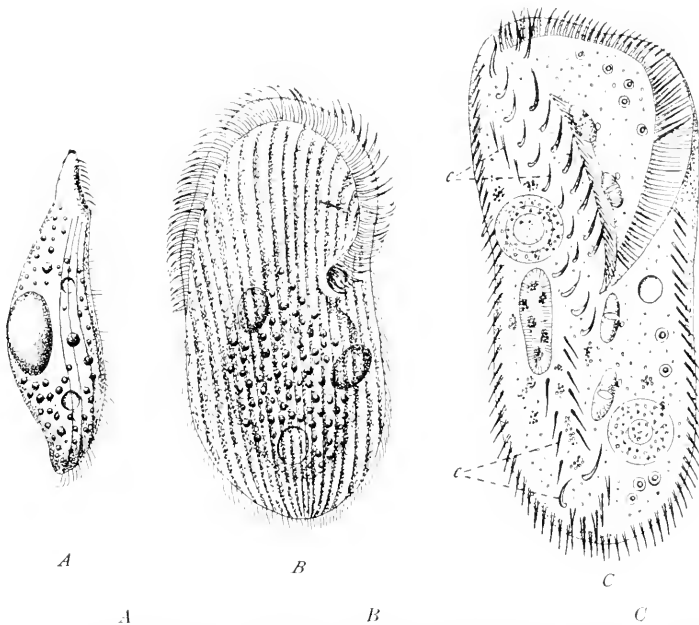


FIG. 207.—A, *Stephanopogon colpoda*; B, *Peritromus emmae*; C, *Onychodromus grandis*; c, cirri. (From Calkins after Bütschli.)

Family 7. **Folliculinidae** Dons 1912.

2. Posterior end and sides of test with sack-like protuberances..... Genus *Mirofolliculina* Dons
Tests without protuberances..... 3
3. Tests attached on broad surface; neck oblique..... Genus *Folliculina* Lamarck
Tests attached at posterior end; tests upright..... 4
4. Test narrow, no central annular furrow
Genus *Pseudofolliculina* Dons
Test plump, with central furrow; neck appears as a short collar..... Genus *Pebrella* Giard

Family 8. **Bursariidae** Perty 1852.

1. Posterior end of peristome straight; parasitic..... 2
Posterior end of peristome bends to right or left; free-living..... 3
2. Peristome opening a narrow slit... Genus *Balantidiopsis* Bütschli
Peristome opening medium wide... Genus *Balantidium*
Clap. and Lach.
3. Posterior end of peristome funnel bends to left..... 4
Posterior end of peristome funnel bends to right..... Genus *Bursaridium* Lauterborn
4. Very large animals with longitudinal fold (tongue) dividing peristomial space in two sections..... Genus *Bursaria* O. F. M.
Medium or small forms with simple peristome..... Genus *Thylacidium* Schewiakoff

Family 9. **Peritromidae** Stein 1867.

1. Ventral surface flat; adoral zone semicircular around anterior left margin. Genus *Peritromus* Stein
Flat surface bent dorsally; anterior to mouth a peristome-like area with meridional fibers to mouth..... Genus *Pediostomum*
Fauré-Fremiet

Family 10. **Lichnophoridae** Stevens 1903.

One genus only—*Lichnophora* Claparede

ORDER 2. **OLIGOTRICHIDA** BÜTSCHLI 1889.*Key to Families*

1. Free-living forms..... 2
Endocommusal forms..... 4
2. Oral part of peristome lies free on ventral surface..... Family 1. HALTERIIDAE
Adoral zone encircles frontal field and mouth region..... 3
3. No house or test..... Family 2. STROMBILIDIDAE
House or test present..... Family 3. TINTINNIDAE
4. With one or two rings of membranelles directed forwards..... Family 4. OPHRYOSCOLECIDAE
With additional bundles of cirri directed backwards..... Family 5. CYCLOPOSTHIDAE

*Key to Genera*Family 1. **Halteriidae** Clap. and Lach. 1859; mod. Kahl.

1. Posterior end without thick, dragging process..... 2
 - Posterior end with long, thick, retractile, protoplasmic process..... Genus *Tontonia* Fauré-Fremiet
2. Highly contractile; Stentor-like; no pre-oral adoral zone.....?Genus *Meseres* Schewiakoff
 - Body not contractile..... 3
3. With equatorial circlelet of long bristles or cirri..... Genus *Halteria* Dujardin
 - Without equatorial circlelet of bristles..... 4
4. Adoral zone with distinct pre-oral section; frontal part of zone surrounds an apical projecting process..... Genus *Strombidium* Clap. and Lach.
 - Adoral zone without special pre-oral section..... Genus *Metastrombidium* Fauré-Fremiet

Family 2. **Strombilidiidae** Kahl 1932.

One safe genus..... Genus *Strombidium* Schewiakoff

Family 3. **Tintinnidae** Clap. and Lach. 1859 (fresh water forms only).*Key to Genera of Fresh Water Forms*

1. Tests gelatinous, delicate; more or less covered by foreign bodies, etc..... 2
 - Tests firm; pseudo-chitin; may be covered by nodules or by algae..... 3
2. Body with rows of distinct cilia; tests very delicate..... Genus *Strombidinopsis* Kent
 - Body with cilia behind peristome only; tests distinct..... Genus *Tintinnidium* Stein
3. Tests cylindrical, without neck part. Genus *Tintinnopsis* Stein
 - Tests with definite neck part..... Genus *Codonella** Haeckel

Family 4. **Ophryoscolecidae** Stein 1858.

1. Adoral zone of membranelles only; no dorsal zone..... 2
 - Adoral and dorsal zone of membranelles... 3
2. Adoral zone in spiral about cytostome; macronucleus elongate..... Genus *Eutodinium* Stein
 - Anterior end uniformly ciliated; macronucleus spherical..... Genus *Lavierella* Buisson
3. Adoral and dorsal zones at about the same level..... 4
 - Dorsal zone posterior to adoral zone..... 14
4. Dorsal zone at right angles to long axis of the body..... 5
 - Dorsal zone nearly parallel to long axis of the body..... Genus *Cunhaia* Hasselmann
5. Forms without skeletal plates..... 6
 - Forms with skeletal plates..... 7
6. Macronucleus beneath dorsal surface of the body; straight..... Genus *Eodinium* Kof. and MacL.
 - Macronucleus beneath right surface; anterior third bent ventrally..... Genus *Diplodinium* Schuberg

* See Kofoid and Campbell (1929) for monographic treatment of Tintinnidae.

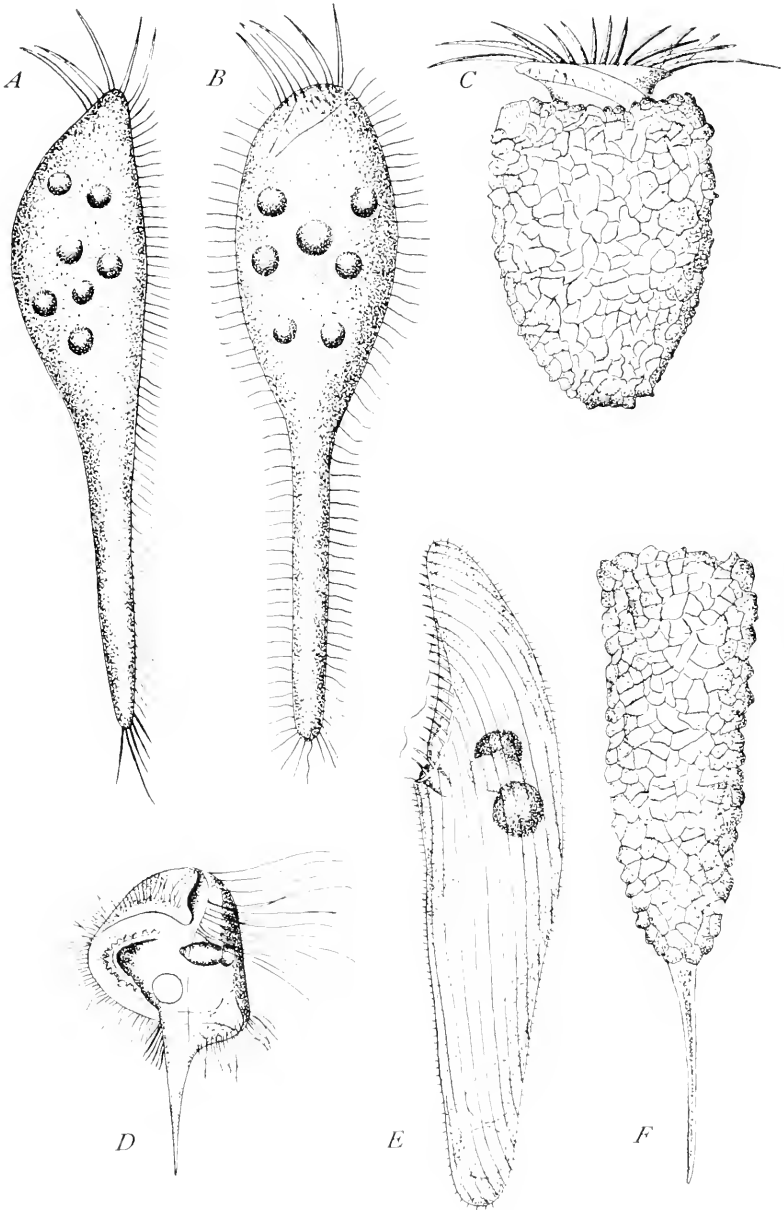


FIG. 208. Types of Ciliata. A and B, *Epiclitus radiosa*; C and F, species of *Tintinnopsis*; D, *Caenomorpha medusula*; E, *Bipharisma undulans*. (A, B, C, E and F, after Calkins; D, after Bütschli.)

Family 4. **Ophryoscolecidae** Stein 1858.

- 7. Forms with one skeletal plate..... 8
 - Forms with more than one skeletal plate.. 10
- 8. Skeletal plate narrow..... 9
 - Skeletal plate very broad beneath the right surface of the body..... Genus *Ostracodinium* Dogiel
- 9. Forms with triangular or rod-like macronucleus; anterior end often bent ventrally..... Genus *Eremoplastron* Kof. and MacL.
 - Rod-like macronucleus with anterior end bent into a hook dorsally..... Genus *Eudiplodinium* Dogiel
- 10. Forms with 2 skeletal plates..... 11
 - Forms with more than 2 skeletal plates... 12
- 11. Macronucleus narrow and rod-like.. Genus *Diploplastron* Kof. and MacL.
 - Macronucleus large with 2 or 3 dorsal lobes Genus *Metadinium* Awerinzew and Mutafova
- 12. Three skeletal plates beneath right ventral surface..... Genus *Enoploplastron* Kof. and MacL.
 - More than 3 skeletal plates..... 13
- 13. Four skeletal plates; 2 right, 1 left and 1 ventral..... Genus *Elyptroplastron* Kof. and MacL.
 - Five skeletal plates; 2 right and 3 left Genus *Polyplastron* Dogiel
- 14. Dorsal zone short and far posterior.. Genus *Opisthotrichum* Buisson
 - Dorsal zone slightly below adoral zone... 15
- 15. Dorsal zone encircling less than half the body..... Genus *Epidinium* Crawley
 - Dorsal zone encircling more than half the body..... 16
- 16. Dorsal zone encircling four-fifths of the body..... Genus *Ophryoscolex* Stein
 - Dorsal zone entirely encircling body.. Genus *Caloscolex* Dogiel

Family 5. **Cycloposthiidae** Poche 1913.

- 1. Posterior cirri in tufts (caudalia)..... 2
 - Posterior cirri in rows..... 6
- 2. Forms with 2 caudalia..... 3
 - Forms with more than 2 caudalia..... 5
- 3. Forms having a spherical macronucleus Genus *Bozasella* Buisson
 - Forms having an elongate macronucleus.. 4
- 4. Body barrel-shaped; caudalia posterior and level..... Genus *Cycloposthium* Bundle
 - Body helmet-shaped; caudalia at different levels..... Genus *Triadinium* Fiorentini
- 5. Forms with 2 dorsal caudalia and 1 ventral Genus *Tripalmaria* Gassowsky
 - Forms with 3 dorsal caudalia and 1 ventral Genus *Protoapirella* Cunha
- 6. Forms with adoral zone and 2 rows of more posterior membranelles..... 7
 - Forms with more than 2 rows of posterior membranelles..... 8

Family 5. **Cycloposthiidae** Poche 1913.

7. Forms with adoral zone and 2 long rows of membranelles in spirals.....Genus *Spirodinium* Fiorentini
Forms with adoral zone and short dorsal rows, 1 caudal and 1 occipital....Genus *Ditoxum* Gassowsky
8. Adoral zone very reduced; 2 rows of membranelles anterior and 2 rows posterior
Genus *Tetratoxum* Gassowsky
Adoral zone prominent..... 9
9. Adoral zone and 3 rows of membranelles, 1 occipital and 2 caudal.....Genus *Cochliatoxum* Gassowsky
Adoral zone and row of membranelles making at least 3 spirals about the body; the row is broken at regular points by skeletal lappets.....Genus *Troglyodytella*
Brumpt and Jouex

ORDER 3. **CTENOSTOMIDA** (LAUTERBORN) KAHL 1931.*Key to Families*

1. Posterior carapace has 4 rows of cilia on left, 2 on right—also 1 row of cilia on left frontal edge.....Family EPALCIDAE
No frontal cilia; posterior row absent on right side; on left side cilia are fused to long cirrus-like groups..... 2
2. Broad, long, ciliated band extends over both broad sides.....Family DISCOMORPHIDAE
Ciliated band is short; extends equally on both sides.....Family MILESTOMIDAE

*Key to Genera*Family 1. **Epalcidae** Wetzel 1928.

1. Only 1 dorsal and 1 ventral row of cilia; number of median teeth usually 4, often indefinite..... 2
Right carapace with 2 median rows of cilia; its median teeth fused to one; 3 teeth in all.....Genus *Pelodinium* Lauterborn
2. At least some anal teeth (left and right) with spines.....Genus *Saprodinium* Lauterborn
Anal teeth all without spines.....Genus *Epalxis* Roux

Family 2. **Mylestomidae** Kahl 1931.

1. Right hind end with 2, left with 1 great notch.....Genus *Atopodinium* Kahl
Notches absent, or very small one on right
Genus *Mylestoma* Kahl

Family 3. **Discomorphidae** Poche 1913.

Only one genus—*Discomorpha* Levander

ORDER 4. **HYPOTRICHIDA** STEIN S. STR.*Key to Families*

1. Adoral zone complete; dorsal bristles present..... 2
Adoral zone reduced to small, encapsulated pre-oral part; on anterior left is an inconspicuous remnant of membranelles which are very small and cirrus-like. Family 3. ASPIDISCIDAE

Key to Families

2. Cirri essentially typical; ventrals may be reduced; marginal rows present; dorsal bristles present. Family 1. OXYTRICHIDAE
- Marginal and ventral rows absent Family 2. EUPLOTIDAE

Key to Genera

Family 1. **Oxytrichidae** Ehr. 1838.

1. Transverse cirri absent (not always easily seen) 2
- Transverse cirri present (not always easily seen) 10
2. Ventral and marginal rows not distinctly spiral 3
- Ventral and marginal rows distinctly spiral, overlapping dorsum 7
3. Long, band form, posterior pointed or rounded (salt lakes) with only 2 feathered frontal membranelles Genus *Cladotricha* Gajevskaja
- Other types 4
4. Frontal cirri not limited to 3 to 6 but are numerous, distributed in rows not distinct from ventral cirri 5
- Frontal cirri reduced to 3; no other cirri 6
5. Small, oval (50 to 100 μ); with long, widely separated bristle-like cirri Genus *Psilotricha* Stein
- Frontal cirri arranged cross-wise over frontal field Genus *Eschaneustyla* Stokes
6. Elongate, narrowed to tail-like end; usually 2 ventral rows Genus *Uroleptus* Engelm.
- Ovoidal forms with 5 to 8 rows of long ventral cirri not different from marginal rows Genus *Kahlia* Horvath
7. Fresh water forms with broad peristome; posterior end short spine-like Genus *Hypotrichidium* Howaisky
- Slender forms with narrow peristome 8
8. Peristome only slightly narrowed; adoral zone short Genus *Strongylidium* Sterki
- Peristome neck-like, narrowed; adoral zone on left side 9
9. Peristome region little or not at all extensible Genus *Stichotricha* Perty
- Peristome region highly distensible Genus *Chaetospira* Lachmann
10. No specialized frontal cirri; ventral rows run to anterior end without cirri specialization 11
- Frontal cirri strongly developed; first 3 especially 16
11. Small, oval, marine forms with very large peristome 12
- Otherwise formed, or not marine 13
12. Transverse cirri small; not continued to posterior end Genus *Caryotricha* Kahl
- Transverse cirri long and stiff, reaching well beyond posterior end Genus *Stylocoma* Gruber

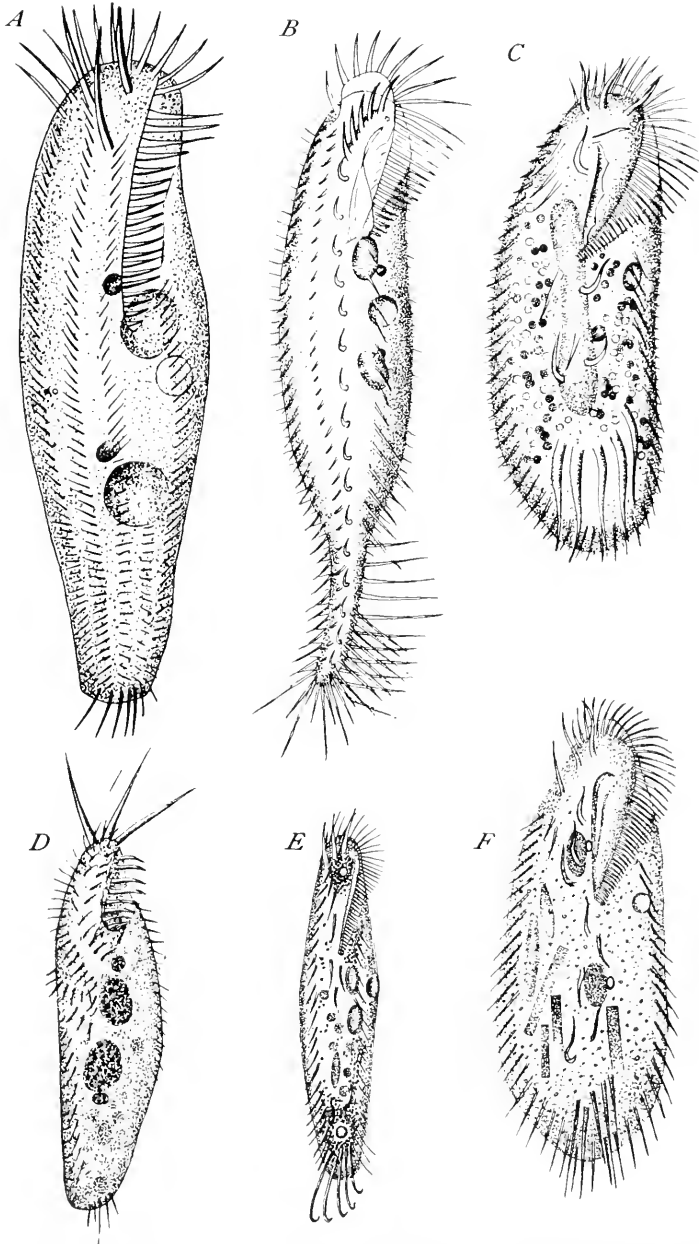


FIG. 209.—Types of Ciliata. A, *Amphisia kessleri*; B, *Uroleptus pisces*; C, *Histrotricha pellionella*; D, *Strongylidium* sp.; E, *Orytricha pellionella*; F, *Orytricha fallax*. (A, after Calkins; B, C, D, E, after Bütschli; F, after Stein.)

Family 1. **Oxytrichidae** Ehr. 1838.

13. Small fresh water forms; long, separated ventrals, no special frontal cirri; dorsal bristles high.....Genus *Balladina* Kowalewski
Form and structure otherwise..... 14
14. With long tail, highly contractile marine forms (Fig. 208).....Genus *Epiclintes* Stein
Form different; if tailed, then moderately contractile—fresh water..... 15
15. Commensal on different species of Hydra
Genus *Kerona* Ehr.
Free-living—not commensal..... 16
16. More than 2 ventral rows of cilia..... 17
One to 3 ventral rows of frontals not distinct.....Genus *Holosticha* (in part see 20)
Wrzesniowski
17. Three ventral rows; frontal rows not much enlarged.....Sub-genus *Trichotaxis* Stokes
More than 3 ventral rows; 3 frontal cirri strongly marked..... 18
18. Ventral cirri all in rows (1 to 6)..... 19
Ventral cirri united in groups..... 21
19. Ventral cirri in 4 or more rows.....Genus *Urostyla* Ehr.
Ventral cirri in 1 to 3 rows or absent..... 20
20. Ventral rows absent; marine form with narrow neck-like peristome.....Genus *Trachelostyla* Kahl
Ventral rows 1 to 3 in number, 3 frontals distinct.....Genus *Holosticha* (in part see 16)
21. Ventral cirri in 1 to 3 rows; post oral and posterior groups of small cirri in addition
Complete ventral rows absent..... 22
22. Complete rows run parallel with long axis... 23
Complete rows run diagonally from anterior right to posterior left..... 25
23. One complete row on left, 2 on right; transverse cirri in complete rows.....Genus *Onychodromopsis* Stokes
The 2 right transverse cirri well behind the 3 left..... 24
24. On each side, one complete ventral row
Genus *Allotricha* Sterki
Two complete ventral rows on right Genus *Pleurotricha* Stein
25. One diagonal ventral row running close to the transverse row (Fig. 210).....Genus *Gastrostyla* Engelmann
Adoral zone placed laterally; ventral rows short, within or just beyond peristome
Genus *Gonostomum* Sterki
26. Posterior end may be drawn out in long thin stalk.....Genus *Ancistropodium*
Fauré-Fremiet
Posterior end never drawn out in long thin stalk..... 27
27. 12 to 15 powerful frontal cirri, 4 macronuclear parts.....Genus *Onychodromus* Stein
8 frontal cirri in 3 groups; macronucleus in 2, rarely in 1 or 4 parts..... 28

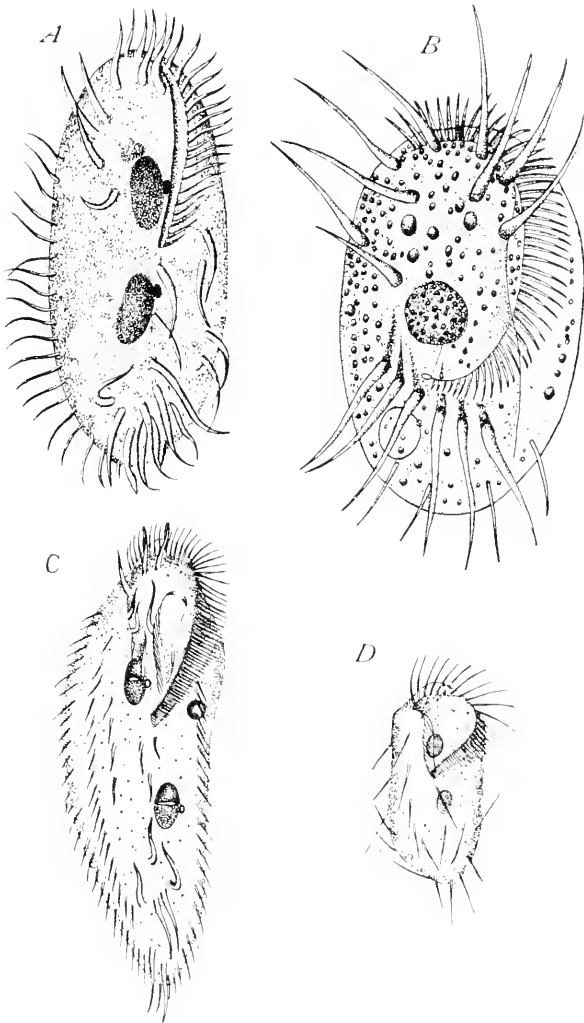


FIG. 210.—Types of Ciliata. A, *Gastrostyla steinii*; B, *Euplotes ramus*; C, *Pleurotricha lanceolata*; D, *Psilotricha acuminata*. (A, B, after Calkins; C, D, after Stein.)

Family 1. **Oxytrichidae** Ehr. 1838.

- 28. Posterior end tail-like or pointed... Genus *Urosoma* Kowalewsky
Posterior end not tail-like or pointed..... 29
- 29. Right peristome edge hook-like and turned
to left, spirally rolled anteriorly... Genus *Steinia* Diesing
Peristome edge does not reach to adoral
zone..... 30
- 30. Body soft, plastic, occasionally contractile. 31
Body stiff..... 33
- 31. Marginal rows continuous posteriorly
Genus *Oxytricha* Bory
Marginal rows broken posteriorly..... 32
- 32. No caudal cirri..... Genus *Tachysoma* Stokes
With caudal cirri..... Genus *Opisthotricha* Kent
- 33. Marginal rows continuous posteriorly; no
caudal cirri..... Genus *Histrio* Sterki
Marginal rows broken posteriorly; with
stiff caudal cirri..... Genus *Stylonychia* Ehrenberg

Family 2. **Euplotidae** Ehr. 1838.

Key to Genera

- 1. Anterior third of body head-like because of
two lateral notches..... Genus *Discocephalus* Ehr.
Anterior third not head-like..... 2
- 2. No special steering cirri near posterior end. 3
Near posterior end 1 or 2 groups of power-
ful cirri..... 5
- 3. Left marginal cirri row continuous; 4 mac-
ronuclei, ellipsoid marine form... Genus *Certesia* Fabre-Dom.
Left marginal cirri absent, or isolated
single ones..... 4
- 4. Frontal part of adoral zone lies in flat
furrow..... Genus *Euplotes* Ehr.
Frontal part of adoral zone separated from
dorsum by deep funnel-like depression
Genus *Crateromorpha*
Perejaslawzewa
- 5. One group of 3 powerful cirri, on right
dorsal..... Genus *Diophrys* Dujardin
Powerful cirri in addition to 3 on right side,
dorsal..... Genus *Uronychia* Stein

Family 3. **Aspidiscidae** Stein 1859.

Only one genus—*Aspidiscus* Ehr.

SUB-CLASS III. **PERITRICHA** STEIN.

- 1. No peristomial trench; attaching disc
ciliated..... Family URCEOLARIIDAE
- 2. With peristomial trench; posterior cilia
temporary..... Family VORTICELLIDAE

Family 1. **Urceolariidae** Stein.

- 1. Forms with smooth attaching ring... Genus *Urceolaria* Stein
Forms with toothed ring..... 2
- 2. With special tactile cilia..... Genus *Trichodina* Ehr.
Without special tactile cilia..... Genus *Acyclochaeta* Zick

Family 2. **Vorticellidae** Ehr.

1. Cup or test-dwelling forms..... 2
 - Without test, with or without stalks, solitary or colonial..... 5
2. Upright; attachment posterior, with or without stalks..... 3
 - Recumbent; attachment lateral..... 4
3. Cup delicate; peristome region cup-like
 - Genus *Ophrydiopsis* Penard
 - Cup thick with or without stalk.... Genus *Colburnia* Ehr.
4. Peristome disc with neck; operculum-like
 - Genus *Lagenophrys* Stein
 - Peristome disc without neck..... Genus *Vaginicola* Lamarck
5. Forms with stalks..... 6
 - Forms without stalks, free-swimming or attached..... 13
6. Stalks contractile..... 7
 - Stalks not contractile..... 9
7. Colonial forms..... 8
 - Solitary forms..... Genus *Vorticella* Linn.
8. Entire colony contracts; stalk threads connected..... Genus *Zoothamnium* Ehr.
 - Individual stalks, only, contract.... Genus *Carchesium* Ehr.
9. Colonial forms..... 10
 - Solitary forms..... 12
10. Great colonies of individuals embedded in jelly..... Genus *Ophrydium* Bory
 - Feathery colonies, individuals not in jelly. 11
11. Peristome region raised on short neck
 - Genus *Opercularia* Stein
 - Peristome region without neck.... Genus *Epistylis* Ehr.
12. Adoral zone with greatly developed membrane..... Genus *Glossatella* Bütschli
 - Adoral zone inconspicuous..... Genus *Rhabdostyla* Kent
13. Free-swimming forms..... 14
 - Attached by posterior end..... 16
14. With digitiform protoplasmic processes
 - Genus *Hastatella* Erlanger
 - Without digitiform protoplasmic processes 15
15. With two caudal threads..... Genus *Astylozoön* Engelmann
 - Posterior ciliated girdle permanent... Genus *Opisthonecta* F. Frem.
16. Posterior end with attaching disc... Genus *Scyphidia* Lachm.
 - No attaching disc; organism rests on posterior end or swims with posterior girdle
 - Genus *Gerda* Clap. and Lachm.

SUB-CLASS IV. **CHONOTRICHA** WALLENGREN.

1. Peristome region funnel-like.... Family SPIROCHONIDAE
 - Peristome region drawn out as two lips

Family CHILODOCHONIDAE

Family 1. **Spirochonidae** Grobben.

1. Peristome spirally wound funnel... Genus *Spirochona* Stein
 - (On gill plates of Gammarus)
 - Peristome not spirally wound..... 2
2. Peristome margin with processes; 1 bud formed (On gill plates of Nebalia)... Genus *Kentrochona* Keuten
 - Several buds formed..... Genus *Kentrochonopsis* Doflein
 - (On gill plates of Nebalia)

Family 2. **Chilodochonidae** Poche.

One genus—*Chilodochona* Wallengren. On mouth parts of crabs (Ebalia and Portunas)

CLASS II. **SUCTORIA** BÜTSCHLI.

- 1. Suctorial tentacles alone present. 2
 Prehensile tentacles in addition to suctorial
 Family EPHELOTIDAE
- 2. Body not bilaterally symmetrical; irregular
 or branched. 3
 Body monaxial; more or less bilateral. 5
- 3. Without "proboscis" or special "arms"
 Family DENDROSOMIDAE
 With retractile proboscis or special "arms" 4
- 4. With retractile proboscis Family OPHRYODENDRIDAE
 With special, tentacle-bearing "arms"
 Family DENDROCOMETIDAE
- 5. Reproduction by internal budding. 6
 Reproduction by external budding
 Family PODOPHRYIDAE
- 6. Pellicle delicate. Family ACINETIDAE
 Pellicle tough, coriaceous. Family DISCOPHRYIDAE

Family 1. **Acinetidae** Bütschli.

- 1. Internal parasites. 2
 External parasites or free-living. 3
- 2. In other Protozoa; no tentacles or suckers
 Genus *Endosphaera* Engelm.
 Horse parasites; with tentacles at opposite
 ends of body. Genus *Allantozoon* Gassovsky
- 3. Parasitic on other suctoria. 4
 Not parasitic on suctoria; or free-living. 5
- 4. Stalk embedded in Acineta or Paracineta
 Genus *Pseudogenemma* Collin
 Parasitic on Ephelota. Genus *Tachyblaston* Martin
- 5. Twelve to 15 finger-form processes, each
 with sucker. Genus *Dactylophrya* Collin
 Without finger-form processes. 6
- 6. Test or cup absent; tentacles in fascicles. 7
 Test or cup present. 8
- 7. Body pyramidal, with stalk (Fig. 117, p.
 228). Genus *Tokophrya* Bütschli
 Form variable; no stalk. Genus *Hallezia* Sand
- 8. Test without free margin, membrane-like
 (Fig. 100, p. 192). Genus *Acineta* Ehir.
 Test cup-like, with free rim or margin. 9
- 9. No definite stalk; test attached by base. 10
 Test attached by definite stalk 11
- 10. Cup attached by entire base. Genus *Solenophrya*
 Clap. and Lach.
 Base of cup narrowed, almost stalk-like
 Genus *Periacineta* Collin
- 11. Cup polyhedral; 1 to 6 central tentacles
 Genus *Acinetopsis* Robin
 Cup not polyhedral; distributed apical ten-
 tacles. Genus *Thaccacineta* Collin

Family 2. **Discophryidae** Collin.

1. One primary tentacle; with or without secondaries 2
With many tentacles 3
2. With stalk Genus *Rhyuchophrya* Collin
No stalk; attachment by protoplasmic body Genus *Rhyncheta* Zenker
3. Suctorial tentacles conical, with enlarged bases Genus *Thaumatoophrya* Collin
Tentacles uniform in diameter 4
4. Tentacles expansile at extremities for food-taking Genus *Choanophrya* Hartog
Tentacles not expansile Genus *Discophrya* Lachmann

Family 3. **Dendrosomidae** Bütschli.

1. Forms with stalk 2
Forms without stalk 3
2. Body much branched, finger-like . . . Genus *Dendrosomides* Collin
Body bar-like, not digitate Genus *Rhabdophrya*
Clap. and Collin
3. Body attached 4
Body free 6
4. Body bilateral or slightly asymmetrical (Fig. 117, p. 228) Genus *Trichophrya*
Clap. and Lach.
Body flat 5
5. With basal stolon; branches erect; often second branches (Fig. 196, p. 477) Genus *Dendrosoma* Ehr.
No stolon; short unbranched processes, fasciated tentacles Genus *Lernacophrya* Perez
6. Body tetrahedral Genus *Tetraedrophrya* Zykoff
Body polyhedral 7
7. With 6 similar protuberances Genus *Staurophrya* Zacharias
With 8 radiate processes, each with a fascicle Genus *Astrophrya* Awerinzew

Family 4. **Dendrocometidae** Stein.

1. Arms branched, each branch with one sucker Genus *Dendrocometes* Stein
2. Arms not branched Genus *Stylocometes* Stein

Family 5. **Ophryodendridae** Stein.

- One genus only Genus *Ophryodendron*
Clap. and Lach.

Family 6. **Podophryidae** Bütschli.

1. Without test or cup 2
With test or cup 3
2. Normally with stalk, attached Genus *Podophrya* Ehr.
Free-swimming or parasitic Genus *Sphaerophrya*
Clap. and Lach.
3. Cup close-fitting, no visible rim Genus *Paracinetia* Collin
Cup not close-fitting, rim visible 4
4. Tentacles numerous; in fascicles Genus *Metacinetata* Bütschli
Tentacles scarce; 1 to 3 Genus *Urnulla* Clap. and Lach.

Family 7. **Ephelotidae** Sand.

- No test or cup; with or without stalk (Fig. 115, p. 226) Genus *Ephelota* Wright
With cup and stalk Genus *Podocorythus* Kent

CHAPTER XIV.

SPECIAL MORPHOLOGY AND TAXONOMY OF THE SPOROZOA.

FORMS adapted to a parasitic mode of life are found in every main group of the Protozoa and several highly pernicious human diseases such as dysentery, Leishmaniasis and trypanosomiasis are due to them. Such forms, however, may be regarded as having arisen as casual parasites which owe their parasitic mode of life to their original power to resist the digestive fluids and other conditions of the animal body. Such adaptations are always possible in normally free-living microorganisms subject to ingestion with food and drink.

Sporozoa are obligatory parasites and free-living forms are unknown. Practically all kinds of animals, even Protozoa, are subject to invasion by one type or other and adaptations are manifold and varied in response to the necessary and often highly specialized conditions of their existence.

In size the Sporozoa vary within wide limits; some are so small that many of them may live together in a single mammalian erythrocyte (*Theileria*, *Babesia*) or in gland cells of different animals (*Microsporidia*). At the other extreme some forms of *Gregarinida* (*Porospora*) grow to a length of 16 mm. In general they are larger than flagellates, smaller than rhizopods and average about the same size as the ciliates.

Form also is variable but fairly consistent within the major groups. Ameboid forms are characteristic of the *Myxosporidia* and of the *Plasmodiidae* of the *Hemosporidia*. *Coccidia* for the most part are spheroidal to ellipsoidal and gregarines elongate ellipsoidal or ovoidal. Fantastic shapes are not uncommon, particularly amongst the *Gregarinida*—star shape in *Astrocytella*, dagger shape, or branched forms in *Aikinetocystis*, etc.

As with parasites generally, a necessary adaptation for the maintenance of species is the power of prolific multiplication. This is realized by the universal method of reproduction by spore formation to which the group owes its name. Such sporulation may occur as multiple reproduction of vegetative individuals without sexual processes or it may follow as a result of fertilization. Asexual and sexual processes give rise to typical alternation of generations in the majority of forms and complicated life histories result.

Nuclei are single in number in Telosporidia and multiple in the majority of Cnidosporidia. In structure they are highly characteristic, particularly in Gregarinida. Here there is a great endosome in vegetative stages of the organism which represents a combination of somatic and germinal chromatin. When ready for sporulation the germinal chromatin leaves the endosome as a small bud and forms chromosomes on a relatively small spindle (Fig. 55, p. 101). The residual mass of endosome and the remainder of the nucleus then disintegrate and disappear. The small aggregate of germinal chromatin together with its division figure thus resembles a micronucleus of the ciliates while the disintegrating portion is equivalent to the macronucleus.

The chromosomes of Telosporidia give more evidence of individuality than do those of any other group of Protozoa. Meiotic phenomena are of two general types—so-called gametic meiosis in which reduction in number of chromosomes occurs during the formation of gametes, and zygotic meiosis in which reduction occurs during the first mitotic division of the amphinucleus. Both types are found in Eugregarinida (Monocystis, Diplocystis, etc.) and Coccidia (Aggregata). The number of chromosomes in gregarines is often uneven (3, 5, 7, etc.) which indicates either zygotic meiosis (Dobell, Jameson) or zygotic synapsis (Naville, see p. 309).

Asexual reproduction may occur by equal division (*e. g.*, *Ophryocystis*, *Babesia*, etc.), by budding which may be exogenous (Myxosporidia) or endogenous (as in the gregarines *Schizocystis* and *Eleutheroschizou*), or by multiple division (Coccidiomorpha). Reproduction following fertilization always involves the formation and the permanent fusion of gametes. These may be isogamous or anisogamous and dimorphic gametes as different as are eggs and spermatozoa of the Metazoa are characteristic of the Coccidia and Hemosporidia. Sexual processes of peculiar type and regarded as self fertilization or autogamy are characteristic of the Cnidosporidia where such processes with resulting sporulation take place in endogenous buds.

Sporulation following fertilization in the majority of forms involves adaptations for preservation of the species during exposure to the conditions external to the definitive host. Such spores are protected against drought and other external conditions by resistant spore membranes or capsules which are opened or dissolved only in the digestive tract of a new host. In the majority of cases such new hosts are individuals of the same species and infection is brought about by eating contaminated food. In many forms, however, the life cycle involves a change of hosts, the metagametic spores developing in one type of animal and the sexual phases of the parasite developing in another type belonging to an entirely different group of the animal kingdom. Thus vegetative stages of the genus *Aggre-*

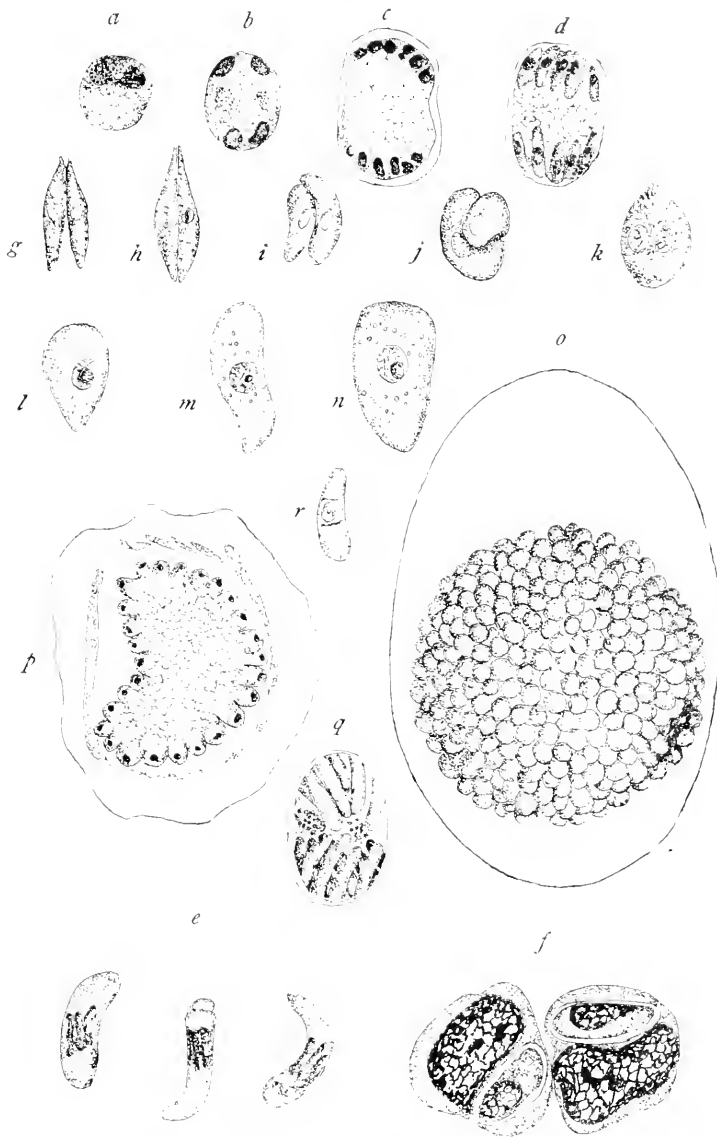


FIG. 211.—*Hepatozoon perniciosum*, a hemogregarine parasite of the rat. *a* to *d*, development of the agamont in the liver cells of the rat; *e*, free parasites in the blood; *f*, encysted parasites in the leukocytes; *g* to *k*, stages of fusion of the gametes in the mite; *l* to *n*, development of the zygote; *o*, sporocyst with sporoblast buds covering the surface; *p*, section of the same; *q*, older sporoblast with sporozoites. (From Calkins after Miller.)

gata develop in the crab (*Portunus depurator*) and the sexual stages in the cephalopod (*Scpia officinalis*); vegetative stages of the malaria organisms, *Plasmodium*, develop in the blood of man or birds and the sexual stages in the mosquito. In these blood-infesting Sporozoa a further adaptation is noted in the loss of the characteristic capsules of the metagametic spores which are inoculated with the bite of the mosquito directly into the blood. In some cases parasites reach the blood by way of the digestive tract and infection is contaminative. The rat parasite *Haemogregarina (Hepatozoön) perniciososa* (according to Miller, 1908) forms its metagametic spores in the rat mite (*Lelaps echidninus*). Such infected mites are eaten by the rat and the spores develop in liver cells through some agametic generations, the agamic spores finally entering the blood where they are taken up by leukocytes in which the parasites encyst. Such encysted spores are taken with the blood into the gut of the mite where sexual phases take place and metagametic spores are formed (Fig. 211).

For characterization of the homologous stages in the very diverse life histories of Sporozoa a special and fairly definite terminology has been adopted by all students of the group beginning with Schaudinn. These terms which are employed in classifications are as follows:

Sporozoite. The final product of metagametic divisions and the beginning of a new life history.

Trophozoite. A vegetative stage which develops from a sporozoite or from a merozoite. (Also termed according to conditions, *agamont*, *gamont* or *schizont*.)

Schizont. A mature trophozoite preparing for multiple or simple division without fertilization. (Also termed *agamont*.)

Schizontocyte. A special type of schizont (or gamont) which by multiple division breaks up into a number of germ-forming centers as in *Caryotropha* and *Klossiella*.

Schizogony. The process of simple or multiple division of a schizont.

Merozoite. A product of schizogony leading to spread of an infection in the same host. (Also called *Agamete*.)

Sporont. A trophozoite destined to form copulating gametes. This may be derived directly (*i. e.*, without schizogony) from a sporozoite as in *Eugregarinida*, or from a merozoite. (Also called *gamont*.)

Sporogony. The process or processes of reproduction leading to the formation of gametocytes and gametes. (Also called *gamogony*.)

Gametocyte. A mother-cell which will produce gametes.

Macrogametocyte. A mother-cell which will produce macrogametes (rare) or develops directly into a macrogamete or female germ cell.

Macrogamete. An inactive (female) cell ready for fertilization.

Microgametocyte. A mother-cell destined to form microgametes.

Microgamete. A motile element (male), equivalent to a spermatozoön.

Gametes. Specialized cells destined to meet and fuse in fertilization.

Gametocyst. A protective covering formed by two gregarines in pseudo-conjugation; not equivalent to oöcyst.

Zygote. A cell formed by the fusion of gametes.

Oöcyst. The hardened fertilization membrane which surrounds the zygote and its products.

Metagamic divisions. Divisions of the zygote leading to the formation of sporoblasts and sporozoites.

Sporoblasts. First products of the division of a zygote. Sporozoite mother-cells.

Sporocyst. Hardened and resistant special capsule of a sporoblast.

Sporozoite. A final product of metagamic divisions.

The significance of these terms will be apparent by illustration with a concrete example for which we may again use the classical case of the life history of *Eimeria (Coccidium) schubergi* as worked out by Schaudinn (1900) (Fig. 212). This is a common intestinal parasite of the familiar centipede *Lithobius*, infection taking place by feeding on contaminated food.

Under the action of the digestive fluids in the centipede the sporozoites are liberated from their protective capsules (oöcyst and sporocyst). A sporozoite penetrates an epithelial cell and grows at the expense of the cell into an agamont (Fig. 212, *a*). When fully grown the nucleus of the parasite divides several times; the protoplasm by multiple division breaks up into small cells about the resulting nuclei the process of nuclear and cytoplasmic division to form these cells being agamogony. The host cell is destroyed and the young cells, known as agametes, are liberated. These agametes make their way by independent gregariform movement to other epithelial cells which they penetrate and in which they repeat the entire agamic cycle, producing in turn new agametes. After five or six days, during which this agamic cycle is repeated resulting in multiple infection of the epithelium, the agametes develop into gamonts or prosexual individuals. Some become large, food-stored cells which, after "maturation" processes form macrogametes directly (*c, f, g*). Others form large cells with clear protoplasm—microgametocytes—which after repeated nuclear divisions give rise to a multitude of microgametes, the process being a form of gamogony. Each microgamete is provided with two flagella by means of which it moves about in the intestinal fluids until it comes in contact with a macrogamete (*h, i, j, s*). The

gametes fuse, a macrogamete being fertilized by a single microgamete (*g*). The fertilized cell resulting from this fusion is the zygote in which the pronuclei fuse. The fertilization nucleus then divides and the two products divide again before the protoplasm divides into four parts, one about each of the nuclei. This process, or metagamogony, results in the formation of four sporoblasts within the sporocyst and each sporoblast has its own individual protective

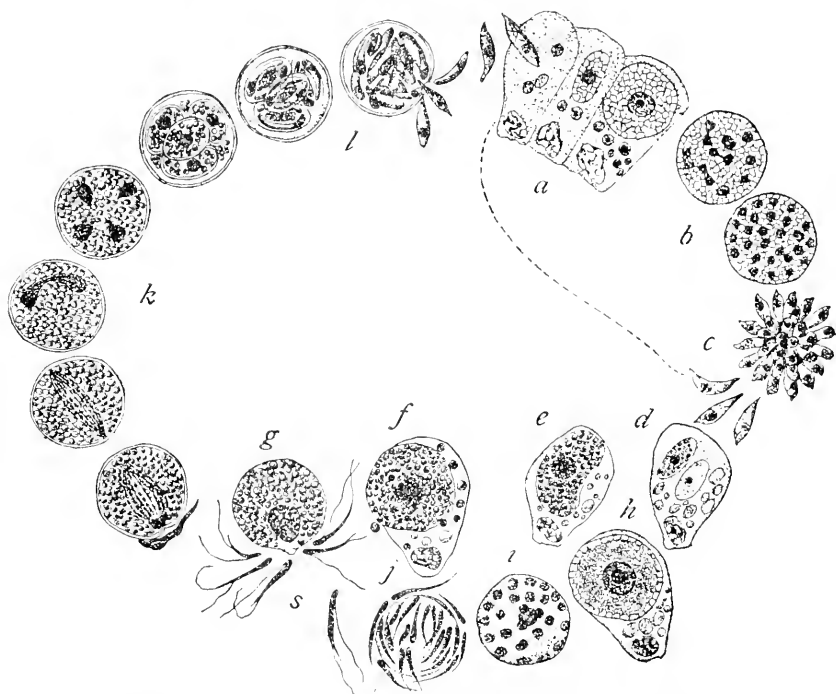


FIG. 212.—*Eimeria schubergi*. Sporozoites penetrate epithelial cells and grow into adult intracellular parasites (*a*). When mature, the nucleus divides repeatedly (*b*), and each of its subdivisions becomes the nucleus of an agamete (*c*). These enter new epithelial cells and the cycle is repeated many times. After five or six days of incubation, the agametes develop into gamonts; some are large and stored with yolk material (*d*, *e*, *f*), others have nuclei which fragment into chromidia which become the nuclei of microgametes (*d*, *h*, *i*, *j*). A macrogamete is fertilized by one microgamete (*g*) and the zygote forms an oöcyst (*k*). This forms four sporoblasts, each with two sporozoites (*l*). (After Schaudinn.)

capsule (*l*). The nucleus of each sporoblast then divides and two independent cells are formed in each sporoblast. These independent cells are the sporozoites. To recapitulate: Sporozoites come from sporoblasts; sporoblasts from zygotes; zygotes from fusion of gametes; gametes from gametocytes, these from gamonts; gamonts from agametes; agametes from agamonts, and agamonts, originally, from sporozoites.

There are thus two complete cycles in the life history of a typical sporozoön, an asexual and a sexual cycle. There are many variations in different types and few life cycles conform exactly with that of *Eimeria*. In the Eugregarines, for example, the asexual cycle is entirely eliminated, the sporozoite developing directly into a gametocyte. In Gregarines also we find a curious process which recalls the phenomenon of conjugation in the Ciliata. It is termed pseudo-conjugation. Two individuals come together side by side or end to end and an envelope is secreted which encloses both indi-

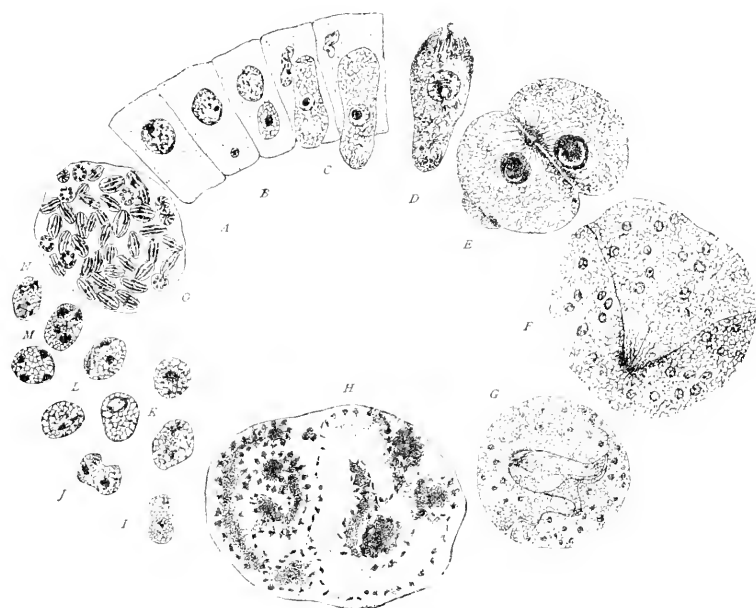


FIG. 213.—*Lankasteria ascidia*. Young sporozoites enter epithelial cells (A, B, C) and grow directly into gamonts (D); two of these unite in pseudo-conjugation (E), and each forms gametes after repeated nuclear divisions (F, G, H). The gametes fuse two and two (I, J, K), and the zygotes undergo three metagametic divisions, forming eight sporozoites (L to O). The parent cells degenerate and the sporocysts are filled with sporoblasts, each with eight sporozoites. (After Siedlecki.)

viduals. This envelope is a gametocyst. Each individual now forms a large number of gametes and those from one individual fuse with the gametes from the other individual and a multitude of zygotes is formed. The actual fertilization membrane becomes the oöcyst and sporocyst and the zygotes divide at once to form sporozoites (Fig. 213).

Invariably parasitic, there is the greatest diversity in sites of parasitism and modes of life of Sporozoa. Gregarines are found only in invertebrates while Coccidiorpha and Cnidosporidia are not

restricted to any particular group. Comparatively harmless types are lumen-dwelling parasites of different organs, particularly of the digestive tract (Gregarinida, Actinomyxida, Cryptosporidium and *Eimeria mitraria* among Coccidia, etc.); more pernicious types are cytozoic (Coccidia, karyozoic in Cyclospora, and Microsporidia) and hematozoic (Hemosporidia) for these involve the destruction of cells and impairment of function. Histozoic forms (Myxosporidia, Sarcosporidia) are likewise pernicious through the formation of great tumor-like cysts in muscles and skin. The massing of cysts in celozoic types often impedes normal activities of the endothelial cells as in the seminal reservoirs of earthworms which frequently contain nothing but cysts, thus virtually effecting castration.

Transmission of Sporozoa from host to host for the most part is by the contaminative method. Enteric parasites develop resistant spores which are passed out with the feces and are ingested sooner or later by other hosts of the same species. Or in some few cases such enteric forms are ingested by hosts of an entirely different animal type. Porospora, for example, is a quite harmless intestinal gregarine of the lobster which forms so-called "gymnospores," either singly (*P. gigantea*) or during pseudo-conjugation (*P. legeri*). These are taken into the digestive tract of the mussel (*Mytilus edulis*) where fertilization occurs. This peculiar history involves some difficulty in classification, for if these gymnospores are gametes as is indicated by *Porospora legeri* then the genus belongs in the Eugregarinida, as is advocated by Reichenow-Doflein; if, on the other hand, they are equivalent to merozoites (agametes) as appears to be the case in *P. gigantea*, then the genus should be classified with the schizogregarines. Until further knowledge is forthcoming we adopt the latter course.

When spores are formed in celomic or body cavities the mode of transmission is less obvious. They may, indeed, be passed out through nephridia or by way of sperm and oviducts or, like coprozoic forms, they may pass unaltered through the digestive tracts of animals which feed upon the normal hosts, to be cast out ultimately with the feces. Minchin suggested that birds may be the main disseminating agent for spores of earthworm gregarines, but it is also probable that dissemination occurs through death of the host or by pinching off infected portions of the organism which then disintegrate. In all such cases and in the great majority of all Sporozoa infection is brought about by swallowing spores, the resistant spore cases of which are dissolved by digestive juices and the germs liberated. These spore coverings for gregarines, coccidia and Cnidosporidia are special adaptations which are undoubtedly useful for protection during the exposed periods in the life cycle. With blood-dwelling parasites such capsules would be fatal, for there is no chemical in the blood to dissolve off the coverings and

liberate the germs, nor would there be any normal way of eliminating such spores if formed in the blood. It is quite possible, of course, that germs may make their way into the blood by way of the digestive tract and this is realized in the Hemoproteidae and in Hemogregarina where final stages in the life history chiefly gametocytes (Hemoproteus) alone are blood-dwelling while other stages occur in the intestinal cells or in the endothelial cells of bloodvessels (Hemoproteus, Haplozoön, Karyolysis). In these cases transmission is brought about by other hosts (flies, mites and leeches) while the definitive host becomes infected by the contaminative method (see also p. 361).

In Plasmodiidae or malaria organisms the digestive tract of the definitive host is not involved in the life history of the parasite. Here the entire vegetative life is in the blood cells of birds or the red blood corpuscles of mammals. No cysts of any kind are formed but the blood, with parasites, is taken into the digestive tract of mosquitoes where fertilization occurs and metagamic products are formed. The final metagamic products—sporozoites—are inoculated by the mosquito directly into the blood (see page 406). Accompanying this type of life history is the formation from hemoglobin of the characteristic pigment melanin (Plasmodium, Hemoproteus) which is absent in forms developing elsewhere than in the blood. Here, also, we note the absence of resistant unchanging membranes (oöcyst, sporocyst) about the zygote which are typical of the majority of Telosporidia. On the contrary, these zygotes produce delicate fertilization membranes which enlarge with growth and development of the zygote which, immediately after fertilization, has the power of independent movement.

Other variations will appear in the discussion of the different groups of Sporozoa as given in the following classification, in which, following the majority of students of the Protozoa, we divide the group into two classes—Telosporidia and Cnidosporidia. The two groups have little in common besides the mode of life of parasites. The Class Telosporidia includes those forms in which the life of the individual comes to an end with sporulation. The Class Cnidosporidia includes those forms in which sporulation occurs in internal buds during the vegetative activity of the individual, sporoblasts being carried about by the still active parent cell.

CLASS I. **TELOSPORIDIA** SCHAUDINN.

Telosporidia are Sporozoa which, with very few exceptions, are intracellular parasites during some phase of the life cycle. A new host is infected by contamination or by inoculation and the young germ—a sporozoite—enters some cell element, an epithelial cell if the parasite is one of the Coccidia, a blood element either blood

corpuscle or blood cell if it is one of the Hemosporidia. The adult forms of Gregarinida are invariably extracellular or lumen-dwelling parasites, young, growing stages alone being intracellular. Adult forms of Coccidiomorpha are persistent intracellular parasites throughout young, adult and reproductive phases. Although some exceptional cases occur in both groups, these are essential differences between the two sub-classes Gregarinida and Coccidiomorpha. All are typically multinucleate in the adult phase.

Reproduction occurs either by agamogony or gamogony, the latter involving fertilization. In one order of the Gregarinida, the Eugregarinida, the sporozoite grows directly into a gamont and asexual reproduction is unknown. In a second order, the Schizogregarinida, agamogony occurs either by equal division, internal budding, or by multiple division. In Coccidiomorpha alternation of generations is the rule and change of hosts is frequent. Multiple division is practically universal.

In both sub-classes the zygote undergoes metagametic divisions. In Gregarinida and in Hemosporidia amongst the Coccidiomorpha, the sporozoites are formed directly by divisions of the zygote; in Coccidia the zygote divides into sporoblasts or sporozoite-forming cells. In all cases except in Hemosporidia the sporozoites formed in each such sporoblast are enclosed in a special capsule—Sporocyst—by which the young organisms are protected against external conditions. Hemosporidia are obligatory parasites in one host or other throughout the entire life cycle otherwise they perish.

SUB-CLASS I. GREGARININA.

The gregarines are typically celozoic or lumen-dwelling parasites of the invertebrates, particularly of annelids and arthropods. They vary in size from 10 μ to 16 mm. (*Porospora gigantea*) and are prone to collect in masses in the intestine, a gregarious habit from which the name of the group is derived. Saprophytic or osmotic in nutrition they apparently do very little if any damage to the host organism, differing in this respect from the intracellular Coccidiomorpha. The most frequent site of parasitism is the digestive tract and the glands opening into it (*e. g.*, *Malpighian tubules*) but the sporozoites of some forms penetrate the wall of the gut and enter the body cavity where they form cysts on the celomic side of the intestinal wall or develop as free forms in the lumen of the seminal vesicles (Monocystidae) or of other parts of the body cavity.

Gregarines are widely varied in form as well as in size but so far as the present accounts go they are similar in their protoplasmic make-up. A peripheral outer layer of lifeless material forms the epicyte which is equivalent to the pellicle or periplast of other Protozoa. This is secreted by the ectoplasm and is frequently

drawn out into attaching organs in the form of filaments, hooks, anchors and knobs. The outer surface is often definitely ribbed, the ribs running longitudinally from end to end of the body. The furrows between the ribs are filled with a gelatinous material derived from a second layer, also lifeless, of the cortex and termed by Schewiakoff the gelatinous layer. The third zone of the body wall is formed by the living ectoplasm which, with the possible exception of *Stomatophora coronata* described by Hesse (1909) as possessing a mouth, peristome and cell anus, forms an unbroken, living, protoplasmic membrane. The endoplasm, or fourth zone, finally, forms the bulk of the organism and contains the single nucleus, usually provided with a large endosome. Paraglycogen, volutin granules and other products of living activity make the endoplasm dense and homogeneous so that it appears white by reflected and black by transmitted light. Crystals of protein-like substance are present in many cases, also crystals which have been identified as calcium oxalate. Between endoplasm and ectoplasm, finally, a system of myonemes may be found in some cases. These, according to Roskin and Levinson (1929), lie in definite canals. The presence of myonemes led to the view that a special myocyte zone is present in addition to the other zones. It is found, however, that in addition to these longitudinal myonemes a second set of circular myonemes is present, lying between the sarcocyte and the endoplasm. A definite myocyte zone, therefore, is absent and myonemes may be found anywhere in the cortex. In *Zygocystis* conspicuous myoneme-like threads originate in the cortex near the anterior end, become free in the posterior third of the body and as free threads trail out behind the posterior end in characteristic manner.

The movement of gregarines has been variously interpreted. In some cases, *e. g.*, *Clepsidrina muniteri*, the organism glides forward without evident contraction of the body; in other cases, *e. g.*, *Monocystis agilis*, forward movement is accompanied by waves of peristaltic contraction and in still other forms there are more or less spasmodic jerks from side to side. The smooth gliding motion, according to Schewiakoff (1894), is due to the secretion of a gelatinous material from the sarcocyte which passes backward along the grooves formed by the ridges of the epicyte. This gelatinous material rapidly hardens on exposure to water, and fresh jelly hardening in turn on this, forces the organism forward. On this interpretation the myonemes play no part. Crawley (1902, 1905), in connection with *Stenophora juli* and *Echinomera hispida*, holds that the slime is not a cause but a result of movement and interprets locomotion as due to the annular contraction of circular myonemes, the organism moving in much the same manner as does a snake. Sokoloff (1912) differs from both Schewiakoff and Crawley

and maintains that the force generated by the secretion of slime is sufficient to send the organism forward on the principle of a sky-rocket.

The majority of observers (Leidy, Lahe, Paehler, Shellack, Dogiel, Cognetti, Roskin and Levinson, etc.) maintain that myonemes alone are responsible for the movements of various types of gregarines, the latest view (Roskin and Levinson, 1929) referring them to the activities of the circular and longitudinal myonemes in much the same way as an earthworm moves through contraction of its longitudinal and circular musculature. The nature of the remarkable threads in *Zygocystis zonata* is not clear. Bowling (1931) observed the thickening of the threads both in living and in fixed material, but whether this indicates a cause or a result of movement is not evident.

Apart from changes in shape due to movements form changes due to development and differentiation are highly characteristic, particularly of the septate gregarines. In all gregarines the early stages in the development of the sporozoite are cytozoic parasites. After a period of growth the partly developed gregarine escapes from the host cell and from that time on lives as a celozoic parasite (Haplocyta). In septate gregarines, however, while the bulk of the young parasite extends into the lumen of the organ, a small portion remains as an anchor in the protoplasm of the host cell. This anchoring part then develops into a specialized structure known as the epimerite which is a characteristic morphological element of the majority of Eugregarinida occurring here and there among the Haplocyta (Diplocystidae, Schaudinnellidae and Rhyncocystidae).

The character of the epimerite is a diagnostic feature of importance in the classification of gregarines. Its development into a long intracellular filament is well shown in Léger and Duboscq's illustration of *Pyxinia moebiuszi* (Fig. 103, p. 201). In other cases it is a mere knob or button within the membrane of the host cell (Stenophoridae), or a knob with recurved hooks as in *Corycella*, *Hoplorrhynchus*, *Sciadophorus*, etc. in short it is a morphological feature of great diversity.

In these septate forms the body is further characterized by the division into chambers (polycystid gregarines of earlier authors) due to the ingrowth of the sarcocyte to form a posterior portion bearing the nucleus and an anterior portion from which the epimerite arises. When the organism approaches maturity these chambers separate from the epimerite, leaving it in the host cell, and as gamonts become free in the lumen. In some rare cases the anterior chamber is also cast off with the epimerite (Genus *Schneideria*), and it frequently becomes a continuous part with the epimerite.

Some forms, notably the Monocystidae, may be highly metabolic;

others move steadily in one direction, a characteristic mode of progression which has given rise to the term gregariniform movement. Motile forms are limited to the free types in the digestive tract or body cavity. Quiescent forms are usually attached to some epithelial cell by the epimerite.

The life history varies from a relatively simple and uncomplicated progression from sporozoite to sporozoite to a complex alternation of generations involving different hosts. The simpler histories are found in the Eugregarinida such as *Monocystis* species or in *Lankesteria ascidiae* (Fig. 213). The latter is a parasite of the digestive tract of the ascidian *Ciona intestinalis* which becomes infected by eating contaminated food. The sporozoites are liberated from the sporocysts and enter epithelial cells where they develop into gamonts. The adult forms are free in the lumen of the gut and are characterized by the possession of a peculiar pseudopodium-like knob which is regarded as a tactile organ. Two of these adults which show evidence of sexual differences (Fig. 214) come together in "pseudo-conjugation." A delicate membrane—gametocyst—is formed and within this membrane each of the individuals forms a large number of gametes. From the great nucleus a smaller nucleus is formed and this divides repeatedly, its products passing to the periphery where small buds, each containing a nucleus, are pinched off as gametes. A gamete from one individual meets and fuses with a gamete from the other. A fertilization membrane is formed which becomes the capsule of the sporoblast. The synkaryon divides three times and eight daughter nuclei are formed which become the nuclei of eight sporozoites. In each sporocyst, therefore, there is a possibility of as many zygotes and sporoblasts as there are gametes formed by one of the original gregarines. The parasites are passed out of the intestine with the feces and further development is inhibited until the sporoblasts are eaten by another host.

A more complex, but still simple, life history involves a change of hosts. The genus *Porospora* appears to be represented by several

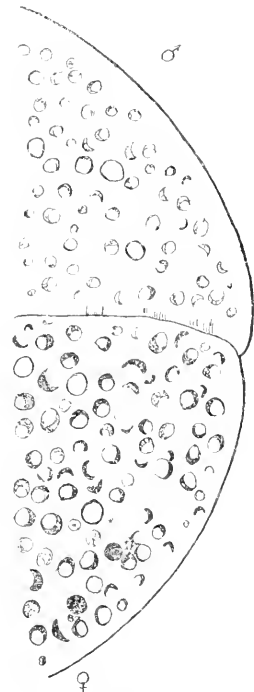


FIG. 214.—*Nina gracilis* in pseudo-conjugation, above male, below female cell. Lipoids (gray) and fats (black) are more abundant in the female than in the male. $\times 500$. (After Joyet-Lavergne, Arch. d'Anatomie Microscopique, courtesy of Masson et Cie.)

species which pass their trophic stages in the digestive tract of crustacea and their sexual stages in mussels. *Porospora gigantea* grows to an enormous size (up to 16 mm.) in the lobster (*Homarus* sp.) where it apparently lives for a long period. Ultimately, and either in association or individually, it becomes spherical and forms a cyst-like ball with a diameter of 3 to 4 mm. The ball then divides into many gametocytes, each with a diameter of from 5 to 8 μ , and

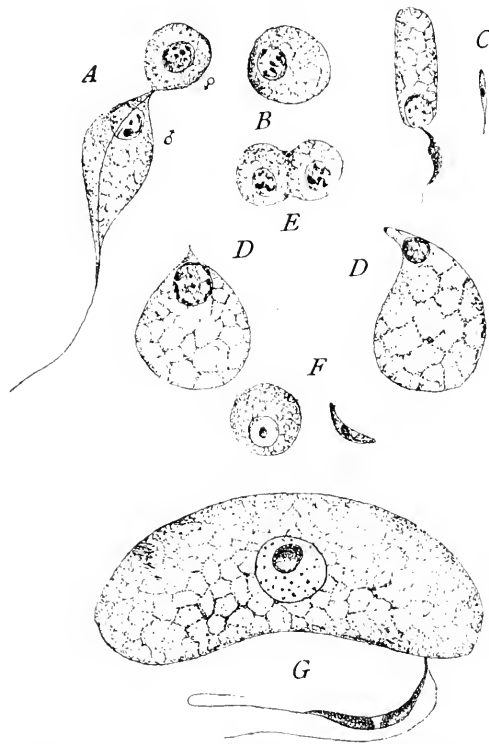


FIG. 215.—Gametes of Gregarines and Coccidia. A, male and female gametes of *Stylorhynchus longicollis*; B, *Monocystis* sp.; C, spermatozoid of *Echinomera hispida*, to the left the two gametes of *Pteroccephalus nobilis*; D, gametes of *Urospora lajidis*; E, of *Gregarina ovata*; F, of *Schaudinella hebrae*; and G, of *Eimeria schubergi*. (From Shellaek after Léger, Cuénot, Brasil, Schnitzler and Schaudinn.)

each gametocyte forms gametes which are arranged radially about a central residual body. The gametes are very small (3μ long by 1μ in diameter) and pass out with the feces into the water with which they enter the digestive tract of the mussel (*Mytilus edulis*) where they unite to form zygotes. Each zygote forms a single sporozoite which is liberated in the gut of the lobster.

The Schizogregarinida are more complicated through the introduction of an asexual reproductive phase in the life history leading

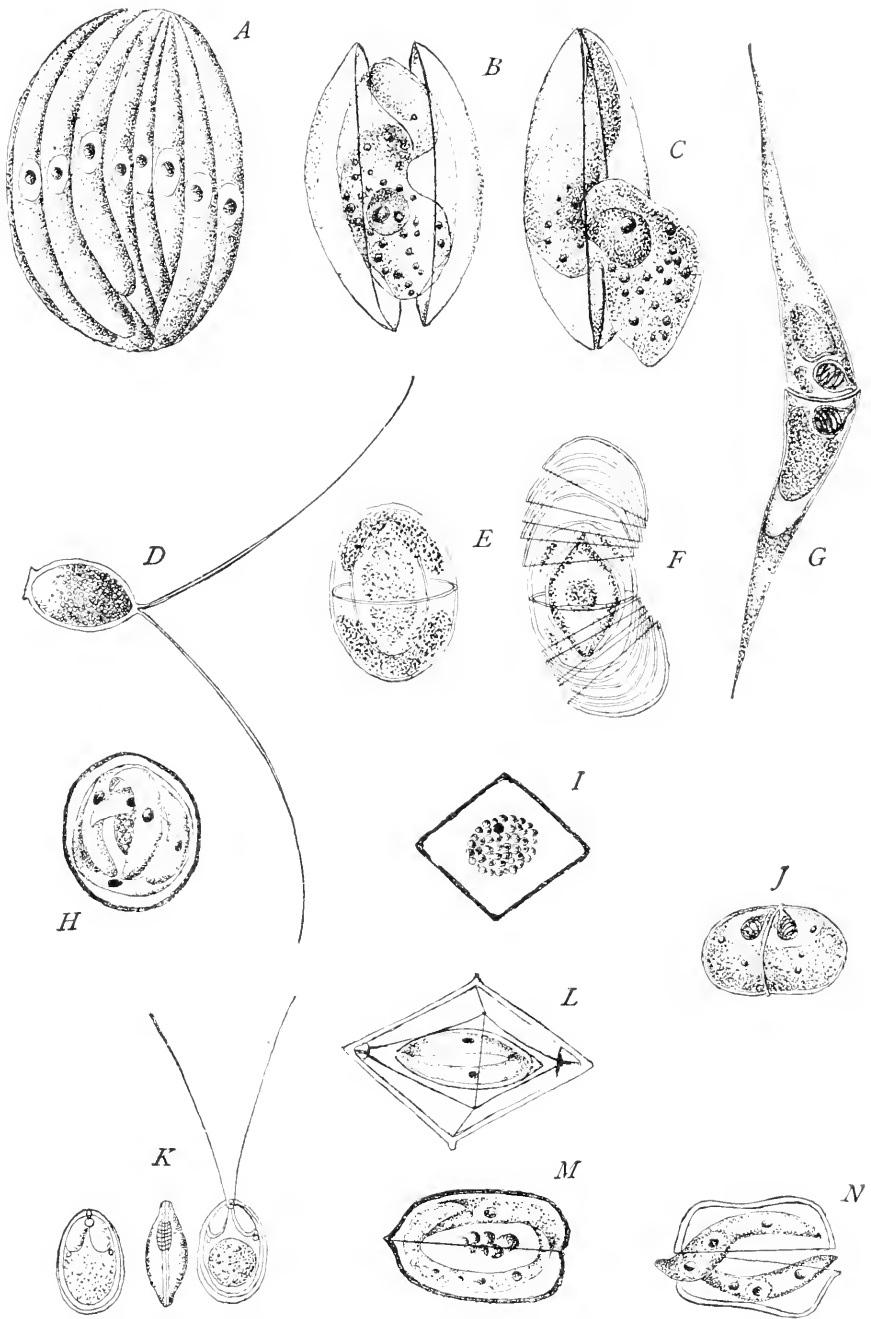


FIG. 216.—Reproductive bodies in Sporozoa. A, agametes of *Barronxoria ornata*; B, C, sporocysts of same with exits of sporozoites; D, tailed sporocyst of *Urospora lagidis*; E, F, sporoblast of *Ophryocystis musuli* with single and multiple spore cases; G, spore of *Ceratomyxa* sp.; H, coccidian sporocyst with four sporozoites; J, spore of *Leptotheca agilis*; K, type of *Myxobolus* spore; L, sporocyst of *Crystallospora crystalloides*; M, N, coccidian sporocyst with two sporozoites. (After Schneider, Wasielesky, Thélohan, Léger and Brasil.)

to spread of the infection in the same host. Under the term "multiplicative reproduction" Doflein distinguishes this phase from the reproduction following fertilization which he calls "propagative reproduction." A relatively simple, but very interesting life cycle is described by Léger in the case of *Ophryocystis mesnili* found in the Malpighian tubules of the beetle *Tenebrio molitor* (Fig. 120, p. 231). Here the asexual cycle is reduced to a process of equal division or multiple division whereby a number of gamonts are formed. These gamonts unite two by two in pseudo-conjugation. The nucleus of each divides twice and one only of the resultant four nuclei becomes the nucleus of a gamete. The two gametes become freed in a brood chamber where they unite and in which the zygote gives rise to a single sporoblast forming eight sporozoites.

In *Schizocystis sipunculi* and in *Eleutheroschizon dubosqui* the asexual cycle is represented by a process of multiple unequal division, the agametes being formed by a process of internal budding (Fig. 119, p. 230).

In some cases, particularly in the cephalont gregarines, specialized sporoblast disseminating tubes known as sporoducts are formed by the gametocysts. These are developed as ingrowths from the cortical protoplasm which in the ripe gametocyst and under the influence of moisture are evaginated as tubular processes through which the sporocysts are emitted (Fig. 125, p. 240). In *Gregarina ovata* they are quite short but reach a considerable length in other species of *Gregarina* and in *Clepsidrina*.

Gamete dimorphism is highly variable in different species of gregarines. Isogametes are produced by some species of *Monocystis*, anisogametes by others although here the differences are slight. Well-marked anisogamy is found in *Pterocephalis nobilis* (Dubosec and Léger) and in *Schandimella heuleae* (Nusbaum), but in general differences in gametes are much less pronounced than in the *Coccidiomorpha* (Fig. 215).

The sporocysts in different species vary widely in form and in sculpturing. The capsule is usually double, consisting of an inner (endospore) and an outer (exospore) capsule, the latter sometimes provided with short spines (*Leanthospora*) or long filaments (*Ceratospora*, Fig. 216). The typical number of sporozoites in a sporocyst is eight, but this is not invariable. They are liberated by action of gastric juices and emerge through preformed openings or by separation of the two valves of the sporocyst. They creep out of the endospore and make their way to epithelial cells within which the first stages of their development occur.

ORDER 1. **Eugregarinida** DOFLEIN EMEND.

The great majority of known gregarines belong to this Order, the agamous individuals living for long periods in the host before unit-

ing in couples to form isogamous or anisogamous gametes. Division or asexual reproduction of any kind is unknown. Only exceptionally are more, or less, than eight sporozoites formed in each sporocyst. They are monocystid (single chambered) or polycystid in structure, the former grouped in the Sub-order Haplocyta, the latter in the Septata.

ORDER 2. **Schizogregarinida** LÉGER (1892).

The Schizogregarinida are parasites of the digestive tract and appended organs of arthropods, annelids and tunicates. They differ from the Eugregarinida in having an asexual or multiplicative cycle, the sporozoite growing into an agamont either as an intracellular or an extracellular parasite. Asexual reproduction occurs by division, internal budding or by multiple division. The life history, gamete formation and metagametic divisions of the zygote vary widely and no characteristic difference marks the sporoblasts from those of the Eugregarinida. Change of hosts is safely established for only one type—the Porosporidae.

SUB-CLASS II. **COCCIDIOMORPHA** DOFLEIN.

While the Gregarinida are practically limited to invertebrate hosts and are typically lumen-dwelling parasites, the Coccidiomorpha are widely distributed in all groups of animals and are typically intracellular parasites in all stages of growth and reproduction. Change of hosts with alternation of generations, while by no means universal, is more common than in the Gregarinida. Agamogony is characteristic of all types and leads to multiple infection with frequently lethal results to the host due to the destruction of multitudes of epithelial or blood cells, to thrombus formation, or to the liberation of toxins. The life cycle varies from relative simplicity to great complexity; gamonts become differentiated into gametocytes which may be recognized as male and female; gametes are anisogamous with rare exceptions; zygotes give rise to sporoblasts which may (Coccidia) or may not (Hemosporidia) be protected by resistant membranes.

ORDER 1. **Coccidiida** LEUCKART EM.

SUB-ORDER 1. **Eimeriina**.

Typically epithelial-cell-dwelling parasites, with exceptions, however, in *Cryptosporidium muris* Tyzzer, *Eimeria mitraria* Laveran and Mesnil and *Orcheiobius herpobdellae* Kunze, which are lumen-dwelling coccidia.

Cellular differentiations are much less numerous than in the gregarines; particularly is this true of the cortex. They are motionless forms without myonemes or other motile organs save flagella

of the microgametes, and cellular processes are generally absent. The endoplasm is usually well stored with products of metabolism, some of which are so characteristic that they have received the name of coccidin. They are all osmotic in nutrition, and infection is always, so far as known, by the contaminative method through the digestive tract. The sporozoite penetrates an epithelial or other definitive cell, grows at the expense of the cell which it ultimately destroys, and forms agametes while still intracellular. *Cyclospora karyolytica* Schaudinn of the ground mole enters the nucleus of the intestinal epithelial cell and as a karyozoic parasite completes its life history.

SUB-ORDER 2. **Hemosporidia** DANILEWSKY, EM. DOFLEIN.

The Hemosporidia are Coccidia-like forms specifically adapted for parasitic life in the blood, particularly of the erythrocytes, although some forms become intracellular parasites of the inner organs. Vertebrates of all classes—mammals, birds, reptiles, amphibia and fish—are subject to infection by one type or other and man is particularly susceptible, the malarial organisms causing serious human diseases which in the tropics are frequently fatal.

Hemosporidia are minute forms, particularly in the agamous stages during which they frequently show highly motile ameboid stages, but in other cases they are more rigid and appear like the hemogregarines. Contractile vacuoles are absent but cytoplasmic non-contractile vacuoles, probably connected with nutrition, are characteristic. Pigmented granules (Melanin) are also characteristic and are formed as a product of hemoglobin break-down and liberated only at periods of reproduction. Other products of metabolism, in the form of toxins, may be liberated at the same time.

Alternation of asexual and sexual generations is the rule, the former taking place in the blood of vertebrates, the latter in the digestive tract of some blood-sucking arthropod, insects in particular. The prevailing opinion is that arthropods were the primary hosts and that parasitism in the blood is the result of adaptation. One such adaptation, and a very essential one, is the absence of protective capsules about the sporozoites. The latter are always formed in the primary or invertebrate host and are transmitted to the vertebrates at the time of drawing blood. A sporozoite penetrates an erythrocyte and grows to an agamont which forms multiple agametes after a definite interval; these agametes are liberated into the blood where other erythrocytes are entered and the asexual cycle is repeated. The parasites thus multiply rapidly by geometrical progression until enough blood elements are destroyed to produce the first marked symptoms of the infection. Hegner and

Taliaferro (1924) estimate about 150,000,000 parasitized blood elements at this time in the case of human malaria, all parasites, if derived from a single infection, undergoing sporulation at practically the same time and liberating their toxin simultaneously into the blood. The pyrexial attacks of chills and fever in human malaria are thus accounted for. Ultimately the agametes develop into gamonts which are usually easy to distinguish from the agamonts and which are frequently differentiated into macrogametocytes and microgametocytes. The gametocytes are taken with the blood into the digestive tract of an invertebrate host (mosquitoes) where the microgametes are formed and where union of gametes occurs. The zygote, like that of some hemogregarines, is motile and makes its way by gregariniform movement to the wall of the gut. These motile zygotes, termed oökinets by Schaudinn, either enter the epithelial cells of the gut or penetrate them and come to rest against the inner membranes of the gut wall. Here a delicate sporocyst membrane is formed and the amphinucleus divides repeatedly without cytoplasmic division until a vast number of nuclei results. The cytoplasm then divides to form as many naked sporozoites as there are nuclei. The delicate sporocyst membrane is ruptured and the sporozoites are liberated into the body cavity from which they are passed into the blood of the vertebrate and the cycle repeated.

The life cycle of the hemosporidian thus has many points of resemblance to that of the coccidian; the same intracellular mode of life, the same asexual generation and agamete formation, the same formation of gametocytes and dimorphic gametes. The microgametes, however, have no flagella, as a rule, but move like spirochetes and the zygote, as noted above, forms naked sporozoites. In many cases, however, there is a reminiscence of sporoblast formation, when, after the amphinucleus has divided for a certain limited number of times, the cytoplasm separates into a number of sporozoite-forming centers. The resemblance to the coccidian would be complete if such centers were provided with definite capsules.

The two families—Hemoproteidae and Plasmodiidae—differ in the site of asexual multiplication. In the former the schizogony cycle occurs in endothelial cells, the merozoites ultimately entering red blood cells of birds where they develop pigment and grow into gametocytes. These are ingested by a biting fly (*e. g.*, *Lynchia*) in which fertilization and sporozoite formation occur in the stomach and body cavity. In Plasmodiidae schizogony occurs in the erythrocytes of mammals and birds.

SUB-ORDER 3. Babesiina.

These are parasites of red blood corpuscles of mammals which differ from Hemosporidiina by the absence of melanin pigment.

They cause epidemic diseases, particularly in cattle (*e. g.*, Texas fever, East Coast fever, etc.). Here, as in Hemosporidiina, there are two families – Babesiidae and Theileriidae, differing again in the site of the asexual cycle. In Babesiidae the parasites reproduce only in red blood corpuscles and only a limited number of division products are formed. In Theileriidae schizogony occurs in endothelial cells where a large number of merozoites are produced.

ORDER 2. Adeleida.

The members of this order differ from the Eimeriina in the absence of flagellated gametes and fertilization of the Eimeria type.

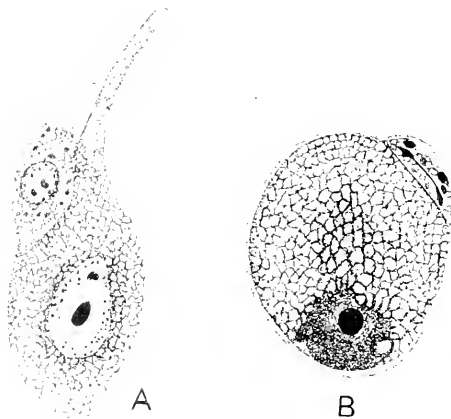


FIG. 217. *Adeleina dimidiata* A. Schu. A, association of macrogametocyte and smaller microgametocyte. B, nuclear divisions in microgametocyte and formation of gametic nuclei. $\times 1100$. (From Doflein after Shellack, Arbeit. aus d. kaiserlichen Gesundheitsamt, courtesy of J. Springer.)

In place of this the sexual process resembles that of pseudo-conjugation in gregarines, without, however, the formation of a gametocyst or a double set of gametes. Two gametocytes, one of which is smaller, unite as in pseudo-conjugation. The microgametocyte may rest cap-like over one pole of the macrogamete (as in *Adelea*), or laterally (as in *Adelina*, Fig. 217). The nucleus of the microgametocyte divides one to three times and one of the products enters the macrogamete and fuses with its nucleus. A rigid fertilization membrane oöcyst as in *Eimeria*, is formed in species of the sub-order *Adeleina*, but in the sub-order *Hemogregarina* the oöcyst is delicate and like that of *Plasmodium* enlarges with growth and development of the zygote. Species of *Adeleina* are intestinal parasites and infection is contaminative. *Hemogregarines* are blood

parasites of vertebrates and are transmitted by leeches, ticks and mites (Fig. 218).

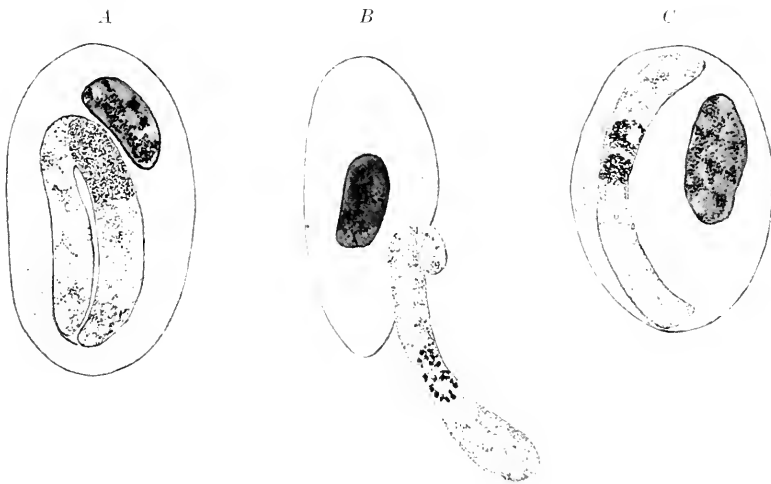


FIG. 218.—Type of Hemogregarines. A, *Haemogregarina stepanovi*; B and C, *Lankesterella ranarum*. (Original.)

CLASS II. CNIDOSPORIDIA DOFLEIN.

The Cnidosporidia form an independent stem of the Protozoa with no recognizable affinities with other groups. They are ameboid and, in the adult stage, usually multinucleated, thus resembling the Mycetozoa. Encapsulated sporoblasts and general mode of life as parasites show some resemblance to the Telosporidia but the life cycle is less complicated, sexual dimorphism and change of hosts being absent. Unlike the Telosporidia reproduction does not bring the life of an individual to an end but takes place more or less continuously throughout the trophic stages, the sporoblasts being carried about with the more or less active organism which ultimately may become a relatively huge mass of spores.

Sporulation and sexual processes are entirely different from analogous activities in the Telosporidia. In a typical form of Myxosporidia in which the ameboid body is multinucleated and the nuclei frequently dimorphic, sporulation begins with a peculiar process of internal budding. An island of protoplasm is formed about two of the nuclei, one of each kind if dimorphic, and this island was termed a pansporoblast by Gurley. This gives rise to two cells, each with 7 nuclei after the 2 nuclei have divided to form 14 nuclei which are now all alike. Two of these 7 nuclei disappear with the formation of a bivalved capsule, 2 of them disappear with the formation of peculiar nematocyst-like capsules termed

polar capsules containing coiled threads, 1 is cast out of the cell and 2 remain as the gametic nuclei which, sooner or later, unite to form one, a process of fertilization frequently interpreted as autogamy (Fig. 164, p. 325).

Sexual processes in Cnidosporidia are so unlike analogous phenomena in other Protozoa that they have long been a puzzle to cytologists as well as matters of controversy to a long list of specialists (Delaisieux, Erdmann, Kudo, Parisi, Auerbach, Mercier, Keysselitz, Schroder, Davis, *et al.*). Thanks to the splendid monograph by Naville (1931) there is a fair prospect that the difficulties will be solved and unanimity established although the phenomena are quite diverse and, in comparison with other Protozoa, most aberrant.

The cnidosporidian trophozoite is an ameboid organism with multiple nuclei and may reproduce by division or by budding (schizogony). There is no alternation of sexual and asexual cycles but the sexual generation is contained in the protoplasm of the trophozoite which develops from a sporozoite.

The activities of the sexual generation are confined to internal buds or spore-forming centers termed pansporoblasts by Gurley (1893). Two nuclei are present at the outset in these endogenous buds and each undergoes division until 14 are present, 7 from each of the original nuclei. The bud then divides into 2 cells, each of which is a sporoblast and each contains 6 nuclei, 1 having been cast out. Two of these 6 form capsules (sporocysts), 2 form nematocysts and 2 remain as pronuclei which subsequently fuse.

From this history it would appear that the endogenous bud represents a zygote and the 2 original nuclei progamete nuclei. Obviously the significance of these nuclei depends upon their previous history. The facts in such histories for different species have been variously interpreted by earlier investigators and find a place in Naville's interpretation. This is based upon his independent study of five different species of Myxosporidia (*Myxobolus guyenoti*, *Chloromyxum leydigi*, *Myxidium incurvatum*, *Sphacromyxa balbianii* and *Sphacromyxa sabrazesi*). In all these species the early divisions of the trophozoite nuclei indicate that there are two types as shown by the mitotic figures. One type represents germinal nuclei with diploid number of chromosomes in the typical division figure. The other type represents vegetative nuclei which divide by amitosis (Naville) or by cryptomitosis (Reichenow). The germinal nuclei after several divisions with the diploid number of chromosomes undergo reducing divisions whereby the number of chromosomes is reduced to one-half.

In *Sphacromyxa sabrazesi* (Fig. 164, p. 325) the two original nuclei of the pansporoblast are different in size. According to Naville this results from two lines of germinal nuclei. In one line

which may be called male, the ultimate division gives rise to four small nuclei, each with the reduced number of chromosomes. In the other line—female—the last two divisions are heteropolar and two so-called “polar bodies” are cast off as in metazoan eggs, leaving one large nucleus with the haploid number. These two haploids, large and small, do not fuse but each divides as stated above and their products become equal in size. Finally the two germ nuclei of the sporozoite unite and thus restore the diploid number characteristic of the species (see also p. 324).

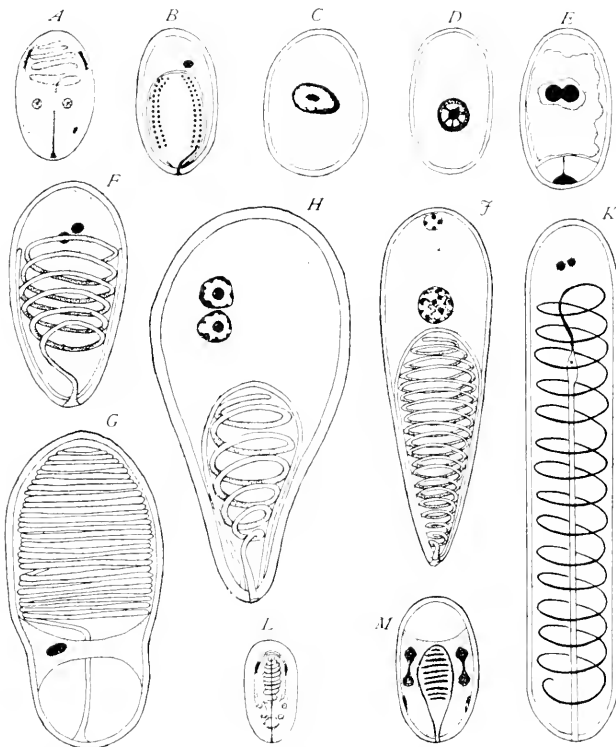


FIG. 219.—Types of Cnidosporidian spores. *A*, *Noscomia apis*, after Fañtham and Porter; *B*, same, after Kudo; *C*, *D*, *E*, different Haplosporidia spores, after Swellengrebel, Perrin, and Swarczewsky; *F*, *Plistophora macrospora*, after Léger and Hesse; *G*, *Plistophora longifilis*, after Schuberg; *H*, *Myxobolus toyamai*, after Kudo; *I*, *Stempellia magna*, after Kudo; *K*, *Mrazekia argoisi*, after Léger and Hesse; *L*, *Nosema bombycis*, after Stempel; *M*, *Thelohanía giardi*, after Mercier. (From Kudo.)

Essentially similar processes occur in Haplosporidia, in Actinomyxida and in Microsporidia but in the latter the nuclei are small and the chromosome history is indefinite.

Sporocysts are bivalved (Myxosporidia) or trivalved (Actino-

myxida) or with a single valve (Microsporidia) and contain one or more polar capsules which recall the stinging cells of the Coelenterata. The threads of the capsules are probably hollow and are spirally wound in the capsule from which they are evaginated under proper conditions. Such threads, the function of which is entirely problematical, may be short or very long, reaching in some cases a length many times that of the sporocyst. The germs can scarcely be called sporozoites since they are not formed as a result of metagametic divisions following fertilization. The term sporoplasm has been used to distinguish the vital, living portion of the spore from the other differentiated parts and will be used here to designate the young germ up to the time of development into the trophic individuals. The spores are built on the same general plan of structure (Fig. 219).

The form assumed by the trophozoites varies with the habitat. Many of the Cnidosporidia are lumen-dwelling, and many are cell-dwelling or tissue parasites. The free forms are characterized by relatively complex organization with ectoplasm, endoplasm and pseudopodia similar to amebae. The pseudopodia may be filiform, lobose or lamellate and locomotion is frequently as active by ameboid movement as in many amebae. Tissue- or cell-dwelling forms are active only in the young stages and according to Doflein may appear in the following conditions: (1) Enclosed in cysts which are formed for the most part by concentric layers of connective tissue derived from the host, and an innermost layer formed by the organism. Huge cysts resulting from association of parasites, and easily visible to the naked eye, are formed in many cases. (2) "Diffuse infiltration," a term used to indicate collections of parasites between tissue cells where they may fill up cavities without doing much or any harm to the host. (3) Intracellular parasites whereby the usually minute organisms live at the expense of the cell host.

ORDER 1. **Myxosporidia** BÜTSCHLI.

The Myxosporidia are the best known of the Neosporidia both as to number of species and life histories. Of the 249 species listed by Kudo (1919) all but 11 are parasitic in fishes, 5 have been found in amphibia, 4 in reptiles, 1 in an insect and 1 in an annelid. They are, therefore, characteristic fish parasites, where they occur both as cellozoic and as histozoic forms, never, according to Davis (1917), in the digestive tract, but the free forms mainly in the gall and urinary bladders, the tissue parasites mainly in the connective and muscular tissues. The free forms produce no evident harmful effects on the host but the tissue parasites are more disastrous, *Myxobolus pfeifferi*, for example, causing costly epidemics amongst food fishes, particularly in the barbel (*Barbus barbus* L.) of Europe.

The free or celozoic forms are the most generalized in structure and the tissue parasites are generally regarded as having been derived from them by adaptation (Auerbach, 1910; Doflein, 1916; Davis, 1917, *et al.*). They are somewhat more numerous than the tissue-dwelling forms, Kudo enumerating 125 species of the former and 114 of the latter while 3 species are apparently transitional and 7 of unknown habitat. The free forms often show a remarkable resemblance to amebae; ectoplasm and endoplasm are usually differentiated, the former, as in some amebae, forming a continuous cortical zone about the organism or, as in other types of amebae, evident in certain regions only. It is occasionally provided with bristle-like processes and the pseudopodia of different types are invariably derived from it (Davis).

The endoplasm is more fluid than the ectoplasm, contains many nuclei and metaplasmic bodies in the form of fat globules, pigment granules and crystalline bodies, in some cases embedded in structures which under the name of spherules (Davis) are sometimes so abundant as to give a characteristic appearance to the organism (Fig. 220).

Like other Sporozoa, the Myxosporidia are highly prolific and adaptations to this end are well marked. Asexual reproduction occurs by simple division or by multiple division (plasmotomy) and by budding. Exogenous budding described by Cohn (1896) in *Myxidium lieberkühni* is regarded by Davis (1916) as abnormal and without significance in reproduction but internal or endogenous budding occurs in *Sinuolinea dimorpha* Davis, where free cells are formed about nuclei in the endoplasm. These cells, called "gemmules" by Davis, escape from the parent organism and develop into individuals (Fig. 121, p. 232).

Propagative reproduction involves the formation of spores and the nearest approach to sexual processes to be found in the Cnidosporidia. The process has been described by various observers and the general agreement of these descriptions indicates a common plan throughout the group. Schröder's account of sporulation in *Sphaeromyxa sabrazesi* Laveran and Mesnil may be selected as an example for the entire Order. This form is parasitic in the sea-horse, *Siphonostoma roundeletii*, and like many others has dimorphic nuclei distinguishable by size and structure. Small areas become differentiated within the endoplasm and contain two nuclei, one of each type. These areas, the so-called pansporoblasts, are the mother-cells of the spores. Each nucleus divides in such order that seven nuclei arise from each; the mother-cell then divides into two cells which are destined to form two spores. Each of these cells has 7 nuclei, 1 of which is cast out as a "reduction" nucleus; 2 are involved in the formation of the two valves of the spore and ultimately disappear; 2 are connected with the elaboration of the polar

capsules and similarly disappear and 2 remain as germinal nuclei. It is generally assumed that these 2 nuclei are descendants of the original dimorphic nuclei of the trophozoite and observations by Schröder (1910), Davis (1916), Erdmann (1911 and 1917), Naville

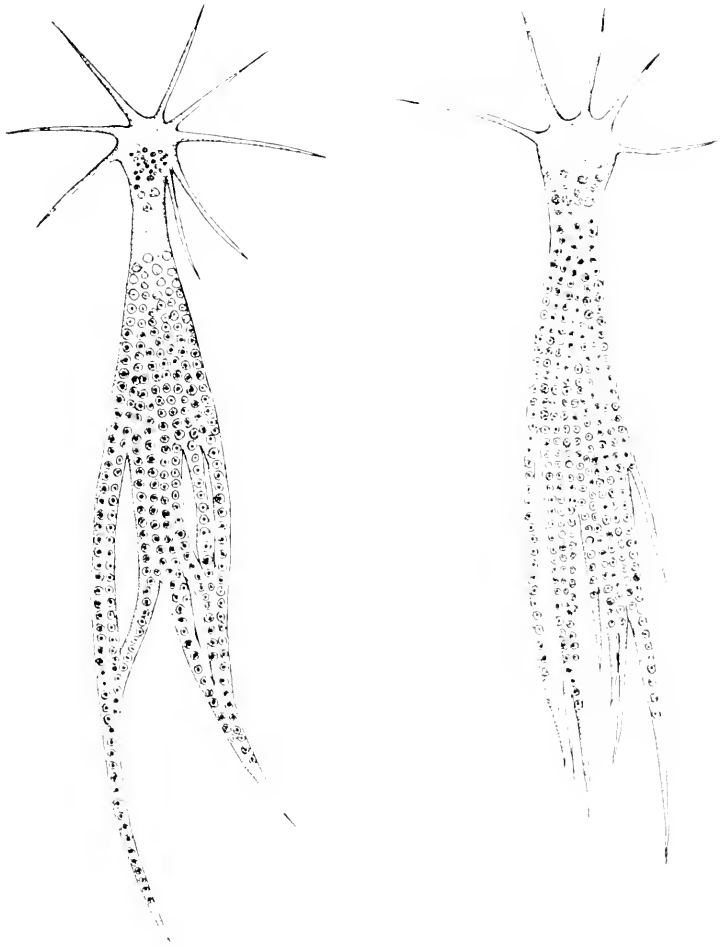


FIG. 220. *Leptotheca scissura*, vegetative individuals with well-developed spherules. (After Davis.)

(1931) leave little doubt that they ultimately fuse in autogamous fertilization (p. 324).

The spores which differ from sporoblasts of the Telosporidia in that they are not formed as a result of fertilization are the most characteristic structures of the Myxosporidia and are much more highly differentiated than are sporoblasts of the former group. They

conform to the same general plan of structure throughout but differ in axial relations and in sculpturing, as well as in number and time of appearance. The spore capsule always consists of two valves which are independently developed and come together with a median suture dividing the spore into right and left halves. In different types the spores may be elongated in the plane of the suture or at right angles to it. The polar capsules with their coiled threads indicate what most authorities regard as the anterior end although spores of the Myxidiidae have thread capsules at each end of the elongated spore (Fig. 164, p. 325). Lateral processes, posterior spines and external sculpturing of various types distinguish the different genera and species and afford a means of classification.

ORDER 2. *Actinomyxida* STOLÇ.

These are Cnidosporidia about which little is known beyond the process of sporulation. In its fully grown condition the entire body may be interpreted as one pansporoblast which is surrounded by a membrane, and which usually produces eight spores, the membranes of which are usually triradiate and drawn out into elaborate spines. Each spore has three polar capsules containing distinct protrusible filaments.

The processes leading to the formation of spores involve fertilization phenomena of a characteristic type. They are essentially similar to those of the Myxosporidia but differ in some important details. A plasmodial stage appears to be absent or represented by a binudeate amebula only, which develops into a spore. The two nuclei divide and form 4 cells, 2 of which disappear with the formation of a membrane within which the other 2 cells lie. Each of these divides, forming 4, 2 of which continue to divide rapidly until 8 are formed, while the other 2 remain large and undivided the two-celled membrane now containing 8 small and 2 large cells. Ultimately the two large nuclei begin to divide in turn until 8 products result and 16 cells, regarded by Caullery and Mesnil (1905) and by Ikeda (1912) as gametes, lie free in the cyst. The two sets of gametes differ slightly in nuclear size and in staining capacity and unite 2 by 2 to form 8 zygotes. The nucleus of each zygote now divides until 6 small nuclei and 1 large one result, the large one destined to form a mass of sporozoites. The 6 small ones arrange themselves in such a manner as to form 3 shell-forming cells, while 3 of them lie within and form 3 polar capsules. The germ-forming cell is not enclosed by the spore-forming cells but lies outside of it and peripherally in the pansporoblast. It divides repeatedly until 8, 32 or many sporozoites result (Fig. 221).

The *Actinomyxida* are parasites of annelids and sipunculids and the spores are invariably triradiate. The anchor or star-form

processes of the capsule are regarded by Doflein as supports in floating, evidence for which is given by Kofoid's observation of these spores in plankton.

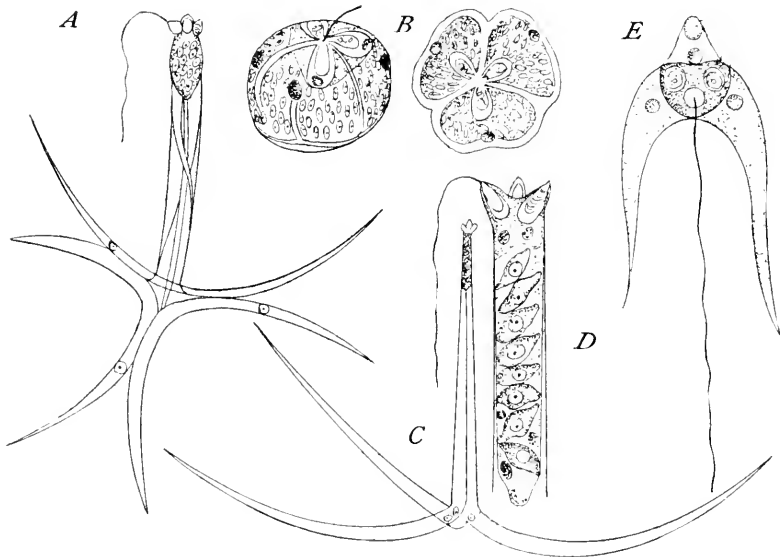


FIG. 221.—Spores of Actinomyxida. A, *Heractinomyxon psammoryctis*, after Stolc; B, *Sphaeractinomyxon stolci*; C, *Triactinomyxon ignotum*; D, same, spore-bearing part enlarged, after Léger; E, *Synactinomyxon tubificis*. (After Caullery and Mesnil.)

ORDER 3. **Microsporidia** BALBIANI.

Probably because of their minute size the organisms included in this Order are incompletely known and many points of structure and of life history are still unknown or controversial. They are practically all cell parasites which enter the host by way of the digestive tract from which they may spread to all tissues of the body, causing epidemics not only in fish but, economically more important, costly epidemics in silkworms (*Nosema bombyces* Naeg.) and honey bees (*Nosema apis* Zander). Pseudopodia and ameboid movement are rarely observed (*Nosema marionis* Thel). Intermediate hosts are unknown.

Agamous reproduction is well established through the observations of many investigators. The agametes are small, uninucleate, and usually with indefinite outlines which scarcely delimit them from the host cell protoplasm; they may have one or several nuclei, and multiply actively by simple division resulting frequently in chain formation through successive nuclear divisions and delayed cell division (Fig. 222). As a result of such agamous reproduction all of the tissues of the host may become infected and myriads of

tissue cells destroyed. In many species tumor-like masses are formed in which the organisms are surrounded by a membrane derived from the host and are thus encapsulated; in other species such membranes are absent. In the majority of cases spread of the infection in the same host comes to an end with sporulation,

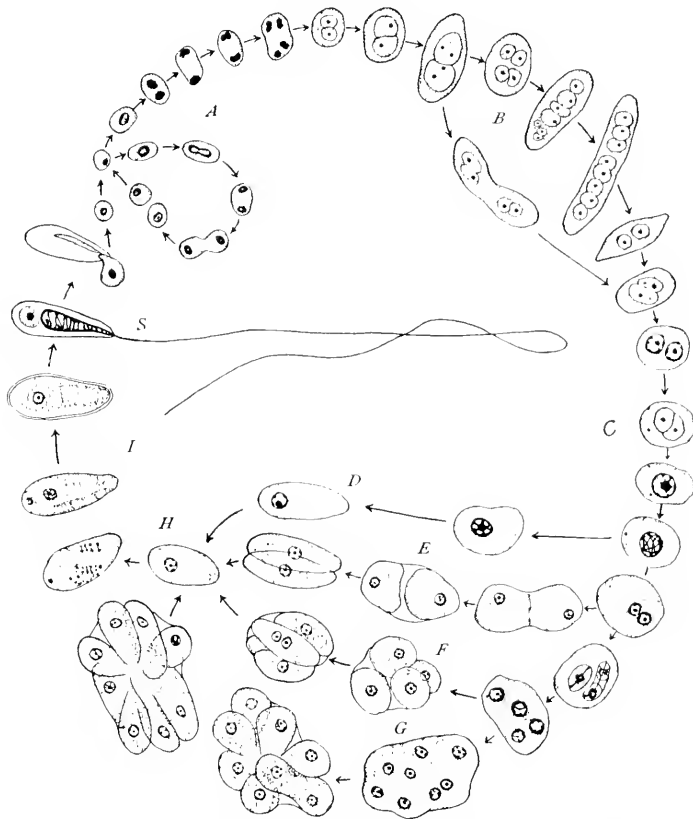


FIG. 222.—*Stimpellia magna*, life cycle. A, Developmental stages of young amebula from spore S; B, stage of nuclear increase; C, formation of sporont; D, formation of a single spore; E, formation of two spores; F, formation of four spores; G, of eight spores; H, development of uninucleated spore with polar capsule. (After Kudo.)

but in some species renewed infection is brought about by the action of the digestive fluids on spores formed in the same organism (Kudo).

Multiple endogenous budding, or fragmentation of the trophozoite into numerous binucleate agametes, is described for some forms (Debaisieux, 1920) and these, as in Telosporidia, ultimately

give rise to the sporulating individuals. The phenomena of sporulation differ widely but there is still much uncertainty in the different accounts at hand. In some cases the trophozoites are said to produce pansporoblasts as in Myxosporidia during the continued vegetative life of the individual (Polysporea). Such cases, included formerly under the name Blastogenea, are regarded as very doubtful

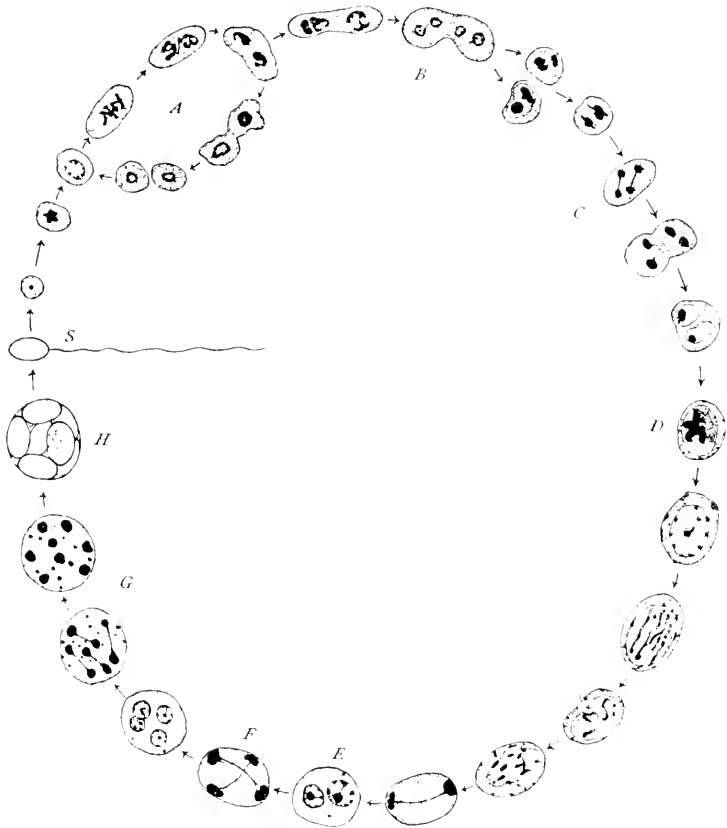


FIG. 223.—*Thecolahania légeri*, life cycle. A, Early stages of sporozoite after leaving the spore S; B, formation of binucleated individuals; C, repeated binary division; D, fusion of the two nuclei to form the sporont; E to H, nuclear and cell divisions to form eight sporoblasts each of which forms one spore. (After Kudo.)

by Doflein. In other cases the trophozoite (pansporoblast?) breaks up into numerous sporulating cells, each of which produces one or more spores (Oligosporea) and in still other cases the entire individual forms a single spore without pansporoblast formation (Monosporea). The absence of pansporoblasts in such cases is regarded as evidence of extreme adaptation on the part of the exclusively cytozoic parasites (*Nosema* species).

The spores on the whole are less complex than those of the Myxosporidia. They are small and ovoidal or bean-shaped and rarely (*Telomyxa* Léger and Hesse, 1910) with more than one polar capsule, in some cases without any. The capsules and threads are invisible or very difficult to see in the living spore (hence cryptozoists), but are demonstrable upon treatment with alkalis. The spore capsule is bivalved in some but consists of a single piece in other species. The history of spore-formation agrees in the main with that of the Myxosporidia but authorities disagree as to details and convincing proof is yet to be demonstrated. Fertilization processes have been described by Mercier (1908, 1909) whereby two isogametes of *Thelohania giardi* fuse to form the pansporoblast. Autogamous union of nuclei prior to spore formation and not, as in Myxosporidia, in the later sporoplasm, has been described by Debaisieux (1913, 1915) in species of *Thelohania* and *Glugea*.

The life history of *Stempellia magna* as given by Kudo (1924) is typical of the Microsporidia (Fig. 222). The polar filament of the spore (*S*) is extruded when the spore reaches the mid-gut of its culicine host; the uninucleate sporoplasm creeps out of the opening made by the cast-off filament, enters a fat cell and becomes an agamont and reproduces by division (*A*). The products ultimately become multinucleated with from four to eight nuclei (*B*); the organisms then breaking up into binucleated cells, the nuclei of which fuse after discarding some chromatin (*C*). This is identified as a sporont which may become transformed into a single spore (*D*), or it may divide into two (*E*), four (*F*) or eight (*G*) sporoblasts, each of which forms a single spore after chromidia formation and reconstruction of small nuclei (*H, I*), some of which take part in the formation of the capsular thread. A more simple life history is shown by *Thelohania légeri* according to Kudo (Fig. 223).

CLASS III. ACNIDOSPORIDIA CEPEDE.

The Sarcosporidia are parasites of vertebrates, particularly mammals, in which the ultimate seat of parasitism is the muscular tissue. There is but one genus—*Sarcozystis*—with several species in pigs (*S. miescheriana* Kühn, 1865, forming "Miescher's tubules"), in sheep (*S. tenella* Railliet, 1886), in cattle (*S. blanchardi* Doflein, 1901), in mice (*S. muris* Blanchard, 1885), in opossums (*S. darlingi* Brumpt, 1913), in monkeys (*S. kortei* Castellani and Chalmers, 1909) and in man (*S. lindemanni* Rivolta, 1878). A species from birds was described by Stiles (1893) under the name of *S. rileyi*.

Sarcosporidia have been studied by a host of observers and an almost equal number of interpretations has been the result. The best-known species is *S. muris* from the mouse in which, beginning with Th. Smith's (1901) inoculation experiments by feeding infected

tissues to mice, the young stages and their development are now known. Observations made by this method of study, particularly by Erdmann (1910, *a, b, c*, and 1914), and by Crawley (1914 and 1916) and Marullaz (1920) permit of a tentative life history of *S. muris* as follows:

Infection occurs by eating infected tissues, or, as Négre (1907) showed, by eating contaminated feces. The germs, regarded by Erdmann (1914) as sporozoites, enter the epithelial cells within an hour to an hour and a half (Crawley and Marullaz). Here, according to Crawley (1914 and 1916), they develop directly into gametocytes which are sexually differentiated. The microgametocytes become practically all nucleus the chromatin of which is distributed in groups of granules about the periphery; each group forms a single microgamete, the spermatozooids being arranged about the periphery very much like the microgametes of a coccidian. The macrogametocytes retain most of their cytoplasm and become macrogametes. The latter are fertilized by a microgamete. The zygotes then give rise to a large number of products (the sporoblasts of Erdmann) which may enter the musculature, or may possibly pass out with the feces (Crawley). Here there is a gap in the accounts of the life history but ultimately the muscles are invaded and asexual multiplication results in a number of sporozoites (Erdmann) groups of which are massed together and kept in place by membranes formed by the host. Upon reinfection these develop again to gametocytes.

It is evident that if this account of the life cycle, the important sexual phases of which are supplied by Crawley, is confirmed by further studies, the Sarcosporidia should not be retained in the Cnidosporidia but, as Crawley suggests, should be placed with the Coccidiomorpha. Until such confirmation is forthcoming the older arrangement is retained.

SUB-PHYLUM SPOROZOA LEUCKART.

CLASS I. **TELOSPORIDIA** SCHAUDINN

Sub-class 1. **Gregarinina (Gregarinae)** Doflein

Order 1. **EUGREGARINIDA** Doflein

- Sub-order 1. *Haplocyta* Lankester
- Family 1. *Monocystidae* Stein
- Family 2. *Zygocystidae* Bhatia
- Family 3. *Diplocystidae* Bhatia
- Family 4. *Schaudinellidae* Bhatia
- Family 5. *Rhynchozystidae* Bhatia
- Family 6. *Stomatophoridae* Bhatia
- Family 7. *Aikinetocystidae* Bhatia
- Family 8. *Syncystidae* Bhatia
- Family 9. *Ganymedidae* J. Huxley
- Family 10. *Urosporidae* Woodcock
- Family 11. *Lecudinidae* Kamm
- Family 12. *Allantocystidae* Bhatia

CLASS I. **TELOSPORIDIA** SCHAUDINNSub-class 1. **Gregarinina** (**Gregarinae** Doflein)Order 1. **EUGREGARINIDA** DofleinSub-order 2. *Septata*Family 1. *Stenophoridae* Léger and DuboscqFamily 2. *Gregarinidae* LabbeFamily 3. *Didymophyidae* LégerFamily 4. *Dactylophoridae* LégerFamily 5. *Actinocephalidae* LégerFamily 6. *Menosporidae* LégerFamily 7. *Stylocephalidae* EllisFamily 8. *Acanthosporidae* LégerOrder 2. **SCHIZOGREGARINIDA** LégerSub-class 2. **Coccidiomorpha** DofleinOrder 1. **COCCIDIIDA** LeuckartSub-order 1. *Eimerina*Family 1. *Cryptosporidiidae* PocheFamily 2. *Selenococcidiidae* PocheFamily 3. *Eimeriidae* LégerFamily 4. *Caryotrophidae* LüheFamily 5. *Aggregatidae* LabbéFamily 6. *Lankesterellidae* ReichenowSub-order 2. *Haemosporidiina*Family 1. *Haemoproteidae* DofleinFamily 2. *Plasmodiidae*Sub-order 3. *Babesina*Family 1. *Babesiidae* PocheFamily 2. *Theileriidae* WenyonOrder 2. **ADELEIDA** LégerSub-order 1. *Adeleina*Family 1. *Adeleidae* LégerFamily 2. *Klossiellidae* WenyonFamily 3. *Dobelliidae* WenyonFamily 4. *Legerellidae* WenyonSub-order 2. *Haemogregarina*Family 1. *Haemogregarinidae*Family 2. *Hepatozoidae*Family 3. *Karyolysidae*CLASS II. **CNIDOSPORIDIA** DOFLEINOrder 1. **MYXOSPORIDIA** BütschliSub-order 1. *Eurysporina* KudoFamily *Ceratomyxidae* DofleinSub-order 2. *Sphaerosporina* KudoFamily 1. *Chloromyxidae* ThelohanFamily 2. *Sphaerosporidae* DavisSub-order 3. *Platysporina* KudoFamily 1. *Myxidiidae* ThelohanFamily 2. *Myxosomatidae* PocheFamily 3. *Myxobolidae* ThelohanFamily 4. *Coccomyxidae* Léger and HesseOrder 2. **ACTINOMYXIDA** StolcFamily 1. *Haploactinomyxidae* GranataFamily 2. *Euactinomyxidae* Granata

CLASS II. **CNIDOSPORIDIA** DOFLEIN

- Order 3. MICROSPORIDIA Balbiani
 - Sub-order 1. *Monocnidea* Léger and Hesse
 - Family 1. *Nosematidae* Labbé
 - Family 2. *Coccosporidae* Kudo
 - Family 3. *Mrazekidae* Léger and Hesse
 - Sub-order 2. *Dicnidea* Léger and Hesse
 - Family *Telomyxidæ* Léger and Hesse

CLASS III. **ACNIDOSPORIDIA** CEPEDE

- Order 1. SARCOSPORIDIA Balbiani
- Order 2. HAPLOSPORIDIA Lühe

KEY TO SUBDIVISIONS AND GENERA OF SPOROZOA.

- 1. Spores with thread capsules. Class 2. CNIDOSPORIDIA
- Spores without thread capsules. 2
- 2. Reproduction ends life of parent organism
 - Class 1. TELOSPORIDIA
 - Reproduction during continued vegetative life. Class 3. ACNIDOSPORIDIA

CLASS I. **TELOSPORIDIA** SCHAUDINN

- 1. Typically celozoic parasites. Sub-class 1. GREGARININA
- Typically cytozoic or hematozoic parasites. Sub-class 2. COCCIDIOMORPHA
(Exception in *Cryptosporidium*)

Sub-class 1. **Gregarinina** (**Gregarinae** Doflein).

- 1. Sporozoites develop into sporonts; no asexual cycle. Order 1. EUGREGARINIDA
- Sporozoites develop into agamonts; with asexual cycle. Order 2. SCHIZOGREGARINIDA

Order 1. **EUGREGARINIDA** Doflein

- 1. Typically with protomerite and deutomerite. Sub-order 2. SEPTATA
- Individuals of one chamber; no protomerite. Sub-order 1. HAPLOCYTA

Sub-order 1. *Haplocyta* Lankester*Key to Families*

- 1. Sporocysts alike at the two poles. 1a
- Sporocysts with dissimilar poles. 8
- 1a. Sporocysts without spines or processes. 2
- Sporocysts with spines at each end
 - Family 8. SYNCYSTIDAE
- 2. Trophozoites without attaching organs. 3
- Attaching organs present. 4
- 3. Trophozoites solitary without free myoneme threads
 - Family 1. MONOCYSTIDAE
- Trophozoites always in pairs; often with free myoneme threads or longitudinal striations. Family 2. ZYGOCYSTIDAE
- 4. Trophozoites with epimerites. 5
- Trophozoites with suckers. 6
- 5. Individuals solitary. 7
- Individuals associated in masses. Family 3. DIPLOCYSTIDAE

Sub-order 1. *Haplocyta* Lankester

Key to Families

- 6. Trophozoites unbranched — 1 terminal sucker Family 6. STOMATOPHORIDAE
- Trophozoites branched, each branch with terminal sucker Family 7. AIKINETOCYSTIDAE
- 7. Male and female gametes well differentiated Family 4. SCHAUDINELLIDAE
- Gametes similar Family 5. RHYNCHOCYSTIDAE
- 8. Sporonts united by ball and socket joint Family 9. GANYMEDIDAE
- Sporonts without ball and socket joint 9
- 9. Sporocysts without funnel at one end 10
- Sporocysts with funnel at one end Family 10. UROSPORIDAE
- 10. Sporocysts tetradral Family 13. TETRAEDROCYSTIDAE Baur
- Sporocysts oval or spindle-shape 11
- 11. Sporocysts oval, one pole thickened Family 11. LECUDINIDAE
- Sporocysts spindle-shape, one side thickened Family 12. ALLANTOCYSTIDAE

SUB-ORDER **Haplocyta** LANK. HOMOPOLARIDEA.

Family 1. **Monocystidae** Aut.

- 1. Trophozoites spherical or oval 2
- Trophozoites much elongated 3
- 2. Trophozoites ovoid, often with button at anterior end Genus *Monocystis* Stein
- Trophozoites spherical, no protuberances Genus *Apolocystis* Cognetti
- 3. Trophozoite elongated; like nematode worm Genus *Nematocystis* Hesse
- Trophozoite elongated; one end swollen; club-shape Genus *Rhabdocystis* Boldt

Family 2. **Zygocystidae** Bhatia 1930.

- 1. Association tête-a-tête; long posterior filaments Genus *Zygocystis* Stein
- Association otherwise 2
- 2. Association side by side; body striations distinct Genus *Pleurocystis* Hesse
- Conjugants form cross; head of one attached to center of the other Genus *Enterocystis* Zwetkov

Family 3. **Diplocystidae** Bhatia 1930.

- 1. Trophozoites spherical or oval Genus *Diplocystis* Kunstler
- Trophozoites small, spatulate Genus *Lankesteria* Mingazzini

Family 4. **Schaudinnellidae** Bhatia.

- One genus with epimerite or free as male and female gamonts Genus *Schaudinnella* Nusbaum

Family 5. **Rhynchocystidae** Bhatia 1930.

- One genus, with metabolic epimerite Genus *Rhynchocystis* Hesse

Family 6. **Stomatophoridae** Bhatia 1930.

- 1. Trophozoites round or oval 2
- Trophozoites ellipsoid or star-shaped 6
- 2. Suckers; without filaments or pseudopodium 3
- Suckers; with filaments or pseudopodium 5

Family 6. **Stomatophoridae** Bhatia 1930.

3. Trophozoites sub-spherical or cup-shape . . . 4
Trophozoites spherical to ovoid; anterior sucker with button Genus *Stomatophora* Drzewiecki
4. Sucker with myonemes directed towards convex side Genus *Craterocystis* Cognetti
Sucker with smooth walls Genus *Alberticella* Cognetti
5. Mobile sucker with pseudopodium and filaments Genus *Choanocystis* Cognetti
Mobile sucker with fringe of filaments, no pseudopodium Genus *Choanocystoides* Cognetti
6. Trophozoites star-shape Genus *Astrocytella* Cognetti
Trophozoites ellipsoidal—suctorial depression anterior Genus *Beccariocystis* Cognetti

Family 7. **Aikinetocystidae** Bhatia 1930.

- One genus in celomic cavities of Eutyphoeus
Genus *Aikinetocystis* Gates

Family 8. **Syncystidae** Bhatia 1930.

- One genus, sp. *S. mirabilis* in body cavity of
Nepa cinerea Genus *Syncystis* A. Schn.

Family 9. **Ganymedidae** J. Huxley 1910.

- One species *G. anaspidis* J. Huxley, in gut of
Anaspis tasmaniae Genus *Ganymedes* J. Huxley

Family 10. **Urosporidae** (1) Woodcock, 1906.

1. Cross-section of epispore, circular 2
Cross-section of epispore, triangular
Genus *Pterospora* Rac. and Labbé
2. Sporocysts without caudal filaments 3
Sporocysts with 1 or 2 caudal filaments 4
3. Sporocysts with funnel at one pole, rounded at other Genus *Gonospora* (2) A. Schn.
Sporocysts with funnel at one pole, flattened at other Genus *Lithocystis* Giard
4. Sporocysts with funnel at one end, one caudal filament Genus *Urospora* A. Schn.
Sporocysts with two rigid, diverging, caudal filaments Genus *Ceratospore* Léger

Family 11. **Lecudinidae** Kamm. (**Doliocystidae** Labbé).

- One genus, species *L. pellucida* (*Doliocystis*)—
gut of *Nereis* Genus *Lecudina* Mingazzini

Family 12. **Allantocystidae** Bhatia 1930.

- One genus, species *A. dasyhelei*, gut of larva
of *Dasyhelea* Genus *Allantocystis* Keilin

Synonymys

1. *Urosporidae* = *Choanosporidae* Dogiel
2. *Gonospora* = *Cystobia* Ming.; *Diptodina* Woodcock; *Kalpidiorhynchus* Cunningham
3. *Lecudina* = *Doliocystis* Léger; *Ophiodina* Ming.

SUB-ORDER 2. **Septata**.

1. Epimerite simple, no hooks or processes . . 2
Epimerite complex, on long necks or with
hooks and processes 4

2. Epimerite a mere knob; sporocysts with definite suture. Family 1. STENOPHORIDAE Léger and Dub.
 - Epimerite variable; sporocysts without suture. 3
 3. Satellites without septum. Family 3. DIDYMOPHYIDAE Léger
 - Satellites with septum. Family 2. GREGARINIDAE Labbé
 4. Sporocysts without bristles or spines. 5
 - Sporocysts with bristles at ends or equator or both. Family 8. ACANTHOSPORIDAE Léger
 5. Sporocysts brown or black; in chains
 - Family 7. STYLOCEPHALIDAE Ellis
 - Sporocysts colorless. 6
 6. Sporocysts crescentic, smooth; epimerite on long protrusible neck. Family 6. MENOSPORIDAE Léger
 - Sporocysts elongate, biconical, cylindrical or ellipsoidal. 7
 7. Epimerite asymmetrical, with finger form processes. Family 4. DACTYLOPHORIDAE Léger
 - Epimerite symmetrical. Family 5. ACTINOCEPHALIDAE Léger
- Family 1. **Stenophoridae** Léger and Duboseq 1904.
- Epimerite rudimentary. Genus *Stenophora* Labbé
 - Epimerite a button on short conical neck
 - Genus *Oöcephalus* Sch.
 - Epimerite a button on small spherical protomerite. Genus *Grenoblia* Hasselmann
- Family 2. **Gregarinidae** Labbé 1899.
1. Gametocysts with sporoducts. 2
 - Gametocysts without sporoducts. 4
 2. Solitary individuals; epimerite a globular knob
 1. Epimerite on short neck. Genus *Leidyana* Watson
 2. Epimerite on long neck. Genus *Gryllotalpia* Hasselmann
 - Individuals associated. 3
 3. Protomerite present in young stages only
 - Genus *Gamocystis* Léger
 - Protomerite in all stages; posterior half yellow-green. Genus *Gregarina* Dufour
 4. Individuals solitary. 5
 - Individuals associated. 6
 5. Protomerite temporary; body spherical, gray. Genus *Sphaerocystis* Leger
 - Protomerite in all stages; posterior half yellow-green. Genus *Cnemidospora* Schneider
 6. Individuals associated in pairs. 7
 - Individuals associated in groups of 2 and more. 10
 7. Endoplasm orange-yellow in color. . Genus *Hyalospora* Schneider
 - Endoplasm not colored. 8
 8. Sporocysts prismatic. Genus *Euspora* Schneider
 - Sporocysts spherical or ovoidal. 9
 9. Sporocysts ovoid, with dark equatorial line
 - Genus *Frenzelina* Leg. and Dub.
 - Sporocysts spherical. Genus *Tettigonospora* Smith

Family 2. **Gregarinidae** Labbé 1899.

10. Individuals in groups of 2 or 3; epimerite
a forked style.....Genus *Uradiophora* Mercier
Individuals in groups of 2 to 12; epimerite
a small papilla.....Genus *Hirmoecystis* Labbé

Family 3. **Didymophyidae** Léger 1892.

- One genus—*D. gigantea* Stein.....Genus *Didymophyes* Stein

Family 4. **Dactylophoridae** Léger 1892.

1. Protomerite long, neck-like.....Genus *Trichorhynchus* Schneider
Protomerite flattened; epimerite long fila-
ments..... 2
2. Protomerite symmetrical; drawn out in two
processes.....Genus *Nina* Grebnecki
Protomerite asymmetrical.....Genus *Echinomera* Labbé

Family 5. **Actinocephalidae** Léger.

1. Sporonts with one or more septa..... 2
Sporonts without septum—protomerite
early lost, deutomerite alone.....Genus *Schneideria*
2. Sporonts with one septum..... 3
Sporonts with several septa
1. Epimerite lobed.....Genus *Rhynchocystis* Keilin
2. Epimerite simple.....Genus *Taeniocystis* Léger
3. Septum convex towards protomerite..... 4
Septum flat..... 6
4. Protomerite with small epimerite bearing
6 long filaments.....Genus *Bothriopsides* Strand
Epimerite without long filaments..... 5
5. Protomerite dilated anteriorly and massive
Genus *Légeria* Labbé
Protomerite a circular, shallow disc. Genus *Colcorhynchus* Labbé
6. Epimerite simple styliform process..... 7
Epimerite without style..... 13
7. Epimerite finger-form, conical or lance-
shape..... 8
Styliform process arises from epimerite
base..... 10
8. Epimerite finger-form changing to flat
button.....Genus *Steinina*
Léger and Duboscq
Epimerite conical or lance-shape..... 9
9. Epimerite a simple sharply-pointed cone
Genus *Stylocystis* Léger
Epimerite lance-shape.....Genus *Pileocephalus* Schneider
10. Epimerite a tuft of bristles on a long neck
Genus *Geneiorhynchus* Schneider
Epimerite without long bristles..... 11
11. Epimerite discoid with style from center.. 12
Epimerite spiny, globular with long apical
style.....Genus *Beloides* Labbé
12. Epimerite long thread-like.....Genus *Pyxinia* Hammerschmidt
Epimerite thick disc with milled border
Genus *Asterophora*
13. Epimerite on long neck-like process of pro-
tomerite..... 14
Epimerite on short neck, or sessile, usually
with hooks..... 15

Family 5. **Actinocephalidae** Léger.

- | | |
|---|--|
| 14. Epimerite button-like with 8 to 10
finger-form processes | } Genus <i>Agrippina</i>
spores ellipsoid
Genus <i>Hoplorhynchus</i>
spores biconical |
| Epimerite cushion-like with short teeth
Genus <i>Phialoides</i> Labbé | |
| 15. Epimerite flat sessile, with 8 to 10 short,
sharp spines | Genus <i>Actinocephalus</i> Stein |
| Epimerite globular or swollen | 16 |
| 16. Epimerite like half-open umbrella | Genus <i>Sciadiophora</i> Labbé |
| Epimerite globular, fluted, or with collar | 17 |
| 17. Epimerite with collar, on short stalk
Genus <i>Discorhynchus</i> | |
| Epimerite without collar; fluted or smooth | 18 |
| 18. Epimerite depressed anteriorly or flat | 19 |
| Epimerite globular, not depressed | Genus <i>Amphoroïdes</i> Labbé |
| 19. Epimerite flat, fluted | Genus <i>Anthorhynchus</i> Labbé |
| Epimerite spheroidal, depressed anterior | 20 |
| 20. Fluting confined to concavity | Genus <i>Amphorocephalus</i> Ellis |
| Fluting deep, on sides | Genus <i>Stictospora</i> Léger |

Family 6. **Menosporidae** Léger 1892.

One genus—*M. polyacantha* Léger Genus *Menospora*

Family 7. **Stylocephalidae** Ellis 1912 (**Stylorhynchidae** Labbé)

Includes *Stylorhynchus* pre-occup.; changed to
Stylocephalus Ellis

- | | |
|--|--------------------------------------|
| 1. Epimerite on long slender neck | 2 |
| Epimerite sessile or on short neck | 3 |
| 2. Spores oval | Genus <i>Sphaerocystis</i> Labbé |
| Spores hat-shape | Genus <i>Stylocephalus</i> Ellis |
| 3. Epimerite a crateriform disc with club-
shaped processes | Genus <i>Lophocephalus</i> Labbé |
| Epimerite a lance-shaped papilla on short
neck | Genus <i>Cystocephalus</i> Schneider |

Family 8. **Acanthosporidae** Léger 1892.

- | | |
|---|---------------------------------|
| 1. Sporocysts with polar but without equa-
torial spines | Genus <i>Corycella</i> Léger |
| Sporocysts with both polar and equatorial
spines | 2 |
| 2. Sporocysts with 2 rows of equatorial spines
Genus <i>Cometoides</i> Labbé | |
| Sporocysts with 1 row of equatorial spines | 3 |
| 3. Epimerite a conical papilla without pro-
cesses | Genus <i>Acanthospora</i> Léger |
| Epimerite spheroidal with 5 to 10 finger-
form processes | Genus <i>Ancyrophora</i> Léger |

Order 2. **SCHIZOGREGARINIDA** Léger 1892.

- | | |
|--|---|
| 1. Sporozoites in sporocysts less than 8 | 2 |
| Sporozoites in sporocysts 8 | 4 |
| 2. Sporozoites in sporocysts 4; many sporo-
cysts | Genus <i>Selenidium</i> Giard |
| Sporozoites in sporocysts 1 | 3 |
| 3. Trophozoite coiled in flat spiral | Genus <i>Spirocystis</i>
Léger and Duboseq |
| Trophozoite not coiled; elongate (2 hosts)
Genus <i>Porospora</i> Schneider | |

Order 2. SCHIZOGREGARINIDA Léger 1892.

4. Sporocysts less than 8..... 5
 - Sporocysts 8 or more..... 7
 5. One sporocyst in gametocyst..... 6
 - Two sporocysts in gametocyst..... Genus *Mattesia* Naville
 6. Schizogony in lumen..... Genus *Ophryocystis* Schneider
 - Schizogony, intracellular..... Genus *Merogregarina* Porter
 7. Intracellular throughout most of cycle... 8
 - Celozoic throughout..... 9
 8. With 16 sporocysts..... Genus *Lipotropha* Keilin
 - With more than 16 sporocysts... Genus *Mensbiera* Bogolavlensky
 9. Trophozoite elongate, worm-like... Genus *Schizocystis* Léger
 - Trophozoite globular..... Genus *Caulteriyella* Keilin
- Eleutheroschizon mesnili* not known in sexual cycle.

SUB-CLASS 2. COCCIDIOMORPHA.

1. Gametocytes develop independently; many microgametes..... Order 1. COCCIDIIDA
- Gametocytes associated in pseudo-conjugation; few microgametes..... Order 2. ADELEIDA

ORDER 1. Coccidiida (Coccidia Leuckart).

1. Zygote and sporoblasts protected by resistant unchanging sporocyst capsules
- Sub-order 1. EIMERIINA
- Zygote with delicate, growing sporocyst
- Sub-order 2. HAEMOSPORIDIINA

ORDER 2. Adeleida.

1. Zygotes with tough, resistant sporocyst; non-motile..... Sub-order 1. ADELEINA
- Zygotes with delicate sporocysts; motile
- Sub-order 2. HAEMOGREGARININA

SUB-ORDER 1. Eimeriina.

Key to Families

1. Growing and multiplicative phases cytozoic..... 2
- Growing and multiplicative phases celozoic..... 5
2. Trophozoite and microgametocyte divide into secondary forms (Schizontocytes)
- Family 4. CARYOTROPHIDAE
- Trophozoite divides into agametes (merozoites)..... 3
3. Zygotes develop directly into sporozoites (asporocystid)..... Family 6. LANKESTERELLIDAE
- Zygotes divide to form sporoblasts..... 4
4. Schizogony in one type of host, sporogony in another..... Family 5. AGGREGATIDAE
- Schizogony and sporogony in the same host
- Family 3. EIMERIIDAE
5. Schizonts and gametocytes intracellular (cytozoic)..... Family 2. SELEXOCOCCIIDAE
- All stages celozoic..... Family 1. CRYPTOSPORIDIIDAE

- Family 1. **Cryptosporidiidae** Poche 1913.
 One genus—*C. muris* Tyzzer 1907. . . . Genus *Cryptosporidium* Tyzzer
- Family 2. **Selenococcidiidae** Poche 1913.
 One genus—*S. intermedium* Léger and Duboseq 1909. . . . Genus *Selenococcidium*
 Léger and Duboseq
- Family 3. **Eimeriidae** Poche 1913.
1. The zygote forms sporozoites directly (1 sporocyst) 2
 The zygote forms more than 1 sporocyst. . . 3
 2. Sporocyst and oöcyst without micropyle
 Genus *Pfeifferinella* Wasielewski
 Sporocyst and oöcyst each with micropyle
 Genus *Caryospora* Léger
 3. The zygote forms 2 sporocysts. 4
 The zygote forms more than 2 sporocysts. . 6
 4. Each sporocyst contains 2 sporozoites
 Genus *Cyclospora* A. Schneider
 Each sporocyst contains more than 2 sporozoites. 5
 5. Each sporocyst has 8 sporozoites. . . Genus *Dorisiella* Ray
 Each sporocyst has 4 sporozoites. . . Genus *Isospora* (1) A. Schneider
 6. The zygote forms 4 sporocysts. 7
 The zygote forms many sporocysts. 11
 7. Each sporocyst has 2 sporozoites. 8
 Each sporocyst has \neq 30 sporozoites. Genus *Angiocystis* Brasil
 8. Sporocysts ellipsoidal or serrated at one end. Genus *Eimeria* (2) A. Schneider
 Sporocysts not ellipsoidal. 9
 9. Sporocysts without neck at one end. . . . 10
 Sporocysts with neck at one end. . . Genus *Jurriina* Léger and Hesse
 10. Sporocyst a double pyramid with short spines. Genus *Crystallospora* Thelohan
 Sporocyst bivalved, opening like pea-pod
 Genus *Goussia* Labbé
 11. Each sporocyst has 1 sporozoite. 12
 Each sporocyst has 2 or more sporozoites. . 13
 12. Sporocysts with radial markings or spines
 Genus *Echinospora* Léger
 Sporocysts smooth. Genus *Barcoxia* Schneider
 13. Each sporocyst has 2 sporozoites. . . Genus *Pseudoklossia*
 Léger and Duboseq
 Each sporocyst has many sporozoites
 Genus *Merocystis* Dakin
1. Synonyms of *Isospora* are: *Diplospora* Labbé, *Klossia* Labbé, *Hyaloklossia* Labbé and *Luertina* Henri and La Blois 1925.
 2. Synonyms of *Eimeria*—*Mitrocystis* Pinto 1927; *Paracoccidium* Lav. and Mesnil; *Orthospora* A. Schm.
- Family 4. **Caryotrophidae** Lühe 1906. . . Genus *Caryotropha* Siedlecki
- Family 5. **Aggregatidae** Labbé 1899.
 One genus; type sp. *A. cherthi* Labbé 1895
 Genus *Aggregata* (1)

Family 6. **Lankesterellidae** Reichenow 1921.

Development takes place in gut cells of lizard;
sporozoites in blood cells. Genus *Shellackia*

Development takes place in endothelial cells
of bloodvessels; merozoites and gameto-
cytes in blood cells of frog. Genus *Lankesterella*

1. Synonyms of *Aggregata*: *Klossia octopiana*, *Benedenia*, *Légeria*,
Eucoccidium, *Légerina*, etc.

SUB-ORDER 2. **Haemosporidiina** (HAEMOSPORIDIA DANILEWSKY).

The entire asexual cycle occurs in the blood
(malaria). Family PLASMODIIDAE

Only gametocytes are present in the blood
Family HAEMOPROTEIDAE

Family 1. **Plasmodiidae** Mesnil 1903.

One genus—*Plasmodium* Marchiafava and Celli

Family 2. **Haemoproteidae** Doflein 1916.

Melanin pigment produced; gametocytes hal-
ter-shape. Genus *Haemoproteus* Kruse

No melanin pigment produced; blood cells
much distorted. Genus *Leucocytozoon* Danilewski

SUB-ORDER 3. **Babesiina** (PIROPLASMODEA).

Schizogony in red blood cells. Family BABESIIDAE Poche

Schizogony in endothelial cells of bloodvessels
Family THEILERIIDAE

Family **Babesiidae** Poche 1913.

One genus with several sub-genera. . . . Genus *Babesia* Starcovici

Family **Theileriidae** França and Borges 1907.

One genus with possible sub-genera. . . . Genus *Theileria* Bettencourt,
França and Borges

ORDER 2. **Adeleida**.

Resistant, unchanging oöcyst. . . Sub-order 1. ADELEINA

With delicate oöcyst, enlarging with growth
Sub-order 2. HAEMOGREGARININA

SUB-ORDER **Adeleina** (ADELEIDAE LÉGER 1911). FAMILIES.

1. Zygote asporocystid. 2

Zygote forms sporocysts. 3

2. Microgametocyte produces many micro-
gametes. Family 3. DOBELLIIDAE

Microgametocyte produces only 4 micro-
gametes. Family 4. LEGERELLIDAE

3. Each sporocyst has a small number (2, 4, 6)
of sporozoites. Family 1. ADELEIDAE

Each sporocyst has many sporozoites
($\neq 30$). Family 2. KLOSSIOLIDAE

Family 1. **Adeleidae**.

1. Each sporocyst has 2 sporozoites. 2

Each sporocyst has 4 or more sporozoites. . 3

2. Macrogametes with finger-form process—
sporocysts few, spherical. Genus *Adelina* Hesse

Macrogametes spheroidal—sporocysts num-
erous, discoid. Genus *Adelea* Schneider

Family 1. **Adeleidae.**

- 3. Sporocysts few (3); 4 to 6 sporozoites
 Genus *Chagasella* Machado
 Sporocysts numerous; 4 sporozoites 4
 - 4. Macrogamete very long; 25 to 30 sporocysts Genus *Orcheobius*
 Schuberg and Kunze
 Macrogamete spheroidal, many sporocysts 5
 - 5. Microgametes 4 in number; 4 sporozoites
 Genus *Klossia* A. Schneider
 Microgametes 2 in number; sporocysts
 with \pm 30 sporozoites Genus *Klossiella*
 Smith and Johnson
- Doubtful genus—*Pneumocystis* Delanoë 1912.

SUB-ORDER 2. **Haemogregarinina.**

Three families, each with a single genus.

- 1. Zygote forms sporozoites without forming sporocysts Genus *Haemogregarina*
 Danilewsky
 Zygote forms several sporoblasts 2
- 2. Sporoblasts produce sporocysts and sporozoites in oöcyst Genus *Hepatozoön* Miller
 Sporoblasts leave oöcyst and develop sporocysts and sporozoites independently
 Genus *Karyolysus* Labbé

CLASS II. **CNIDOSPORIDIA** DOFLEIN.

- 1. Spores large, with valves; polar capsules visible *in vivo* 2
 Spores small; membrane one piece; capsules invisible *in vivo* Order 3. MICROSPORIDIA
- 2. Spore membrane bivalved; 1, 2 or 4 polar capsules Order 1. MYXOSPORIDIA
 Spore membrane trivalved; 3 polar capsules Order 2. ACTINOMYXIDA

ORDER 1. **Myxosporidia** BÜTSCHLI.

- 1. Spores elongated at right angles to sutural plane Sub-order 1. EURYSPORINA
 Spores spheroidal, oval or elongated in sutural plane 2
- 2. Spores spherical or sub-spherical; no iodophilus vacuole Sub-order 2. SPHAEROSPORINA
 Spores with sutural plane in long axis or oblique to it Sub-order 3. PLATYSPORINA

SUB-ORDER 1. **Eurysporina** (EURYSPOREA KUDO).

One family Family CERATOMYXIDAE

Family **Ceratomyxidae** Doflein.

- 1. Spore valves conical and hollow . . . Genus *Ceratomyxa* Thelohan
 Spore valves otherwise 2

Family **Ceratomyxidae** Doflein.

2. Valves hemispherical or rounded... Genus *Leptotheca* Thelohan
Valves otherwise..... 3
3. Spores spheroidal or ovoidal in front view;
flattened in side view..... Genus *Mitrospora* Fujita
Spores otherwise..... 4
4. Spores pyramidal..... Genus *Myxoproteus* Doflein
Spores oval in side view; front view isosceles triangle with convex sides... Genus *Wardia* Kudo

SUB-ORDER 2. **Sphaerosporina** (SPHAEROSPORA KUDO).Spore with 4 polar capsules..... Family **CULOROMYXIDAE**Spores with 2 polar capsules..... Family **SPHAEROSPORIDAE**Family 1. **Chloromyxidae** Thelohan.One genus..... Genus *Chloromyxum* MingazziniFamily 2. **Sphaerosporidae** Davis.

1. Spores with 1 polar capsule..... Genus *Unicapsula* Davis
Spores with 2 polar capsules..... 2
2. Spores with sinuous sutural line... Genus *Sinuolinea* Davis
Sutural line not sinuous..... Genus *Sphaerospora* Thelohan

SUB-ORDER 3. **Platysporina** (PLATYSPOREA KUDO).

1. Spores without iodophilous vacuole..... 2
Spores with an iodophilous vacuole

Family 3. **MYXOBOLIDAE**

2. Spores with 1 polar capsule..... Family 4. **COCCOMYXIDAE**

Spores with 2 or 4 polar capsules..... 3

3. One polar capsule at each of 2 poles

Family 1. **MYXIDIIDAE**

Two or 4 polar capsules all at one end

Family 2. **MYXOSOMATIDAE**Family 1. **Myxidiidae** Thelohan.

1. Polar filaments long and fine..... 2
Polar filaments short and thick... Genus *Sphaeromyxa* Thelohan
2. Spores fusiform with pointed or rounded
ends; polar capsules oppositely directed
Genus *Myxidium* Bütschli
Spores fusiform usually with truncated ends;
polar capsules obliquely directed. Genus *Zschokkella* Auerbach

Family 2. **Myxosomatidae** Poche.

1. Spores without posterior processes; 2 polar
capsules..... 2
Four anterior polar capsules; with long
posterior processes..... Genus *Agarella* Dunkerly
2. Spore ovoidal, flattened, somewhat elongate..... Genus *Myxosoma* Thelohan
Spore circular to oval in front view... Genus *Lentospora* Plehn

Family 3. **Myxobolidae** Thelohan.

1. Each valve of spore prolonged in long
process..... Genus *Henneguya* Thelohan
Valves without posterior processes... Genus *Myxobolus* Bütschli

ORDER 2. **Actinomyxida** STOLÇ.

With 2 spore membranes, outer trivalved,
inner one piece..... Family 1. HAPLOACTINOMYXIDAE
With only 1 membrane which is trivalved

Family 2. EUACTINOMYXIDAE

Family 1. **Haploactinomyxidae** Granata.

One genus only..... Genus *Tetractinomyxon* Ikeda

Family 2. **Euactinomyxidae** Granata.

1. Spore with posterior processes..... 2
Spore rounded, no posterior processes... 4
2. Spores with 2 posterior processes... Genus *Synactinomyxon* Stolç
Spores with 3 or 6 posterior processes... 3
3. Anchor-shape, with 3 posterior processes

Genus *Triactinomyxon* Stolç

Anchor-shape, with 6 posterior processes

Genus *Hexactinomyxon* Stolç

4. Spores spherical..... Genus *Sphaeractinomyxon*
Caulley and Mesnil

Spore globular; each valve swollen to
hemisphere..... Genus *Neoactinomyxon* Granata

ORDER 3. **Microsporidia** BALBIANI.

Spores with 1 polar capsule..... Sub-order 1. MONOCNIDEA

Spores with 2 polar capsules..... Sub-order 2. DICNIDEA

SUB-ORDER 1. **Monocnidea** LÉGER AND HESSE.

1. Spores elongate, tubular or cylindrical
Family 3. MRAZEKIIDAE
Spores spheroidal or ovoidal..... 2

2. Spores oval to pyriform..... Family 1. NOSEMATIDAE
Spores spheroidal..... Family 2. COCCOSPORIDAE

Family 1. **Nosematidae** Labbé.

1. Sporont becomes a single sporoblast..... 2
Sporont forms more than 1 sporoblast... 3
2. The single sporoblast form a single spore

Genus *Nosema* Naegeli

The single sporoblast forms 2 spores. Genus *Glugea* Thelohan

3. Each sporont forms 16 or more sporoblasts 4
Each sporont forms less than 16 sporoblasts..... 5

4. Sixteen sporoblasts formed..... Genus *Duboscqia* Perez
More than 16 sporoblasts formed... Genus *Plistophora* Gurley
5. Sporonts produce 1, 2, 4 or 8 sporoblasts

Genus *Stempellia* Léger and Hesse

Sporonts produce 4 or 8 sporoblasts..... 6

6. Four sporoblasts produced..... Genus *Gurleya* Doflein
Eight sporoblasts produced..... Genus *Thelohania* Henneguy

Family 2. **Coccosporidae** Kudo.

One genus only..... Genus *Coccospora* Kudo

Family 3. **Mrazekiidae** Léger and Hesse.

1. Spores straight or slightly bent..... 2
Spores distinctly curved..... 3

Family 3. **Mrazekiidae** Léger and Hesse.

2. Spores straight, tubular; basal part of thread runs through cell, thread coils around it..... Genus *Mrazekia* Léger and Hesse
- Spores slightly bent, no thickened basal thread..... Genus *Oösporea* Flu
3. Spores spirally bent..... Genus *Spiroglugea* Léger and Hesse
- Spores bent U-shape..... Genus *Toxospora* Kudo

SUB-ORDER 2. **Dicnidea** LÉGER AND HESSE.

One family—TELOMYXIDAE; one genus—*Telomyxa* Léger and Hesse

CLASS III. **ACNIDOSPORIDIA** CEPEDA.

Here (provisionally) are *Sarcosporidia* with one genus *Sarcocystis* and *Haplosporidia* about which there is still some doubt as to their Sporozoan nature and affinities. The following list of genera without definite taxonomic position is given for completeness:

Amphiacantha Caullery and Mesnil, parasitic in Gregarine Lecudina.

Amphiamblis Caullery and Mesnil, parasite in gregarine of worm *Capitella*.

Anurosporidium Caullery and Chappellier, parasite of Trematode in *Donax* sp.

Bertramia Caullery and Mesnil, a body cavity parasite of worms and rotifers.

Caulleryetta Dogiel, parasitic in the gregarine *Selenidium* in *Travisia forbesi*.

Dermocystidium Perez, cyst-forming parasite in fish and amphibia.

Haplosporidium Caullery and Mesnil, different species parasitic in marine annelids, fresh water oligochaetes, nemerteans and Chiton.

Helicosporidium Keilin, spiral parasite in insects.

Icthyophonous Plehn and Mulsow, plasmodium-like, causes tumors in fish.

Ichthyosporidium Caullery and Mesnil, forms tumors in fish.

Lymphocystis Woodcock, forms globular masses in fish.

Lymphosporidium Calkins, in fish and oligochaete worms.

Metschnikovella Caullery and Mesnil, several species parasitic in gregarines.

Rhinosporidium Ridewood and Fantham, causes nasal tumors in man (India).

Urosporidium Caullery and Mesnil, body cavity parasites of *Syllis gracilis*.

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INDEX.

A

- ABNORMALITIES, artificial production of, 345
- Acantharia, 440
- Acanthocystis*, Fig. 75, p. 139
budding, Fig. 50, p. 95
centroblepharoplast in division, Fig. 50, p. 95
food-getting by Protozoa, Fig. 97, p. 186
mitosis, 120; Fig. 67, p. 121
- Acanthoeca spectabilis*, Fig. 178, p. 419
- Acanthosporidae, Key, 563
- Acineta* sp., Fig. 100, p. 192
tuberosa, endogenous budding, Fig. 117, p. 228
- Acinetidae, Key, 523
- Aenidosporidia, 555
Key, 570
- Acrasida, 447
Key, 462
- Actinoboliva radians*, Fig. 91, p. 163
feeding, 189
isolation cultures, 254
- Actinobolinidae, Key, 494, 562
- Actinomonas mirabilis*, Fig. 174, p. 412
- Actinomoxida, 351
development, 551
Key, 569
- Actinophrys sol*, axial filaments, Fig. 66, p. 120
fertilization, 277; Fig. 142, p. 278
maturation, Fig. 157, p. 309
meiosis, 102
vitality graph, Fig. 134, p. 257
- Actinopoda, 436
- Actinosphaerium*, centrosomes, 122; Fig. 68, p. 123
cichorii, axial filaments, 122
nucleus, Fig. 23, p. 50
- Adelea*, pseudo-conjugation, 275
- Adeleida, 544
Key, p. 566
- Adelina dimidiata*, pseudo-conjugation, Fig. 140, p. 275; Fig. 217, p. 544
- Adolph, oxygen consumption, 175
- Adoral zone, 157
left and right wound, 181
- Agamogony, 233
- Age and differentiation, résumé, 282
and division rate, table of, 207
of parents and vitality of offspring, 339
reduced vitality, 269
- Aggregata iberthi*, chromosomes, Fig. 56, p. 102
zygotic meiosis, 310
- Aggregatidae, Key, 566
- Aikinetocystidae, Key, 560
- Akaryomastigont, 414
- Alexeieff, *Chilomonas*, 68
chondriome, 73
division types, 89
kinetoplast, 114
- Allantocystidae, Key, 560
- Allogromia*, normal, Fig. 10, p. 32
- Alternation of generations in *Polystomella*, 236
- Altmann, structure of protoplasm, 43
- Alverdes, isolated cilium, 124
seat of sensory reaction, 128
- Ameba*, rejuvenescence by merotomy, 239
- Amebae*, 453
Key, 466
- Amebae of man, non-pathogenic, 396
- Amebic dysentery, 387, 388
history of, 388
- Amebida, 455
Key, 466
- Amino-acid nutrition, 200
- Amoeba crystalligera*, division, 96
dysenteriae, 390
proteus, Fig. 3, p. 22
Golgi apparatus, Fig. 39, p. 78
R.Q., 174
vespertilio, division, 96; Fig. 52, p. 97
- Amoebidae, 455
Key, 466
- Amoeboid movement, 180
- Amphileptidae, Key, 497
- Amphimixis, Weismann, 324
- Amphimonadidae, Key to genera, 425
- Amphisia lessleri*, Fig. 88, p. 159
Fig. 209, p. 518
- Ancistrumidae, Key, 503
- Animaleula, 17
- Anisogametes, 274, 276
- Anisospores in Radiolaria, 279

- Anoplophryidae, Key, 489
Anthophysa vegetans, colony, 39; Fig. 21, p. 40
 Anti-digestive ferments, 359
 Aphrothoraca, Key to genera, 460
 Arago, gametocyte formation in *Plasmodium*, Plate II opp. p. 408
Arcella vulgaris, life cycle of, 236
 origin of nucleic acid, Fig. 36, p. 71
 Arcellidae, 458
 Key, 467
 Arcyriidae, Key, 465
 Armourochetidae, Key, 464
 Arndt division in *Hartmannella*, 213
 Hartmannella klitzkei, 106
Askenasia elegans, Fig. 84, p. 153
Aspidisca, Fig. 90, p. 161
 Aspidiscidae, Key, 521
 Assimilation, products of, 203
 Astomida, Key, 489
Aulacantha scolymantha, chromosomes, Fig. 53, p. 98
 Austin, *Uroleptus mobilis*, 256
 Autogamy, 322
 in *Cnidosporida*, 324
 Awerinzew, autogamy, 324
 Axopodia, 434
 Axopodium, 145; Fig. 78, p. 146
 Axostyle, 144
 division of, Fig. 77, p. 145
- B**
- BABES, volutin, 72
 Babesiidae, Key, 566
 Babesia, 513
 Key, 566
 Bacillary dysentery, 391
 Baitsell, *Plurotricha lanceolata*, graph, 251
 Stylonychia pustulata, 253
Balantidium, 401
 coli, copulation, 275
 neuronotor system, 129
 Balbiani, merotomy, 55, 219
 Baneroff, reversibility of phase, 180
 Basal body, 107
 granules, ciliates, 123
 Basichromatin, 57
 Becker, reducease, 175
 Beers, *Amoeba* gastric vacuole, 189
 Bélař, *Actinophrys sol*, maturation, 308
 Bodo oratus, Fig. 29, p. 62
 division of *Lophomonas*, Fig. 105, p. 211
 fertilization in *Actinophrys sol*, Fig. 142, p. 278
 Karyosome, 51
 lipoplasts in *Actinophrys*, 316
 substances in nucleus, 57
 vitality graph of *Actinophrys sol*, Fig. 134, p. 257
 Benda, chondriome, 73
 Benedict, uric acid in *Paramecium*, 177
 Berthold, amoeboid movement, 180
Blepharisma undulans, Fig. 208, p. 514
 pseudo-membrane, 157
 R.Q., 174
 Blepharocoridae, Key, 503
 Blepharoplast, 107
 Blepharoplastless trypanosomes, 114
 Bioecidae, Key to genera, 424
 Bignami, relapse in malaria, 344
 Bilateral symmetry, Figs. 17, 18, pp. 35, 36
 Binucleata, 113
 Bioblast, 43
 Biparental inheritance, 350
 Bishop, *Spirostomum*, 25
 Bistadiidae, 141, 455
 Key, 466
Bodo laetereae centrioles, 63; Fig. 33, p. 65; Fig. 34, p. 66
 parabasal, Fig. 62, p. 116
 oratus, kinetic elements, Fig. 29, p. 61
 species, Fig. 76, p. 143
 Bodonidae, Key to genera, 426
 Boeck, blepharoplast in *Chilomastix*, 109
 and Drbohlav, *Endameba* cultures, 393
 Bogert, *Aulacantha*, 68
 scolymantha, Fig. 53, p. 98
 Botsford, merotomy, 178
 Boveri, centronucleus type, 61
 "spheres of influence" and division, 205
 Boveriidae, Key, 503
 Bowen, Golgi apparatus function, 179
 Bowling, *Zygocystis*, 536
 Brandt, function of contractile vacuole, 176
 Brasil, budding in *Elcatheoschizon*, 229; Fig. 119, p. 230
 Gonospora varia, 94
 Brazilian trypanosomiasis, 384
 Bresslau, artificial membranes, 193
 silver line system, 80
 temporary cysts, 267
 Bresslau and Serenin, Feulgen reaction, 57
 parabasal Feulgen reaction, 118
 Bresslau and Tektin, protrichocysts, 135, 137
 Brown, *Dinennympa*, Fig. 176, p. 414
 Brown and Golgi in *Amoeba proteus*, 78
 Bruce, tsetse flies and trypanosomes, 381
 Brumpt, copulation in *Balantidium*, 275
 Budding, 225
 division, 214
 endogenous, 228
 exogenous, 226

- Budding in Myxosporidia, 230
 in *Spirochona*, 227
 in Sporozoa, 229
 terminal in ciliates, 227
- Bunge, anaerobic forms, 24
- Bursaria truncatella*, Fig. 94, p. 169
- Bursariidae, Key, 512
- Bütschli, amoeboid movement, 180
 frontal field, 168
 protoplasmic structure, 42; Fig. 15, p. 36
 Verjüngung, 329
- Bütschliidae, Key, 495
- Buxtonella*, 401
- C**
- CALKINS, *Actinobolina* feeding, 189
 chondriome, 75
 division of double *Uroleptus*, 246, 247
 monster formation, Fig. 108, p. 216
 origin of double *Uroleptus*, 244
 split conjugants, 284
Uroleptus halseyi motorium, 129
- Calkins and Bowling, gametogamy in *Glaucoma*, Fig. 200, p. 485
Glaucoma motorium, 129
- Calkins and Gregory, selection in *Paramecium*, 348; Fig. 168, p. 349
- Callimastigidae, Key to genera, 430
- Calonympha grassii*, 115; Fig. 63, p. 117
- Calonymphidae, Key to genera, p. 432
- Calymma, 439
- Campanella umbellaria*, 125
- Camptonema nutans*, pseudopodia, 122
- Canalicular system, Fig. 101, p. 194
- Cannibalism, 185
- Capillitium in Mycetozoa, 238
- Carbohydrate digestion, 198
- Carchesium polypinum*, colony, 38
 gastric vacuole history, Fig. 102, p. 196
- Carrel, tissue culture, 210, 258
- Caryotropha mesnili*, food-getting, Fig. 103, p. 201
- Caryotrophidae, Key, 565
- Casagrandi and Barbagallo, 389
- Castellani, human trypanosomiasis, 381
- Catalase as stimulus to division, 206
- Caulery and Mesnil, Actinomyxida development, 551
 autogamy in Actinomyxida, 326
- Causey, chondriome, 76
 origin of Golgi bodies, 79
- Cavulae, 42
- Cell division, 204
- Cellulose digestion, 199
- Central granule of Heliozoa, 119
- Centrioles, 63, 107
- Centriolepharoplast, 117
 arising from nucleus, Fig. 50, p. 95
- Centrodemesose, 62
- Centronucleus type, 61
- Centropyxis aculeata*, chromidia, 69
 fertilization, 277
- Centrosomes, 122
- Ceratomyxidae, Key, 567
- Cerecomonadidae, Key to genera, 427
- Chagas, *Schizotrypanum*, 383
- Chalarothorax, Key to genera, 460
- Chambered shells, Fig. 19, p. 38
- Chambers, periplast, 135
 physical conditions in *Amoeba*, 180
- Chambers and Dawson, pseudo-membranes, 157
- Chatin, chitin, 137
- Chatton, abnormalities, 345
 anisospores in Radiolaria as parasites, 279
 contractile vacuole, 179
 environment and conjugation, 287
Glaucoma scintillans, 266
 isolation cultures, 250
 life cycle in ciliates, 256
 mesomitosis, 89
Pansporella, 386
 thigmotrichia, 483
 yellow cells, 441
- Chatton and Courier, *Schizotrypanum*, 383
- Chatton and Lalung-Bonnaire, *Löschia*, 389
- Chatton and Lwoff, *Ellobiophrya donacis*, Fig. 104, p. 202
Foellingeria, 399
 silver line system, 81
- Chatton, Lwoff and Monod, origin of mouth at division, Fig. 114, p. 224
- Chatton and Perard, Pycnothricidae, 400
- Chejfec, bacteria eaten by *Paramecium caudatum*, 185
 longevity of single *Paramecium* individual, 259
- Chemistry of protoplasm, 43
- Child, senescence, 209
- Chilodochonidae, Key, 523
- Chilodon*, mouth of, 168
 sp., Fig. 30, p. 62
uncinatus, Fig. 112, p. 222
 mutation, 351
- Chilomastigidae, Key to genera, 431
- Chilomastix centrioles*, 63
mesnili, Fig. 60, p. 110
 cyst, 23
- Chilomonas paramecium*, contractile vacuole, Fig. 95, p. 181
- Chitin, 133-137
- Chlamydonon mucosynuc*, hyaline band, 124
- Chlamydotodontidae, Key, 498
- Chlamydroplys stercorica*, Fig. 189, p. 458
- Chloromyxidae, Key, 568
- Chlorophyll forms, 18
- Choanocca perplexa*, Fig. 178, p. 419

- Choanoflagellate collar, 164
 Choanoflagellates, distribution of, 26
Choenia teres, Fig. 191, p. 472
 Chondriochonts, 73
 Chondriome, 73
 Chondriomites, 73
 Chonotricha, Key, 522
 Chromatin, 54
 Chromatoid bodies, 395
 Chromidia, p. 69
 Chromosomes in *Uroleptus*, 321
 origin, 88
 meiotic, 100
 Cilia, 152
 and membranes of Infusoria, Fig. 69, p. 124
 composite, 155
 replacement, 223
 structure, Fig. 82, p. 152; Fig. 83, p. 153
 Ciliary beat, 127
 Ciliata, anal modifications, 164
 cytostomes, 164
 division zone, 215
 myonemes, 124
 oral modifications, 164
 position of mouth of, 167
 Ciliates, amiconucleate, 477
 commensal, 397
 distribution, 26
 parasitic, 397
 symbiotic, 397
 Cirri, 157
 anal, 158
 caudal, 158
 frontal, 158
 marginal, 158
 movement, 160
 substitution, 223
 tactile, 161
 types, 479
 ventral, 158
Cladomonas fruticulosa, colony, 38
 Clathrostomidae, Key, 501
Clathrudina, colony, 38
 delegans, stalk origin, 148; Fig. 80, p. 139
 Cleveland, *Paramecium* cysts, 24
 symbiotic flagellates, 203
 wood digestion by flagellates, 199
 Cleveland and Sanders, excystation of *Endamoeba dysenteriae*, 395
Climacostomum virens, frontal field, 169
 myophanes, neurophanes, Fig. 71, p. 128
 Clowes, permeability, 172
 reversibility of phase, 180
 Club root in cabbages, 386
 Cnidosporidia, 545
 Key, p. 567
 spore types, Fig. 219, p. 547
 Coccidia, effects produced, 404
 lumen-dwelling forms, 541
 Coccidiida, 541
 Coccidiida, Key, 564
 Coccidiomorpha, 541
 Key, 564
 Coccidiosis in chickens, 405
Coccidium (cimeria) schubergi, centriole, 63
 Coccosporidae, Key, 569
 Cochliopodium, normal, Fig. 9, p. 31
 Cochlosomidae, Key to genera, 431
Codosiga botrytis, origin of flagellum, 107; Fig. 59, p. 108
 pulcherrimus, Fig. 92, p. 165
 ramosum, colony, 38; Fig. 20, p. 39
Cornomorpha medusula, Fig. 208, p. 514
 Cohn, budding in Myxosporidia, 227
 Colepidae, Key, 494
Coleps hirtus armature, Fig. 73, p. 136
 cilia structure, Fig. 82, p. 152
 division, 215
 Collars in choanoflagellates, 165
 Collin, origin of basal granules, 122
Collinella, Fig. 172, p. 400
Collodictyum triciliatum, nuclear division, Fig. 51, p. 96
 Colonies, 18, 21, 38
 Colony types, 38
Colpidium colpoda, canalicular system, 194; Fig. 101, p. 194
 Colpodidae, Key, 501
Comatricha nigra, Fig. 184, p. 447
 Commensals, 202
 Composite ciliary organs, 155
 Concephthiriidae, Key, 501
 Concrement vacuoles, 171
Condylostoma patens, Fig. 206, p. 511
 Condylostomidae, Key, 510
 Conjugation and encystment, graph, Fig. 137, p. 268
 and environment, 286
 conditions for, 285
 disorganization at, 311
 effects of salts on, 288
 endogamous, 286
 reorganization after, 312
 survival value of, 333
 tests, 267
 unfavorable effects on *Paramecium*, 332
 "Conscious" activities, 189
 Contaminative infection, 360
 Contractile vacuoles, 170
 and Golgi apparatus, 79
 function, 176
 membrane of, 178
 supposed functions, 177
 Contraction in ciliates, 125
 Coordinating fibers, 127
 systems in protozoa, 183
 Coprozoic protozoa, 357
 Copulation and conjugation, 274
Cornuspira, type of shell, Fig. 19, p. 38
 Cortex, 132
 zonal differentiation, 152
 Cortical differentiations, 135

- Cosmovici, canalicular system, Fig. 101, p. 194
- Costia necatrix*, ectoparasite, 359
- Councilman and Laffleur, *Amoeba dysenteriae*, 390
- Cowdry, functions of mitochondria, 76
- Craig, toxins in endameba, 363
- Craspedomonadidae, Key to genera, 424
- Crawley, gregarine movement, 535
Sarcocystis, 556
- Cribrariidae, Key, 464
- Cribidia caryophthalmi*, Fig. 61, p. 111
gerridis, Fig. 169, *G*, p. 366
leptocoridis, Fig. 61, p. 111
subulata, Fig. 170, p. 368
- Cryptobia* sp., parabasal, Fig. 62, p. 116
- Cryptocysts in Microsporidia, 555
- Cryptosporidiidae, Key, 565
- Crystalline excretory products, 177
- Ctenostomida, Key, 516
- Cups, houses, etc., 137
- Cushman, Foraminifera, 452
- Cutler, division-rate and food, 206
Endameba cultures, 393
- Cutler and Crump, soil forms, 25
- Cyathodiniidae, Key, 503
- Cyathosoma striatum*, Fig. 179, p. 420
- Cyclidium glaucoma*, Fig. 199, p. 482
cilia structure, Fig. 83, p. 183
- Cyclonympha mirabilis*, Fig. 180, p. 429
- Cyclonymphidae, 428
- Cycloposthiidae, Key, 515
- Cycloposthium bipalmatum*, conjugation, Fig. 141, p. 276
interchange of nuclei, Fig. 146, p. 293
- Cyclosis, 150
- Cylospora karyolytica*, nuclear parasite, 542
- Cyctotrichium gigas*, Fig. 81, p. 151
ovatum, Fig. 191, p. 472
sphaericum, Fig. 84, p. 153
- Cysts, air-borne, 23
endomixis, 267
- Cytomeres, 227
- Cytoplasmic elements of fundamental organization, 68
kinetic elements, 104
list, 69
- Cytostome in taxonomy, 481
- D**
- DA CUNHA and MUNIZ, parabasal Feulgen reaction, 118
- Dactylophoridae, Key, 562
- Dallinger, adaptations to heat, 343
- Dallinger and Drysdale, enduring modifications, 343
- Daniel, respiration quotient, 174
- Darling, dysentery, 393
- Dauermodificationen, 344
- Davis, autogamy, 324
Leptotheca, Fig. 220, p. 550
Sphaerospora dimorpha, Fig. 121, p. 232
- Dawson, abnormalities, 346
cannibalism, 185
isolation cultures, 256
- Dawson and Belkin, oil digestion, 199
- Debaisieux, fertilization in Cnidosporidia, 326
Microsporidia, 553, 555
- Dedifferentiation with division, 263
- de Garis, monster production, 261
- Degen, function of vacuole, 176, 178
- Dehorne, *Paramecium* chromosomes, Fig. 57, p. 103
- Delage and Herouard, flagellum action, 142
- Demboska, cirrus regeneration, 164
cirrus removal, 223
- Dendrocometidae, Key, 524
- Dendrosoma elegans*, Fig. 196, p. 177
- Dendrosomidae, Key, 524
- Derived nuclear structures, 81
organization, cytoplasmic, 101
definition of, 45
- Desmothoraea, Key to genera, 461
- Development, 241
embryos of Suctoria, 243
- Devescovina*, parabasal, Fig. 62, p. 116
- Devescovinidae, Key to genera, 431
- Dianemidae, Key, 466
- Diastatic ferments, 196
- Dicnidea, Key, 570
- Dicraspedella stokesi*, Fig. 178, p. 419
- Dictyostelidae, 449; Key, 462
- Dictyostelium*, Fig. 185, p. 448
- Dictyotic moment, 134
- Didinium nasutum*, food-getting, 185
rhizoplasts, 155
swallowing *Paramecium*, Fig. 98, p. 189
- Didymiidae, Key, 463, 494
- Didymophyidae, Key, 562
- Dicentameba fragilis*, 396
- Differentiation, age, 269
and organization, 260
cyclical, 266
gametic, 274
inter-divisional, 260
maturity, 271
youth, 266
- Diffluence, 30
- Diffugia lobostoma*, Fig. 190, p. 159
- Diffugiidae, Key, 468
- Digestive fluids, 193
in gastric vacuoles, 195
use of indicators, 193
- Dikaryomastigina, 422
Key to genera, 431
- Dileptus*, beef-fed, Fig. 25, p. 52
gigas, Fig. 6, p. 27; Fig. 194, p. 474
division, 91; Fig. 46, p. 92
nuclear division, 217

- Dileptus gigas*, starvation, 172
regeneration, 45
- Dimastigamoeba histadialis*, kinetic element, 107
gruberi, Fig. 13, p. 34
- Dimorpha mutans*, Fig. 13, p. 34
Fig. 79, p. 148
- Dimorphic nuclei, 84
origin after conjugation, 315
- Dinenympha fimbriata*, Fig. 176, p. 415
- Dinenymphidae, Key to genera, 430
- Diophrya lieberkühni*, Fig. 84, p. 153
- Diophrys appendiculata*, Fig. 89, p. 160
- Diphasic forms, 34
- Diploceyctidae, Key, 559
- Diploceyctis schneideri*, zygotic meiosis, Fig. 158, p. 310
- Diplodinium caudatum*, Fig. 2, p. 20
motorium, 129
interchange of nuclei, Fig. 146, p. 293
- Diplocca placita*, Fig. 178, p. 149
- Diplosiga socialis*, Fig. 92, p. 165
- Discomorpha pectinata*, silver line system, Fig. 41, p. 80; Fig. 42, p. 80
- Discomorphidae, Key, 516
- Discophryidae, Key, 524
- Distribution of Protozoa, 23
- Division and reorganization, 209
in Mastigophora, 210
in Sarcodina, 213
modes, 209
of protoplasmic granules, 208
- Dobell, amebic dysentery, 388
axostyle function, 144
kinetoplast, 114
Protozoa as organisms, 18-19, 40
zygotic meiosis, 310
- Dobell and Jameson, chromosome aggregates, Fig. 56, p. 102
- Doflein, *Amoeba respertilio*, Fig. 52, p. 97
anaerobic forms, 24
axostyle function, 144
chromidia, 70
Codosiga botrytis, 109
digestive fluids as toxins, 193
free nuclei formation, 88
Karyosome, 51
Plasmodroma and Ciliophora, 411
pole plates, 66
primitive form, 141
stereoplasm and rheoplasm, 42
stereoplasmatic axis, 435
- Dogiel, concreted vacuoles, 171
gametic nuclei as spermatozoa, 276
ophryoscolecin, 139
polymerization, 38
Schizocystis sipunculii, 229
- Donovan, organism of kala azar, 369
- Dujardin, 22
classification, 140
diffluence, 30
sarcodae, 433
- Dreyer, skeleton formation, 138
skeletons, Fig. 12, p. 33
- Driesch, architektonik, 173
- Drüner, causes of division, 205
- Duboseq and Grassé, Golgi apparatus, 79
- Duke, sites of trypanosome development, 382
- Dutton, human trypanosomiasis, 381
- Dysentery, amebic, 387
- Dysteriidae, Key, 498

E

- EBERLEIN, silica in ciliates, 125
- Echinomera hispida*, gametes, Fig. 141, p. 281
- Ectoparasites, 359
- Ectoplasm, 132
- Eimeria schubergi*, cycle, Fig. 173, p. 403
gametes, Fig. 144, p. 281; Fig. 215, p. 538
- Eimeriidae, Key, 565
- Eimeriina, 541
Key, 564
- Elaters in Mycetozoa, 239
- Elutheraschizon dubasqui*, budding, 229; Fig. 119, p. 230
- Ellis, choanoflagellates, Fig. 178, p. 419
food ingestion by Choanoflagellates, 188
- Ellobiophrya donacis*, anchorage, Fig. 104, p. 202
- Elpatiewsky, chromidia, 69
endogenous budding in *Accella*, 228
fertilization in *Accella*, 277
life cycle of *Accella*, 236
- Emerson, respiration quotient, 174
- Euchelys pupa*, Fig. 191, p. 472
- Encystment, 23
- Endameba in insects, 386
in man, 387
in vertebrates, 387
- Endamoeba coli*, 396
nuclear division, Fig. 26, p. 53
cultures, 393
dysenteriae, Fig. 31, p. 62
cycle, 395
ex-cystation, 395
synonyms, 393
trophozoite and cysts, Fig. 171, p. 394
gingivalis, 396
intestinalis, Fig. 24, p. 51
- Endamoebidae, 455
Key, 466
- Endobasal bodies, 49
body, 53, 60
- Endoenzymes and toxins, 198
- Endomixis, 252, 317
- Endoparasitic protozoa, 359
- Endoplasm, 132

Endosome, defined, 50
 Endotoxins, 197
 in protozoa, 363
Endotrypanum schaudinni, Fig. 169 II,
 p. 366
 Energid theory, 205
 Engelmann, chemiotaxis in fertiliza-
 tion, 291
 neural fibers, 131
 Enriques, isolation cultures, 250
 stalk formation, 193
Entamoeba coli, 391
 histolytica, 391
 Entodiniomorpha, 402
 Entz, *Actinobolus radians*, 162
 origin of basal granules, 123
 polytoma, 107
 Epaleidae, Key, 516
Ephelota, exogenous budding, Fig. 115,
 p. 226
 tentacles, Fig. 198, p. 480
 Ephelotidae, Key, 524
Epiclontes, Fig. 208, p. 514
Epistylis, myonemes, 125; Fig. 70, p.
 126
 umbellaria, colony, 58
 fertilization, Fig. 143, p. 280
 Erdmann, reorganization, 341
 Sarcocystis, 556
 Euaetnomyxidae, Key, 569
 Euciliata, Metcalf, 398
Eudorina elegans, 266
Euglypha alveolata, budding division,
 214
 cyst, Fig. 4, p. 23
 normal, Fig. 9, p. 31
 Euglyphidae, 458; Key, 469
 Eugregarinida, 540; Key, 558
 Euplasmodida, 449; Key, 463
Euplates charon, Fig. 89, p. 160
 patella, absorption bands, Fig. 48,
 p. 94
 merotomy and reactions, Fig.
 96, p. 182
 microdissection, 129; Fig. 72,
 p. 130
 rannus, Fig. 210, p. 520
 Euplotidae, Key, 521
 Eurysporina, Key, 567
 Evans, *Trypanosoma*, cause of Surra,
 381
 Excretion, 176
 products, effects on Protozoa 200
 Excretory granules, 197
 Exosporia, Key, 463

F

FANTHAM, soil protozoa, 354
 Fantham and Porter, fertilization in
 Cnidosporidia, 326
 Fat and oil digestion, 199
 Fatigue in protozoa, 181

Fauré-Fremiet, chondriome, 73
 ciliate types, Fig. 84, p. 153
 Fellers and Allison, soil protozoa, 354
 Fermor, endomixis in *Stylonychia*, 319
 Fertilization, effect of initial contact,
 292
 phenomena, 285
 processes of, 292
 Feulgen and Rossenbeck, nuclear reac-
 tion, 57
 Filopodia, 150, 435
 Flagella, 140, 141
 number and arrangement, 113
 Flagellata, adaptations, 419
 classification, 421
 with suckers, Fig. 179, p. 420
 Flagellates of soil, 354
 list of, 355
 parasitic, 364
 Flagellum, insertion, Fig. 59, p. 108
 Flemming, structure of protoplasm, 43
 Flexner, bacillary dysentery, 391
 Foetingeriidae, 399
 Key, 499
Folliculina ampulla, Fig. 94, p. 169
 contraction, 125
 Folliculinidae, Key, 510
 Food-catching by Protozoa, 185
 Food-getting by Protozoa, 183
 organoids, 162
 Foraminifera, 450
 alternation of generations, 452
 arenaceous tests, 450
 distribution, 26
 porcellanous tests, 450
 tests, types of, Fig. 187, p. 452
 Forde, Gambia fever, 381
 França, sensory flagella, 127
 Francé, choanoflagellate collar, 164
 Frontal fields, 168
Frontonia leucas, Fig. 93, p. 167
 division zone, 217
 Frontoniidae, Key, 505
Fuligo varians, chemistry of, 14
 Fundamental organization changes, 83
 definition of, 45

G

GAMBIA fever, 381
 Gametes, defined, 529
 of Gregarinida and Coccidia, Fig.
 144, p. 281
 Gametochromidia, 70
 Gametocyte, defined, 528
 Gamogony, 233
 Ganymedidae, Key, 560
 Garnjobst, temporary cysts, 267
 Gastric vacuole formation, 195
Gastrastyla steinii, Fig. 210, p. 520
 Gatenby, function of mitochondria, 77
 Gelei, contractile vacuole, 179
 Gemmation, 225

- Giardia*, bilateral symmetry, 36; Fig. 17, p. 37
- Gibbs and Dellinger, selection in Protozoa, 181
- Glaessner, diastatic ferments, 196
- Gläser, centrioles, 63
- Glaucoma*, Fig. 205, p. 504
frontata, Fig. 8, p. 29
 conjugation, Fig. 201, p. 486
 (*Dallasia*) gametogamy, Fig. 200, p. 485
scintillans, basal bodies, 124
 origin of posterior mouth at division, Fig. 114, p. 224
- Glutathion, 175
 and mitochondria, 77
- Glycogen, 133
 at conjugation periods, 290
 in *Pelomyxa*, 198
- Goette, chromidia, 69
- Goldfuss, Protozoa, 17
- Goldschmidt, chromidia, 69, 87; Fig. 25, p. 88
- Golgi apparatus, 69, 77
 in flagellates, 416
 bodies and contractile vacuole, 79
 types of malaria organisms, 406
- Gonder, enduring modification in *Trypanosoma*, 344
- Goodey, *Prowazekia salmans*, 110
 soil forms, 25
 protozoa, 354
- Gourret and Roeser, distribution, 26
- Granata, *Haplosporidium*, 94
- Grassé, parabasal, 119
- Grassi, dysentery, 389
 malaria and mosquitoes, 407
- Grassi and Feletti, genera of malaria organisms, 406
- Greenleaf, effect of crowding on division, 206
- Greenwood and Saunders, digestion in gastric vacuoles, 195
- Gregarina cancuta*, sporoducts, Fig. 125, p. 240
ovata, gametes, Fig. 215, p. 538
- Gregarinida, epimerite types of, 243
 protomerite, 242
- Gregarina, 534
 Key, 557
- Gregarines, epicyte in, 534
 epimerite, 536
 movement, 535
 myonemes in, 535
 pseudo-conjugation, Fig. 213, p. 531
 sex differences, Fig. 214, p. 537
- Gregory, chromosomes in *Oxytricha*, 319
Tillina magna, vitality, 253
Uroleptus response to chemicals at different ages, 246, 257
- Grenacher, central granule, 119
- Griffin, fibers in *Euplotes*, 131
- Griffin, reorganization in ciliates, 221
- Griffiths, function of contractile vacuole, 176
- Gromiidae, Key, 469
- Grosse-Allerman, *Amoeba terricola* feeding, 188
- Gruber, environment effects, 178
- Gruby, *Trypanosoma*, 381
- Guilliermond, mitochondria, 77
 volutin, 72
- Gunther, skeleton, 125
- Gurwitsch, inadequacy of term cell, 19, 40
- Guttulinidae, 448
 Key, 462
- Gymnostomida, Key, 491

H

- HABITAT groups, 352
 anaërobic types, 353
 mesosaprobic types, 352
 oligosaprobic types, 352
 saprobic types, 353
- Haackel, Protista, 18
 Radiolaria classification, 438
- Hacmogregarina stepanowi*, Fig. 218, p. 545
- Haemoproteidae, Key, 566
- Halteriidae, Key, 513
- Hamburger and Buddenbrock, distribution, 26
- Haploactinomyxidae, Key, 569
- Haplocyta, Key, 558
- Haptophrya*, colony, 38
- Hartmann, *Arceella*, 70
 Binucleata, 112
 cell and protozoa, 21
 centrioles in *Endameba*, 63
 chromidia, 70
Endamoeba africana, 392
Eudorina, 266
 Karyosome, 51
 necessity of conjugation, 329
 Polyenergic, 71
 rejuvenescence by merotomy, 239
- Hartmann and Chagas, *Spongomonas*, 94
- Hartmann and Nägler, autogamy, 323
Sappinia, 94
- Hartmannella klitzkei*, Fig. 58, p. 106
 division, 213
- Hartog, function of vacuole, 176
- Hartog and Dixon, pepsin-like ferments, 196
- Haughwout, *Pentatrichomonas*, food, 193
- Hegner, selection in *Arceella dentata*, 347
- Heidenhain, causes of division, 205
 two kinds of chromatin, 57
- Heitzmann, structure of protoplasm, 43
- Hefiozoa, 437
 central granule, 119

- Heliozoa, distribution, 26
 with centroblepharoplast, Fig. 50,
 p. 95
Helkesimastix faecicola, copulation, 276
 Hematozoic parasites, 360
 Hemosporidia, 406
Hepatozoön, cycle, Fig. 211, p. 527
 hosts, 361
 Heredity and variation, 342
Herpomonas musca-domesticae, Fig.
 170, p. 368
 muscarum, Fig. 169 B, p. 366
 parabasal, Fig. 62, p. 116
 Hertwig, *Actinosphaerium cichbornii*,
 centrosomes, 122; Fig. 68, p. 133
 chromidia, 55
 and chromidial net, 69
 duality of chromatin, 56
 immortality, 341
 Microgamia socialis, Fig. 107, p.
 214
 nucleoplasmic relation, 205
 pole plates, 65
 Radiolaria, classification, 438
 split conjugants, 284
 Herzfeld, reorganization at division,
 264
 Heterochromosomes of *Trichonympha*
 campanula, 99
 Heterotrichida, Key, 508
Hexactinomyxon, Fig. 221, p. 552
 Hirschler, Golgi and mitochondria, 77
Histrio pellionella, Fig. 88, p. 159; Fig.
 209, p. 518
 Hofer, *Ameba* anchorage at feeding,
 186
 merotomy, 55
 periplast, 135
 reaction of fragments, 183
 Hogue, environment effects, 178
 Hologametes, 274
 Holomastigotidae, Key to genera, 428
Holophrya, Fig. 191, p. 472
 discolor and myonemes, Fig. 69, p.
 124
 Holophryidae, Key, 491
 Holotricha, Key, 488
 Holozoic nutrition, 181
 Homogeneous endobasal bodies, 61
 Hopkins, oxidation and reduction
 potential, p. 174
Hoplitophrya, Fig. 202, p. 492
 Hoplitophryidae, Key, 490
 Hoplonymphidae, 428
 Horning, chondriome, 73, 75, 76
 Howland, membrane of contractile vac-
 uole, 178
 oxygen consumption, 175
 pH of gastric vacuoles, 196
 test for uric acid, 177
 Hübener, endotoxins in *Trypanosoma*,
 198
 Huber, cysts of *Endamoeba dysenteriae*,
 392
 Hulpieu, effect of oxygen on *Ameba*,
 175
 Hunger satisfaction and fatigue, 190
 Huxley, nature of life, 173
Hyalosphenia, Fig. 188, p. 157
 Hyman, pseudopodia formation, 180
 Hymenostomida, Key, 503
 Hypocomicidae, Key, 503
 Hypostomina, Key, 491, 498
 Hypotrichidae, Key, 516
- I**
- ICHTHYOPHITHIRIUS, fish parasite, 359
 Icthiocromidia, 70
 Howaisky, endomixis in *Stylonychia*,
 319
 Immaturity, 254
 Immortality in Protozoa, 311
 Immunity, 363
 passive, 364
 Indicators in digestion, 193
 Infraciliature, 82
 Infusionsthier, 17
 Infusoria, division in, 215
 Key, 488
 taxonomy, 471, 486
 tentacles in, 162, 163, 180
 tests, 471
 Inoculative infection, 360
 Intestinal flagellates of man, 384
 Intoshellinidae, Key, 490
 Intracellular kinetic elements, 60
 Invertebrate hosts of parasitic forms,
 364
Iodamoeba, Prowazek, 397
 Irritability, 179
 Isogametes, 274, 276
 Isolation cultures, 248
 with carnivorous ciliates, 253
Isospora in man, 405
 Isospores and anisospores as parasites,
 279
 in Radiolaria, 279
 Isotrichidae, Key, 503
 Ivanic, endomixis in *Chilodon*, 319
 macronucleus, 93
- J**
- JAHN, mycetozoa, 271
 James, dysentery, 393
 Jameson, *Buxtonella*, 401
 zygotic meiosis, 310
 Janicki, division of *Lophomonas*, 212
 karyomastigont, Fig. 175, p. 414
 parabasal, 111, 114
 Jennings, conjugation and division
 rate, 332
 tests, 287
 motor response in Protozoa, 181
 physical conditions in *Ameba*, 180

- Jennings, seat of sensory reaction, 128
 selection in *Acella*, 348
 split conjugants, 284
 variations in *Paramecium*, Fig. 167, p. 342
- Jepps and Dobell, *Dicatanocha*, 396
- Jirovec, parabasal Feulgen reaction, 118
- Joenidae, Key to genera, 428
- Jollo, endomixis and environment, 340
 enduring modifications, 344
- Joukowsky, cannibalism, 185
 isolation cultures, 252
- Joyet-Lavergne, chondriome and sex, 76
 Golgi in metozoa, etc., 79
 Glutathion and mitochondria, 175
Ninia gracilis, sex, Fig. 214, p. 537
- K**
- Kahl, protrichocysts, 135
- Kalmus, respiration, 174
- Kantbak, extractives from Trypanosomes, 198
- Kartulis, dysentery, 390
- Karyomastigont, 414
- Karyosome, endosome, 51
- Kepner and Taliaferro, purpose in protozoan activity, 181
- Kerona pediculus*, Fig. 89, p. 160
- Keuten, *Euglena*, 61
- Key to genera of flagellates, 423
- Keysselitz, *Myxobolus autogamy*, 324
 somatic structures in *Myxobolus*, 240
- Khainsky, chromidia, 70
 digestion, 195
- Kidder, *Concophthirus*, 98
molorum, 129
- Kinetic elements in ciliates, 121
 in cytoplasm, 105
- Kinetonucleus, 112
- Kinetoplast, 114
- King and Gatenby, Golgi apparatus, 78
- Kingsbury, mitochondria and respiration, 76
- Kite, physical conditions in *Amba*, 180
- Klebs, primitive form, 141
- Klein, cilia structure, Fig. 82, p. 152;
 Fig. 83, p. 183
 silver line system, 80
- Kofoid, axostyle function, 144
 chromidia, 55, 70
 free nuclei formation, 88
 function of parabasal, 111, 115
 neuromotor system, 105
Trichomonas, 117
- Kofoid and Swezy, blepharoplast, 109
 centroblepharoplast, 117
Endamoeba dysenteriae, nuclei, 394
- Kofoid and Swezy, mitosis in *Trichonympha campanula*, 99
 parastyle, 114
Streblomastix, Fig. 16, p. 3
Trichomonas augusta, 110
- Kofoididae, 428
- Koidzumi (Teratonympha), *Cyclo-nymphea*, Fig. 180, p. 429
- Kolkwitz, habitat groups, 352
- Kossel, chemistry of chromatin, 56
- Kränzlin, origin of elaters, 446
- Krogh, oxidation reduction potential, 174
- Krukenberg, pepsin-like ferments, 196
- Kudo, Myxosporidia distribution, 549
Stenpellia magna, cycle, Fig. 222, p. 553
Thalobania cycle, Fig. 223, p. 554
- Kuschakewitsch, chromidia, 70
- L**
- Labyrinthulidae, 443
 Key, 461
- Lackey, sewage protozoa, 357
- Laerquaria olor*, elasticity, 162
 types, Fig. 85, p. 156
- Lamprodermidae*, Key, 464
- Lang, types of pseudopodia, 434
- Lankesterella ranarum*, Fig. 218, p. 545
- Lankesterellidae, Key, 566
- Lankesteria ascidiacae*, cycle, Fig. 213, p. 531
- Lapage, cannibalism, 185
- Lauterborn, sarpopelic forms, 24, 353
- Laveran, kinetonucleus, 113
 malaria, 406
 transmission of malaria, 407
- Laveran and Mesnil, sarcocystin, 197
- Lavoisier, respiration, 174
- Learning in Protozoa, 181
- Lebedew, chromidia, 70
- Leber, endotoxins in *Trypanosoma*, 198
- Leecudinidae, Key, 560
- Ledenmüller, Infusionsthier, 17
- Léger, *Ophryocystis mesnili*, 229; Fig. 120, p. 231
 origin of mammalian trypanosomes, 361
- Léger and Duboseq, chromidia, 69
- Leidy, *Endamoeba*, 386, 389
- Leishman, organism of dum dum fever, 369
- Leishmania donovani*, Fig. 169 E, F, p. 366
 transmission, 371
- Leishmaniasis, 367
- Leishmaniasis, types of, 369
- Lambdion bullinum*, Fig. 199, p. 482
- conchoides*, Fig. 87, p. 158
 undulating membranes, 157;
 Fig. 87, p. 158
- Lembidae, Key, 508

- Lembus pusillus*, Fig. 204, p. 502
 Lepeshkin, chemistry of *Fuligo*, 44
Leptomonas cleuoccephali, Fig. 65, p. 119; Fig. 169 A, p. 366
Leptotheca scissura, Fig. 220, p. 550
 Lewis, mammalian trypanosomes, 384
 Levander, distribution, 26
 Liceidae, Key, 465
Lichnaspis giltochii, Fig. 182, p. 440
 Lielmophoridae, Key, 512
 Life and Death, Weismann, 248
Lionotus fasciola, Fig. 203, p. 496
 feeding, Fig. 99, p. 188
 food-getting by, 186
 procerus, 86
 wrzesniowskii, Fig. 203, p. 496
 Lipoplasts in *Actinophrys*, 316
 Lister, chromidia, 69
 Lobopodia, 150, 435
 eruptive type, Fig. 78, p. 146
 Looper, nucleoplasmic relation, 205
 Lophomonadidae, Key to genera, 428
Lophomonas blattarum, division, Fig. 105, p. 211
 division, 212
 Lösch, *Amoeba coli*, 388
 Löschia, 389
 Losina-Losinsky, feeding reactions, 189
Loxcephalus granulatus, Fig. 205, p. 504
Loxodes rostrum, Fig. 203, p. 496
 Loxodidae, Key, 497
Loxophyllum, Fig. 203, p. 496
 Lund, function of contractile vacuole, 176
 Lundgardh, karyolymph, 59
 Lwoff, *Leptomonas cleuoccephali*, 119
 parabasal Feulgen reaction, 119
 temporary cysts, 267
 Lynch, contractile vacuole function, 179
 Lysin, reaction of host, 363
- M**
- McCulloch, origin of parabasal, Fig. 61, p. 111
 McDonald, motorium, neuromotor apparatus, 129
 MacDougall, *Chilodon uncinatus*, Fig. 112, p. 222
 mutation in *Chilodon*, 351
 pharyngeal baskets, 476
 MacNeal, endotoxins in *Trypanosoma*, 198
 Macrochromatin and microchromatin, 484
 Macrogametes, 272
 Macronucleus, beaded, reorganization of, 218
 formation, 85
 reorganization of, 217
 Macrospheric and microspheric tests, 152
 Maier, basal bodies of membranes, 124
 Malaria organisms, sporulation, 238
 types and reproduction, Plate I, p. 408
 Mammalian trypanosomes, origin of, 364
 Manson, transmission of malaria, 407
 Marchiafava and Celli, *Plasmodium*, 406
 Martin, endotoxins in *Trypanosoma*, 198
 soil protozoa, 354
 Martin and Robertson, axostyle function, 144
 Marullaz, *Sarcocystis*, 556
 Maryniidae, Key, 501
 Massart, contractile vacuole, 176
 Massive nuclei, 50
 Mast, *Amoeba*, gastric vacuole, 189
 Dalium cyst, 267
 isolation cultures, 256
 and Pusch, learning in Protozoa, 181
Mastigamoeba aspera, Fig. 174, p. 412
Mastigella viteca, chromidia, Fig. 45, p. 88
Mastigina, chromidia, Fig. 45, p. 88
 Mathews, physiology, 172
 Maturity, 255
 Maupas, action of tentacles, 191
 cannibalism, 185
 conditions of conjugation, 285
 isolation cultures, 249
 rejuvenescence, 329
 senility and division, 330
 Suctoria feeding, 163
 vitality graph of *Stylonychia*, Fig. 165, p. 331
 Mayor, autogamy, 324
 Meiosis, genetic, 307
 in Sporozoa, 526
 zygotic, 309
 Melanin, 134, 533
 in malaria, 409
 Membrane of nucleus, 59
 Membranelles, 155
 Membranulae, 155
 Memory in Protozoa, 181
 Mengheni, conditions of encystment, 290
 Menosporidae, Key, 563
 Mercier, fertilization in Cnidosporidia, 326
 in *Thelohanina*, 555
 Merotomy and rejuvenescence in *Amoeba*, 239
 Uronychia, Fig. 135, p. 262
 Merozoite, defined, 528
 with Golgi apparatus, Fig. 40, p. 79
 Mesnil, chromidia, 70
 kinetoneucleus, 113
Mesolinium, tentacles, Fig. 198, p. 480
 Mesomitosis, 89

- Metabolic gradient in *Ameba*, 180
types, 135
- Metachromatic bodies, 72
- Metacyclic trypanosomes, 382
- Metacystidae, Key, 494
- Metaganic divisions, defined, 529
- Metalnikoff, choice of food, 189, 190
digestion in gastric vacuoles, 195
Paramecium, vitality, 253
selection in Protozoa, 181
- Metamastigota, 422, 427
- Metaplastids, 133
- Metcalf, macrochromatin, 481
Opalinidae, 397
- Metopidae, Key, 509
- Metopus sigmoides*, Fig. 206, p. 511
- Metschnikoff, acid digestion, 196
- Meves, chondriome, 73
- Meyer, volutin, 72
- Meyerhof, oxidation-reduction potential, 174
- Michelson, *Paramecium* cysts, 24
- Microdissection of *Euploes patella*, 131
- Microgametes, 272
- Microgametocyte, defined, 529
- Microgromia socialis* colony, Fig. 107,
p. 214
division, 214
- Micronucleus, 85
division, 218
- Microsporidia, 552
Key, 569
- Microthorax sulcata*, Fig. 204, p. 502
- Middleton, effect of increased temperature, 344
- Miescher, chemistry of chromatin, 56
- Miescher's tubules, 555
- Miller, *Hepatozoön* cycle, Fig. 211, p. 527
history of *Hepatozoön*, 361
- Minchin, cellular grade, 18
digestion, 195
endosome, 50, 51
kinetoneucleus, 113
origin of cellular grade, 87
parabasal of little owl Trypanosome, 112
source of blood parasites, 360
- Minchin and Thompson, life history of *Trypanosoma lewisi*, 233
- Minot, chromatin and sex, 272
- Mitochondria, 69, 73
and respiration, 76
of *Opalina* in division, Fig. 38, p. 75
- Mitosis, 89
- Monadidae, Key to genera, 426
- Monoenidea, Key, 569
- Monocystidae, Key, 559
- Monocystis*, meiosis, 309
rostrata, chromosomes, 99; Fig. 55,
p. 100
- Monodinium balbianii*, Fig. 84, p. 153
- Monokaryomastigina, 422, 430
- Monster formation, 264
- Monsters and reduced vitality, Fig. 138, p. 270
- Moody, *Actinobolina radicans*, 162
isolation culture of *Spathidium*, 251
- Moore and Breinl, kinetoneucleus, 113
- Motile organoids, 139
organs, renewal, 221
- Motor response in Protozoa, 181
- Motorium in ciliates, 129
- Mouth, origin at division, Fig. 111, p. 224
shifting, ciliates, Fig. 15, p. 36
- Mouton, trypsin-like ferments, 196
- Mrazekiidae, Key, 569
- Mulow, meiosis in *Monocystis*, 309
Monocystis rostrata, 99; Fig. 55, p. 102
- Multiple nuclei, 84
- Mutations, arising after treatment in sensitive periods, 345
- Mycetozoa, 445
aethalia, 450
capillitium in, 271, 446
elaters in, 446
Key, 462
life history, 445
microcysts, 445
peridium in, 271, 446
sclerotium in, 271
spore formation, 237
- Mylestonidae, Key, 156
- Myonemes of ciliates, 124
- Myoplanes, 128
- Myophrisks of Radiolaria, 127
- Myriaphrys paradoxa*, Fig. 197, p. 478
- Myxamebae, 445
- Myxidiidae, Key, 568
- Myxobolidae, Key, 568
- Myxobolus Pfeifferi*, autogamy, Fig. 164, p. 325
- Myxollagellates, 445
- Myxogastres, Key, 463
- Myxopodia, 435
stereoplasmatic axis, 435
- Myxopodium, Fig. 78, p. 146
- Myxosomatidae, Key, 568
- Myxosporidia, 548
budding, 230
development, 551
Key, 567

N

- NAEGLER, centrioles, 63
promitosis, 89
- Nassonov, Golgi apparatus, 79
and contractile vacuole,
Fig. 95, p. 171
in contractile vacuole,
178
- Nassula aurea*, Fig. 195, p. 475
- Nassulidae, Key, 498

- Naville, autogamy in Cnidosporidia, 326
 meiosis in Cnidosporidia, 546
- Xereshheimer, coordinating fibers, 161
 myophanes and neurophanes, 128
- Neuromotor apparatus, 129
 system, 105
- Neurophanes, 128
- Nicolle, culture medium, 366
Leishmania infantum, 369
- Nicollela*, Fig. 172, p. 400
- Nicollellidae, conjugation, 287
- Nina gracilis*, sex difference, Fig. 214, p. 537
- Nirenstein, digestion in gastric vacuoles, 195
 gastric vacuole formation, 195
- Nodosarine type of Foraminifera, origin, Fig. 186, p. 451
 of shell, Fig. 19, p. 38
- Noguchi, serological work with *Leishmania*, 363
- Nosematidae, Key, 569
- Noxy and MacNeal, culture medium, 366
 endotoxins in Trypanosomes, 198
- Nuclear derivatives during division, 88
 reorganization, 217
 structure of fundamental organization, 49
- Nuclearia delicatula*, Fig. 183, p. 444
- Nuclei with pole plates, 65
- Nuclein, 65
- Nucleoplasmic relation, 205
- Nucleus, 49
 cytoplasm changes at conjugation, 290
 formation, 84
 placenta, 84
- Nutrition of Protozoa, 183
- Nyctotherus*, 401
cordiformis, Fig. 206, p. 511
 basal bodies, 124
oralis, Fig. 81, p. 151
- O**
- OICOMONADIDAE, Key to genera, 423
- Oicomonas*, food-getting by Protozoa, Fig. 97, p. 186
- Oils and fats, 133
- Oken, Urtiere, 17
- Old age in *Uroleptus*, 255
- Oligotrichida, Key, 512
- Onychodromus grandis*, Fig. 207, p. 511
- Oocyst, defined, 529
- Oogamy in Coccidiomorpha, 280
- Opalinidae, fertilization, 484
 Metcalf, 397
- Operculina* shell, Fig. 74, p. 138
- Ophrydium*, colony, 38
versatile, 21
- Ophryocystis mexuili*, gamete formation, 229; Fig. 120, p. 231
- Ophryodendridae, Key, 524
- Ophryoglena flava*, Fig. 205, p. 504
- Ophryoglenidae, Key, 507
- Ophryoscolecidae, 401, 513
- Ophryoscolecina, 139
- Opisthodon mucimicnsis*, Fig. 191, p. 472
- Oral baskets, 167
 replacement at division, 222
- Orcadellidae, Key, 465
- Organization and differentiation, 260
 defined, 47
- Orthodon hamatus*, Fig. 93, p. 167
- Overton, permeability, 172
- Oxidation-reduction potential, 174
- Oxychromatin, 57
- Oxygen, source of, 174
- Oxymonadidae, Key to genera, 432
- Oxytricha*, Fig. 209, p. 518
 chromosomes, 319; Fig. 162, p. 320
jullax, Fig. 88, p. 159
pellionella, Fig. 88, p. 159
- Oxytrichidae, Key, 517
- P**
- Pachysocca longicollis*, Fig. 178, p. 419
- Pansporella perplexa*, 386
- Pansporoblasts as endogenous buds, 232
- Parabasal bodies, 60
 body, 110, 111
 types of, 416; Fig. 62, p. 116
 Feulgen reaction, 118
- Paradesmose, 118
 in *Trichonympha campanula*, 99; Fig. 54, p. 100
- Paraglycogen, 133
- Paramebidae, 456
 Key, 467
- Parameciidae, Key, 501
- Paramecium aurilia*, endomixis, Fig. 161, p. 318
barsaria, Fig. 204, p. 502
caudatum, fertilization in, Fig. 139, p. 273
 first meiotic, Fig. 57, p. 103
 Golgi bodies and contractile vacuole, Fig. 95, p. 171
 in depression, Fig. 145, p. 283
 in division, pole plates, Fig. 35, p. 67
 nucleus, Fig. 23, p. 50
 trichocysts, Fig. 193, p. 474
- cilia structure, Fig. 82, p. 152
- cyst, Fig. 5, p. 24
- merotomy, Fig. 108, p. 216
- monster formation, Fig. 108, p. 216
- oxygen consumption, 175
- patricium*, Fig. 204, p. 502
- variations in size, Fig. 167, p. 342
- Parasites, carriers of, 362

- Parasites, effect of, on hosts, 362
 Parasitic flagellates, Haptomonad stages, 367
 Nectomonad stages, 367
 Protozoa, 358
 Parasitism, sites of, 360
 Parastyle, 114
 Paratrichidae, Key, 503
 Parthenogenesis, 316
 and rejuvenescence, 340
 in Paramecium, 251
 Pascher, chromatophores of *Paulinella*, 442
Paulinella "chromatophores," 442
 Peebles, merotomy, *Paramecium*, 264
Pelomyxa binucleata, nucleus, Fig. 23, p. 50
 Penard, types of Heliozoa, Fig. 75, p. 139
 Pepsin-like ferments, 196
Peranema trichophora, Fig. 3, p. 22
 Peranemidae, Key to genera, 424
 Perichenidae, Key, 465
 Periplast, 135
 Peristome, 156
 Peritricha, Key, 521
 Peritromidae, Key, 512
Peritromus emmae, Fig. 89, p. 160; Fig. 207, p. 511
 Peters, effect of oxygen on *Colpidium*, 175
 Pfeiffer, sarcocystin, 197
 transmission of malaria, 407
 Pheodium, 134
Phalarsterium digitatum, colony, 39; Fig. 22, p. 41
 Pharyngeal baskets, 167, 475
Phialonema cyclostoma, flagellum insertion, 109; Fig. 60, p. 110
 Philasteridae, Key, 507
 Physaridae, Key, 463
 Physiological balance, 19
 Physiology, 172
Phytomonas davidi, Fig. 169 C, p. 366
Phytomyxa, 449
 Key, 462
 Pigments, 134
Piniaciophora spicules, Fig. 75, p. 139
 Plagiopylidae, Key, 500
 Plagiotomidae, Key, 510
 Plasmodiidae, Key, 566
Plasmodiophora brassicae, 386
Plasmodium falciparum, gametocytes, Plate 11, p. 409
 formation, 271
 malariae, sporulation, Fig. 124, p. 238
 Marchiafava and Celli, 406
 species, 406
 vivax, sporulation, Fig. 124, p. 238
 Plasmodroma, 411
 Platin, 58
 Platysporina, Key, 568
Pleuronema chrysalis, Fig. 199, p. 482
 Pleurostomina, Key, 491, 497
Pleurotricha lanceolata, Fig. 210, p. 520
 vitality graph, Fig. 132, p. 251
 Plimmer, endotoxins in *Trypanosoma*, 198
Plocozia vitrea, Fig. 76, p. 143
Podophrya cyst, Fig. 4, p. 23
 flava, infraciliature, Fig. 43, p. 81
 tentacles, Fig. 198, p. 480
 sp., Fig. 100, p. 192
 Podophryidae, Key, 524
 Pole plates, 65
 Poljansky, *Barsaria* conjugation, 315
 Polycystid gregarine, development, Fig. 126, p. 242
 Polyenergid theory, 71
 Polykaryomastigina, 422
 Key to families, 432
Polymastix, parabasal, Fig. 62, p. 116
Polystomella crispa, alternation of generations, Fig. 123, p. 235
 chromidia, 69
 nucleus, Fig. 23, p. 50
 Ponselle, immunity, 364
 Popoff, abnormalities, 345
 division zones, 264
 nucleoplasmic relation, 205
Porospora, cycle, 538
 gymnospores, 532
 taxonomy, 532
Poteriodendron, 21
 petiolatum, Fig. 177, p. 418
 Predatory forms of protozoa, 185
 Primitive forms, 141
 Prociliata, Metcalf, 398
 Promitosis, 89
Proodon, Fig. 202, p. 492
 Prostomina, Key, 490, 491
 Proteomyxa, 443
 Key, 461
Protospongia, colony, 38
 Protista, 18
 Protoplasm, death of, 227
 Protoplasmic structure, 39
 Prototrichiidae, Key, 466
 Protozoa as organisms, 19
 definition of, 17
 distribution of, 23-25
 form relations, 30
 habitat of, 22
 measurements of, 27
 relation to other groups, 18
 size, form and appearance, 26
 the individual, 241
 Prottrichocysts, 135
 Prowazek, division of *Herpetomonas*, 211
 fibers in *Euplotes*, 131
 granules in digestion, 196
 Iodamoeba, 397
 Mastigamoeba invertens, 109
 parabasal, Fig. 62, p. 116
 Pseudochitin, 133-137
Pseudochlamys, Fig. 188, p. 457

Pseudodifflugia, Fig. 11, p. 33
 Pseudomembranes, 157
 Pseudopodia, 145
 as organs of locomotion, 150
 formation, 180
 Pseudopodiospores, 236
Psilotricha acuminata, Fig. 210, p. 520
Pteroccephalus nobilis, gametes, Fig. 144,
 p. 281; Fig. 215, p. 538
Ptychostoma bouasae, Fig. 179, p. 420
 Pure lines and series, 250
 Puschkarew, common air cysts, 23
 Dimastigamoeba bistadialis, 107
 Pütter, reactions to stimuli, 181
 Pycnothricidae, 400
 Key, 409
Pycnothrix, Fig. 172, p. 400
 Pylea of central capsule, 438
 Pyronine action on *Trypanosoma brucei*, 114
Pyxinia moebiuszi, epimerite for food-
 getting, Fig. 103, p. 201

R

RADIOLARIA, 438
 central capsule of, 438
 distribution of, 26
 isospores and anisospores, 279
 myophrisks, 127
 spore formation, 237
 types of, Fig. 181, p. 439
 yellow cells of, 441
Radiophya limnodrili, terminal bud-
 ding, Fig. 116, p. 227
 Raffel, conjugation and division rate,
 332
Raphidiophrys pallida, Fig. 75, p. 139
 Reducase and oxidation, 175
 Rees, *Paramecium motorium*, 129
 Regaud, function of mitochondria, 77
 Regeneration of fragments without
 micronuclei, 225
 Reichenow, Feulgen reaction with volu-
 tin, 72
 nucleal reaction, 57
 Reichenowellidae, Key, 510
 Rejuvenescence after parthenogenesis,
 340
 by division, 209
 by merotomy, 238
 Maupas, 329
 Reorganization and vitality, 328
 bands of *Euplotes*, 94
 cytoplasmic, 218
 in *Chilodon uncinatus*, 222
 in ciliates, 221
 in *Uronychia*, 222
 of cytoplasm at division, 218
 Reproduction, 204
 multiplicative, 540
 propagative, 540
 Respiration, 174
 quotient, R.Q., 174

Reticulariidae, Key, 465
 Reversibility of structures, 21, 48
 Reynolds, selection in *Arcella polyppora*,
 347
 Rheoplasm, 42
 Rhizomastigidae, Key to genera, 423
 Rhizopoda, Key, 461
 Rhizopodia, 148, 442
 Rhizopods, parasitic forms, 385
 Rhodesian trypanosomiasis, 383
 Rhlumbler, amoeboid movement, 180
 food ingestion by Protozoa, 186
 importation, 189
 spumoid structure, 42
 Rhynechoeystidae, Key, 559
 Richardson and Horning, chondriome,
 74
 Robertson, age and vitality, 269
 catalase stimulating division, 206
 environment and vitality, 256, 258
 Feulgen reaction, 57
 parabasal, 118
 Rogers, cultivation of *Leishmania*, 369
 Root, selection in *Centropyxis*, 317
 Rosenau, paroxysm toxins, 197
 Rosenbusch, kinetonucleus, 113
 Roskin and Levinson, gregarine myco-
 nemes, 535
 Ross, malaria and mosquitoes, 407
 Rotifers, desiccation, 45

S

SACUS, energid theory, 205
 Saedeleer, choanoflagellate collar, 164
 food ingestion by Choanoflagel-
 lates, 188
Salpingoeca marinus, Fig. 92, p. 165
 Sandon, soil Protozoa, 353
Sappinia, Fig. 185, p. 418
 diploidea, 95
 autogamy, Fig. 163, p. 323
 Sappiniidae, 447
 Key, 462
 Sapropelic fauna, 24
 flagellates, 356
 Saprozoic nutrition, 199
 Sarcocystin, 197
Sarcocystis muris, life history, 556
 species, 555
 Sarcode, 433
 Sarcodietyum, 439
 Sarcodina, chitin in, 433
 nuclei in, 434
 pseudopodia types of, 434
 taxonomy, 433
 Sarcomatrix, 439
 Schaeffer, *Ameba* anchorage at feeding,
 186
 choice of food, 189, 190
 periplast, 135
 pseudopodia, 150
 selection in protozoa, 181

- Schaudinn, *Actinophrys sol*, Fig. 66, p. 120
Camponema movement, 147
 chemiotaxis in fertilization, 291
 chromatin and sex, 272
 chromatida, 69
 cycle of *Eimeria schubergi*, Fig. 212, p. 530
 division of *Acanthocystis*, 213
 dysentery, 391
 endobasal bodies, 62, 63
 fertilization in *Actinophrys*, 277
 in *Centropyxis*, 277
 life cycle of *Eimeria*, 259
 pole plates, 65
 sex in *Cyclospora*, 280
Trichosphaerium, 457
 Trypanosome of owl, 112
- Schaudinella *heuleae*, gametes, Fig. 144, p. 281; Fig. 215, p. 538
- Schaudinnellidae, Key, 559
- Schewiakoff, *Acantharia*, 440, 441
 budding division in *Euglypha*, 214
 gregarine movement, 535
 excretion, 177
 mitosis in *Euglypha*, 98
- Schizocystic *sipunculii*, budding, 229; Fig. 119, p. 230
- Schizogony, defined, p. 528
- Schizogregarinida, 541
 Key, 564
- Schizont, defined, 528
- Schizontocyte, defined, 528
- Schizontocytes, 227
- Schizotrypanum cruzi*, 383
- Schmahl, reorganization at division, 264
- Schröder, *Epistylis*, Fig. 70, p. 126
 myonemes, 125
 somatic structures in *Actinomyxida*, 240
Sphaeromyxa autogamy, 324
- Schultz, physical conditions in *Ameba*, 180
- Schultze, division of *Ameba*, 213
- Schumacher, volutin, 72
- Schulostomidae, Key, 500
- Sclerotium, 446
- Scopula, 359, 483
- Secretions as toxins, 193
- Seizing organ, *Didinium*, 163
- Selection and variations, 347
- Selenococcidiidae, Key, 565
- Senescence and division, 330
 "Sensing" at a distance by *Ameba*, 189
- Sensory cilia and flagella, 127
- Septata, Key, 560
- Sergent, immunity, 364
- Serological work, 363
- Sewage Protozoa, list of, 357
- Sex, definition of, 272
 in *Cyclospora karyolytica*, 280
- Shapiro, pH of gastric vacuoles, 196
- Sharp, *Diplodinium*, Fig. 2, p. 20
- Sharp, motorium, neuromotor apparatus, 129
 skeletal structure, 125
- Shellackia*, hosts, 361
- Shells and tests, 137
- Shiga, bacillary dysentery, 391
- Siebold, v., unicellular organisms, 17
- Siedlecki, *Lankesteria*, Fig. 213, p. 531
 schizontocyte formation, 227
- Silver line system, 69, 80
 origin of mouth at division, Fig. 114, p. 224
- Skin as barrier to infection, 360
- Slime moulds, 445
- Slonimski and Zweibaum, excretory granules, 197
- Smith, *Sarcocystis muris*, 555
- Soil Protozoa, 353
- Sokoloff, gregarine movement, 535
- Somatella, formation of, 233, 418
- Somatic structures and death, 239
- Somatochromidia, 70
- Spathidiidae, Key, 495
- Spathidium spathula*, feeding, Fig. 99, 188
 food-getting by Protozoa, 186
 increased vitality after conjugation, 332
 vitality graph, Fig. 133, p. 252
- Sphaeractinomyxon*, Fig. 221, p. 552
 autogamy, 326
- Sphaerastrum* with centrolepharoplast, Fig. 50, p. 95
- Sphaeromyxa sabrazesi*, autogamy, Fig. 164, p. 325
- Sphaerospora dimorpha*, endogenous buds in, Fig. 121, p. 232
- Sphaerosporidae, Key, 568
- Sphaerosporina, Key, 568
- Spicule formation and alveolar structure, Fig. 12, p. 33
 types, Fig. 75, p. 138
- Spiral types, Fig. 16, p. 36
- Spirochonidae, Key, 522
- Spirohemidae, 431
- Spirostonidae, Key, 510
- Spirostonum ambiguum* nuclei, 86
 contraction, 125
terres nuclei, 86
- Spirotricha, Key, 508
- Spirozonidae, Key, 500
- Split conjugants, 284
- Spongomonas*, centrioles, 63
splendida, division, 94; Fig. 49, p. 95
 reorganization at division, 212
- Sporamebidae, Key, 467
- Spore formation, 233
 in *Mycobolus*, 210
 of Radiolaria, 237
- Sporetia, 70
- Sporoblast, defined, 529
- Sporocyst, defined, 529
 types, Fig. 216, p. 539

- Sporoducts, 240
 age differentiations, 270
- Sporogony, defined, 548
- Sporont, defined, 528
- Sporozoa, 525
 form, 525
 gametes in, Fig. 215, p. 538
 nuclei, 526
 parasites of man, 402
 size, 525
- Sporozoite, defined, 528, 529
- Sporozoites with Golgi apparatus, Fig. 40, p. 79
- Stamiewicz, fat digestion, 199
- Staurojoeeniidae, 428
- Stenomitidae, Key, 461
- Stempel, fertilization in Cnidosporidia, 326
- Stempellia magna*, life cycle, Fig. 222, p. 553
- Stenophoridae, Key, 561
- Stentor coeruleus*, myophanes, neurophanes, 128
 cilia and myonemes, Fig. 69, p. 124
niger, basal bodies, 124
polymorpha, Fig. 81, p. 151
 regeneration, 45
- Stentoridae, Key, 510
- Stentorin, 134
- Stephanocca ampulla*, Fig. 178, p. 419
- Stephanomyxa silvestri*, Fig. 175, p. 414
- Stephanopogon*, Fig. 207, p. 511
- Stephens and Fantham, trypanosomiasis, 384
- Stercome, 450
- Stereoplasm, 42
- Stern, central granule of Heliozoa, 120; Fig. 67, p. 121
 division of *Acanthocystis*, 213
 pH of medium, 353
- Steudel, thymonucleic acid, 57
- Stichotricha secunda*, Fig. 192, p. 473
- Stiles, *Sarcocystis rileyi*, 555
- Stocking, conjugation and division-rate, 332
- Stolç, glycogen in *Pelomyxa*, 133, 198
- Stomatophoridae, Key, 559
- Strasburger, energid theory, 205
- Strebloplastigidae, 431
- Strelkow, *Tripalmaria*, Fig. 14, p. 35
- Strombidiidae, Key, 513
- Stromyglidium*, Fig. 88, p. 158; Fig. 209, p. 518
- Stylocephalidae, Key, 563
- Stylonychia*, cirrus structure, Fig. 82, p. 152
mytilus, Fig. 3, p. 22
 vitality graph, Fig. 165, p. 331
pustulata, vitality graph, Fig. 165, p. 331
 senescence, Fig. 130, p. 249
- Stylorhynchus longicollis*, gametes, Fig. 144, p. 281; Fig. 215, p. 538
- Suckers in flagellates, 420
- Suctorina, ciliated embryos, 228
 embryos, development of, 243
 endogenous budding, Fig. 117, p. 228
 food-taking in, 191
 Key, 523
- Surra, trypanosome disease of horses, 381
- Swarzewski, chromidia, 69
 endogenous budding in *Acella*, 228
 fertilization in Cnidosporidia, 326
Vahlkampfa, 62
- Symbionts, 202
 in ciliates, 476
- Synactinomyxon*, Fig. 221, p. 552
- Syneystidae, Key, 560

T

- TALIAFERRO, serological work, 363
- Taylor, dedifferentiation with division, 263
 merotomy in *Euplotes patella*, Fig. 96, p. 182
 microdissection, 129; Fig. 72, p. 130
- Taxonomic structures, 132
- Taxonomy of Flagellates, 111
- Tektin, 135
- Telosporidia, 533
 Key, 557
- Ternetz, amino-acid nutrition, 200
- Testacea, 456
 Key, 467
- Tetramitidae, Key to genera, 130
- Theileriidae, Key, 566
- Thelobania legeri*, life cycle, Fig. 223, p. 554
- Thigmotricha, 483
- Thon, seizing organ of *Dilinium*, 163
- Thymonucleic acid formula, 57
- Tintinnidae, distribution, 26
 Key, 513
- Tintinnopsis*, Fig. 208, p. 511
- Tissue-cell culture, 210, 258
- Tolophrya cyclopani*, 228; Fig. 118, p. 229
quadripartita, Fig. 3, p. 22
 endogenous budding, Fig. 107, p. 228
- Toxins, 197, 363
- Tracheliidae, Key, 497
- Trachelius orum*, Fig. 93, p. 167
- Trachelocerca*, Fig. 202, p. 492
 contraction, 125
- Trailing flagellum, 112
- Triactinomyxon*, Fig. 221, p. 552
- Trichiidae, Key, 465
- Trichites, 134, 166, 473
- Trichocysts, 134, 473

- Trichomonadidae, Key to genera, 430
- Trichomonas augusta*, division, Fig. 77, p. 145
distribution in man, 361
- Trichonympha campanula*, Fig. 64, p. 118
mitosis, Fig. 54, p. 100
- Trichonymphidae, Key to genera, 428
- Trichopelmidae, Key, 501
- Trichophrya salparum*, Fig. 100, p. 192
- Trichosphaerium*, alternation of generations, p. 457
- Trichosporidae, Key, 500
- Trichostomida, Key, 499
- Trimastigamoeba philippincensis*, kinctic element, 107
- Trimastigidae, Key to genera, 427
- Tripalmaria dogieli*, Fig. 14, p. 35
- Trophochromatin, 56
- Trophonucleus, 112
- Trophozoite, defined, 528
- Tropisms, 181
- Trypanosoma cruzi*, origin of parabasal, Fig. 61, p. 111
parabasal, Fig. 62, p. 116
enduring modifications, 344
flagellum insertion, 109; Fig. 61, p. 111
gambicse, Fig. 169 D, p. 366
genus, 371
lewisi, somatella formation, 233; Fig. 122, p. 234
life history, 382
list of species and hosts, 372-381
rhodesiensis, 383; Fig. 169 I, p. 366
stations in insects, 382
- Trypanosomiasis, clinical history, 383
in man, 381, 383
- Trypanosomidae, Fig. 169, p. 366
Key to genera, 424
- Trypsin-like ferments, 196
- Tschenzoff, *Euglena viridis*, 61
- Tsetse flies and trypanosome transmission, 381
- Tubiferidae, Key, 465
- Turner, motorium, 129
reorganization bands, 93; Fig. 48, p. 94
- Tyzzar, chicken coccidiosis, 405
Cochlosomidae, Fig. 179, p. 420
- U**
- UHLENHUTH, endotoxins in *Trypanosoma*, 198
- Undulating membrane, 142
ciliata, 157
- Unequal division, 225
- Unger, vacuole activity, 284
- Ureolariidae, Key, 521
- Uroecetrum turbo*, Fig. 205, p. 504
- Uroleptus*, bilateral symmetry, 36; Fig. 18, p. 37
- Uroleptus halseyi*, chondriome, Fig. 37, p. 74
nuclear cleft, 92; Fig. 47, p. 93
- mobilis*, Fig. 1 (Frontispiece)
centriole, 63; Fig. 32, p. 64
division of double individual, Fig. 128, p. 246; Fig. 129, p. 247
division of macronucleus, Fig. 110, p. 220
encystment period, 267
formation of nuclei, 84
fusion of macronuclei, Fig. 109, p. 219
graph of vitality, Fig. 131, p. 251
isolation cultures, 254
metagamic divisions, Fig. 160, p. 314
nuclear fusion, Fig. 159, p. 313
old age, Fig. 7, p. 28
optimum vitality for nine years by conjugation, Fig. 166, p. 334
origin of double individual, 244; Fig. 127, p. 245
origin of Macronucleus, Fig. 27, p. 58
- pisces*, Fig. 81, p. 151; Fig. 209, p. 518
- Uronychia transfuga*, division zone, 217
merotomy, Fig. 113, p. 223; Fig. 135, p. 262
structure, Fig. 111, p. 221
- Urospora lagidis*, gametes, Fig. 144, p. 281; Fig. 215, p. 538
- Urosporidae, Key, 560
- Urthiere, 17
- V**
- Vahlkampffia limax*, chromidia, Fig. 28, p. 59
division, Fig. 106, p. 212
nuclear division, Fig. 26, p. 53
- Valkanov, origin of *Clathrulina* stalk, 148
- Vampyrellidae, 444
Key to genera, 462
- van Herwerden, volutin, 73
- Variation and heredity, 342
- Verjüngung, Bütschli, 329
- Verworn, cilia beat, 127
effect of oxygen on *Colpidium*, 175
merotomy, 55
respiration, 174
- Vesicular nuclei, 50
- Vianna, cause of espundia, 369
- Viereck, *Eudamoeba tetragena*, 392
- Visseher, trichocysts, 475
- Vitality, 214
and reorganization, 328
intensity and endurance of, 333
and renewal of, 335

- Vitality, measure of, 248
 of parent and offspring series,
 Table, p. 336
 Volutin grains, 69, 72
 von Leeuwenhoek, discovery of Protozoa, 17
 Vonwiller, protoplasmic structure, 43
Vorticella, frontal field, 169
 structures, Fig. 86, p. 158
 type, Fig. 86, p. 158
 Vorticellidae, fertilization, 279; Fig. 143, p. 280
 Key, 522

W

- WAGNERELLA, axial filaments, 122
borcalis, division, 213
 Wailes, Pseudogromiinae, Key, 469
 Walker and Selhards, dysentery, 393
 Wallengren, reorganization in ciliates, 221
 Washburn, tropisms, 181
 Weatherby, uric acid, 177
 Weininger, sex, 272
 Weinkand, anti-digestive ferments, 359
 Weismann, amphimixis, 329
 germ and soma, 48
 Life and Death, 248
 Wenrich, *Actinobolina vorax*, 162
 Wenyon, grouping of trypanosomes according to site in insect, 382
 kinetoplast, 114
 Wenyon and O'Connor, *Endolimax nana*, 397
 Werbitzski, enduring modification in *Trypanosoma*, 344
 trypanosomes without kinetoneucleus, 114
 Wetzell, cavulae, 42
 Whitman, inadequacy of term cell, 19, 40
 Whitmore, dysentery, 393
Trimastixamoeba philippinensis, 107
 Willis, reaction of fragments, 183
 Wilson, division of granules, 208
 Wilson, C., *Dimastixamoeba grabcei*, 107; Fig. 59, p. 108
 Winter, chromidia, 69
 Woithe, endotoxins in *Trypanosoma*, 198
 Wolff, soil Protozoa, 354
 temporary cysts, 267

- Woodcock, kinetoneucleus, 112
 and Lajage, copulation in flagellates, 276
 Woodruff, effects of excretion products, 200
 isolation cultures, 250
 rejuvenescence after endomixis, 340
 succession of Protozoa, 23
 survival value of conjugation, 333
 Woodruff and Erdmann, endomixis, 317
 parthenogenesis, 251
 Woodruff and Spencer, rejuvenescence in *Spathidium*, 332
 sensing, 189
Spathidium, vitality, 252, 255
 Wortmann, cellulose digestion in Foraminifera, 199
 Wright, organism of tropical ulcer, 369

Y

- Yocom, myophanes and neurophanes, 128
 Young, cirrus regeneration, 161
 endomixis and environment, 310
 merotomy in *Urographia*, 225, 263

Z

- ZOÖMASTIGOPHORA, 412, 121
 Zoösporidae, 444
 Key to genera, 461
Zoöthamnium alternans, 21
arbuscula, 21
 colony, 38
 contraction, 125
 Zuelzer, chromidia, 70
 division of *Wagnerella*, 213
 enduring modification in *Ameba*, 343
 environment effects, 178
Wagnerella, 122
 Zumstein, amino-acid nutrition, 200
 Zweibaum, chondriome, 75
 dis-organization significance, 311
 protoplasmic make-up at conjugation, 290
 Zygoecystidae, Key, 559
Zygoecystis zonata, caudal threads, 536
 Zygote, defined, 529

