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PROTOZOÖLOGY

BY

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Illustrated with 125 Engravings
and 4 Colored Plates

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PREFACE.

IT is my purpose, in the present volume, to discuss some of the old and some of the new problems in biology as illustrated by the lowest forms of animal life, the protozoa, the subject matter being founded on a course of Lowell Institute lectures given in the fall and winter of 1907. Interest in these organisms, of late, has centred mainly in the practical side until, to many biologists and to most medical men, protozoölogy implies the science dealing with pathogenic protozoa. Protozoölogy has a broader scope than this, and it is one purpose of this book to check, if possible, the limiting tendency and to point out again the important part that the protozoa play in the problems of modern biology. This is the more necessary because, in my opinion, in the application of biological principles which underlie the vital phenomena of free and parasitic forms alike, will be found the most valuable data for the more practical sides of protozoölogy. Here in these mere specks of animated jelly, which rarely measure more than the hundredth part of an inch, we find, in their simplest forms, the manifold processes of the living organism. Digestion and assimilation; respiration, with its dual action of oxidation and renewal; excretion and secretion; irritability and fatigue; reproduction, together with the unfathomed mystery of fertilization and inheritance, all find expression in these simple animals and raise the lowest protozoön immeasurably above the most complex of non-living substances. With such vital processes reduced to their lowest terms in these protozoa, we should expect to find a wealth of material for the study of life phenomena which in the higher animals are masked under a cloak of differentiated structures, and the study of these more general functions should form the basis for explanations or interpretations of the more specialized adaptations which are characteristic of pathogenic forms. This more comprehensive field, as I understand it, is the scope of modern protozoölogy.

The researches of Louis Pasteur, in connection with fermentation, souring of wine, and the silkworm disease, led him to many reflections and conclusions as to the nature of various contagious and hereditary diseases. Perhaps more than any other single research, his investigations, begun in 1865, on the cause and prevention of silkworm epidemics (to which De Quatrefages had given the name of pèbrine,

because of the characteristic black spots), led him to the belief that many human ills are similarly due to minute and microscopic forms of life, and so paved the way for the later generalization which now dominates medicine—the germ theory of disease. Ahead of his times in recognizing the present-day axiom that epidemics are ended by prevention rather than by individual treatment, Pasteur patiently advised and demonstrated, in connection with the silk industry, that perfect silkworms and moths would not develop from eggs having pèbrine corpuscles on them. It is of no importance that these corpuscles were not recognized by him as the spores of a protozoön, but the important results which followed their discovery, and which led to increased length of human life, and to the mitigation of human and of animal suffering throughout the civilized world, make an increasingly substantial monument to the patience, courage, and virility of this man of pure science, who, by the apotheosis of scientific method, proved these unknown corpuscles to be the cause of this silkworm disease.

The recently opened chapter of the protozoan diseases of man might have been earlier studied had these observations of Pasteur upon the spores of *Nosema bombycis* been followed up. The parasitic protozoa were known and the free-living forms had been brought into prominence in scientific circles through the controversies over the cell theory and the theory of spontaneous generation, but more than thirty years were to elapse before general acceptance of the first human disease attributable to protozoa.

The other minute organisms, bacteria and yeasts, whose presence Pasteur had demonstrated in his experiments on fermentation and spontaneous generation, were not neglected. In the hands of R. Koch the means of studying bacteria were perfected, and "culture" methods were introduced which soon raised bacterial research to the dignity of an independent branch of biological science. The ease with which bacteria could be studied, thanks to these methods, and the rapidly increasing list of bacterial diseases, seemed to divert the attention of specialists from the pursuit of protozoan diseases and to confine it to research on those of bacterial origin. Attempts were repeatedly made, however, to cultivate protozoa as the bacteria are cultivated, on artificial media, but until the present decade such efforts, for the most part, were fruitless. The difficulties in applying the artificial culture method to the protozoa are due, essentially, to the differences in their mode of nutrition. Some of them, indeed, are similar to the bacteria in being saprophytic or saprozoic (to use Blanchard's expressive term), absorbing liquid or dissolved proteid matter through the body wall. Such forms lend themselves to the culture method, and the success of Novy and MacNeal and others with trypanosomes, herpetomonads, etc., in artificial liquid media follows from this nutritional characteristic. Other forms of protozoa, as, for example, the parasitic amebæ, may or

may not lend themselves to the culture method, and then only upon the condition of having other living things as food. Many observers have found that intestinal amebæ, and others that feed on bacteria, will thrive on solid culture media provided the latter are seeded with bacteria, and this fact is of the greatest importance in obtaining material for study. Other amebæ cannot be cultivated in this way, and it is quite probable, as Lühe maintains, that many parasitic protozoa, especially the intracellular parasites, such as the coccidia, will never be successfully cultivated.

There is need, furthermore, of caution in studying protozoa under such artificial conditions, for they are extremely sensitive to variations and are readily adapted to new conditions. The reactions, both morphological and physiological, of protozoa under such conditions of study require careful control.

The study of protozoa, therefore, even when it is possible to apply bacteriological methods, is fundamentally different from the study of bacteria as at present carried on. The latter, dependent upon growth conditions, colony formation, reactions to media, etc., are essentially physiological and based upon the functions of the organisms. The study of protozoa, on the other hand, is essentially morphological, or based upon the structures of the protozoan cell, and involves the changes in cell structures which an individual undergoes during various phases of vitality. Hence it becomes necessary, first of all, to know the life history of the protozoön and the fundamental modifications which its protoplasm assumes. Modern protozoölogy, therefore, has demanded as a basis for genera and species of protozoa a knowledge of the complete life cycle, and as a basis for classification not the structures of the single cells, but the structures which the protoplasm may assume throughout its entire life history from fertilization to death or until the next fertilization.

The present volume, finally, does not aim at being an exhaustive treatise on the protozoa; it aims, rather, to give an introduction to the study of modern protozoölogy as seen from the author's point of view; and for numerous omissions, incomplete references, etc., he can only plead the excuse of a large subject crowded into a limited space.

G. N. C.

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PROTOZOÖLOGY.

CHAPTER I.

GENERAL ORGANIZATION OF THE PROTOZOA.

A PROTOZOÖN is a primitive animal organism usually consisting of a single cell, whose protoplasm becomes distributed among many free living cells. These reproduce their kind by division, by budding, or by spore formation, the race thus formed passing through different form changes and the protoplasm through various stages of vitality collectively known as the life cycle.¹

FIG. 1

Types of protozoa. *A*, *Ameba proteus*, a rhizopod (after Calkins); *B*, *Peranema trichophorum*, a flagellate (after Bütschli); *C*, *Stylonychia mytilis*, a ciliate with specialized cilia (after Bütschli); *E*, *Tokophrya quadripartita*, a suetorian (after Bütschli); *D*, *Pyxinia*, sp., a polycystid gregarine with primate and deutomerite (after Wasielewsky); *c*, contractile vacuole; *e*, epithelial host cell; *n*, nucleus; *v*, food vacuole.

It is quite impossible within the limits of a small volume to give a detailed or even adequate account of the many sides of interest of the unicellular animals. The wide range in habitat, from the purest waters of lake or sea to the foulest ditch, or the adaptation to environments varying in character from a mountain stream to the semifluid substance of an epithelial nerve or muscle cell, has brought about

¹ Definition by Calkins, 1906.

manifold varieties of protozoön structure. To describe all of these modifications under one or a few headings, and to attempt to formulate general laws from the different and often highly complicated life histories, is out of the question. Nevertheless, in spite of the structural modifications and special adaptations to particular modes of life, it is possible to group the different kinds of protozoa in four definite types, first outlined by the French microscopist Felix Dujardin in 1841. Three of these types—sarcodina, mastigophora, and infusoria—are based upon the form of the locomotor organs, pseudopodia, flagella, and cilia respectively, while the fourth type—sporozoa—including the gregarinida, first recognized as unicellular organisms by Kölliker in 1845, are devoid of motile organs, and are invariably parasitic in mode of life (Fig. 1).

A. GENERAL MORPHOLOGY.

While the different kinds of protozoa are undoubtedly the simplest animals known to us, they comprise at the same time some of the most complicated forms of cells, and the protoplasmic differentiations within these cells are frequently highly developed. In some cases these modifications are so highly evolved that we have little reason to regard such cells as units of structure comparable with the tissue cells of higher animals and plants, but should look upon them as composed of still more elementary vital units, and to this extent the cell theory, when applied to them, is inadequate.

The wide distribution of the protozoa and their varied modes of life lead to the greatest possible differences between them and even within the limits of the same class. No one form is characteristic of any type, but in all cases where the body is plastic and subjected to an even environmental pressure, as in floating, or in intracellular, quiescent forms, the body is spherical (homaxonic), readily changing, however, into an elongate or monaxonic form when the organism moves or is subjected to a current. In all divisions, when for any reason the surrounding medium becomes unsuitable, or in some cases for purposes of digestion or reproduction, the organisms secrete a thick and resistant covering of chitin, and they remain thus "encysted" until conditions are again suitable, and such cysts are usually spherical.

The size of protozoa likewise varies within wide limits. Some of them are on the very limits of vision, and some, apparently, are invisible, even when the eyes are assisted by the highest powers of the microscope. Thus, the organism causing yellow fever, and thought to be a protozoön, is so minute that it has never been seen, although its habitat and its general history are well known. Other protozoa, on the other hand, are relatively enormous single cells, a *Pelomyxa palustris*

or a *Bursaria truncatella*, reaching the size of 2 mm. (one-twelfth of an inch), while the parasitic gregarine *Porospora gigantea* of the lobster's gut attains the length of 16 mm., or two-thirds of an inch.

Unlike the majority of bacteria, the size of any given species of protozoa often varies within wide limits, and this in the same environment. The reasons for this difference are numerous, sometimes it is due to starvation, sometimes to developmental condition, and sometimes to the variations in vitality at different periods in the life history. Thus, two cells from the same culture of dileptus species may be mistaken for different species, the difference between them being so great,

FIG. 2

Dileptus, sp. Two sister cells. *A*, normal individual with macronucleus in form of scattered chromatin granules (chromidia); *B*, individual starved for several days. From photographs taken with same magnification.

and due solely to the lack of food in one case (Fig. 2). This divergence in size is particularly noticeable in the parasitic forms, where many factors influence the development of the cell.

Many forms of protozoa, especially the flagellated types, have acquired the habit of association into colonies, and with such association have gained the economy which comes from division of labor, so that here in the colony forms may be found the first step in the differentiation of cell aggregates and the nearest approach of protozoa to the metazoa. Such colonies have been designated according to their mode of formation, gregaloid, spheroid, arboroid, and catenoid

colonies. A gregaloid colony arises by the adventitious union of previously separated cells. Thus, many of the so-called "agglomera-

FIG. 3

Uroglana americana, Calkins, a spheroid colony, consisting of monads embedded in a gelatinous matrix.

FIG. 4

Codosiga cymosa Sav. Kent, an arboroid colony of Choanoflagellates. (After Kent.)

tions" of spirochetes and trypanosomes are gregaloid colonies brought about by some adverse condition of the environment. A spheroid

colony is a more perfect compound individual in which the cells are embedded and held together in a common gelatinous matrix (Fig. 3). An arboroid colony is one formed by continuous division of cells which remain attached at some point, such colonies often being large dendritic branched aggregates (dinobryon, epistylis, carchesium, etc., Fig. 4). A catenoid colony, finally, is formed by the union of two or more cells end to end or side by side.

(a) **Protoplasmic Structure.**—The body of a protozoön is made up of a somewhat gelatinous, diaphanous substance, to which Dujardin, in 1835, gave the name “sarcodé,” but which M. Schultze, in 1863, showed to be identical with the substance “protoplasm” of higher plants and animals, and named by von Mohl in 1846. The minute structure of this protozoön protoplasm appears to be little more than a fine network, the meshes of which are sometimes minute and narrow, as though compressed, and sometimes large and open. The substance of the walls of the meshwork appears to differ noticeably from that within its spaces, the former more dense and made up of fine granules (microsomes), the latter more fluid and containing granules of considerable size. Microchemical reactions show that these granules differ in chemical composition, and that some are reserve food particles, others reserve matters for one use or other, and that still others are waste matters. This protoplasmic make-up, which Bütschli ('92) compared with a foam structure (Schaumplasma), was described by him as consisting of fine drops of a liquid alveolar substance, enclosed within the meshes of a continuous interalveolar substance, also liquid but of a different density. Each alveolus he compared with a bubble in a foam structure; the air of the bubble corresponding to the alveolar, the walls to interalveolar, substance.

While the inner protoplasm of all protozoa is probably alveolar in nature, there is considerable variation in structure due to the great variations in size of the alveoli and of the granules contained within them. In some forms (*e. g.*, in the heliozoön actinospherium) the vacuoles are so large as to give a parenchymatous appearance to the cell, but in others they are so minute as to give a uniformly dense appearance; between these two typical cases fall the remainder of the types of protozoa. The granules within the walls of the alveoli are equally variable in size; in some cases they are very minute, corresponding, apparently, to the fine elementary granules which Altmann ('94) regarded as the basis of all protoplasm, while in other cases they are obviously of different kinds. There is reason to believe that some of these interalveolar granules are endowed with a specific function, and that some of them underlie the various motor activities of the cell (“kinoplasm” of Strasburger; “ergastoplasm” of Prenant). It is certain that the protoplasmic alveoli tend to condense toward the periphery of the cell, the condensation due, apparently, to the loss of

the more fluid alveolar substance, while the specific kinetic elements, if present, are concentrated. Such an hypothesis might very well account for the contractility of the ectoplasm of an ameba or for the various locomotor appendages of flagellated and ciliated forms (see page 29).

It is on the basis of these protoplasmic modifications that the protozoa are grouped into classes, orders, and finer subdivisions, and the most important of these have to do with the changes undergone by the outer protoplasm. This is the part of the cell that comes in contact with the surrounding medium, and this is the part, therefore, if any, which becomes changed by such contact. Being on the outside, it is the region of the cell for food ingestion, and we find it differentiated into mouth parts and into protoplasmic modifications for the procuring and directing of food. It is also the seat of motion, and may be differentiated into a great variety of motile organs which are so characteristic that classification is based mainly upon them. These motile organs, all of which may be traced back to a similar primitive type, may become modified into complex organs of the cells, while the function of locomotion is frequently changed into that of food getting, or into a sensory function of touch. It is an interesting point in this connection that the sensory apparatus arises in the outer or cortical plasm as a response of protoplasm to the surrounding medium, and it is significant that in all higher animals the sensory and nervous systems arise from the outermost layer of cells, the ectoderm.

In many protozoa, especially among the simpler rhizopods and some of the sporozoa, there may be no distinction between the inner and the outer protoplasm. Such cases, however, are exceptional, for in the majority of protozoa a well-marked ectoplasm can be distinguished. In most cases the difference appears to be mainly in the presence or absence of granules, their distribution depending upon the density of the plasm. No great morphological value can be placed upon this regional difference, for it appears to be only an index of the physical condition of the protoplasm. In *Ameba proteus*, for example, the outer layer is dense and the granules of the alveoli are forced into the more fluid endoplasm, but in *pelomyxa* the protoplasm appears to be everywhere the same in density and the granules penetrate to the very periphery. In some of the rhizopods, especially the shelled forms, the distribution of granules according to density is so marked that several zones can be made out. In this connection it is significant that in the artificial mixtures which Bütschli so successfully made to imitate protoplasm, a similar regional differentiation into outer and inner structures could be distinguished, a result due in this case to surface tension.

(b) **Membranes, Shells, and Tests.**—It is possibly due to such a tendency of protoplasm to stiffen under the influence of surface tension

in water that we may turn for an explanation, first pointed out by Gruber ('81), of the outer condensation of protoplasm resulting in the numerous types of membranes and tests of the rhizopods or of the outer coverings of the protozoa in general. The simplest form of membrane is an almost invisible cuticle of extreme delicacy, and it would be difficult to say whether such coverings are due to the physical change of the protoplasm or to secretion of a covering material which gradually hardens in the water (as cysts are formed). In the ordinary forms of ameba, at any rate, the pellicula is merely a hardening or condensa-

FIG. 5

A, *Englypha alveolata*; B, *Cochliopodium*.

tion of the outer zone, and in the different species of ameba all grades may be distinguished up to the relatively thick membranes of *Ameba tentaculata* or *Ameba actinophora*. In other forms of protozoa there is a gradual increase in density from within outward and the body of the cell is covered by a living membrane which may become complicated by the addition of muscular fibrils (myonemes), sensory or tactile organs (cirri), or various protective structures like hooks, spines, and tentacles (Figs. 5 and 6).

Like many of the cells which constitute the tissues of higher animals,

the protozoön has the power of manufacturing by chemical processes, over and above those which are devoted to nutrition, various products which are secreted just within or outside the peripheral protoplasm, where they may form a protective armor in the shape of shells, or tests. The materials thus formed within the cell body may be chitin (as in the case of *Arcella vulgaris* or in any other shelled rhizopod where the shell material is always laid down upon a chitin base); cellulose (as

FIG. 6

Ceratium tripos, a dinoflagellate. (After Stein.)

in the dinoflagellates); calcium carbonate (as in the foraminifera); or silica (as in the radiolaria). The secretions may take the form of definite plates, as in dinoflagellates, of continuous deposits, or of symmetrical skeletons which are often very complex. When the deposit is regular and continuous the shell material is added to the chitin membrane, the walls growing thicker with age of the organism; but when the material is deposited at one time (dictyotic moment),

as in the radiolaria, the deposit follows the contour of the protoplasmic alveoli and gives rise to skeletons often of extreme beauty (Fig. 8). In a number of fresh-water rhizopods the bulk of the shell material is not secreted, but the test is composed of foreign particles, such as

FIG. 7

Types of marine rhizopod shells (Reticulariidae, Carpenter).

diatom shells, sand, mud, or detritus of any kind, all fused together and to a chitinous substratum by means of a mucilaginous cement secreted by the inner protoplasm.

These shells and skeletons after death of the organisms sink to the bottom of ponds, lakes, or seas, where they may form thick beds of

FIG. 8

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

calcium carbonate (as in globigerina ooze), or silica (as in radiolaria ooze). Such beds have been thrown up from time to time in the past by volcanic upheavals, forming more or less extensive areas of protozoan land in which foraminifera or radiolaria may be easily identified.

(c) **Plastids.**—In addition to the basic substances making up the fluid protoplasm there are larger or smaller granules of different kinds embedded in the alveolar or interalveolar material; these granules may be food particles ready for assimilation, waste particles waiting for excretion, metaplastic particles like oil drops, pigment grains, and the like, or foreign particles like sand grains, calcium, silica, etc., to be used in building shells or stalks.

The plastids that are formed in a great many protozoa, especially in those types which lie on the boundary line between the lower plants and the protozoa, may have a considerable economic importance. Many of them are starchy in nature, *i. e.*, formed products to be used

FIG. 9

A complex polythalamous shell (schematic) of *Operculina*. (After Carpenter.) The shell is represented as cut in different planes to show the distribution of the canals (a' , a'' , a'''); c , c , c , the outer chambers with double walls (d , d , d), one of which is shown in section (g). The chambers communicate by apertures at the inner ends of the septa (e), and by minute pores (f). The outside (b) of the shell is marked by the radial septa.

as food; others are starch-forming centres or pyrenoids, which are usually embedded in plastids of large size, called chromatophores from the color they possess. These colors, due to some form of chlorophyl, may be bright green like the foliage of higher plants, or red, orange, yellow, brown, or black, according to the nature of the materials which combine with the chlorophyl. When great numbers of these color-bearing protozoa are massed together the result is a brilliantly colored area; red snow, for example, being due to aggregates of *hematococcus*, the red coming from the color of the minute chromatophore in each small cell. Similarly, great patches on the sea may be colored orange by the presence of *noctiluca*, or red by *peridinium*, while

drinking waters are not infrequently made unsightly because of the red coloring matters of *Euglena sanguinea*, or of the yellow coloring matters of dinobryon or uroglena.

In some cases the pigment is due to collections of waste materials stored up in the cell, products of proteid metabolism held in reserve for some useful purpose, or to be voided to the outside. The black pigment of metopus or of tillina is a waste product of this nature, while the yellow to brown pigment of some of the colony forms is utilized in building the stalk.

The fats, oils, and other metaplastic products, stored up in these minute cells, minute as they are in the individual, are, collectively, a great nuisance, or, in some parasitic forms, may be a menace to the life of the host. Potable waters are frequently rendered unfit to drink because of the odors and tastes due to these products of protozoan vitality. Such odors are rarely due to putrefaction of the organisms, but rather to the liberation of the minute drops of oil upon disintegration of the cell bodies. As crushing a geranium leaf causes minute drops of oil to be thrown into the air, giving the fragrant perfume of the plant, so disintegration of a uroglena colony, crushed by the pressure in pumps and mains, liberates the minute oil drops stored up in the inner protoplasm, but the cod-liver oil smell which they give to the water is far from fragrant. Such water is harmless so far as the health is concerned, but very offensive to the esthetic sense. So characteristic are these metaplastic products, that many kinds of protozoa can be recognized in drinking waters simply by the odors they impart.

The oils, which in the majority of cases, like fat, are probably a reserve store of nutriment, may, in some cases, become useful for purposes of protection. An interesting case of a possible protecting function is that of noctiluca, where the particles of oily matter are rapidly oxidized upon exposure to the air, resulting in a brilliant flash of light, and giving one great source of the phosphorescence in the sea. The possibility of a protective function comes from the fact that the fatty material is thrown out of the body upon irritation, and the flash of light may scare away small enemies.

Other plastids that are used for purposes of protection are trichocysts and trichites. These are minute structures derived from the nucleus (Mitrophanow, 1904) and arranged radially about the entire periphery, as in paramecium, frontonia, etc., or in certain regions only, as in dileptus or chilodon. When the organism is irritated the contents of the capsules are thrown out with considerable force, and the poison which they contain is strong enough to paralyze any single-celled opponent, or, possibly, as Mast ('09) suggests, they form, after their discharge, a dense protective envelope which cannot be penetrated by small enemies. Sometimes they are used as weapons

of offence as well as protective organs, and the minute hunters stalk about with them in search of prey (see page 77).

(d) **Vacuoles.**—The other formed structures of the inner protozoan body are the vacuoles. These for the most part are mere fluid-filled spaces, but in many cases they possess a definite and permanent form and are frequently complicated in structure.

The vacuoles are either storage or contractile vacuoles. The former are minute improvised stomachs, and in them the food matters are digested. The latter are the more complex structurally, varying from simple spaces, which fill with fluid and empty to the outside in rhythmic periods, to great branching canal systems with storage reservoirs and contractile vesicles, the excretory system permeating the entire inner protoplasm with a network of vessels.

(e) **Nuclei, Chromatin, and Chromidia.**—At the present time no one who has made a careful study of protozoan cells accepts Haeckel's view ('66) that some forms of unicellular animals are without nuclei (Monera). It is, indeed, true that there are many forms in which nuclei, in a morphological sense, are not permanently retained, but the essential part of the morphological nucleus—the chromatin—is invariably present. Sometimes this chromatin is distributed uniformly throughout the cell (the "distributed nucleus" of tetramitus, dileptus, etc.), but usually it is concentrated about a central body (division centre) having some of the attributes of a centrosome, or it is confined within a firm nuclear membrane.

Within the last four years there has developed an ever-growing tendency to recognize in protozoa two distinct types of nuclei. These are distinguished from one another in the majority of cases not by any structural characteristics, but by their functions in the cell. One type, the trophonucleus, has to do with the ordinary vegetative functions of metabolism. The other type, which may be designated the *karyogonad*, or simply the gonad nucleus, has no function in ordinary metabolism, but is the source of chromatin forming the nuclei of conjugating gametes. In a broad sense, therefore, the karyogonad represents the germ plasm of protozoa.

The forms assumed by the chromatin in these two types of nuclei vary within wide limits. In many cases both are included within one common nuclear membrane, and are separated from one another only at periods of maturation in preparation for fertilization (most gregarines, coccidia, and many flagellates). In other cases the gonad nucleus becomes separated from the trophonucleus at an earlier period in the life history of the individual, and appears in the cytoplasm in the form of distributed chromatin granules (idiochromidia of many different genera, "chromidialnetz," etc.) or as compact and homogeneous nuclei (micronuclei of infusoria, "secondary" or gametic nuclei of sarcodina).

The trophonuclei also may be permanently distributed in the form of chromatin granules, or, under certain conditions of the environment, may assume this condition (chromidia formation). The former is characteristic of the vegetative nucleus of some infusoria (*e. g.*, *dileptus*, Fig. 2), the latter as a result of starvation or overfeeding, or other abnormal environmental condition (*e. g.*, "chromidia" formation in *actinospherium*, Hertwig). (For further discussion of the significance of chromidia formation, see page 115.)

In addition to the chromatin elements which enter into the make-up of nuclei, there are specific materials of the cell which apparently underlie the kinetic functions of protozoa. In some cases these are aggregated into definite nucleus-like bodies to which the name kinetonucleus (Woodcock) has been applied (*e. g.*, in *trypanosoma* and other flagellates). Such organs of the cell will be considered at greater length in the following section.

(f) **Kinoplasm.**—The question as to a specific motor or kinetic substance in the cell has been repeatedly raised in general cytology and is still unsettled. Strasburger has long maintained that the plant cell possesses such a specific kinetic substance, which he termed "kinoplasm" and which enters into the formation of mitotic figures, flagella, cilia, and the peripheral zone of protoplasm. It is, according to him, a substance which forms all of the motor organs and underlies all of the physical activities of the cell. Similarly for animal cells, Boveri ('88) early pointed out that the astrospheres and other parts of the spindle figure are composed of a substance apparently quite different from the rest of the protoplasm, and suggested the term "archoplasm" for it. Subsequent observers have amplified this view and some, notably Prenant, have endeavored to show that archoplasm, or, in a larger sense, kinoplasm, is not only specific, but a kind of "superior" protoplasm, self-perpetuating and distinct. Wilson ('00), summing up the evidence for and against such a view in relation to metazoan cells, comes to the conclusion that such substances, if they exist in the cell, represent a more or less persistent but not permanent phase, or product, of cellular metabolism. (*The Cell*, page 323.)

Prenant's point of view is probably the most satisfactory in connection with the protozoan cell, for here the specific substances are more persistent than in the higher animal cells, and in most cases they assume the form of definite, active, kinetic bodies closely associated with the mechanism of nuclear division and of locomotion. To this body of the protozoan cell, whether within or without the nucleus, the non-committal term "division centre" has been applied (Calkins, 1898).

In many different kinds of protozoa this division centre remains inside the nucleus, giving rise to what Boveri has called the "centro-nucleus" type. It is almost universally found among the represen-

tatives of the flagellated and ciliated protozoa, and a characteristic form is found in *Euglena viridis* and its allies (Fig. 10). Here a definite intranuclear body is surrounded by chromatin granules, and when the cell is ready to divide, this division centre, like a centrosome, divides first and the chromatin elements are separated into two equal groups, each half following one of the centres. In this case, and in some of the infusoria (e. g., *Paramecium aurelia* [caudatum]) the division centre seems to be formed from a specific substance, and it appears to be a permanent body in the cell, retaining its individuality from generation to generation.

FIG. 10

Mitosis in *Euglena*. (From Wilson after Keuten.) A, preparing for division; the nucleus contains a division centre surrounded by chromatin granules; B, formation of an intranuclear "central spindle;" C, later anaphase, and D, telophase stage.

Much more enlightening, however, are the conditions in the heliozoa. Here, in many cases, there is a central granule in the geometrical centre of the cell, which was early noted by Grenacher ('69) and Schultze and called by the former the "Centralkorn." The axial filaments of the pseudopodia centre in this granule, which divides like a centrosome prior to division of the cell, while the axial filaments radiate out on all sides like the astral fibers of a mitotic figure. Bütschli ('92) was the first to compare this body with a centrosome, and the view was quickly accepted by cytologists, while the most complete

observations regarding its history have been made by Schaudinn ('96) in the case of *acanthocystis* and *sphastrum* (Fig. 11).

This central granule or division centre, while thus apparently permanent in the adult forms of heliozoa, must be regarded as a product of protoplasmic changes which have their seat in the nucleus. This is clearly shown by the formation of the central body in small cells of the above organisms that have been produced by budding. Schaudinn has shown that in the formation of these buds the nucleus divides by amitosis, after which the daughter nuclei migrate to the periphery of the cell, where they are budded off with a small amount of cytoplasm.

FIG. 11

Nuclear division and budding in *Heliozoa*. (After Schaudinn.) *A*, vegetative cell of *Sphastrum* with the axial filaments focussed in a central granule (centrosome); *B*, *D*, division of nucleus in *Acanthocystis*; *E*, *F*, flagellated and ameboid buds of *Acanthocystis*; *G*, exit of the centrosome from the nucleus.

In some cases as many as twenty-four buds are thus formed by the same animal, although this is an unusual number. The history of these buds is somewhat different in different cases. In the simplest ones the bud merely drops off of the parent and remains on the bottom for some days, where it moves about by ameboid motion. These buds contain no portion of the original division centre, nor does a new division centre arise in them until about five days after their formation, when in each bud a new division centre makes its appearance inside the nucleus, from which it migrates to the cytoplasm, where it takes up its position in the geometrical centre of the cell and gives rise to the

axial filaments, and with their formation the young organism for the first time assumes the appearance of a heliozoön (Fig. 11, *F*, *G*).

These structures of the protozoa certainly justify, if any do, the use of the term kinoplasm. Not only are they connected with the activity of the cell in division, but they are also closely identified with the motile organization of the cell. In heliozoa, as already pointed out, they are the centres for the formation of the axial rays of the pseudopodia, which vary in motile power from practically quiescent appendages in forms like *Actinophrys sol*, through a slight elasticity in forms like *acanthocystis* to vigorously vibratile appendages in *artodiscus*, which cause the minute organism to dance about the field on the tips

FIG. 12

Dimorpha mutans. (After Schoutedan.) Two flagella and radiating axial filaments centring in the extranuclear division centre.

of its pseudopodia. The similarity between these axial filaments and flagella of the flagellated organisms is well shown in the case of *Dimorpha mutans*, in which the majority of the axial filaments are similar to those of other heliozoa, but two of them remain uncovered by streaming protoplasm and whip about in the surrounding water like the vibratile lashes of the flagellates. One of these flagella, according to Schoutedan ('07) serves to anchor the animal, while the other provides a food current (Fig. 12). In such cases the close connection of these axiopodia with flagella is clearly shown and may well help to point out the course of evolution of heliozoa and flagellates, perhaps the former from the latter.

The actual participation of such division centres in the formation

of the more active motile organs is well shown in the flagellated protozoa. In the majority of cases where the morphology has been minutely studied, the flagellum has been traced either to such a basal body or to the nucleus, while in some forms, notably in trypanosoma, the materials of the vibratile or undulating membrane, of the flagellum, which forms its edge and continues beyond the cell as a free whip, and of the contractile myonemes are all derived from such a division centre called by Woodcock the "kinetonucleus," which, in some cases at least, has some of the attributes of a morphological nucleus.

In many cases this active substance of the division centre is confined to the nucleus, where it may be in the form of a definite and permanent body, as in euglena and its allies, or it may be diffused throughout the nucleus as in actinophrys and actinospherium. The substance of the axial filaments of such forms is derived from the nucleus by a nuclear secretion, as Schaudinn has clearly shown in the case of *Camptonema nutans*. All of these, however, are characteristically quiet forms, and the activity of the division centre is shown only in the process of nuclear division. In *Actinophrys sol* a typical spindle with centrosomes and fibers is formed as in the metazoa and all from the substance of the nucleus.

There seems to be unmistakable evidence, therefore, that the substance of the division centre is formed within the nucleus and that a definite body, or condensation of this substance, occurs at certain periods of vitality and has a more or less continuous existence as such. This body makes its appearance in the bud of acanthocystis, during mitosis of actinophrys and during reorganization of the cell after fertilization in trypanosoma and its allies. It divides as do the nuclei, and like a centrosome has a certain individuality in the cell.

In certain other types of protozoa the substance of the division centre may be permanently outside of the nucleus. This is the case in the rhizopod parameba and in the flagellate noctiluca, while in the latter there is good evidence to show that the material is diffused throughout the cell body during vegetative phases. It is not too imaginative to think of a diffusion of this material throughout protozoan cells generally, as it may be diffused through the nucleus, and it is conceivable that the basal bodies of cilia, the substance of the contractile centres of flagella and myonemes are, like the basal bodies of flagella or the *Centralkorn* of the heliozoa, only local condensations of such kinoplasm, which, in the long run, must be traced back to the nucleus.

B. ORGANS OF LOCOMOTION OF PROTOZOA, AND CLASSIFICATION.

As Dujardin ('41) early pointed out, the motile organs of protozoa offer a natural basis for classification, which, with proper subdivisions, is quite adequate to satisfy all of the requirements of a natural system. Within the last year or so some confusion has arisen because of the different forms an organism may assume at different periods of its life history. *Herpetomonas (Leishmania) donovani*, the cause of kala azar, for example, has an intracellular non-motile phase in addition to a free-living, flagellated phase, and in such a form it is conceivable that some difficulty might arise as to whether the organism should be classified as a sporozoön or as a flagellate. Such exceptions, however, do not offer insuperable difficulties, and may, indeed, serve a useful purpose in pointing out the path of evolution which the organisms in question have undergone. They do not in any way destroy the value of the motile apparatus as a basis for classification.

Dujardin outlined three of the four great divisions of the protozoa, while the fourth, the Sporozoa, was named by Leuckart in 1879. The first group of protozoa was characterized by Dujardin as "animals provided with variable processes" (pseudopodia); the second as "animals provided with one or several flagelliform filaments" (flagella); and the third as "ciliated animals." Gregarinida, belonging to the fourth group, were the first protozoa to be regarded as single cells, Kölliker ('45) regarding them as such.

The finer subdivisions of these several groups are made chiefly according to the variations in the structure of the motile organs, the Sarcodina, for example, are here subdivided into two classes, the Rhizopoda and the Actinopoda, according as the pseudopodia are amorphous or ray-like. These classes in turn are divided into subclasses, the former into Reticulosa, Mycetozoa, Foraminifera, and Amebea, the latter into Heliozoa and Radiolaria.

Some subdivisions of the protozoa deserve especial mention because the organisms included, occupy an anomalous position in the scale of living things. One such group, the Mycetozoa, is sometimes placed as a group of rhizopods, sometimes as fungi. In their simplest forms these organisms are minute cells with lobose pseudopodia, which are soft and miscible and fuse upon coming together. Such fusions result in great accumulations of protoplasm known as plasmodia, which may assume a variety of shapes and may become so highly differentiated as to resemble higher metaphytes much more than single celled protozoa. Another such group, the Phytoflagellida, have long been the subject of academic wrangling as to the boundary line between animals and plants. Similarly, the Spirilloflagellata are today the

subjects of contention between bacteriologists and protozoölogists. Little satisfaction, however, comes from such wrangling, and there is little practical value in connection with these hypothetical boundary lines beyond setting the limits to text-book or monograph.

Pseudopodia, and Classification of the Sarcodina.—In many respects pseudopodia are the simplest forms of motile organs. They are merely prolongations or outflowings of the cell protoplasm, the external expressions of internal physical forces which biologists have tried in vain to analyze. In the inner protoplasm of nearly all kinds of protozoa, the almost fluid cell contents with granules of various kinds, food more or less digested, and with waste products, are in a constant movement or cyclosis. In the more highly differentiated forms of protozoa, this flow is quite confined to the inner protoplasm, the firm cell membrane preventing an outward manifestation of the forces which cause the flow. In the shell-less Sarcodina, however, there is no firm outer covering, and the peripheral protoplasm gives way at the points of least resistance and an outward flow of protoplasmic stuff is the result, this flow ceasing with the exhaustion of the particular force which caused it, while a new point of rupture gives rise to a new pseudopodium. Thus the motile organs of these low types are inconstant, endlessly changing centres of protoplasmic energy, which have defied the physicist, the chemist, and the biologist. Not all pseudopodia are of this simple type, however, and some of them have a permanent form with supporting skeletal elements. The former, transitory kind, are characteristic of the ordinary rhizopods such as ameba, arcella, diffugia, etc., which are familiar to the novice as "the lowest forms of animal life," and they appear and disappear again with an ever-fascinating, inexplicable regularity. These are the so-called lobose, "lobopodia," or finger-form pseudopodia.

The second, more permanent kind of pseudopodia, are sometimes called axiopodia, because of the presence of a stiff axial filament, composed of condensed protoplasm similar to acanthin or chitin, which runs through the axis of the pseudopodium. These pseudopodia, characteristic of the class Actinopoda, stand out, ray-like, from all sides of the usually spherical animal, and give a peculiar radiating appearance which led the early students of the group to call them the sun-animals, a name which Haeckel, with characteristic felicity, turned into Heliozoa. In these the protoplasmic flow leads to no change in configuration of the motile organ, but courses outward on one side of the pseudopodium and backward on another.

The central axis belonging, as shown above, to the category of kinoplasmic substances, has a certain amount of elasticity, and may bend and straighten again with considerable force, and thus the pseudopodium becomes a more or less vigorous organ of locomotion, an acanthocystis rolling over and over with a slow vibration of the elastic

filaments, while an artodiscus dances about the field with an energetic, but erratic movement due to the springiness of the tips of its axiopodia.

FIG. 13

Lichnaspis giltochii, Haeck. One of the Actipylea. (After Haeckel.) The spines are arranged in accordance with the Müllerian law as follows: *a, a, a, a*, northern polar spines; *b, b, b, b*, northern tropical spines; *c, c, c*, — equatorial spines; *d, d, d, d*, southern tropical spines; and *e, e, e*, southern polar spines.

In some forms both flagella and these pseudopodia exist at the same time, as in dimorpha or in myriophrys, while in the former the one may change into the other. These axiopodia, therefore, are of con-

siderable interest from a theoretical point of view, and indicate a possible line of evolution which the protozoa may have followed in the past (Fig. 14).

The Heliozoa possessing these axiopodia are not very numerous nor are there many species; they are never parasitic and are mainly confined to fresh water, only a few being found in the sea. Another group, however, closely allied to the Heliozoa, the Radiolaria, are exclusively marine. More than four thousand species of these marine forms are known, and they are provided for the most part with the same kind of pseudopodia as those of the Heliozoa, while the great majority of them possess supporting skeletons of acanthin or silica, often exquisitely designed (Fig. 13).

FIG. 14

Myriophrys paradoxa, Pénard. (From Lang after Pénard.) Heliozoön with axiopodia and flagelliform cilia.

Still another type of pseudopodia which may be considered intermediate between the lobose and the filose types is the reticulose type, so called from the side streams of protoplasm which start from the central streams and fuse or anastomose with other pseudopodia forming a network or reticulum of protoplasm. The calcareous shells of these forms are usually perforated, so that their pseudopodia have easy access to the surrounding medium. Such perforations gave rise to the term foramen- or window-bearing, and under the name Foraminifera these rhizopods have been known ever since D'Orbigny gave the name in 1826. In addition to the function of locomotion, the pseudopodia of these forms become a trap for diatoms, other protozoa or larval stages of higher forms, the sticky protoplasm making escape very difficult, while the struggles of the prey stimulate an additional flow of protoplasmic secretions by which digestion takes place.

PHYLUM PROTOZOA.¹

Subphylum **SARCODINA**. Protozoa showing no connections with the bacteria, usually of simple structure and characterized mainly by motile organs in the form of changeable protoplasmic processes—the pseudopodia.

Class I. **RHIZOPODA**. Sarcodina without axial filaments in the pseudopodia, which may be lobose, filose, or reticulose.

Subclass 1. **Proteomyxa**. Minute organisms with soft, miscible pseudopodia, which anastomose upon touching; the cells unite at times to form plasmodia; frequently parasitic.

Typical genera: *Gymnophrys*, Cienkowski, 1876; *Pontomyxa*, Topsent, 1893; *Vampyrella*, Cienkowski, 1876; *Pseudospora*, Cienkowski, 1876; *Plasmodiophora*, Woronin, 1878; *Nuclearia*, Cienkowski, 1876.

Subclass 2. **Mycetozoa**. Pseudopodia-forming single cells which fuse to form plasmodia, the latter often of great complexity. There are so many characteristics of the fungi in the organisms of this group that their systematic position is unsettled; botanists include them with the fungi as a primitive group under the name *Myxomycetes* or slime moulds.

Order 1. **ACRASIE**. The single cells unite to form a common mass, but the cells do not fuse, hence a pseudoplasmodium is formed which is enclosed in a gelatinous mantle.

Typical genera: *Copromyxa*, Zopf, 1885; *Acrasis*, Van Tieghem, 1880; *Dictyostelium*, Brefeldt, 1869.

Order 2. **FILOPLASMODIA**. The aggregated cells are not firmly united, but remain connected for the most part by delicate threads of protoplasm.

Typical genera: *Labyrinthula*, Cienkowski, 1876; *Chlamydomyxa*, Archer, 1875.

Order 3. **MYXOMYCETES**. The aggregation of the cells is here complete and often results in the formation of complex fructifications in which hygroscopic threads play an important part in scattering the often flagellated spores.

Typical genera: *Fuligo*, Haller, 1768; *Craterium*, Trentepol, 1797; *Stemonitis*, Gleditsch, 1753; *Didymium*, Schrader, 1797.

Subclass 3. **Foraminifera**. Rhizopoda with fine branching and anastomosing pseudopodia which form an irregular network around the entire body or parts of it. Shells, when present, are calcareous, provided with many pores (*Perforina*) or without pores (*Imperforina*), and consist of one chamber (*Monothalamous*) or of many chambers (*Polythalamous*). Rigid diagnoses are here impossible, for the limits of the orders are ill-defined, and in some cases it is difficult to accurately place organisms which are sometimes grouped as foraminifera, sometimes as test-bearing amebæ. The classification adopted here is that of Lister, 1903.

Order 1. **GROMIDA**. (Fresh-water test-bearing forms removed.) The cell covering is simple and for the most part without calcareous deposits; chitinous and single chambered.

Typical genera: *Gromia*, Dujardin, 1835; *Microgromia*, Hertwig, 1874; *Diplophrys*, Barker, Shephardella, Siddall, 1880; *Platoum*, F. E. Sch., 1877.

Order 2. **ASTRORHIZIDA**. Lister recognizes four families. Here the test is composite, large, and monothalamous; the walls are formed of chitin with firmly attached particles of sand, mud, sponge spicules, etc.

¹ The classification adopted for a group of animals or plants in which life histories are but little known and relationships obscure must be of a tentative nature, and the one here suggested, while indicating relationships as they appear with our present knowledge, is only a snap shot, as it were, of a growing subject and makes no claim of finality.

Typical genera: Astrorhiza, Sandahl, 1857; Syringammina, Brady, 1884; Pilulina, Carpenter, 1862; Saccammina, Sars, 1868; Rhabdammina, Sars, 1868; Haliphysema, Bowerbk., 1862; Marsipella, Norman, 1878.

Order 3. LITUOLIDA. Lister recognizes four families. Here the test is arenaceous, usually regular, mono- or polythalamous. Lister notes that it comprises sandy isomorphs of certain types of hyaline or porcellaneous forms.

Typical genera: Lituola, Lamarck, 1801; Rheophax, Montfort, 1808; Haplophragmium, Reuss, 1860; Hippocrepina, Parker, 1870; Polyphragma, Reuss, 1860; Cyclammina, Brady, 1884; Loftusia, Brady, 1884; Parkeria, Carpenter, 1862.

Order 4. MILIOLIDA. Lister recognizes six families. Here the test is typically calcareous and hyaline, but may be covered with sand or detritus.

Typical genera: Cornuspira, M. Sch., 1854; Spiroloculina, D'Orb., 1826; Triloculina D'Orb., 1826; Vertebralina, D'Orb., 1826; Articulina, D'Orb., 1826; Peneroplis, Montfort, 1810; Orbiculina, Lamarck, 1801; Orbitolites, Lamarck, 1801; Alveolina, D'Orb., 1826; Keramosphera, Brady, 1884; Nubecularia, DeFrance.

Order 5. TEXTULARIDA. Lister recognizes three families. Here the chambers are arranged in one or two series, which may be alternate, spiral, or irregular; arenaceous and with or without a perforated calcareous basis.

Typical genera: Textularia, DeFrance, 1824; Valvulina, D'Orb., 1826; Virgulina, D'Orb., 1826.

Order 6. CHILOSTOMELLIDA. Lister has three genera. The test is calcareous, polythalamous and finely perforated.

Typical genera: Chilostomella, Reuss, 1860; Allomorphina, Reuss, 1860.

Order 7. LAGENIDA. Lister recognizes four families. Here the test is similar to the last save for the monothalamous shell, which, however, may be compound by the union of chambers end to end in a straight or curved series. Canals and canalicular skeleton wanting.

Typical genera: Lagena, Walker, and Boys, 1784; Nodosaria, Lam., 1801; Polymorphina, D'Orb., 1826; Ramulina, R. Jones, 1875.

Order 8. GLOBIGERINIDA. Not divided into families. The test is perforated and calcareous, with few chambers arranged in a spiral. Canals and canal system absent.

Typical genera: Globigerina, D'Orb., 1826; Orbulina, D'Orb., 1826.

Order 9. ROTALIDA. Lister recognizes three families. The test is calcareous and perforated, with all of the chambers visible from one aspect, and arranged in a spiral; some of the more highly developed forms with canal system.

Typical genera: Spirillina, Ehr., 1841; Discorbina, Parker and Jones, 1862; Calcarina, D'Orb., 1826; Rotalia, Lamarck, 1801; Tinoporus, Carpenter, 1857; Carpenteria, Gray, 1858.

Order 10. NUMMULITIDA. Lister recognizes three families. Here the test is calcareous, filled with tubules, and bilaterally symmetrical (except Amphistegina), and with canal system in the higher forms.

Typical genera: Fusulina, Fischer, 1829; Polystomella, Lamarck, 1822; Operculina, D'Orb., 1826; Nummulites, Lamarck, 1801; Orbitoides, D'Orb., 1826 (Fig. 9, p. 26).

Subclass 4. Amebea. Here are included the more common forms of rhizopods with blunt or lobose pseudopodia which do not anastomose on touching one another, a physiological character which indicates a well-marked difference in the different types of rhizopods. The protoplasmic body may bear shells or not.

Order 1. GYMNAMEBIDA. Here the body is uncovered, although there is, in many cases, a tendency of the peripheral plasm to harden into a denser, membrane-like zone which approaches the simpler forms of tests.

Typical genera: Ameba auct. Parameba, Schaudinn, 1896; Trichosphaerium, Schneider; Hyalodiscus, Hert. and Lesser, 1874; Chromatella, Frenzel, 1892; Pelomyxa, Greeff, 1874; Dactylosphaera, Hert. and Lesser, 1874; Nucleophaga, Dangeard, 1895.¹

Order 2. TESTACEA. The ameboid organisms here are covered by definite membranes or tests composed of different materials cemented to a chitinous base. The pseudopodia are protruded through the single opening of the shell and may be simply lobose or branched, but do not anastomose.

Typical genera: Arcella, Ehr., 1838; Cochliopodium, Hert. and Lesser, 1874; Hyalosphaeria, Stein, 1857; Quadrola, F. E. Sch., 1875; Diffugia, Leclerc, 1815; Euglypha, Dujardin, 1841; Trinema, Dujardin, 1836; Campascus, Leidy, 1877.

Class 2. **ACTINOPODA**. Sarcodina provided with fine, ray-like pseudopodia which are supported by a central axial filament corresponding to the kinetic material of flagella.

Subclass 1. **Heliozoa**. Typically fresh-water forms of actinate protozoa in which there is no trace of a chitinous central capsule separating ectoplasm and endoplasm.

Order 1. **APHROTHORACA**. Naked forms of heliozoa (except during encystment).

Typical genera: Actinophrys, Ehr., 1830; Myxastrum, Haeckel, 1870; Actinosphaerium, Stein, 1857; Actinolphus, F. E. Sch., 1874.

Order 2. **CHLAMYDOPHORA**. Heliozoa with a soft gelatinous or felted fibrous covering.

Typical genera: Heterophrys, Archer, 1865; Sphaerastrium, Greeff, 1873.

Order 3. **CHALARATHORACA**. Heliozoa with a silicious covering made up of separate or loosely connected spicules.

Typical genera: Pompholyxophrys, Archer, 1869; Raphidiophrys, Archer, 1870; Pinacocystis, Hert. and Lesser, 1874; Acanthocystis, Carter, 1863; Diplocystis, Pénard, 1890.

Order 4. **DESMOTHORACA**. Heliozoa with a covering of one piece perforated by numerous openings.

Typical genus: Clathrulina, Cienk., 1867.

Subclass 2. **Radiolaria**. Actinopoda in which the inner protoplasm is separated from the outer by a firm chitinous "central capsule" perforated in different ways for the intercommunication of inner and outer parts. Exclusively salt-water forms, living at the surface, suspended at various depths, or near the bottom. Classification based upon Haeckel's magnificent monograph in the Challenger reports.

Division A. **Porulosa**. Spherical (homaxonic) organisms with spherical central capsule perforated by numerous scattered pores of minute size.

Legion 1. **Peripylea** (Spumellaria). The central capsule is perforated by evenly scattered pores; a skeleton is usually present consisting of scattered silicious spicules, fused spicules, or a latticed network.

Order 1. **COLLIDA** (following Brandt, 1902). Solitary forms with or without skeletogenous spicules.

Typical genera: Thalassicolla, Huxley, 1851; Actissa, Haeckel, 1887.

Order 2. **SPHEROZOE** (Brandt). Colony building forms with or without skeletogenous spicules.

Typical genera: Collozoum, Haeckel, 1862; Collosphaera, J. Müll, 1855.

Order 3. **SPHEROIDA**. Skeleton present as one or several concentric spherical latticed or reticulate structures.

¹In this group I would place, provisionally, the organisms of smallpox (Cytoryctes variolæ), of rabies (Neuroryctes hydrophobiæ), and the allied organisms which Prowazek (1908) includes in his group Chlamydozoa.

Typical genera: Haliomma, Ehr., 1838; Actinomma, Haeckel, 1862.

Order 4. PRUNOIDA. Haeckel recognizes seven families. With spheroidal, ellipsoidal to cylindrical skeleton, single or concentric, sometimes constricted.

Typical genera: Ellipsoidium, Haeck., 1887; Druppula, Haeck., 1887.

Order 5. DISCOIDA. Haeckel recognizes six families. The skeleton and central capsule are discoidal to lenticular.

Typical genera: Cenodiscus, Haeck., 1887; Heliodiscus, Haeck., 1887.

Order 6. LARCOIDA. Haeckel recognizes nine families. The skeleton is ellipsoidal with asymmetrical axes, in some cases forming almost a spiral.

Typical genera: Larcarium, Haeck., 1887; Pylonium, Haeck., 1881.

Order 7. SPHEROPYLIDA (Dreyer). Peripylea having in addition to the distributed pores one basal or a basal and an apical opening to the central capsule.

Typical genus: Spheropyle, Dreyer, 1888.

Legion 2. Actipylea (Acantharia). Porulose forms in which the pores are aggregated in definite areas; the skeleton usually consists of twenty spines of acanthin radiating from the centre of the organism in a regular order (Müllerian law). Branches from these spines may unite to form a latticed shell.

Order 8. ACTINELLIDA. Haeckel recognizes three families. The radial spines are more numerous than twenty.

Typical genus: Xiphacantha, Haeckel, 1862.

Order 9. ACANTHONIDA. Haeckel recognizes three families. The twenty spines are arranged in regular order (four equatorial, eight tropical, and eight polar), all are equal in size.

Typical genus: Acanthometron, Müller, 1855.

Order 10. SPHEROPHRACTA. Haeckel recognizes three families. With twenty equal, quadrangular spines and a complete fenestrated shell.

Typical genus: Dorataspis, Haeckel, 1860.

Order 11. PRUNOPHRACTA. Haeckel recognizes three families. The twenty radial spines are unequal, and an ellipsoidal, lenticular, or doubly conical shell is present.

Typical genus: Thoracaspis, Haeck., 1860.

Division B. Osculosa. Radiolaria with monaxonic form and with the pores of the central capsule limited to an area on the base, or to one such primary basal area and two secondary, apical areas; these perforated areas of the central capsule are termed oscula.

Legion 3. Monopylea (Nassellaria). The central capsule is subspherical to ovoid, consists of a single layer of chitin, and is perforated only at one pole. The skeleton is silicious.

Order 12. NASSOIDA. Haeckel recognizes only one family. Skeleton absent.

Typical genus: Nassella, Haeck., 1887.

Order 13. PLECTOIDA. Haeckel recognizes two families. A complete latticed shell is never formed, the skeleton consisting of three or more spines radiating from one point below the central capsule or from a central rod.

Typical genus: Triplecta, Haeck., 1881.

Order 14. STEPHOIDA. Haeckel recognizes four families. The skeleton consists of fused spines forming one or more rings.

Typical genus: Lithocircus, Müller, 1856.

Order 15. SPYROIDA. Haeckel recognizes four families. The skeleton consists of a sagittal ring and a latticed shell furrowed in the sagittal plane; in some cases a lower chamber is added to the shell.

Typical genus: Dictyospiris, Ehr., 1847.

Order 16. BOTRYOIDA. Haeckel recognizes three families. Skeleton similar to the preceding, but having in addition one more wing-like process or lobe and one or more additional chambers.

Typical genus: Lithobotrys, Ehr., 1844.

Order 17. CYRTOIDA. Haeckel recognizes twelve families. Skeleton similar to the preceding, but minus lobes or furrows.

Typical genus: Theconus, Haeckel, 1887.

Legion 4. **Cannopylea** (Pheodaria). The chitinous central capsule is double, with a spout-like main opening at one pole and frequently with one or more accessory openings at the opposite pole. The skeleton is silicious and the spicules or bars are often hollow. The extracapsular protoplasm contains an accumulation of dark pigment granules (pheodium).

Order 18. PHEOCYSTINA. Haeckel recognizes three families. The skeleton consists of distinct spicules or is absent altogether; the central capsule is in the centre of the spherical body.

Typical genus: Aulactinium, Haeckel, 1887.

Order 19. PHEOSPHERIA. Haeckel recognizes four families. The skeleton is a simple or double latticed sphere, and the central capsule is in the geometrical centre.

Typical genus: Oroslena, Haeck., 1887.

Order 20. PHEOGROMIA. Haeckel recognizes five families. The skeleton is a simple latticed shell with a large opening at one pole; the central capsule is excentric, lying in the aboral half of the cell.

Typical genera: Pharyngella, Haeckel, 1887; Tuscarora, Murray, 1876; Haeckeliana, Murray, 1879.

Order 21. PHEOCONCHIA. Haeckel recognizes three families. The skeleton consists of two valves opening in the same plane as the three openings of the central capsule.

Typical genus: Concharium, Haeck., 1879.

Flagella and Classification of the Mastigophora.—Flagella do not present as many striking variations in form as do pseudopodia. Nevertheless, several different types exist. The simplest form assumed is a slight, tapering filament broadest at the base and ending in an invisibly fine tip. It moves constantly, the tip forming a circle, while undulations or waves pass from base to extremity. In other types of flagella the tip alone moves, while the base is a conspicuous filament without undulation, the whole flagellum resembling a whip stock with lash. It is a remarkable sight to see a peranema, for example, with its stiff whip base, dragged along by the propelling movement of the tip end of the slender lash.

In some forms of mastigophora the flagellum appears to be flattened out until it is quite band form. This is the case in some species of peridinium, where the band is drawn out to a pointed end, or in other cases it retains the same width throughout.

In many of the flagellates there is but one flagellum attached at one end of the cell as in peranema or euglena. In other cases there are two, and these may be of similar or dissimilar length. In bodo and in most of the colony forming flagellates like dinobryon, synura, uroglena, etc., one is much shorter than the other. In many forms of bodo the longer flagellum trails along on the substratum so that the cell has the appearance of sliding along on a runner (Fig. 15). In some forms, especially the parasitic flagellates, this sliding flagellum has apparently fused with the cell membrane, projecting outward from

one end as a trailing flagellum and forming a definite seam down one side of the cell body (trypanoplasma). This seam in *Trypanophis grobbeni* becomes an undulating membrane, while in trypanosoma

FIG. 15

Free-living flagellates with trailing flagellum. (After Calkins.) A, C, D, *Bodo caudatus*, Stein; B, *Bodo globosus*, Stein; E, *Anisonema vitrea*, Duj.

the anterior flagellum has disappeared apparently, leaving only the undulating membrane and the distal flagellum as motile organs. Finally, in spirocheta, especially in *Spirocheta balbianii*, both free flagella have disappeared, leaving only the undulating membrane, while in some species of spirocheta even this remnant of the motile apparatus has disappeared, leaving the organism with no visible means of locomotion. As such forms of spirocheta move with great freedom, it is not incredible that the remnant of the contractile element is still retained within the membrane of the cell.

In a number of forms the flagella are numerous and distributed uniformly around the body. Many of these types are of doubtful systematic position and are placed by some students of the group in the class ciliata while others regard them as flagellates. The nature of the flagellum in such cases justifies the mastigophora affinities, for they are long and undulating and have the characteristic flagellum movement. Such is the case in multicilia, actinobolus, myriophrys, etc., and in parasitic forms like trichonympha, pyrsonympha, etc. Other features of the cell body, however, such as the nuclei, trichocysts, etc., indicate relationship with the infusoria, and to classify such questionable forms as one or the other type shows the artificial character of even the best system of classification. The difficulty is one that is constantly met with by systematists, and in this case it serves a useful purpose by indicating the very close connection between the ciliated and the flagellated protozoa.

The single flagellum is usually inserted deep within the substance of the body, sometimes, as in euglena, at the base of an opening at the end of the body; this opening, known as the flagellum fissure, is the means of exit of the waste matters of the cell, thrown out by the contraction of the contractile vacuole. The flagellum originates deep within the substances of the protoplasm and usually in the vicinity of the nuclear membrane. The energy constantly freed by protoplasmic oxidation is here concentrated, apparently, in the constantly moving material of the flagella. The contractile material, formed within the nucleus or at its periphery, as in the case of *Camptonema mutans*, is of similar nature to the material of the heliozoön axial filaments as shown in the case of dimorpha, and is associated in some way with the material of the mitotic figure or division centre, as shown by its origin from the blepharoplast in herpetomonas, crithidia, trypanosoma, and trypanoplasma. The flagellum, therefore, is an element of the cell formed from the active or kinetic substances that are intimately associated with the nucleus. It is not merely a periplastic or membrane prolongation which may arise at any point on the cell periphery, but is much more deeply involved in the protoplasmic make-up. The real flagellum is permanent, thrown off and reproduced again, only at times of cell division. This point has importance in view of the ques-

tionable nature of the so-called flagella of certain parasites belonging to the genus *spirocheta*, many of which are said to have flagella. These so-called flagella are apparently variable structures, for in

FIG. 16

Trypanosoma raia. (After Robertson.) Forms observed in the digestive tract of the leech *Pontobdella muricata*. A, mature specimen from blood of skate; B to F, stages in the development of the flagellum from the kinetoplast, and change in position of the latter in relation to the nucleus.

many cases as "diffuse flagella" they appear not only at the ends of the cell, but at different points about the periphery, and there seems to be no uniformity about their distribution. This is said to be the case in

Spirocheta duttoni and in *Spirocheta gallinarum*. It is not improbable that such diffuse and variable filaments, and with them perhaps the so-called flagella of some bacteria, are mere transitory structures of the cell, which, like the filaments sometimes seen on the outer side of a diatom's shell, owe their origin not to any formed structural element of the cell, but to some unformed exudation of a gelatinous nature, or to disintegration of the cell membrane, or to some other fortuitous cause. Whatever future research may show them to be, the so-called flagella of these forms are as yet much too indefinite and too uncertain to be taken as a basis for specific differences (see p. 223, and Fig. 88).

The various modes of origin of true flagella, as distinguished from these transitory filaments, have recently been studied by Dobell ('08), who makes out four distinct types, as follows: One, in which the flagellum arises directly from the nucleus (cf. axiopodia of actinophrys or dimorpha); a second, in which the flagellum base is united to the nucleus by a connecting filament, the "zygoplast," as in monas; a third, in which the flagellum arises from a basal granule which is independent of the nucleus, as in copromonas, herpetomonas, etc.; and a fourth, in which the flagellum arises from a special "motor" nucleus, the "kinetonucleus," as in trypanosoma (Fig. 16).

CLASSIFICATION OF THE MASTIGOPHORA.

Subphylum. **MASTIGOPHORA.** Protozoa in which the kinoplasm is concentrated in the form of one or more vibratile or undulating motile processes, called flagella, or in a kinetonucleus which may lie inside or outside of the trophonucleus. Simplest forms closely related to bacteria.

Class 1. **ZOÖMASTIGOPHORA.** Flagellated forms in which animal characteristics are predominant.

Subclass. **Lissoflagellata.** "Smooth" flagellates, *i. e.*, without protoplasmic collars.

Order 1. **SPIROCHETIDA.** Organisms, often pathogenic, of somewhat uncertain position because of incomplete knowledge of flagella and life history; spiral in form, the turns of the spiral more or less plastic; nuclei unknown or distributed as in bacteria; division either transverse or longitudinal, sometimes both.

Typical genera: *Spirocheta* Ehr., 1833; (?) *Treponema*, Schaudinn, 1905; (?) *Spiroschaudinina*, Sambon, 1907.

Order 2. **MONADIDA.** Organisms of simple structure, the body being often plastic or even ameboid and with one or more flagella at one end (so-called "anterior" end); there is no distinct mouth opening, the food materials being ingested by a soft area of protoplasm at the base of the flagellum; in some cases the organisms are saprozoites.

Family *Rhizomastigidae*: Simple organisms with one or two flagella and with an ameboid body capable of forming pseudopodia which may be lobose, as in rhizopods, or axial, as in heliozoa; food taking is assisted by flagellum and pseudopodia.

Typical genera: *Mastigameba*, Schultze, 1875; *Dimorpha*, Gruber, 1881; *Actinomonas*, Kent, 1880; *Mastigophrys*, Frenzel, 1891.

Family *Cercomonadidæ*: The organisms are frequently plastic and changeable in form, but unable to form pseudopodia; there is but one flagellum with a flagellum-fissure at the base; nutrition is holozoic, saprozoic, or parasitic.

Typical genera: *Cercomonas*, Dujardin, 1841 (a very uncertain genus); *Herpetomonas*, Kent, 1880, ("including Donovan-Leishman bodies"); *Crithidia*, Léger, 1904; *Oikomonas*, Kent, 1880; *Copromonas*, Dobell, 1908.

Family *Codonecidæ*: Small colorless monads which secrete and remain in gelatinous or membranous cups.

Typical genera: *Codoneca*, James-Clark, 1866; *Platytheca*, Stein, 1878.

Family *Bikecidæ*: Minute organisms of peculiar shape, the basal broader portion bearing a tentacle-like process; nutrition is holozoic; the individuals single or colony forming.

Typical genera: *Bicoseca*, James-Clark, 1867; *Poteriodendron*, Stein, 1878.

Family *Heteromonadidæ*: Small colorless monads possessing one or more accessory flagella in addition to the primary one; they frequently form large but delicate colonies upon a common stalk.

Typical genera: *Monas*, Stein, 1878; *Dendromonas*, Stein, 1878; *Anthophysa*, St. Vincent, 1824; *Rhipidodendron*, Stein, 1878.

Order 3. *HETEROMASTIGIDA*. A small group comprising various kinds of flagellated forms which are sometimes naked and plastic, sometimes provided with a highly differentiated membrane. The essential morphological characteristic is the possession of two or more flagella, one or two of which are directed downward and backward, while the other is directed forward and used in locomotion.

Typical genera: *Bodo*, Stein, 1878; *Phyllomitus*, Stein, 1878; *Oxyrrhus*, Dujardin, 1841; *Anisonema*, Dujardin, 1841; *Trimastix*, Kent, 1881.

Order 4. *TRYPANOSOMATIDA*. Organisms of elongate, usually pointed form, and of parasitic mode of life; with one or two flagella arising from a special "motor" nucleus, and with an undulating membrane provided with myonemes running from the kinetonucleus to the extremity of the cell; one of the flagella is attached to the edge of this membrane throughout its length, and may terminate with the membrane or be continued beyond the body as a free lash.¹

Typical genera: *Trypanosoma*, Gruby, 1841; *Trypanoplasma*, Lav. and Mesnil, 1904; *Trypanophis*, Keysseltz, 1904.

Order 5. *POLYMASTIGIDA*. Organisms characterized by numerous flagella, frequently arranged in groups, and with one or many mouth openings usually at the bases of the flagella.

Tribe 1. *Astomea*. Organisms with many flagella uniformly distributed, and without special mouth openings.

Typical genera: *Multicilia*, Cienk., 1881; *Grassia*, Fisch., 1885.

Tribe 2. *Monostomea*. Organisms with mouth opening at the base of the group of from four to six flagella.

Typical genera: *Collodictyon*, Carter, 1865; *Trichomonas*, Donné, 1837; *Megastoma*, Grassi, 1881; *Tetramitus*, Perty, 1852.

Tribe 3. *Distomea*. Organisms with two mouth openings at the bases of the two groups of flagella.

Typical genera: *Hexamitus*, Dujardin, 1838; *Trepomonas*, Dujardin, 1839; *Spirosonema*, Klebs, 1893; *Urophagus*, Klebs, 1893.

¹ The conclusions of Novy, MacNeal, and Torrey (1907) that *herpetomonas*, *crithidia*, and *trypanosoma* are synonyms cannot be accepted on the basis of cultural methods alone; when the life history of these parasitic forms is known in detail will be time enough to speak of synonyms, and as the important structural characteristic which the membrane represents far outweighs the cultural characteristics, it is better to hold to the older view and thus to prevent further complications in what is already almost a hopelessly complicated group.

Tribe 4. *Trichonymphinea*. Parasitic forms of the digestive tract covered with a coating of long flagella.

Typical genera: *Trichonympha*, Leidy, 1877; *Pyrsonympha*, Leidy, 1877; *Jenia*, Grassi, 1885.

Order 6. **EUGLENIDA**. Large forms of flagellates possessing one or two flagella, a contractile often complicated body wall, a mouth and pharyngeal opening at the base of the flagellum through which the contractile vacuole opens to the outside; chromatophores are often present and colony forms are not uncommon.

Family *Euglenidæ*: The organisms are elongate with more or less pointed ends and usually with one flagellum. The membrane is marked with spiral stripings indicating the course of the myonemes. Red eye spots, and green chromatophores are usually present. Pyrenoids and paramylum granules usually present in abundance.

Typical genera: *Euglena*, Ehr., 1830; *Trachelomonas*, Ehr., 1833; *Phacus*, Nitsch, 1816.

Family *Astasiidæ*: The body is elongate and usually provided with a striped membrane and otherwise similar to *Euglena*, but there are no eye spots and the body is always colorless.

Typical genera: *Astasia*, Ehr., 1838; *Rhabdomonas*, Fres., 1858.

Family *Peranemidæ*: The body is either stiff or plastic, and is usually symmetrical.

Typical genera: *Peranema*, Dujardin, 1841; *Petalomonas*, Stein, 1859.

Order 7. **SILICOFLAGELLIDA**. Organisms with a peculiar lattice-like skeleton of silica, one flagellum, and simple structure. Parasitic on radiolaria.

Typical genus: *Distephanus*, Stöhr, 1881.

Subclass 2. **Choanoflagellata**. Simple flagellated protozoa with a well-defined and characteristic protoplasmic collar surrounding the base of the flagellum. They frequently form colonies in which the cells are embedded in a gelatinous or a chitinous matrix.

Typical genera: *Monosiga*, Kent, 1880; *Codosiga*, James-Clark, 1867; *Proterospongia*, Kent, 1880; *Diplosiga* (with two collars), Frenzel, 1891; *Phalansterium*, Cienk., 1870.

Class II. **PHYTOMASTIGOPHORA**. Flagellated forms in which the plant characteristics, if not predominant, are clearly marked. Here are classified the majority of complex colony forming types, but the single cells are invariably of simple structure, possessing eye spots, pyrenoids, and yellow, green, or brown chromatophores.

Subclass 1. **Phytoflagellata**. In this group the organisms have yellow or green chromatophores.

Order 1. **CHRYSOFLAGELLIDA**. With yellow chromatophores.

Typical genera: *Chromulina*, Cienk., 1870; *Dinobryon*, Ehr., 1838; *Hyalobryon*, Lauterborn, 1899; *Mallomonas*, Perty, 1876; *Synura*, Ehr., 1833; *Uroglena*, Ehr., 1833; *Chrysosphaerella*, Lauterb., 1899; *Cryptomonas*, Ehr., 1831; *Chilomonas*, Ehr., 1831 (without chromatophores).

Order 2. **CHLOROFLAGELLIDA**. With green chromatophores.

Typical genera: *Chlorogonium*, Ehr., 1835; *Polytoma*, Ehr., 1838; *Hemato-coccus*, Agardh., 1828; *Phacotus*, Perty, 1852; *Gonium*, O. F. Müller, 1773; *Pandorina*, St. Vincent, 1824; *Eudorina*, Ehr., 1831; *Pleodorina*, Shaw, 1894; *Platydorina*, Kofoid, 1899.

Subclass 2. **Dinoflagellata**. Organisms with yellow or brown pigment, two or more flagella, and an outer shell of cellulose secreted in the form of plates. The body is usually cut by furrows, of which the transverse is the more important; one flagellum lies in this furrow, while the other is extended in advance of the organism. The two flagella combine to give a rotation and forward movement at the same time.

Order 1. ADINIDA. Dinoflagellates without furrows, the two flagella free in the water, the transverse with movement the same as though the furrow were present.

Typical genera: *Prorocentrum*, Ehr., 1833; *Exuviella*, Cienk., 1882.

Order 2. DINIFERIDA. Dinoflagellates with furrows, one transverse, the other longitudinal.

Family 1. *Peridinidae*. The transverse furrow is without wide ledges and the shell may be absent.

Typical genera: *Peridinium*, Ehr., 1832; *Ceratium*, Schrank, 1793; *Glenodinium*, Ehr., 1835; *Gymnodinium*, Stein, 1878.

Family 2. *Dinophysidae*. The borders of the cross furrow are developed into great ledges, making a deep furrow for the flagellum.

Typical genera: *Dinophysis*, Ehr., 1839; *Cithiristes*, Stein, 1883; *Amphidinium*, Clap. and Lach., 1859; *Ceratocorys*, Stein, 1883; *Triposolenia*, Kofoid, 1906.

Order 3. POLYDINIDA. The order consists of but one genus, *Polykrikos*, Bütschli, 1873, which is characterized by a naked body, by several transverse furrows and flagella, by macro- and micronuclei, and nematocysts.

Subclass 3. *Cystoflagellata*. Marine protozoa, which are plant-like in having a highly parenchymatous body, a single nucleus and a firm membrane. The young forms pass through a dinoflagellate stage in development.

Three genera: *Noctiluca*, Suriray, 1836; *Leptodiscus*, Hertwig, 1877; *Craspedotella*, Kofoid, 1905.

Cilia, and Classification of the Infusoria.—Cilia are quite different from flagella, being shorter and moving with a sharp stroke in one direction and with a slower, non-forceful recovery in the opposite direction. Like the flagellum, the cilium is thicker at the base and tapers to a fine point, while it owes its contractility to the presence of a

FIG. 17

Aspidisca hexeris, Quen. An hypotrichous ciliate with brushes of fused cilia.
(After Calkins.)

filament of kinetic granules placed along one edge of the cilium, the contraction of this thread furnishing the power of the cilium, while the synchronous contraction of thousands of similar cilia furnishes the motive power of the organism.

In some forms, as in *dileptus* or *paramecium*, and the majority of

the largest forms of protozoa, the cilia are distributed evenly over the entire cell body. But in some cases they are limited to one-half of the body, as in halteria; in others to the ventral surface only, as in gastrostyla, oxytricha, and the hypotrichida in general, while in others they are reduced to a single girdle of cilia about the mouth, as in vorticella and its allies.

An interesting feature in the comparative anatomy of infusoria is the fusion of simple cilia into motile organs of a more complicated type. Sometimes a bundle of cilia are grouped together in a small brush-like organ, as in aspidisca, where the constituent elements of the bundle can still be made out (Fig. 17). In other forms, as oxytricha, the bundles are more tightly fused to form compact motile organs, which are sometimes used for walking and running, or sometimes they are differentiated for feeling, and so constitute an elemen-

FIG. 18

Pleuronema chrysalis, Ehr., with well-developed undulating membrane. (After Calkins.)

tary sensory apparatus. Again, the cilia are fused into continuous sheets, or membranes, which provide currents for bringing food toward the mouth, as in pleuronema or lembus (Fig. 18).

Rows of small membranes, called membranelles, are found in three of the four orders of ciliata. These are always placed around the oral or peristomial cavity, and their synchronous beating brings a constant food-bearing current toward the mouth. In some cases, as the vorticella group, the cilia have quite disappeared, leaving, under ordinary vegetative conditions, only this row of membranelles.

In one subdivision of the infusoria, the suctoria, the cilia disappear after a short embryonic life of the individual, and their place is taken by protoplasmic prolongations called tentacles. Some of these tentacles are hollow and provided with a suction cap, so that food may be drawn through them into the inner protoplasm. Others are sharp

pointed and are used by the animal as piercing needles for penetrating the membranes of the victims that are caught for food.

The more than superficial resemblance of these suctoria to the heliozoa gives a clue to the possible evolution of the infusoria from sarcodina. We have seen that in forms like myriophrys, cilia and pseudopodia are equally distributed around the body. We have also seen that the central axis of such pseudopodia and flagella are of the same type, and are probably homologous structures; furthermore, we have seen that in actinobolus, projectile tentacles armed with trichocysts can be thrown out at any point on the periphery. These facts indicate the possibility of a common ancestry of the infusoria

FIG. 19

Cilia and myonemes of infusoria: *a*, *b* and *e* after Johnson; *c*, *d*, *f* and *g* after Bütschli. The surface view of *Stentor ceruleus* (*c*, *e*) shows rows of cilia inserted on the borders of canal-like markings, each of which contains a myoneme (*d*). These are more clearly shown in the optical section (*f*). In *Holophrya discolor* (*g*) the canals and myonemes are inserted deeper in the cortical plasm. *a*, the membrane of *Stentor ceruleus* under pressure.

from a heliozoön-like ancestral race, represented in present-day forms by types like myriophrys, hypocoma, ileonema, and mesodinium, which have both tentacles and cilia. From such an ancestral group the ciliata may have arisen by losing the tentacles and adapting the cilia to the various needs of the cell, while the suctoria may have arisen by loss of the cilia and development of the tentacles to meet all of the needs of the cell, the cilia appearing in the embryos of the suctoria as reminiscences of the earlier ciliated condition of the race.

These motile organs of the protozoa, with the exception of the flagella, are products of the cortical protoplasm, the flagella retaining

the same origin from the nucleus that the axial rays of the filose pseudopodia have. Cilia, however, arise from small basal bodies called microsomes, which have a nuclear origin and belong apparently in the same category of kinetic stuffs as the substance of flagella. In many of the infusoria these granules are arranged in definite lines or rows, forming threads of contractile substance which lie immediately below the cuticle. These threads, called myonemes, are in reality primitive muscle elements, and their sudden contraction resembles the action of the complicated muscle bundles of the metazoa (Fig. 19).

Subphylum **INFUSORIA**. Protozoa in which the motor apparatus is in the form of cilia, either simple or united into membranes, membranelles, or cirri. The cilia may be permanent or limited to the young stages. With two kinds of nuclei, macronucleus and micronucleus. Reproduction is effected by simple transverse division or by budding. Nutrition is holozoic or parasitic.

Class I. **CILIATA**. Infusoria provided with cilia during all stages. Reproduction is brought about typically by simple transverse division. Mouth and anus are usually present. The contractile vacuole is often connected with a complicated canal system.

Order 1. **Holotrichida**. Ciliata in which the cilia are similar and distributed all over the body, with, however, a tendency to lengthen in the vicinity of the mouth. Trichocysts are always present, either distributed about the body or limited to a special region.

Suborder 1. **GYMNOSTOMINA**. Holotrichida without an undulating membrane about the mouth, which remains closed except during food-taking intervals.

Family 1. *Encheliniidae*. The mouth is always terminal or subterminal, and is usually round or oval in outline. Food taking is usually a process of swallowing.

Typical genera: *Holophrya*, Ehr., 1831; *Urotricha*, Clap; and Lach., 1858; *Enchelys*, Hill, 1752, Ehr., 1838; *Spathidium*, Duj., 1841; *Chenia*, Quennerstadt, 1868; *Prorodon*, Ehr., 1833; *Dinophrya*, Bütschli, 1888; *Lacrymaria*, Ehr., 1830; *Trachelocerca*, Ehr., 1833; *Actinobolus*, Stein, 1867; *Ileonema*, Stokes, 1884; *Plagiopogon*, Stein, 1859; *Coleps*, Nitsch, 1827; *Tiarina*, Bergh, 1879; *Stephanopogon*, Entz, 1884; *Didinium*, Stein, 1859; *Mesodinium*, Stein, 1862; *Bütschlia*, Schuberg, 1886.

Family 2. *Tracheliniidae*. The body is distinctly bilateral or asymmetrical, with one side, the dorsal, slightly arched. The mouth may be terminal or subterminal, or the entire mouth region may be drawn out into a long proboscis. An esophagus or gullet may or may not be present; when present, it is usually supported by a specialized framework.

Typical genera: *Amphileptus*, Ehr., 1830; *Lionotus*, Wrzesniowski, 1870; *Loxophyllum*, Duj., 1841; *Trachelius*, Schrank, 1903; *Dileptus*, Duj., 1841; *Loxodes*, Ehr., 1830.

Family 3. *Chlamydodontidae*. The general form is oval or kidney-shaped. The mouth is almost always in the posterior region. The pharynx is supported by a rod-apparatus or a smooth, firm tube.

Subfamily 1. *Nassulinæ*. Ciliation is complete.

Typical genera: *Nassula*, Ehr., 1833.

Subfamily 2. *Chilodontinæ*. The body is generally flattened, and the cilia are stronger on the dorsal side, or are confined to that region.

Typical genera: *Orthodon*, Gruber, 1884; *Chilodon*, Ehr., 1833; *Chlamydodon*, Ehr., 1835; *Opisthodon*, Stein, 1859; *Phascolodon*, Stein, 1857; *Scaphidio-*
don, Stein, 1857.

Subfamily 3. *Erviliinae*. The cilia are confined to the ventral surface or to a portion of it. The posterior end invariably possesses a movable style arising from the posterior ventral surface.

Typical genera: *Egyria*, Clap and Lach., 1858; *Onychodactylus*, Entz., 1884; *Trochilia*, Duj., 1841; *Dysteria*, Huxley, 1857.

Suborder 2. TRICHOSTOMINA. In addition to the general coating of cilia there is an undulating membrane or membranes at the edge of the mouth or in the pharynx. The mouth is always open.

Family 1. *Chiliferidae*. The mouth is in the anterior half of the body or close to the middle. The pharynx when present is short. The so-called "peristome area" leading to the mouth is absent or only slightly developed.

Typical genera: *Leucophrys*, Ehr., 1830; *Glaucoma*, Ehr., 1830; *Dallasia*, Stokes, 1886; *Frontonia*, Ehr., 1838; *Ophryoglena*, Ehr., 1831; *Colpidium*, Stein, 1860; *Chasmatostoma*, Engelmann, 1862; *Uronema*, Duj., 1841; *Urozona*, Schewiakoff (Bütschli), 1888; *Loxocephalus*, Kent, 1881; *Colpoda*, Müller, 1773.

Family 2. *Urocentridae*. The mouth, with a long, tubular pharynx, is in the centre of the ventral side. The cilia are confined to two broad zones around the body at each end.

Typical genera: *Urocentrum*, Nitsch, 1827.

Family 3. *Microthoracidae*. Small asymmetrical forms, with the mouth invariably in the hinder portion. The cilia are always more or less dispersed, sometimes limited to the oral region. There may be one or two undulating membranes.

Typical genera: *Cinetochilum*, Perty, 1849; *Microthorax*, Engelmann, 1862; *Ptychostomum*, Stein, 1860; *Ancistrum*, Maupas, 1883; *Drepanomonas*, Fresenius, 1858.

Family 4. *Paramecidae*. The mouth is sometimes in the anterior, sometimes in the posterior, half of the body, and is accompanied by a large, triangular "peristome area," running from the left anterior edge of the body to the mouth.

Typical genera: *Paramecium*, Stein, 1860.

Family 5. *Pleuronemidae*. The mouth is at the end of a long peristome, which runs along the ventral side; the body is dorsoventrally or laterally compressed. The entire left edge of the peristome is provided with an undulating membrane which occasionally runs around the posterior end of the peristome to form a pocket leading to the mouth. The right edge of the peristome is provided with a less developed membrane. There may or may not be a well-developed pharynx.

Typical genera: *Lembadion*, Perty, 1849; *Pleuronema*, Duj., 1841; *Cyclidium*, Ehr., 1838, a subgenus of the preceding; *Calypotricha*, Phillips, 1882; *Lembus*, Cohn, 1866.

Family 6. *Isotrichidae*. The body is more or less plastic, but not contractile. The cuticle is thick and provided with evenly distributed cilia. The mouth is posterior and accompanied by a distinct pharynx. They are parasites in the digestive tract of ruminants.

Typical genera: *Isotricha*, Stein, 1859; *Dasytricha*, Schuberg, 1888.

Family 7. *Opalinidae*. The form is oval, and the body may be short or drawn out to resemble a worm. They are characterized mainly by the absence of mouth and pharynx.

Typical genera: *Anoplophrya*, Stein, 1860; *Hoplitophrya*, Stein, 1860; *Disco-phrya*, Stein, 1860; *Opalinopsis*, Fættinger, 1881; *Opalina*, Purkinje and Valentin, 1835; *Monodontophrya*, Vejdowsky, 1892.

Order 2. *Heterotrichida*. Ciliata characterized by the possession of a uniform covering of cilia and an *adoral zone*, consisting of short cilia fused together into membranelles.

Suborder 1. *POLYTRICHINA*. Heterotrichous ciliates provided with a uniform coating of cilia.

Family 1. *Plagiotomida*. The peristome is a narrow furrow, which begins, as a rule, close to the anterior end, and runs backward along the ventral side to the mouth, which is usually placed between the middle of the body and the posterior end. A well-developed adoral zone stretches along the left side of the peristome, and it is usually straight.

Typical genera: *Conchophthirus*, Stein, 1861; *Plagiotoma*, Duj., 1841; *Nyctotherus*, Leidy, 1849, a subgenus; *Blepharisma*, Perty, 1849; *Metopus*, Clap. and Lach., 1858; *Spirostomum*, Ehr., 1835.

Family 2. *Bursarida*. The body is usually short and pocket-like, but may be elongate. The chief characteristic is the peristome, which is not a furrow, but a broad triangular area, deeply insunk, and ending in a point at the mouth. The adoral zone is usually confined to the left peristome edge, or it may cross over to the right anterior edge.

Typical genera: *Balantidium*, Stein, 1867; *Balantidiopsis*, Bütschli, 1888; *Condylostoma*, Duj., 1841; *Bursaria*, O. F. Müller, 1773; *Thylakidium*, Schewiakoff, 1892.

Family 3. *Stentorida*. The peristome is relatively short and limited to the front end of the animal, so that its plane is nearly at right angles to that of the longitudinal axis of the body. The adoral zone of cilia either passes entirely around the peristome edge, or ends at the right-hand edge. The surface of the peristome is spirally striated and provided with cilia. Undulating membranes are absent.

Typical genera: *Climacostomum*, Stein, 1859; *Stentor*, Oken, 1815; *Folliculina*, Lamarck, 1816. *Genera incertæ sedis*: *Cenomorpha* (*Gyrocorys*, Stein), Perty, 1852; *Maryna*, Gruber, 1879.

Suborder 2. *OLIGOTRICHINA*. Heterotrichous ciliates characterized by the reduced cilia, which are limited to certain localized areas.

Family 1. *Lieberkühnida*. This name was given by Bütschli for certain little-known forms, which were at first considered young *Stentors*.

Family 2. *Halteriida*. The peristome has no cilia, and only a few scattered ones can be found on the ventral and dorsal surfaces.

Typical genera: *Strombidium*, Clap. and Lach., 1858; *Halteria*, Duj., 1841.

Family 3. *Tintinnida*. The body is attached by a stalk to a theca. Inside of the adoral zone of membranelles is a ring of cilia (paroral cilia).

Typical genera: *Tintinnus*, Fol., 1889; *Tintinnidium*, Kent, 1881; *Tintinnopsis*, Stein, 1867; *Codonella*, Haeckel, 1873; *Dictyocysta*, Ehr., 1854.

Family 4. *Ophryoscolecida*. Heterotrichous ciliates characterized by a thick cuticle and deep funnel-like peristome. The posterior end is provided with distinct spine-like processes, while the terminal anus is provided with a well-defined anal tube.

Typical genera: *Ophryoscolex*, Stein, 1859; *Entodinium*, Stein, 1859; *Diplo-dinium*, Schuberg, 1888.

Order 3. *Hypotrichida*. Ciliata in which the cilia are limited to the ventral surface of a dorsoventrally flattened body; they are frequently fused to form larger appendages, the cirri, and an adoral zone of membranelles. The dorsal surface is frequently provided with bristles. A pharynx may be absent or but slightly developed.

Family 1. *Peritromida*. The peristome is but slightly marked off from the remaining frontal area. The cilia on the ventral surface are uniform in size and arrangement, and are not differentiated into cirri.

Typical genera: *Peritromus*, Stein, 1862.

Family 2. *Oxytrichida*. The peristome is not always distinctly marked off from the frontal area. In the most primitive forms the ciliation on the ventral sur-

face is similar to that of the preceding family. Almost invariably in these primitive forms some of the anterior and some of the posterior cilia are fused into large and more powerful appendages, the cirri, which are distinguished as the *frontal* and *anal* cirri, respectively. In the majority of forms all of the cilia are thus differentiated; strong marginal cirri are formed in perfect rows, and ventral cirri in imperfect rows. In addition to the adoral zone of membranelles, there is an undulating membrane on the right side of the peristome, and, in some cases, a row of cilia between the membrane and the adoral zone. These are the paroral cilia, and they form the paroral zone.

Typical genera: Trichogaster, Sterki, 1878; Urostyla, Ehr., 1830; Kerona, Ehr., 1838; Epiclintes, Stein, 1862; Stichtotricha, Perty, 1849; Strongylidium, Sterki, 1878; Amphisia, Sterki, 1878; Uroleptus, Stein, 1859; Sparotricha, Entz, 1879; Onychodromus, Stein, 1859; Pleurotricha, Stein, 1859; Gastrostyla, Engelmann, 1862; Gonostomum, Sterki, 1878; Urosoma, Kowalewsky, 1882; Oxytricha, Ehr., 1830; Stylonychia, Stein, 1859; Actinotricha, Cohn, 1866; Ballardina, Kowalewsky, 1882; Psilotricha, Stein, 1859; Tetrastyla, Schewiakoff, 1892; Holosticha, Wrzesniowski, 1877.

Family 3. *Euplotida*. Hypotrichous ciliates, which are characterized mainly by the considerable reduction of the cilia, frontal, marginal, and ventral cirri; the anal cirri, on the other hand, are always present. The macronucleus is band-formed.

Typical genera: Euplotes, Stein, 1859; Certesia, Fabre-Domergue, 1885; Diophrys, Duj., 1841; Uronychia, Stein, 1857; Aspidisca, Ehr., 1830.

Order 4. *Peritrichida*. Ciliata usually of cylindrical or cup-like form, in which the cilia are reduced, as a rule, to those which form the adoral zone, but secondary rings of cilia may be present.

Family 1. *Spirochonida*. Peritrichous ciliates in which the peristome is drawn out into a curious funnel-like process, either simple or rolled. They are parasitic forms in which reproduction by budding is characteristic.

Typical genera: Spirochona, Stein, 1851; Kentrochona, Römpel, 1894; Kentrochonopsis, Doflein, 1897.

Family 2. *Lichnophorida*. In addition to the adoral zone, there is a secondary circlet of cilia around the opposite end. The adoral zone is a left-wound spiral. A single genus *Lichnophora*, Claparède, 1867, which is parasitic on various marine arthropods.

Family 3. *Vorticellida*. Attached or unattached forms of peritrichous ciliates, in which the adoral zone, seen from above, forms a right-wound spiral (dextro-tropic). A secondary circlet of cilia around the under end may be present either permanently or periodically.

Subfamily 1. *Urceolarina*. Vorticellidæ having a permanent secondary circlet of cilia which incloses an adhesive disk, and without a peristome fold.

Typical genera: Trichodina, Stein, 1854; Cyclochæta, Jackson, 1875; Trichodinopsis, Clap. and Lach., 1858.

Subfamily 2. *Vorticellidina*. Peritrichous forms without a permanent secondary circlet of cilia, and provided with a peristome fold which can be contracted sphincter-like to inclose the peristome.

Typical genera: Scyphidia, Lachmann, 1856; Gerda, Clap. and Lach., 1858; Astylozoön, Engelmann, 1862; Vorticella, Ehr., 1838; Carchesium, Ehr., 1830; Zoothamnium, Stein, 1854; Glossatella, Bütschli, 1888; Epistylis, Ehr., 1830; Rhabdostyla, Kent, 1882; Opercularia, Stein, 1854; Ophrydium, Ehr., 1838; Cothurnia, Clap. and Lach., 1858; Vaginicola, Clap. and Lach., 1858; Lagenophrys, Stein, 1851.

Subclass 2. *Suctorioria*. Infusoria having no cilia during the adult stages, but provided with them during the embryonic period. In a few cases the cilia are retained. They have tentacles of various kinds, some adopted for sucking, some for piercing.

Family 1. *Hypoconidæ*. These are unattached forms of Suctoria with a permanently ciliated ventral surface, and with one suctorial tentacle. Reproduction is effected by cross-division. A single genus, *Hypocoma*, Gruber, 1884.

Family 2. *Urnulidæ*. A family of small attached forms, with or without a cup or theca; with one or two, rarely more, simple tentacles. Swarm-spores holotrichous.

Typical genera: Rhyncheta, Zenker, 1866; Urnula, Clap. and Lach., 1858.

Family 3. *Metacinetidæ*. Thecate forms; the base of the cup is drawn out into a long stalk, and the walls are perforated for the exit of the tentacles. A single genus, *Metacinetæ*, Bütschli, 1888.

Family 4. *Podophryidæ*. Stalked or unstalked forms of more or less globular shape. The tentacles are numerous and distributed about the entire surface or limited to the apical region; some of them are knobbed, others pointed and have a prehensile function.

Typical genera: Spherophrya, Clap. and Lach., 1858; Endosphæra, Engelmann, 1876; Podophrya, Ehr., 1838; Ephelota, Str. Wright, 1858; Podocyathus, Kent, 1881.

Family 5. *Acinctidæ*. The individuals are naked and stalked, or thecate and stalked or unstalked. The tentacles are numerous, usually knobbed and all alike. Reproduction is effected by inner or *endogenous* budding, which may be simple or multiple. The swarm spores are usually peritrichous, but may be holotrichous or hypotrichous.

Typical genera: Tokophrya, Bütschli, 1888; Acinetæ, Ehr., 1833; Solenophrya, Clap. and Lach., 1858; Suctorella, Frenzel, 1891.

Family 6. *Dendrosomidæ*. Suctoria without stalks or theca. The tentacles are numerous, all alike, and knobbed and grouped in distinct tufts; they may be simple or branched. Reproduction by endogenous division; the swarm spores are peritrichous.

Typical genera: Trichophrya, Clap. and Lach., 1858; Dendrosoma, Ehr., 1838; Staurophrya, Zacharias, 1893.

Family 7. *Dendrocometidæ*. Sessile suctoria resting upon the entire basal surface or upon a portion of it raised as a stalk. The numerous tentacles are short and knobbed, and distributed over the entire apical surface or localized upon branched arms. Spore formation is endogenous; the swarm spores peritrichous.

Typical genera: Dendrocometes, Stein, 1867; Stylocometes, Stein, 1867.

Family 8. *Ophryodendridæ*. Stalked or sessile forms possessing numerous long, rarely knobbed tentacles, which are supported upon proboscis-like processes of the apical side. Reproduction is brought about by endogenous budding. The swarm spores are peritrichous.

Typical genera: Ophryodendron, Clap. and Lach., 1858.

PROTOZOA WITHOUT MOTILE ORGANS, AND CLASSIFICATION OF THE SPOROZOA.

To state that the sporozoa are without motile organs is not strictly accurate, for many of them have well-developed myonemes (gregarines) and move with a vermiform motion. Others have, at times, the power of progressing by means of pseudopodia (many of the neosporidia). Nor is the method of reproduction (spore formation) any less equivocal, for many forms reproduce by simple division as well as by spore formation (schizogregarinida). This division, therefore, more than

any other of the unicellular animals must be regarded as provisional only and comprising numerous heterogeneous groups of organisms which can be more accurately classified only after the full life histories are made out. Some of these groups are obviously related to the mastigophora through the blood-dwelling flagellates, and others are equally related to the sarcodina. Two divisions only, the gregarinida and the coccidiidia, may be accepted as sufficiently definite to constitute an acceptable division of the protozoa. At the present time, Schaudinn's grouping into telosporidia and neosporidia cannot be bettered, although evidence is accumulating to show that the latter group is entirely artificial.

SUBPHYLUM SPOROZOA.—Parasitic protozoa without motile organs, but capable of moving from place to place by structural modifications of one kind or other. Reproduction either simple or multiple, but mainly by spore formation, which is either asexual (schizogony) or sexual (sporogony).

The following classification of sporozoa is based upon Labbé's "sporozoa," and upon "sporozoa" in Lankester's *Treatise on Zoölogy*, Part I, Introduction and Protozoa. Second fascicle, with additions and changes necessary for the present work and to bring the classification up to date.

Subphylum **SPOROZOA.**

Class I. **TELOSPORIDIA**, Schaudinn. Sporozoa in which sporulation ends the life of the individual.

Order 1. **Gregarinida.** Cœlozoic telosporidia reproducing usually by spore formation alone, and after the fertilizing union of but slightly different gametes.

Suborder 1. **SCHIZOGEGARINÆ.** Gregarines reproducing by division or by multiple budding in addition to spore formation.

This interesting group, which is continually being added to by various observers, was until quite recently represented by only those supposedly ameboid forms known as the Amebosporidia. The investigations begun by Lèger and carried on by Lèger, Dubosq, Dogiel, Brasil, and others of recent date have shown that the supposed ameboid processes are actually unchangeable, serving more as attaching organs and for the purpose of absorbing food than for the purposes of locomotion.

There is no question that these forms are gregarines; and from the very characteristic types included here there is some hope of ultimately getting light upon the closer relationships of the entire group of sporozoa to other groups of protozoa.

Genus 1. *Schizocystis*, Lèger, 1900. Type species *S. gregarinoides*, Lèger, from the intestine of larva of *Ceratopogen* sp. The trophozoites are somewhat similar to *Monocystis*, but differ in reproducing by the formation of a group of internal buds, which, as merozoites, leave the parent cell and grow into new trophozoites; these finally couple up, fertilization and sporulation result, and octozoic spores are finally formed, as in *Monocystis* (Fig. 76).

Genus 2. *Ophryocystis*, A. Sch., 1884. Many species are known, most of which are parasites in the Malpighian tubules of beetles. The organisms have characteristic pseudopodia-like processes for purposes of attachment, and the trophozoites reproduce by simple division or by multiple division. Sporulation ultimately takes place, the process differing in different cases (Fig. 80).

Genus 3. *Selenidium*, Giard, 1884; emend Caullery et Mesnil, 1899. The body is attenuated and worm-like, and marked externally by longitudinal striæ due to the ectoplasmic myonemes. Epimerite conical and slender. Parasites of polychetes and numerous species are recorded.

Suborder 2. EUGREGARINÆ, Lèger. Reproduction here is limited apparently to sporulation, division occurring, if at all, within the host cell and during the young stages.

Tribe 1. *Acephalina*, Kölliker. Eugregarines in which there is no division into chambers and in which at no stage is there an epimerite.

Genus 4. *Monocystis*, Stein, 1848. The trophozoites are often highly contractile owing to the peristalsis brought about by the contractions of ectoplasmic myonemes. Spores boat-shaped and octozoic. Many species from worms and entomotraca, a typical species, *M. agilis* may be found almost invariably in the seminal reservoirs of the common earthworm, and excellent stages in sporulation and fertilization may be easily obtained.

Genus 5. *Zygocystis*, Stein, 1848. The trophozoites are usually found in pairs or groups of three. Typical species, *Z. cometa*, Stein, found in the seminal vesicles and body cavity of the earthworm *Lumbricus agricola*.

Genus 6. *Zygosoma*, Labbé, 1899. The trophozoite has typical and characteristic finger-like processes and is usually found in couples. Sporulation unknown. Typical species, *Z. gibbosum*, Greeff, 1880, in the gut of *Echiurus pallassii*.

Genus 7. *Pterospora*, Racovitza and Labbé, 1896. The piriform trophozoites are always associated in couples. The spores have dissimilar poles and the episore is drawn out into lateral processes. One species, *P. maldaneorum*, R. and L., from the celomic cavity of maldanid worms.

Genus 8. *Cystobia*, Mingazzini, 1891. The trophozoites are large and irregular in form and usually have two nuclei due to the early fusion of two individuals. The spores are heteropolar, and the episore is drawn out into chimney-like projections at one pole. One species, *C. holothuriæ*, A. Sch., from the blood-vessels and body cavity of holothurians.

Genus 9. *Lithocystis*, Giard, 1876. The trophozoite is characterized by an endoplasm filled with crystals of calcium oxalate. The episore has long processes. A single species from the celomic cavities of various echinids.

Genus 10. *Ceratospora*, Lèger, 1892. The trophozoites fuse by their truncated ends and give rise to spores without encysting. The spores are characterized by long spinous processes (Fig. 20). A single species, *C. mirabilis*, Lèger, from the body cavity of *Glycera*.

Genus 11. *Urospora*, A. Schn., 1875. The spores are characterized by the presence of a long caudal filament at one pole. Several species from the body cavities of oligochetes, nemertines, sipunculids, and other marine invertebrates.

Genus 12. *Gonospora*, A. Schn., 1875. The trophozoites are quite variable in form and give rise to heteropolar spores bearing from one to several tooth-like processes at one pole, and rounded at the other. Four species from the body cavities of polychetous worms.

Genus 13. *Syncystis*, A. Schn., 1886. The spores are ovoid or boat-shaped, with spines or processes at each extremity. One species, *S. mirabilis*, A. Schn., from fat body and celom of species of *Nepa*.

Genus 14. *Diplocystis*, Kunstler, 1887. The trophozoites fuse precociously to form spherical masses of gregarines in the body cavity of crickets and cockroaches. The spores are either spherical or oblong.

Genus 15. *Lankesteria*, Mingazzini, 1891. The spores are more or less flattened or spatulate, oval in outline, and octozoic. Type species, *L. ascidiæ*, Lank, from the gut of *Ciona intestinalis*.

Genus 16. *Callintrochlamys*, Frenzel, 1885. The trophozoites have a central constriction but no septum dividing the body into protomerite and deuto-

merite; they are covered by a fur-like fringe of processes resembling cilia. The spores are unknown. Type species, *C. phronimæ*, Frenz., from the gut of *Phronima sedentaria*.

Genus 17. *Ancora*, Labbé, 1899. The trophozoite has a peculiar anchor-like form by reason of two lateral bulgings of the body. Spores unknown. Species, *A. sagittata*, Leuck, from the gut of *Capitella capitata*.

Other genera provisionally placed here are: *Pleurozyga*, Mingazzini, 1891, from ascidians; *Ophiodina*, Mingazzini, 1891, from *Bonellia*; *Köllikerella*, Labbé, 1899, from *Staurocephalus*; *Lobianchella*, Mingazzini, 1891, from *Alciope*.

Tribe II. *Cephalinæ*, Delage. Eugregarines possessing an epimerite at some stage of the life history, either in the adult phase or in the temporary young phases. The body is usually divided by a septum into protomerite and deutomerite, and the trophozoites are frequently associated in couples arranged tandem, each couple consisting of primite and satellite. The tribe consists mainly of parasites of the gut of various forms of arthropods.

Legion A. *Gymnosporæa*, Lèger. The sporoblast mother cells give rise directly to sporozoites which do not form in sporocysts or specially protected sporoblasts.

Family 1. *Aggregatidæ*, Labbé. With sporozoites grouped irregularly about a number of residual masses.

Genus 18. *Aggregata*, Frenzel, 1885. With the characteristics of the family. Several species from various crustacean hosts.

Family 2. *Porosporidæ*, Labbé. Special centres of sporozoite formation are present (sporoblast centres), but they lack the protective sporocysts.

Genus 19. *Porospora*, A. Schn., 1875. Trophozoite with small button-like epimerite; cells very large (up to 16 mm.) and usually solitary. One species, *P. gigantea*, Van Beneden, from gut of the lobster.

Legion B. *Angiosporæa*, Lèger. The sporocysts are well developed and usually double coated to form endospore and episporæ.

Family 3. *Gregarinidæ*, Labbé. Trophozoites with simple epimerites; sporocysts with or without sporoducts. Spores oval or barrel-shaped, and united in strings in species with sporoducts.

Genus 20. *Gregarina*, Dufour, 1828. Cysts with sporoducts; epimerite small, conical, or knobbed (see Fig. 81, p. 191). Many species widely distributed in digestive tracts of various insects.

Genus 21. *Gamocystis*, Lèger, 1892. The trophozoite has a temporary epimerite. Cyst with sporoducts. Spores cylindrical and elongated. From gut of cockroach and other insects.

Genus 22. *Eiromocystis*, Lèger, 1892. The sporonts unite to form aggregates of several individuals. The spores are ellipsoidal. Cysts without sporoducts. One species, *E. polymorpha*, Lèger, from the gut of insects.

Genus 23. *Hyalospora*, A. Schn., 1875. Cysts without sporoducts. Spores pointed at each end and bulging in middle. Gut of *Petrobius* sp.

Genus 24. *Euspora*, A. Schn., 1875. Spores prismatic, cysts without sporoducts. One species, *E. fallax*, from gut of *Rhizotrogus estivus*.

Genus 25. *Spheroecystis*, Lèger, 1892. Body spherical, protomerite temporary, cysts without sporoducts, spores oval. One species, *S. simplex*, Lèger, from the gut of *Xyphon pallidus* larva.

Genus 26. *Cnemidospora*, A. Schn., 1882. The epimerite is large and lancet-shaped; sporonts solitary with globular protomerites. No sporoducts. Spores ellipsoidal, with thick spore cysts. One species, *C. lutea*, A. Schn., from the gut of *Glomeris*.

Genus 27. *Stenophora*, Labbé, 1899. Sporonts large, with small protomerite. Cyst without sporoducts; spores fusiform with dark sutural line. One species, *S. juli*, Franz, from gut of species of millipedes.

Family 4. *Didymophyidæ*, Lèger. The sporonts always associated in pairs, the protomerite of the satellite disappearing, thus giving the appearance of an organism with three chambers and two nuclei.

Genus 28. *Didymophyes*, Stein, 1848. The epimerite has the form of a spike. Cysts open by simple rupture liberating the oval spores. Four species.

Family 5. *Dactylophorida*, Lèger. The epimerite is asymmetrical and irregular, with digitiform processes. Sporocysts open by simple rupture or by the swelling of a residual mass of plasma termed a "pseudocyst."

Genus 29. *Rhopalonia*, Lèger, 1893. The epimerite is irregular and asymmetrical, bearing finger-formed prolongations. The trophozoite is solitary and with traces only of a protomerite. One species, *R. geophili*, Lèger, from gut of *geophilus* sp.

Genus 30. *Echinomera*, Labbé, 1899. The trophozoite massive and oval in outline; epimerite persistent and spiked, the point bearing small transitory digitiform processes. Cysts open by simple rupture. One species, *E. hispida*, A. Schn., from gut of *Lithobius forficatus*.

Genus 31. *Trichorhynchus*, A. Schn., 1882. Protomerite truncated with an elongated and conical top. Cysts with oblong, wart-like protuberances. Cysts open by the swelling of laterally placed pseudocysts. Spores not in strings. One species, *T. pulcher*, A. Schn., from the gut of *Scutigera*.

Genus 32. *Pterocephalus* A. Schn., 1887. Protomerite extends beyond the deutomerite on the two sides and is divided into two lobes by a constriction; the two lobes are provided with sharp papillæ, and are united on one side and so curved as to form a coiled horn. The spores are oval and associated obliquely in strings. One species, *P. nobilis*, A. Schn., from gut of *Scolopendra*.

Genus 33. *Dactylophorus*, Balb., 1889. The protomerite is dilated excentrically and bears epimerite with digitiform processes. Sporonts are solitary and elongated; cysts spherical and spores cylindrical; cysts open by swelling of lateral pseudocyst. One species, *D. robustus*, Lèger, from the gut of *Cryptops hortensis*.

Family 6. *Actinocephalidæ*, Lèger. Sporonts always solitary with simple, symmetrical, or irregular appendages. Cysts open by simple rupture. Spores biconical, cylindrical, or navicular. Parasitic usually in the gut of carnivorous arthropods.

Group A. *Sciadiophorinæ*, Labbé, 1899. Protomerite umbrella-shaped, and with radiating ridges. Spores biconical and with central swellings, the opening at the equator by simple dehiscence, while the endospore opens terminally.

Genus 34. *Sciadiophora*, Labbé, 1899. The epimerite is large and flattened and with the characteristics of the group. Three species from digestive tracts of phalangidæ.

Group B. *Anthorhynchinæ*, Labbé, 1899. Spores ovoid with pointed ends; joined in strings; equatorial opening.

Genus 35. *Anthorhynchus*, Labbé, 1899. Epimerite in form of a large grooved knob or button. One species from gut of *Phalangium opilio*.

Group C. *Pileocephalinæ*, Labbé, 1899. Epimerite simple and regular; cysts open by simple rupture; spores usually biconical.

Genus 36. *Pileocephalus*, A. Schn., 1875. Epimerite simple and regular and somewhat lance-like. Cysts open by simple rupture, spores biconical.

Genus 37. *Amphoroides*, Labbé, 1899. Epimerite spiked or rounded; protomerite very short and cup-like. Spores biconical. One species, *A. polydesmi*, Lèger, from the gut of *Polydesmus*.

Genus 38. *Discorhynchus*, Labbé, 1899. Epimerite large and discoid, with a distinct rim; protomerite larger than the deutomerite, which is regularly cylindrical and truncated posteriorly. Cysts spherical, spores biconical and slightly bent. One species, *D. truncatus*, Lèger, from gut of *Sericostoma* sp.

- Group D. *Stictosporinae*, Labbé, 1899. Spores biconical, with points slightly incurved and with papillæ on the endospore.
- Genus 39. *Stictospora*, Lèger, 1893. Epimerite with globular head depressed ventrally, and covered with ribs which project posteriorly as spikes. Spores biconical. One species, *S. provincialis*, Lèger, from the gut of *Melolontha* and *Rhizotrogus* larvæ.
- Group E. *Actinocephalinae*, Labbé, 1899. Epimerite always with appendages. Spores regular, navicular or subnavicular, biconical or cylindrical.
- Genus 40. *Schneideria*, Lèger, 1892. Sporont has but one chamber; epimerite a thick plate bordered by rib-like thickenings. Spores somewhat thickened and biconical. Two species, *S. mucronata*, Lèger, from gut of larvæ of *Bibio marci*, and *S. caudata* from gut of larva of *Sciara nitidicollis*.
- Genus 41. *Asterophora*, Lèger, 1892. The epimerite is a circular ridge with ribs surrounding a prominent central papilla. The protomerite is as large or larger than the deutomerite. Sporonts solitary; spores cylindrical with conical extremities. Two species, *A. mucronata*, L., and *A. elegans*, L., from the intestines of larvæ of insects.
- Genus 42. *Stephanophora*, Lèger, 1892. Epimerite large and in form of a convex disk with a crown of digitiform processes. Spores cylindrical with conical ends. One species, *S. lucani*, Stein, from gut of *Dorcus* sp.
- Genus 43. *Bothriopsis*, A. Schn., 1875. Epimerite in form of a large lens-shaped knob with non-motile processes. Sporonts highly developed and very motile. Spores biconical and thickened. One species, *B. histrio*, A. Schn., 1875, from the gut of *Hydaticus* sp.
- Genus 44. *Coleorhynchus*, Labbé, 1899. Sporont with sucker-like protomerite extending over deutomerite. The convex septum projects into the protomerite. Cysts open by simple rupture; spores navicular. One species, *C. heros*, A. Schn., from gut of *Nepa cinerea*.
- Genus 45. *Legeria*, Labbé, 1899. Protomerite enlarged and club-like, with invading septum, as above. Spores with thick sporocysts and subnavicular in form. One species, *L. agilis*, A. Schn., from gut of *Colymbetes* sp.
- Genus 46. *Phialoides*, Labbé, 1899. Complex epimerite consisting of a discoid retractile cap surrounded by a circular ridge with collar-like membrane, with ridges ending in triangular teeth. Sporonts solitary, massive; spores biconical and thickened. One species, *P. ornata*, Lèger, from the gut of *Hydrophilus* larvæ.
- Genus 47. *Geniorhynchus*, A. Schn., 1875. Epimerite in the form of a disk which bears fine pointed teeth and is carried on a long neck. Spores subnavicular. One species, *G. monnieri*, A. Schn., from intestines of nymphs of libellulidæ.
- Genus 48. *Actinocephalus*, Stein, 1848. Epimerite sessile or borne on neck-like process, and is provided with hooks and spines. Spores biconical. Several species from digestive tracts of beetles.
- Genus 49. *Pyxinia*, Hammerschmidt, 1838. Epimerite in the form of a cup with rim surrounding a central spine. Many species (Fig. 73).
- Genus 50. *Beloides*, Labbé, 1899. Epimerite in the form of a disk or knob and bearing about ten teeth in addition to a long spike. Spores navicular or oval. Two species parasitic in the gut of species of *Dermestes*.
- Genus 51. *Stylocystis*, Lèger, 1899. Trophozoite non-septate; epimerite in the form of a long spine which is usually curved. Sporonts solitary with biconical spores. One species, *S. precox*, Lèger, from the intestine of the larva of *Tanypus* sp.
- Family 7. *Acanthosporida*, Lèger, 1892. Sporonts always solitary; epimerite simple or with appendages; cysts open by simple rupture; spores ornamented with bristles at the poles or at the equator. Parasites of carnivorous insects.
- Genus 52. *Corycella*, Lèger, 1892. Protomerite spherical and somewhat dilated.

Epimerite a knob with a crown of eight large and recurved hooks. One species, *C. armata*, Lèger, from the gut of *Gyrinus natator*.

Genus 53. *Acanthospora*, Lèger, 1892. Sporonts solitary and of elongate oval form. Epimerite a conical obtuse knob; spores oval with four bristles at each end and a circlet of spines about the equator. Three species, *A. pileata*, Lèger, from the gut of larva of *Omoplus*, a typical species.

Genus 54. *Ancyrophora*, Lèger, 1892. Sporonts solitary; posterior part pointed. Epimerite a knob with appendages in the form of recurved hooks. Spores biconical with polar tufts and six equatorial bristles. Two or more species from carnivorous beetles.

Genus 55. *Cometoides*, Labbé, 1899. Epimerite a spherical knob flattened centrally and bearing a circlet of flexible filaments. Spores with a bunch of bristles at each pole and two circlets of bristles about the equator. Two or more species from the larvæ of beetles.

Family 8. *Menosporidæ*, Lèger, 1892. Sporonts solitary, epimerite symmetrical, with appendages and connected with the protomerite by a long neck. Cysts spherical, opening by simple rupture. Spores in form of crescents more or less curved.

Genus 56. *Menospora*, Lèger, 1892. Epimerite cup-like and bordered by hooks. One species, *M. polyacantha*, Lèger, 1892, from gut of *Agrion puella*.

Genus 57. *Hoplorhynchus*, Carus, 1839. Epimerite in the form of a disk with sharp teeth. One species, *H. oligacanthus*, Sieb., from the gut of *Calopteryx virgo*, larva.

Family 9. *Stylorhynchidæ*, A. Schn., 1886. Epimerite symmetrical with or without appendages. Cysts with two envelopes and pseudocyst. Brown or black-colored spores in strings.

Genus 58. *Lophocephalus*, Labbé, 1899. Epimerite sessile, cup-like, with fringe of vesicular appendages. Protomerite compressed. Cysts irregular, sub-spherical. One species, *L. insignis*, A. Schn., in gut of *Helops striatus*.

Genus 59. *Cystocephalus*, A. Schn., 1886. Epimerite vesicular, with short neck. One species, *C. algerianus*, A. Schn., from gut of *Pimelia* sp.

Genus 60. *Ooccephalus*, A. Schn., 1886. Epimerite a rounded knob on a short neck. One species, *O. hispanus*, A. Schn., from the gut of *Morica* sp.

Genus 61. *Spherorhynchus*, Labbé, 1899. Epimerite small, spherical or oval, and carried on a long cylindrical neck constricted deeply below the epimerite. One species, *S. ophioides*, A. Schn., from the gut of *Acis* sp.

Genus 62. *Stylorhynchus*, Stein, 1848. Epimerite small and knob-like, borne on an elongated neck of the protomerite. Deutomerite of the sporont much elongated; protomerite rounded. Two or three species, the most typical being *S. longicollis*, Stein, from the gut of *Blaps mortisaga*.

Family 10. *Doliocystidæ*, Labbé, 1899. Epimerite regular and simple; no trace of a septum. Spores oval with a polar thickening. Marine annelids.

Genus 63. *Doliocystis*, Lèger, 1893. No trace of septum; oval spores, and sporocysts with polar thickenings. Two or three species, the most typical *D. pellucida*, Kölliker, from the gut of *Nereis* sp.

Other genera referred to this division by Labbé, Minchin, and other systematists are: *Nematoides*, Mingazzini, 1891, from the gut of cirrhipedes; *Ulivina*, Mingazzini, 1891, from the gut of *Audouinia filigera*; *Sycia*, Lèger, 1892, from gut of same.

Order 2. **Coccidiidia**. Cell-infesting sporozoa which usually reproduce by schizogony and by sporogony, thus giving a life cycle with an alternation of asexual and sexual generations. After fertilization the oosphere forms sporoblasts which may or may not (asporocystea) be covered by a sporocyst membrane, and which may each become transformed into one or several sporozoites.

Suborder 1. **ASPOROCYSTINEA**. Coccidiidia in which the sporoblasts have no

sporocysts. Here, if we were to be strictly consistent, we would advise, with Minchin, the inclusion of the malaria-causing organisms, and group the other hemosporidia with the genera included under the Sporocystinea. But it does not seem opportune at the present time to give up the old group Hemosporidia, at least not until the questionable "binucleate" forms have been worked out in complete detail.

Following Minchin, in naming the families according to the more characteristic of the contained genera, we have the following:

Family 1. *Eimeridæ* (Asporocystidæ, Lèger). Sporocysts absent, the sporozoites being naked in the parent cell (gymnosporous).

Genus 1. *Eimeria*, A. Schn., 1875. (Syn., Lègerella Mesnil.) With the characters of the family. One species, *E. nova*, A. Schn., from the Malpighian tubules of Glomeris.

Family 2. *Isosporidæ* (Disporocystidæ, Lèger). The oösphere forms two sporoblasts each with sporocysts (chlamydo-spores).

Genus 2. *Cyclospora*, A. Sch., 1881. Each sporoblast forms two sporozoites. *C. glomericola*, A. Schn., 1881, intestine of Glomeris sp., and *C. caryolytica*, Schaudinn, from the intestine of moles.

Genus 3. *Diplospora*, Labbé, 1893. Spores tetrazoic; many species occurring in birds, snakes, lizards, and frogs.

Genus 4. *Isospora*, A. Schn., 1881. Spores polyzoic (?). *I. rara*, A. Schn., from the black slug, *Limax cinereo niger*.

Family 3. *Coccidiidæ*, (Tetrasporocystidæ, Lèger). The fertilized cell produces four sporoblasts with sporocysts (chlamydo-spores).

Genus 5. *Coccidium*, Leuckart, 1879. The dizoic spores are spherical or oval. Many species almost entirely limited to vertebrate hosts, and found in nearly all orders. Here, also, belong some questionable forms, such as *Paracoccidium prevoti*, Lav. and Mes., from the frog.

Genus 6. *Crystallospora*, Labbé, 1896. The spores are dizoic and the sporocysts in the form of a double pyramid placed base to base. One species, *Cr. crystalloides*, Thél., from the intestine of Motella tricurata of Roscoff (Fig. 20, L).

Family 4. *Klossidæ*, (Polysporocystidæ, Lèger). The fertilized cell contains many sporoblasts (chlamydo-spores).

Genus 7. *Barroussia*, A. Schn., 1885. Spores spherical and monozoic; sporocyst smooth. Many species, a good type being *B. ornata*, A. Schn., from the gut of *Nepa cinerea* (Fig. 20, C).

Genus 8. *Echinospora*, Lèger, 1897. Spores monozoic, oval, and with spinous sporocyst. Typical species, *E. labbei*, Lèger, from gut of *Lithobius mutabilis*.

Genus 9. *Diaspora*, Lèger, 1898. Spores, as above, but sporocysts not bivalve and with micropyle at one end. *D. hydatidea*, Lèger, from gut of *Polydesmus*.

Genus 10. *Adelea*, A. Schn., 1875. Spores dizoic with smooth, spherical or flattened sporocyst. Many species, a typical one, *A. ovata*, A. Sch., from gut of *Lithobius*.

Genus 11. *Minchinia*, Labbé, 1896. Spores dizoic, with oval sporocysts drawn out into long polar filaments. *M. chitonis*, Lankester, 1896.

Genus 12. *Eucoccidium* ("Benedenia"), Lühe, 1902. Spores trizoic, schizogony absent. *E. eberthi*, Labbé, from Sepia.

Genus 13. *Klossia*, A. Schn., 1875. Spores tetrazoic or polyzoic, and with spherical sporocysts.

Genus 14. *Caryotropha*, Siedlecki, 1902. Twenty, more or less, sporoblasts, with twelve sporozoites in each. Sporocysts spherical. One species, *C. mesnili*, Sied., from the spermatogonia of *Polymnia nebulosa*.

Genus 15. *Klossiella*, Smith and Johnson, 1902. Sporoblasts polyzoic, sporocysts subspherical thirty to thirty-four sporozoites. One species, *K. muris*, S. and J., from the kidney of the mouse.

FIG. 20

Types of spores. (After Wasielewsky, A. Schneider, Thélohan.)

Questionable genera of coccidiida are the following:

Hyaloklossia, Labbé, 1896, from the frog.

Goussia, Labbé, 1896, from various species of fish. Usually classed as *Coccidium* species (Fig. 20, *M*, *N*).

Bananella, Labbé, 1895, from the gut of *Lithobius*. Usually classed with *Coccidium*.

Rhabdospora, Laguesse, 1895; *Gonobia*, Mingazzini, 1892; *Pfeifferella*, Labbé, 1899; *Molybdis*, Pachinger, 1886; *Cretya*, Mingazzini, 1892; *Gymnospora*, Moniez, 1886, are all probably species of *Coccidium*.

Order 3. Hemosporidia, Danilewsky. Blood dwelling sporozoa cytozoic or celozoic in mode of life in the blood constituents, and with or without alternation of hosts. A somewhat heterogeneous collection of parasitic protozoa with obscure affinities, pointing in part toward the flagellates, in part toward the coccidia. For convenience, and purely as a temporary matter, we follow Minchin in dividing the order into two suborders, *Acytosporea* and *Hemosporea*, the former including those blood-dwelling forms which seem to bear some relationship to *Crithidia* and *Herpetomonas*, the latter including the more *Coccidia*-like forms.

Suborder A. ACYTOSPOREA. The trophozoite is an intracellular or intracorpuseular parasite which usually completes its schizogony within the host cell. The sexual cycle is completed in the digestive tract or body cavity of some intermediate host—in all known cases some species of blood-sucking arthropod, usually an insect or arachnid.

Genus 1. Plasmodium, Marchiafava and Celli, 1885. The organisms of human malaria are all referred to this genus. The characteristic morphological features are the presence of melanin pigment, oval merozoites grouped around a central residual body, and spherical or crescentic gametes. Sporogony in the gut and body cavity of mosquitoes of the genus *Anopheles*. Three species generally recognized *P. vivax*, Grassi and Feletti, 1892, the cause of tertian fever, with schizogony every forty-eight hours. *P. malariae*, Lav., 1880, the cause of quartan fever, with schizogony every seventy-two hours. *P. immaculatum* Gr. and Fel., 1892, the cause of pernicious malaria, with subspecies according to Craig and others, exhibiting quartan and tertian characteristics. This last species is generally held to be a distinct genus under the name *Laverania*, Gr. and Fel., 1890, but Schaudinn's contention that crescentic instead of spherical gametocytes is an insufficient distinction for generic difference is rapidly gaining ground, and we follow it here. Minchin's remark (footnote, p. 267, 1903), that the popular names given to the malaria-causing parasites ("tertian," "quartan," and "pernicious") are more intelligible and less misleading than the so-called scientific names, is confirmed by Lühe, but it seems to us that such confusion is only further aggravated by their retention of the generic name *Laverania*. In addition to the species of *Plasmodium* causing human malaria, Laveran described a species from the blood of apes under the name of *P. kochi*, and Lühe places in the same species the blood parasites of chimpanzees from Kamerun.

Subgenus. Hemoproteus, Kruse, 1890. The cause of bird malaria. Merozoites and schizogony as in the preceding, sporogony in the digestive tract and body cavity of mosquitoes of the genus *Culex*. Gametocytes bean-shaped. The various species of this genus are now commonly referred to the genus *Plasmodium*. Common in birds.

Genus 2. Babesia, Starcovici, 1893. (Syn., *Pyrosoma*, Smith and Kilb.; *Piroplasma*, Patton.) An intracorpuseular parasite of mammalian blood. Trophozoites usually piriform, without pigment, and reproducing by simple division or by budding within the blood corpuscle. Transmission by ticks and sporogony in the latter's gut.

Many species: In man, *B. hominis*, Manson, the disputed cause of Rocky Mountain Spotted Fever; in cattle, *B. bovis*, Babes, 1888, and *B. bigemina*, Smith and Kilbourne, 1893, and *B. parvum*, Theiler, 1904; in sheep, *B. ovis*, Babes, 1892; in horses, asses, and mules, *B. equi*, Laveran, 1901; and in dogs, *B. canis*, Piana and Galli-Valerio, 1895.

Some genera of questionable taxonomic value are referred to this suborder. Among them *Polychromophilus*, Dionisi, 1900, and *Achromaticus*, Dionisi, 1900, from the blood of bats of the genera *Vespertilio* and *Vesperugo*, must be temporarily placed. The former is characterized by the presence of pigment similar to that of *Plasmodium*, while in the latter such pigment is absent.

Suborder B. **HEMOSPOREA**, Minchin, 1903. Intracellular blood parasites usually called Hemogregarines, which become free in the blood. Alternation of hosts in some cases, but apparently not in all. Parasites mainly in cold-blooded animals.

Genus 3. *Laankesterella*, Labbé, 1899. (Syn., *Drepanidium* Lank., 1882.) The parasite is not more than three-quarters of the length of the blood cell of the frog in which it lives. Many species in many different species of frogs and toads. Life history not yet satisfactorily worked out; according to Hintze, it is completed in the frog's blood and digestive tract; according to Billet, it involves a trypanosome phase analogous to that of *Halteridium*, as described by Schaudinn (*Hemoproteus*). Further observations are much needed.

Genus 4. *Hemogregarina*, Danilewsky, 1885. The body of the parasite exceeds the length of the blood cells of reptiles which it infests, and is bent in the form of the letter U. Life history unknown, although varied observations have been recorded in connection with the ten or more species that have been described (see Lühe).

Genus 5. *Hepatozoon*, Miller, 1908. A liver cell, and blood parasite of rats. Schizogony in liver cells, engulfing and encapsulation in leukocytes, dissolution of capsule and copulation of gametes in the digestive tract of the intermediate host (a gamasid mite, *Lelaps echidninus*); sporulation in the body cavity of the mite, ingestion of the mite and its parasites by rat, penetration of gut wall by sporozoites and new infection of liver cells. One species, *H. perniciosum*, Miller, 1908 (Fig. 106, p. 271).

Class II. **NEOSPORIDIA**, Schaudinn. Sporulation of the ameboid parasites takes place during the activity of the parent cell and without interfering with the vegetative processes. Celozoic, histozoic, or cytozoic parasites, mainly of vertebrate hosts, and especially of fish.

Order 1. **Myxosporidia**, Bütschli. Relatively large neosporidia reproducing by pansporoblast formation, the spores provided with polar capsules containing more or less easily seen threads.

Suborder 1. **DISPOREA**, Doflein, 1901. One pansporoblast containing two spores, produced by each trophozoite. Spores wider than long. Trophozoites floating freely in the fluids of various organs of fish hosts and frog hosts.

Family 1. *Ceratomyxida*, Doflein. With the characters of the suborder.

Genus 1. *Ceratomyxa*, Thélohan, 1892. The two valves of the spore produced into long attenuated processes. About nine species, mostly from the gall-bladders of fishes (Fig. 20, G).

Genus 2. *Leptotheca*, Thélohan, 1895. Valves of the spore not drawn out into long processes. The sporoplasm completely fills the spore membranes. About six species from the gall-bladders of fishes and the kidneys of frogs (Fig. 20, J).

Suborder 2. **POLYSPOREA**, Doflein. More than two spores, usually a great number, produced in each pansporoblast. The spores are longer than wide.

(The characteristics distinguishing these two suborders are not very definite, and some more natural system should be worked out with further knowledge of the group. Under the polysporous forms, for example, the genus *Sphaerospora* is exceptional in having at least one disporous species and in having nearly spherical spores.)

- Family 2. *Myxidiidae*, Thèlohan, 1892. The trophozoites are typically free-living parasites in the fluids of the internal organs of their hosts; the spore has two polar capsules.
- Genus 3. *Sphaerospora*, Thèlohan, 1892. With spherical spores. Four or five species, mostly from fish kidneys.
- Genus 4. *Myxidium*, Bütschli, 1882. Spores navicular, with polar capsules at each end. Seven or more species from kidney and gall-bladder of fishes and tortoises.
- Genus 5. *Spheromyxa*, Thèlohan, 1892. Spores navicular with truncated ends and a polar capsule at each extremity. Polar filaments are short and thick, and somewhat conical in form. Three species from the gall-bladder of fishes.
- Genus 6. *Cystodiscus*, Lutz, 1889. Trophozoites without ameboid movement or changes of form; spores symmetrical with the sutural plane running obliquely from one extremity to the other and with a polar capsule at the extremities of the oblique suture. One species, *C. immersus*, Lutz, from the gall-bladder of toads and *Cystignathus* in Brazil.
- Genus 7. *Myxosoma*, Thèlohan, 1892. Spores flattened and ovoid in form and with the polar capsules crowded together at the narrow extremity. One species, *M. dujardini*, Thèl., from the gills of *Scardinius* sp.
- Genus 8. *Myxoproteus*, Doflein, 1898 (*Myxosoma ambiguum* of Thèlohan and Labbé). Spores somewhat pyramidal with spinous processes from the base of the pyramid. One species, *M. ambiguus*, from the bladder of *Lophius piscatorius*.
- Family 3. *Chloromyxidae*, Thèlohan, 1892. Spores with four polar capsules.
- Genus 9. *Chloromyxum*, Mingazzini, 1890. With the characters of the family. Several species (six or seven) known and distinguished by presence of appendages and distribution of polar capsules.
- Family 4. *Myxobolidae*, Thèlohan, 1895. Typical histozoic parasites rarely found in the ameboid form but usually as cysts filled with spores. Usually polysporous, the spores with one or two polar capsules. The sporoplasm contains vacuoles which are stained a reddish brown by iodine.
- Genus 10. *Myxobolus*, Bütschli, 1882. Spores ovoid or flattened into an ellipse. Polar capsules single or double. A great many species (about forty) known, and found in some organ or other of various fishes, and usually in the connective tissue of such organs. The genus is usually split up into three divisions, the first of which contains the aberrant forms *M. piriformis* and *M. unicapulatus* from the tench, with a single polar capsule and with pear-shaped spores. In the second are species with spores having polar capsules of dissimilar size. In the third are the great majority of the species referred to this genus, all with polar capsules of similar form and size (Fig. 20, K).
- Genus 11. *Henneguya*, Thèlohan, 1892. Ovoid spores with two polar capsules, the sporocyst prolonged into two long caudal processes which are not penetrated by the sporoplasm. Four species from stickleback, pike, and perch.
- Genus 12. *Hoferella*, Berg, 1898. Spores broad and compressed with two tail-like processes at the posterior end. One species, *H. cyprini*, Dofl., from the carp.
- Order 2. *Microsporidia*, Balbiani, 1883. The trophozoites are more or less ameboid; the spores are very minute, piriform, and with only one polar capsule which is invisible in the fresh state. They are typically parasites of inverte-

brates and usually of crustacea and other arthropods, where they are typically cytozoic.

Family 5. *Glugeidae*, Thélohan, 1892. With the characters of the order.

Group A. *Polysporogenea*, Doflein, 1898. The trophozoite produces many pansporoblasts, each of which gives rise to many spores.

Genus 13. *Glugea* (*Nosema*), Thélohan, 1891. With the characters of the group. Many species which are not satisfactorily worked out. The most famous species is *G. bombycis*, which caused the destructive epidemic among silkworms from 1850 to 1865.

Group B. *Oligosporogenea*, Doflein, 1898. The trophozoite produces but one single pansporoblast.

Genus 14. *Gurleya*, Doflein, 1898. The pansporoblast produces four spores. One species, *G. tetraspora*, Dofl., from *Daphnia maxima*.

Genus 15. *Thélohania*, Henneguy, 1892. The pansporoblast produces eight spores contained in small spherical or fusiform vesicles. Five species recorded, all from the muscles of crustacea.

Genus 16. *Pleistophora*, Gurley, 1893. The pansporoblasts produce more than eight spores. Many species, some of fish, but mostly of invertebrates.

Order 3. *Actinomyxidia*, Stölz, 1890. Sporozoa consisting of a double cellular envelope, three polar capsules, and eight spores arranged in ternary symmetry.

Genus 1. *Hexactinomyxon*, Stölz, 1899. Spores in anchor form, with six branches. *H. psammoryctis*, Stölz, 1899, in the intestinal epithelium of *Psammoryctes barbatus*.

Genus 2. *Triactinomyxon*, Stölz, 1899. Spore in anchor form, with three branches. *T. ignotum*, Stölz, 1899, in the intestinal epithelium of *Tubifex tubifex*, Müller.

Genus 3. *Synactinomyxon*, Stölz, 1899. Spores associated in a common envelope. *S. tubificis*, Stölz, 1899, in the intestinal epithelium of *Tubifex rivulorum*, Lam.

Genus 4. *Spheractinomyxon*, Caull. and Mesnil, 1904. Spores spherical and without wing-like prolongations. *S. stolci*, C. and M., 1904, in the body cavity of marine oligochetes (*Clitellus arenarius*, O. F. M.), etc.

Order 4. *Haplosporidia*. Caull. and Mesnil, 1899. A group of little-known parasites with obscure affinities and undetermined life histories. Caullery and Mesnil, 1905, group them in three somewhat ill-defined subdivisions, as follows:

Family 1. *Haplosporidiidae*, C. and M., 1905. Parasites of ameboid form, which reproduce by encapsuled merozoites, which may or may not be ornamented by spines or processes. Genera *Haplosporidium* and *Urosporidium*, with six species enumerated by C. and M., all parasites of annelids.

Family 2. *Bertramiidae*, C. and M., 1905. With two genera, *Bertramia* and *Ichthyosporidium*, and with four species parasitic in annelids, rotifers, and fish.

Family 3. *Celosporidiidae*, C. and M., 1905. With three genera, *Celosporidium*, Mesnil and Marchoux, 1898; *Polycaryum*, Stempel, 1901; and (?) *Blastulidium*, Ch. Perez, 1903, mainly parasites of copepods. Doubtful forms, including the genera *Schewiakowella*, C. and M., 1905, parasite of *Cyclops*, etc.; *Chytridiopsis*, A. Schneider, 1884, parasite of *Tenebrio molitor* and of *Blaps*; *Celosporidium*, Crawley, of *Blattella germanica*; *Lymphosporidium*, Calkins, 1898; and *Rhinosporidium*, Minchin and Fantham, the cause of nasal tumors in man.

Order 5. *Sarcosporidia*. Sporozoa in which the initial stage is passed in muscle cells of vertebrates. Great sac-like spore cases are formed (Miescher's tubules) with double membranes. Genus, *Sarcocystis*, Lankester, 1882 (Fig. 79, p. 186).

CHAPTER II.

PHYSIOLOGICAL ACTIVITIES OF THE PROTOZOA.

EHRENBERG, in 1838, entitled his monumental work on the protozoa *Die Infusionsthierchen als vollkommene Organismen* (*The Infusoria as Complete Organisms*). Despite the great improvements that had been made in the microscope, and the vast collection of facts that had accumulated in connection with the structures of the protozoa, Ehrenberg's point of view was but slightly advanced beyond that of Leeuwenhoek one hundred and fifty years before. "Animalcula," said Leeuwenhoek, "which swim in stagnant waters, and which are no longer than the tails of the spermatric animalcula, are provided with organs similar to those of the highest animals. How marvellous must be the visceral apparatus shut up in such animalcula!" Ehrenberg sought to make out the various organs in this "visceral complex," and with great ingenuity managed to find digestive tract, kidney, brain, heart, ovary, and other organs characteristic of metazoa. The red, so-called "eye spots" were regarded by him as eyes, and the colorless lens upon which they frequently lie was interpreted as a cerebral ganglion, or brain. The contractile vacuole became, for him, a beating heart, and the collecting canals formed the vessels. The macronucleus was an ovary, the gastric vacuoles stomachs, while various chance inclusions were regarded as organs of one kind or another.

While Leeuwenhoek's and Ehrenberg's interpretation made out these primitive animals as marvels of creation in miniature, how much more marvellous are the facts as we know them today and summed up in the statement that the functions of all of these organs of the highest animals are performed within the single cell! The protozoön has no digestive tract, but it seizes food, digests and assimilates it, and grows in size through the addition of such food. It has no heart or circulatory system, and yet it distributes the digested food throughout the body, takes in oxygen, and throws off carbon dioxide as does every many celled animal. It has no kidney, but disposes of the waste matters of oxidation none the less, and so every function of the highest metazoa finds its counterpart in the vital activities of the primitive forms. Nor is the importance of these simpler processes of the protozoa any the less, in that they come very close to the ordinary physical and chemical processes that we are familiar with in non-living matter. As complete organisms, therefore, in a sense quite different from that

meant by Ehrenberg, the protozoa today offer a field of research in physiology that is quite unique, for while they epitomize the vital activities of the higher animals, these activities are of such simple types that they may be more easily observed and correlated with the ordinary reactions in physics and chemistry, reactions which we do not associate with the vital processes of the higher animals.

The warning may not be out of place here that despite the simplicity of function in the protozoön, and the analogy with reactions in the inorganic world, there is, nevertheless, a power of acting as a whole, a power of coördination combined with factors of adaptation and

FIG. 21

Food-taking. *A*, after Pénard; *B* and *C*, after Bütschli. *A*, *Raphidiophrys elegans*, H. and L.; *B*, *Oikomonas termo*, Ehr.; *C*, *Didinium nasutum*, O. F. M.; *f*, food particles.

evolution, which permit of development into more and more complicated structural units, which arises, per se, in all protoplasm, and raises it immeasurably above the most complex of non-living substances; this power of adaptation is an inherent characteristic of living matter, transcending physical or chemical analysis, and justifying, perhaps, the often abused term vitalism. It must not be forgotten that, notwithstanding the simplicity of the single functions, the protozoa are units exhibiting a complex of these activities and an harmonious working of them all, no less surely than fish, bird, or mammal. In studying these simple functions it is well not to forget that each belongs in the same category of activities as the functions of much more highly

evolved organs. Consciousness, for example, an attribute of the brain and central nervous system in general, is not seen as such in the protozoa, but its prototype irritability, with the coördinated responses to stimuli, is common to every protozoön, and such stimuli sometimes lead to reactions on the part of the protozoön which are often apparently directed toward a given end until we are tempted to interpret them as conscious acts. While most of the actions of protozoa are reactions to external stimuli, many are combinations of reactions that do not lend themselves to analysis. Such, for example, is the apparent choice of food or of building material for shells and tests, or the complex reactions that are frequently involved in the avoidance of some obstruction. Not infrequently such reactions have been interpreted as evidence that the protozoön acts wilfully, or with a certain amount of intelligence of the end to be accomplished, and they are frequently cited as examples of conscious activity on the part of these primitive forms. Many of these so-called conscious acts can be explained by the ordinary physical laws of fluids, and while one cannot deny that the protozoön's actions may be conscious, it seems much more probable that these activities are the fundamental, often physical or chemical, reactions which serve in evolution as the starting point for the infinitely more complex activities which we call our consciousness.

In all animals there is a certain amount of work done in the daily life, and the energy put into such work comes from the oxidation, or physiological burning, of the body protoplasm. There is, therefore, a constant waste of protoplasmic material which goes off as work done, as heat, or as residual waste matters comparable with the smoke and ashes of physical combustion. Such waste is made good by the addition of new raw materials in the form of food, which is made over into new protoplasm. The phenomena of waste and renewal are usually spoken of together under the name of metabolism—waste as destructive, repair as constructive, metabolism. Food getting, therefore, becomes the first necessity of the living thing, and the chief end toward which the fundamental structures of the body are directed, and this, whether in the highest mammal or the lowest protozoön, becomes the chief economic problem to be solved (Fig. 21).

The methods employed by different kinds of living things are widely varied, and the great problem is apparently well solved in many different ways. Green plants are the starting point for all living things, for they manufacture not only their own food, but indirectly the food for all other living things. This they are able to do because of the chlorophyl or green colored matter which they possess and which has the power to utilize the energy of sunlight in reducing CO_2 and manufacturing starch out of water and carbon. The further changes of the starch into more complex substances, and these into protoplasm of the plant, are buried in the obscurity of unknown chemical processes

which take place in the plant's protoplasm. Animals solve the problem of nutrition by living on plants, or by eating other animals which, either directly or indirectly, live on plants. Still other types live as parasites upon other animals, some, like the intestinal worms, using freely the foods that are prepared by, and for the use of, the host, while others, like some insects, suck the blood, or, like trichina, invade the cells and tissues, and live at the expense of the living protoplasm.

In the group of protozoa all of these methods of food getting are found. Many forms possess chlorophyl, and like the green plants,

FIG. 22

Synura uvella, a colony of phytoflagellates, often a source of disagreeable odors and tastes in drinking waters. (After Calkins.)

manufacture their food directly from simple elements. These protozoa are of considerable theoretical interest, for they stand upon the borderline between the animal and the plant kingdoms, and are sometimes classed as one, sometimes as the other. They are thus involved in what has been one of the most contested of biological problems, the limits of the animal and plant kingdoms, and the problem is the more difficult because some types of this intermediate group may on occasions make their food, while at other times they eat like undoubted animals and take in solid food (*Chromulina flavicans*, and some forms of dinoflagellata). The problem has but little significance in the present

day, for biologists recognize that it is only an academic matter after all, and merely affords further evidence of the artificiality of classification.

It is to these intermediate forms that we must turn for the causes of odors and tastes, which occasionally make potable waters unfit to drink. As shown in the previous chapter, the metaplastic products of vital activity are sometimes stored up in the cell as oils or fats, which, when liberated in a water supply, give rise to offensive odors and tastes (Fig. 2). Like all organisms which make their food, these suspended protozoa require salts of different kinds. Many such salts are normal to drinking waters, the nitrites and nitrates being almost invariably present, and these are the very salts most needed for the maintenance of these forms of life. Hence, it follows that if an infected water supply can be freed from an excess of such nitrogen-holding salts, the protozoa will disappear. If inlet and outlet of a given water supply are closed, the organisms soon exhaust the available food elements and die.

While some forms of protozoa are thus holophytic, like the green plants, others combine the holophytic with the animal, or holozoic method, while still other protozoa, and, indeed, the great majority of them, are entirely holozoic. They seize their food in the form of other minute living things and digest it in much the same way that higher animals do, all of the organs of the cell playing some part in the process. Food-getting, therefore, more than any other function of the body, has been the most influential in leading to morphological development.

Seizure of food is one of the most interesting of the protozoön processes, and is frequently accompanied by such complicated reactions on the part of the minute animal as to suggest wilful activity. In other cases it is quite mechanical, as, for example, in choanoflagellates, or in many ciliates. In these the motile organs, flagella, or cilia, create a current in the surrounding water toward the mouth, and this carries with it bacteria or minute pieces of disintegrated plant or animal matter. In *Vorticella campanula* and its allies the apparatus is most highly developed for this method of food taking. A powerful adoral zone of membranelles creates a vortex current toward the oral or vestibular opening, while within the vestibule a long, undulating membrane carries the current to the mouth opening. The protoplasmic area around the mouth is furnished with contractile muscle threads or myonemes, so that when any irritating object comes with the food current, the entire vestibular area, adoral zone and all, contracts into the cell body, while the myonemes of the distended stalk contract at the same time and draw the body away from the offending region. In other ciliates, like paramecium, colpidium, oxytricha, etc., the process is essentially the same except that the animal is not

attached nor provided with contractile fibrils. In all of these ciliated forms there is a definite and frequently very complicated mouth opening, but in the flagellated forms, as a rule, there is no permanent mouth, the entire anterior end of the cell forming a receptive area for food products swept toward it in the current created by the flagellum. This is a vortex current caused by the undulations of the long flagellum, which, at the same time, moves in such a way as to describe a cone whose apex is at the base of the flagellum and base at the tip. In some cases, as in the collared flagellates or choanoflagellata, the flagellum moves inside a protoplasmic, collar-like membrane, which, like a pseudopodium, can be thrown out or retracted by the animal. The surfaces of this collar are sticky, and small particles move down it to the floor of the collar pit, where they are taken into the body.

As the flagella and cilia are in constant action, and as the mouth is always open for more, these protozoa become, as Maupas pointed out, the gluttons, par excellence, of the animal kingdom, while the oral apparatus becomes strikingly modified and diversified.

Not all protozoa, however, are so persistent in food taking, and many of them, while provided with a mouth opening, keep the mouth shut until a food particle is to be eaten. Such forms live upon larger things than bacteria, and with them eating involves a regular swallowing process. In some cases this is combined with the food-getting activity of the flagella or cilia, and large particles of solid proteid matter, either in the form of small organisms or of disintegrated fragments of plant or animal brought with the current, are seized by protoplasmic processes, as in *Oikomonas termo*, or the mouth opens to swallow them, as in *Didinium nasutum*. There seems to be a remarkable power of distention in these mouth openings, for a didinium can take in an organism quite as large as itself (Fig. 21).

In those forms of protozoa belonging to the group suctoria there is no mouth opening, nor flagella or cilia to create food currents, but the animals are provided with tentacles, often twice as long as the diameter of the body, with which they seize passing organisms. Once seized, the victim struggles for a short time and then becomes quiet, as though paralyzed. Its protoplasmic contents are then sucked into the body of the captor, or, in some forms, the protoplasm of the captor passes into the body of the victim and there digests its meal.

Many protozoa set a trap for their victims, so that they become entangled as in a spider's web. This is the case with the majority of the great group of rhizopods, especially the foraminifera and radiolaria, where the pseudopodia form a network of branching protoplasm, or a forest of protoplasmic spines, in which the streaming of granules is constant, passing from the inner protoplasm of the shell outward to the farthest tip of the pseudopodia. The sticky character of the pseudopodia makes it difficult for any small animal to break away,

while its struggles furnish the stimulus for an accumulation of more protoplasm about it, and this, armed with digestive fluids, soon kills

FIG. 23

Allogromia, sp., with pseudopodial net and two diatoms. (After Calkins.)

the prey, which is then digested without even the formality of carriage into the shell of the captor (Fig. 23).

Other rhizopods, as an ameba, throw out pseudopodia under the stimulus of the touch of some other living animal or plant. These

FIG. 24

Actinobolus radians with tentacles partially retracted and with five ingested halterias; swimming. (After Calkins.)

surround the victim, which frequently does not begin to struggle until ensheathed in a wall of protoplasm, from which it rarely escapes. Large animals like rotifers, and relatively large plants like the desmids are thus captured and digested.

While most of the protozoa thus far described wait until the prey

comes to them, and take what they can get, others are predatory and go in search of food. These are the most interesting of all protozoa, for they are occasionally too fastidious, apparently, to take the ordinary run of the microscopic wilds, but seem to select their food with all the care of a gourmand. They are usually armed with offensive weapons in the form of trichocysts, which may be shot out from the surface of the body, or carried javelin-like, at the extremities of projectile tentacles. One of the most interesting of these types is *Actinobolus radians*, one of the most primitive and one of the surest of hunters (Fig. 24). "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 (Entz, 1883). When at rest, the mouth is directed downward, and the tentacles are stretched out in all directions, forming a minute forest of plasmic processes, among which smaller ciliates, such as urocentrum, gastrostyla, etc., or flagellates of all kinds may become entangled without injury to themselves and without disturbing the actinobolus or drawing out the fatal darts. When, however, an *Halteria grandinella*, with its quick and jerky movements, approaches the spot, the carnivore is not so peaceful. The trichocysts are discharged 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 contracted until the victim is brought to the body, where by action of the cilia it is gradually worked around to the mouth and swallowed with one gulp. Within the short time of twenty minutes I have seen an actinobolus thus capture and swallow no less than ten halterias." (Calkins, *The Protozoa*, p. 50.)

The complicated processes involved in this act of food-getting would certainly justify an Ehrenberg in the belief that actinobolus is capable of wilful actions to a certain end, and that in the apparent choice of food, and skill in bringing it down, it shows a high order of intelligence. It would be a natural tendency to interpret such activities in terms of our own consciousness, but it is much more probable that simple physical or chemical laws of attraction are at the bottom of it all, halteria possessing an attraction for the darts of actinobolus analogous to that between an iron filing and a magnet, or between various chemical elements.

In all of the above cases solid food is taken into the body of the protozoön and there disintegrated and digested. Many other protozoa have no mouth opening nor chromatophores to manufacture their food, but absorb it through the general surface of the body, as does a tapeworm. Such protozoa, like some of the lower plants, are saprophytes and get their nutrition in the proteid matter from disintegrating

plant and animal tissues, dissolved in the water. Other saprophytes live upon the juices in blood or other fluids of the animal body which are similarly taken in by osmosis; these, however, belong to the group of parasites or commensals, the difference between the two being largely one of degree only, a parasite exerting some deleterious effect upon the host, while a saprophyte and a commensal are harmless. In all such cases the protozoa multiply in the region, such as a water supply, or the fluids of the body, where food is most abundant and

FIG. 25

Digestion in a foraminiferon. (After Verworn.) A-E, successive stages in the disintegration of a ciliate (Colpoda) in a pseudopodium of Lieberkühnia.

where they are least disturbed by environmental factors. Thus, we would account for the immeasurable swarms of chilomonas in a meat infusion, or quantities of opalina in the frog's rectum, or the myriads of cytoryctes and neuroryctes in skin and brain of victims of smallpox and rabies.

In the higher animals solid food materials are taken into the food receptacles of the body, where a secretion from the lining epithelial cells is poured upon them, the food matter not coming in close contact

with the secreting cells. In the protozoa the solid food is taken directly into the living cell, and the processes of digestion are all within the living matter. Such a method is known as intracellular digestion, as contrasted with intercellular digestion of the higher animals (Fig. 25).

When a rotifer or other small animal is enwrapped by the pseudopodia of an ameba, or swallowed by an actinobolus or other predatory form, a certain amount of water is taken in with it so that the victim moves freely within the body of its captor and in its normal water environment. The water, with victim, forms a gastric vacuole or an "improvised stomach," and is surrounded on all sides by a wall of living protoplasm, and this soon begins to pour a secretion into the vacuole. With the first changes in chemical nature of the surrounding water the prey begins to struggle, and ceases its efforts to escape only when killed by the secretion. This, according to the researches of Fabre-Domergue, Meissner, le Dantec, and others, is acid in nature, but, beyond the fact that it is some mineral acid probably hydrochloric as in other animals, nothing is known as to the exact chemical nature of this digestive fluid. Whatever it is, its manufacture is intimately connected with the chromatin material of the nucleus, for Hofer and Verworn have shown that digestion does not take place when the nucleus is absent. This was determined by cutting an ameba into two parts, one of which contained the nucleus, the other, a recently ingested animal. The enucleated protoplasm retained its vitality for from nine to fourteen days without any change in the gastric vacuole; the nucleated fragment, on the other hand, soon recovered from the operation and began to digest as usual. It is probable that the minute particles of nucleoproteids that are constantly arising in the neighborhood of the nucleus contain digestive ferments which stimulate the formation of the mineral acid in the vicinity of the gastric vacuole.

In those protozoa in which the mouth is continually open, as in paramecium, vorticella, dileptus, bursaria, etc., the food is usually minute forms of unicellular algæ, or, most often, bacteria. These are collected in water in the protoplasm at the base of the vestibular opening until a great number are massed together, or until the vacuole has assumed a certain size. It is then caught up in the flow of protoplasm on the interior of the organism, and dragged away from the mouth, while a new vacuole begins to form. The process of digestion in one of the bacteria-eating vorticellids, carchesium, has been studied by Greenwood, who found that the aggregate of bacteria passes into a region of protoplasm in the immediate vicinity of the horseshoe-shaped nucleus, where the water disappears, leaving the bacteria in close contact with the protoplasm. This state of "storage" lasts for from one to twenty hours, and during the time the many separate or individual bacteria are massed together into a compact ball of food. This mass is then again surrounded by fluid, this time having a decidedly acid

reaction. Through the action of this acid the compact mass of bacteria is broken into minute fragments, which ultimately mix with the protoplasm as digested food. Although nothing further is definitely known about it, it is quite probable that the product of this digestive action is the formation of soluble peptones similar to the products of proteid digestion in the higher animals. This is rendered the more probable because of the extraction of a pepsin-like ferment from the myxomycete *Fuligo varians* by Krukenberg, and from the huge ameboid rhizopod *Pelomyxa palustris* by Dixon and Hartog.

The problem of the nature of the digestive processes in protozoa has an interest in connection with other questions of more vital importance. The nature of the digestive reaction in phagocytes in response to the food matters supplied are involved in the general subject of intracellular digestion. While the initial experiments of Engelmann, Metchnikoff, Le Dantec, Greenwood, and others showed that there is an acid reaction in the gastric vacuoles of certain forms of protozoa, their conclusion that digestion here is entirely due to the action of some ferment-like pepsin acting in an acid medium were apparently premature. The extraction by Krukenberg from fuligo, and by Dixon and Hartog from pelomyxa, of a digestive ferment which dissolves proteid in an acid medium, undoubtedly lends support to their view. But, on the other hand, Mouton ('02) extracted a digestive ferment from ameba which dissolves gelatin and fibrin in an alkaline medium, while Mesnil and Mouton ('03) extracted a similar ferment from paramecium. These observers, therefore, insist that the digestive fluid is more like trypsin than like pepsin.

An intermediate position was taken by Metchnikoff ('03), who, on the basis of repeated observations, claimed that the reactions in the paramecium vacuole are first acid and then alkaline. Feeding paramecium with powdered alizarin, which is colored reddish violet in an alkaline medium in which paramecium lives, he found that the vacuoles are at first of this same color. In from five to fifteen minutes the color changes from red to yellow, showing an acid reaction, and this, after from ten to fifteen minutes more, is changed again to the red, showing an alkaline reaction. Not all vacuoles are thus colored, a few giving the alkaline reaction throughout. Metchnikoff concluded, therefore, that proteid digestion in these protozoa follows the same course as in higher animals, a ferment acting in an alkaline medium following one which acts in an acid medium.

Nierenstein, repeating these experiments, confirmed Metchnikoff's observations, but came to the conclusion that the acid medium plays no part in the actual digestion of the food, serving merely to kill the living organisms taken in. Metchnikoff, however, in a later publication maintains that the bacteria swell in the acid medium and thus undergo the first steps in the process of digestion. These results differ to some

extent from those obtained by Greenwood in the case of carchesium, where the acid reaction is not forthcoming until after the "state of storage," a state varying in length of time from one to twenty hours. The chemical reactions in the later periods were not observed.

The protozoön, therefore, like phagocytes, evidently has the power of secreting different kinds of ferments in response to the stimulus of different kinds of living food particles. Not only proteolytic, but other kinds of ferments as well are formed in the various types of protozoa, although not by all kinds. Thus, some types of protozoa are able to create starch dissolving ferments similar to the diastatic ferments of higher animals, or fat emulsifying ferments similar to steapsin. In many forms, however, the starch grains, like other indigestible parts, are thrown out of the body untouched (Greenwood, Fabre-Domergue, Meissner).

The granules that are formed by the breaking down of food particles through the digestive process are ultimately distributed by means of the protoplasm streaming to all parts of the protozoön. Some are probably converted directly into protoplasm by an assimilative process that is as little understood in these forms as in the metazoa, a process involving synthetic changes whereby the relatively complex food elements are built up into still more complex protoplasmic molecules, thus leading to the repair of waste and to growth. Other granules are not immediately assimilated, but are stored up in the protoplasm as a reserve of nutriment. In these cases it is impossible to say whether such granules are utilized directly as fuel for functional activity through oxidation, or whether they are first built up into protoplasm and the protoplasm itself, or its products, oxidized. In all protozoa these reserve matters are present, giving the characteristic granular appearance to the protoplasm of these forms, and their disappearance may be easily followed by starving the individual. A paramecium, for example, when normal and active, has a characteristic granular appearance, while numerous gastric vacuoles are distributed throughout the inner protoplasm. When it is starved these granules disappear first of all, and then, with continued starvation, the protoplasmic network is used as a source of energy for the active animal, and great vacuoles appear which increase in size with starvation, while the size of the cell decreases to an eighth or a sixteenth of the normal volume, the macronucleus alone, although frequently fragmented, retaining its normal volume.

It often happens that some one of the many functions of metabolism fails to act, and the organism suffers from the failure to assimilate or from lack of oxidative ferments. I have frequently seen *Paramecium aurelia* so filled with these reserve food granules that its protoplasm appeared dense and black under the microscope (Fig. 26). In such cases there are no gastric vacuoles, food taking and movement stop,

division stops, and the animal, unless treated, invariably dies. The trouble seems to be due to the lack of oxidative processes, possibly because the nucleus fails to provide the necessary ferments. The tension is relieved and activity again started up by treating such an organism with salts like potassium chloride or potassium phosphate, or with the more complicated salts contained in an extract of pancreas. It is possible that in the latter case the extracts from the pancreas have some direct effect upon the granules in question, but such an explanation cannot account for the successful results with the simple potassium salts, and it seems more probable that the explanation lies in the fact that the stimulants act directly upon the nucleus and cause it to resume a neglected function. This conclusion is borne out by the fact that the tension is first relieved in the immediate vicinity of the nucleus (Fig. 26), and then progressively toward the ends of the organism.

FIG. 26

Paramecium aurelia in condition of protoplasmic "stability" (extreme left) and resumption of normal "labile" condition as a result of treatment with salts.

The inner processes of digestion are entirely unknown in the saprophytic forms of protozoa and in the parasitic forms, but there is reason to believe that it is taken up at the point of granule formation in other, holozoic, forms. In parasites like trypanosoma living in blood lymph the nourishment is probably derived from the digested food materials carried by the blood and upon which the organisms, presumably, live as saprophytes. Such forms are quite different, physiologically, from intracellular or intracorpuseular parasites, such as coccidia, malaria organisms, etc., which live upon the substance of the cells or blood corpuscles.

The free-living forms of protozoa are almost constantly at work; they are usually in motion, either in progressive movement, or, by action of their flagella or cilia, are creating currents toward the mouth. The energy for such work comes from the breaking down of complex molecules of protoplasm or possibly of digested food, which is accomplished by oxidation or physiological burning. The products of such combustion, as in physical combustion, are kinetic energy, heat, and residual matter, and the latter, like ashes, must be disposed of, or by accumulation they hinder and ultimately prevent the normal processes. The ordinary products of such physiological activity are solid or fluid matters consisting mainly of water, some mineral substances, urea, and a gas, carbon dioxide. In higher animals the former are disposed of through the medium of the skin in part, but mainly through the activity of the kidney, while the latter are thrown out through the skin and lungs, or gills. In protozoa, while there is the same need of elimination of the waste materials, there is in many forms no especial organ for the purpose, elimination of urea and of carbon dioxide taking place, as in some intestinal parasitic worms, by osmosis through the general surface of the body. Such is the case in all of the foraminifera and radiolaria, and in individual cases among the other types of protozoa. In other forms of protozoa, however, there may be special organs for the disposal of such waste matters. These are the contractile vacuoles which fill with fluids from the interior of the cell and then contract, emptying their contents to the outside through a minute pore, as in the majority of infusoria, or breaking through the outer wall of protoplasm at any point where the vacuole may be at the time of contraction, as in amebæ. The fluids of these contractile vacuoles are supposed to hold urea in solution as well as carbon dioxide, the experiments of Griffiths ('89) indicating the presence of urea, while biologists generally agree that carbon dioxide must also be present in the fluids discharged, although in no case has this been proved. Another function of the contractile vacuole may be, as Hartog early pointed out, the regulation of the tension in protoplasm and surrounding water and the prevention of large disruptive vacuoles through the constant addition of water taken in by the crystalloids of the cell. Whatever may be the function of the vacuole, it becomes a very important element of the cell in the more complicated forms of protozoa, and is frequently associated with long, branching feeding canals, which to Ehrenberg were evidences of a vascular system, since they ramify through the protoplasm, collecting fluid which is emptied into the contractile vacuole. While the function of such contractile vacuoles is elimination of waste or regulation of density, they cannot be absolutely necessary to protozoa, nor the sole means of disposing of waste materials, since great numbers of protozoa are without them.

Oxygen, necessary for the various processes of oxidation, is taken in through the general surface of the body and from the surrounding water. Little or nothing is known regarding its action in the protozoan cell.

Irritability.—"This liberation of energy is the 'response' to an action of itself inadequate to produce it, and has been compared not inaptly to the discharge of a cannon, where foot-tons of energy are liberated in consequence of the pull of a few inch-grains on the trigger, or to an indefinitely small push which makes electric contact; the energy set free is that which was stored up in the charge. This capacity for liberating energy stored up within, in response to a relatively small impulse from without, is termed 'irritability;' the external impulse is termed the 'stimulus.'" (Hartog, 1906, p. 8.) The sensitiveness or irritability of protozoan protoplasm has been a favorite branch of protozoön research, and is especially interesting in the light of comparative psychology, for here is the prototype of higher animal consciousness. It is manifested in a great variety of ways, and the manifestations have been grouped into categories called taxes or tropisms. Nearly all of these reactions take the form of motion in some form or other, and are usually called out in response to stimuli, which may be of various kinds. Mechanical stimuli, light and heat rays, electricity, diffusing chemical substances, all exert some effect on the movements of protozoa, sometimes toward the source of stimulation (positive taxis), sometimes away from it (negative taxis). It is this irritability of protoplasm that frequently saves the life of the small organism, or provides it with food. Positive thigmotaxis is the name given to that reaction of a paramecium, for example, when it approaches and adheres to some larger object where its bacterial food may be concentrated; positive chemiotaxis is the reaction shown in the sudden extension of the tentacles of *actinobolus*; positive or negative aërotaxis is that reaction whereby the organism so places itself in a medium that irritability is reduced to a minimum, and so on, all movement probably being a response to stimuli which owe their origin either to external or internal causes, the latter due, perhaps, to the varying conditions of hunger, fatigue, and the like.

The most extensive and illuminating observations on this aspect of protozoan physiological activity have been made by Jennings, and the results of his long studies on the behavior of lower organisms are well stated in his own words in the following theses (Jennings, 1906, p. 261):

1. "First, we find that in organisms consisting of but a single cell, and having no nervous system, the behavior is regulated by all the different classes of conditions which regulate the behavior of higher animals. In other words, unicellular organisms react to all classes of stimuli to which higher animals react. All classes of stimuli which

may affect the nervous system or sense organs may likewise affect protoplasm without these organs. Even the naked protoplasm of ameba responds to all classes of stimuli to which any animal responds. The nervous system and sense organs are, therefore, not necessary for the reception of any particular classes of stimulations.

2. "The reactions produced in unicellular organisms by stimuli are not the direct physical or chemical effects of the agents acting upon them, but are indirect reactions, produced through the release of certain forces already present in the organism. In this respect the reactions are comparable with those of higher animals. It is true for ameba as well as for more differentiated protozoa.

3. "In the protozoa, as in the metazoa, the structure of the organism plays a large part in determining the nature of the behavior. There are only certain acts which the organism can perform, and these are conditioned by its organization; by one of these acts it must respond to any stimulus. If the behavior of the metazoa is comparable in this respect to the action of a machine, the same comparison can be made for the behavior of the protozoa.

4. "Spontaneous action—that is, activity and changes in activity induced without external stimulation—takes place in the protozoa as well as in the metazoa. Both vorticella and hydra, as we have seen, spontaneously contract at rather regular intervals, even when the external conditions remain uniform. Continued activity is the normal state of affairs in paramecium and most other infusoria. The idea that spontaneous activity is found only in higher animals is a totally erroneous one; action is as spontaneous in the protozoa as in man.

5. "In unicellular organisms, without a nervous system, certain parts of the body may be more sensitive than the remainder, forming thus a region comparable to a sense organ in a higher animal. Whether such a part may become more sensitive to one form of stimulation while insensitive to others, as in higher organisms, seems not to have been determined.

6. "Conduction occurs in organisms without a nervous system. This is, of course, seen in the fact that a stimulus limited to one part of the body may cause a contraction of the entire body, or a reversal of cilia over the entire body surface. A strongly marked case is the contraction of the stalk in vorticella, when only the margin of the bell is stimulated.

7. "Summation of stimuli occurs in protozoa, as in metazoa. This is shown most clearly in Statkewitsch's experiments with induction shocks. Weak induction shocks have no effect until frequently repeated.

8. "In the unicellular animal, as in that composed of many cells, the reaction may change or become reversed as the intensity of the stimulus increases, though the quality of the stimulus remains the

same. Such a change in reaction has sometimes been claimed as a specific property of the nervous system. The protozoa ameba and stentor, as well as the metazoan planaria, move toward sources of weak mechanical stimulation, away from sources of strong stimulation.

9. "In the protozoa, as in the metazoa, the reaction may change while the stimulus remains the same; that is, the animal may respond at first by a certain reaction; later, while the stimulus remains the same, by other reactions. This has been shown in detail in the account of stentor. The change may consist in either a cessation of the reaction or in a complete alteration of its character. These changes are, as a rule, by no means due to fatigue, but are regulatory in character. The behavior thus depends on the past history of the organism. For such modifications of behavior a nervous system is then unnecessary.

10. "In the protozoa, as in the metazoa, the reactions are not invariably reflexes, depending only on the external stimulus and the anatomical structure of the organism. The reaction to a given stimulus depends upon the physiological condition of the organism. In stentor we could distinguish at least five different conditions, each with its characteristic reaction to the given stimulus.

11. "In unicellular, as well as in multicellular, animals we find two chief general classes of reactions, which may be designated as positive and negative. The positive reaction tends to retain the organism in contact with the stimulus, the negative to remove it from the stimulus. In many classes of stimuli we can distinguish an optimum condition. A change leading from the optimum produces a negative reaction, while a change leading toward the optimum produces no reaction, or a positive one. The optimum from this standpoint usually corresponds, in a broad way, to the optimum for the general interests of the organism. These relations hold equally for protozoa and metazoa.

12. "In both the protozoa and the metazoa that we have studied, the behavior is based to a considerable degree on a selection of certain conditions through the production under stimulation of varied movements. When the organism is subjected to an irritating condition, it tries many different conditions or many different ways of ridding itself of this condition, until one is found which is successful.

"Altogether, there is no evidence of the existence of differences of fundamental character between the behavior of the protozoa and that of the lower metazoa. The study of behavior lends no support to the view that the life activities are of essentially different character in the protozoa and metazoa. The behavior of the protozoa appears to be no more and no less machine-like than that of the metazoa; similar principles govern both."

Growth and Reproduction.—In all of the constructive processes of the cell there is no doubt that the nucleus plays the most important part, and that it is, in a sense, the directive centre of activi-

ties. This is shown by the behavior and history of enucleated fragments, which, as we have seen, cannot digest food; other functions are similarly crippled by removal of the nucleus, and movement itself is greatly impaired. A contractile vacuole will reform and will contract to a certain extent in enucleated protozoa, but it will not act normally and soon ceases to contract, swelling then, with the continued addition of fluids, until the cell bursts, as in the characteristic phenomenon of diffuence.

When the constructive activities of the protozoan body exceed the destructive, and when the addition of new raw material exceeds the waste, new protoplasm is added to the old and growth results. The dimensions of the cell are increased in all directions, the increase taking place in the fluid protoplasm apparently throughout all parts of the cell at the same time, a process of growth by intussusception. The mere accumulation of reserve food granules plays no part in growth, all growth ceasing when the cell becomes packed with them, but must take place only after the necessary constructive changes have converted such reserve stores into protoplasm. Growth continues until the cell has attained to a more or less definite, optimum size, and then it divides into two or more small cells according to the species.

The explanation of growth is one of the unsolved problems of biology, and we get but little nearer the solution in the case of protozoan organisms than in the higher forms of life. We know, indeed, that growth ceases with the elimination of the nucleus, hence, we conclude that the nucleus is a necessary factor in the process. Growth in the protozoa can be controlled in a variety of ways, and we know that certain conditions of temperature, of density, and the like, are necessary. While the explanation of the finer processes of growth is far away, the solution of the problem of cell division is almost equally remote, and no theory yet propounded satisfies the conditions as we see them in the various forms of life. Spencer's theory of volume and surface is very seductive; indeed, it may be a step toward the final solution. Briefly stated it predicates that a normal relation exists between the protoplasm and the nucleus of the cell, and, if the form remains the same, this relation is disturbed by growth, for the surface of the organism increases as the square of the diameter, while the volume increases as the cube. Hence it results that the mass increases faster than the surface which provides the means of interchange with the environment (absorption and the like). The changed ratio of surface to mass of protoplasm, according to Spencer and his followers, brings about internal changes which result in cell division. But after this theory is stated, we know nothing more about the ultimate causes of cell division than we did before. When the nature of the changes is understood, the reason for cell division will naturally follow. Leaving aside the causes of cell division, and looking at the phenomena

alone, we find a far more satisfactory state of affairs, for the details of the process are known in many different cases.

Whatever the causes of cell division may be, whether limits of growth or sun spots, the fact is established that the first indications of the process in the majority of cases are found in the nucleus. Here we are dealing with a universal biological phenomenon, the division of a cell, and the protozoa are interesting in this connection because of the variations in the process which they present, and also because the structures involved are less complicated than those of higher animal and plant cells, and, therefore, more easily analyzed. In all tissue cells of normal character, division is brought about through the medium of a peculiar structure of the nucleus known as the mitotic or karyokinetic figure. Under ordinary vegetative conditions of the cell, the

FIG. 27

A micronucleus of *Paramecium aurelia* in division.

nucleus contains chromatin substance in the form of granules arranged in a more or less definite network or reticulum. Prior to cell division these granules become rearranged in a much wound thread or spireme, and later the spireme thread is divided across into a number of short chromatin elements known as the chromosomes, the number of such chromosomes being constant for all of the cells of the same species of animal or plant. The number of these chromosomes in no way indicates the degree of differentiation of the organism, nor its position in the animal or plant scale, some protozoa, for example, having a larger number of chromosomes than does man. In the ordinary process of mitosis these chromosomes are arranged in the centre of a spindle-formed nuclear figure consisting of fibers of kinetic substance focussed at two poles, these poles characterized by the presence of

small granules of deeply staining substance, the centrosomes. The centrosomes, spindle fibers, and chromosomes, to which the spindle fibers are attached, are collectively known as the mitotic figure, and few cells that are known divide without the formation of this mitotic figure, or some modification of it (Fig. 27). It represents, therefore, the mechanism of cell division, and further, since the hereditary characteristics are now known to be connected in some way with the chromosomes, the mitotic figure becomes the mechanism of heredity. The chromosomes, while in the equator of this mitotic figure, or in some cases even before the mitotic figure is formed, are divided by a cleft which passes from end to end through the centre, and the two halves, as the daughter chromosomes, are apparently drawn apart by the mechanism of the mitotic figure; the cell body is then divided into two daughter cells by a constriction or cleft passing through the middle; the nuclei reform their characteristic reticular condition, and the two cells are then ready for further processes of digestion, assimilation, and growth.

Ever since 1883, when Roux first called attention of biologists to the extreme care with which the chromosomes were halved and distributed to the daughter cells, and especially since the publication of Weismann's classical essays on the nature and constitution of the germ plasm, these elements of the cell have been recognized as the physical basis of inheritance, and their mode of origin and complete history have been the chief subject for study by cytologists. Not only the chromosomes, but the entire spindle figure as the mechanism by which they are divided, has also demanded the attention of biologists.

In this branch of biological research the protozoa have played an important part, for in these cells we find the simplest types of the division figure and the simplest forms of the chromosomes, while cell division is found in every conceivable form, sometimes strikingly similar to the division of a metazoan cell, as in some heliozoa, sometimes so highly modified as to be regarded as a type by itself, as in the budding forms.

Cell division, therefore, which Spencer interpreted as marking the limit of growth of a cell, is inaugurated through some change in the relations of nucleus and cytoplasm, and some change which is entirely unknown. In many protozoa the process is so different from tissue-cell division that other names are given to it. We recognize: (1) Simple binary division of the cell into equal parts, or simply cell division. (2) Unequal division of the cell, the smaller part being pinched off from the larger as a bud. This is known as budding or gemmation, and is only a slight modification of cell division. (3) Spontaneous division of the cell into four or more, frequently a great number of daughter elements, each with a portion of the original cell nucleus, the process being known as spore formation or sporulation.

These various modifications of the process of division or reproduction in its broadest sense may be conveniently summarized as follows:

FIG. 28

Trypanosoma gambiense; stages in longitudinal division. Original from a preparation by F. W. Baeslack.

In a number of protozoa, the cell before division draws in or throws off its motile organs, rounds out into a sphere, and then divides into two equal parts. This is the case in some of the heliozoa, a nuclearia, for example, which is very plastic with freely moving and often branching pseudopodia, becomes spherical and then divides through the middle, the entire operation, as seen under the microscope, taking not more than a minute.

The process becomes more complicated in those forms with complex motile organs. In some cases, as in some forms of trypanosoma, the flagellum is divided throughout the entire length, but in other cases the basal body alone divides, a second flagellum being formed

from the free half, while in still other cases it is discarded before division, and, as in *Copromonas*, each daughter cell creates a new one (Fig. 28). Similarly with the infusoria, some forms like *Paramecium*, *Colpidium*, etc., have a cover of uniform cilia which are retained during the act of division; indeed, the organisms swim vigorously throughout the entire process, but in other forms, as *Euplotes patella*, *Oxytricha*, *Stylonychia*, etc., the more complex motile organs are discarded and formed anew by the daughter cells (Wallengren) (Fig. 29).

In the flagellate *Noctiluca miliaris* (Fig. 30), the division is accompanied by very complicated nuclear changes, and a division figure is formed which recalls the mitotic figure of the metazoan cells. The chromatin in the ordinary conditions of the cell is contained in a few

FIG. 29

Euplotes patella in division. The macronucleus is not quite divided, the daughter nuclei being connected by a delicate strand.

large chromatin reservoirs or karyosomes; these disintegrate prior to division, and the granules thus formed collect in lines, the chromosomes, which are oriented toward one pole of the nucleus. At this pole, but on the outside of the nuclear membrane, lies a large centrosome or division centre, which divides during the time of disintegration of the karyosomes and forms a central spindle between the two halves. The nuclear membrane next disappears in the region between the chromosomes and the spindle, but is retained elsewhere, and special spindle fibres grow out from each of the division centres and become attached to the ends of the chromosomes. The division centres then move apart and the chromosomes are drawn asunder, each having divided through the middle.

An entirely different mode of division is found in some of the more simple flagellates. *Euglena*, for example, divides without any rupture of the nuclear membrane and without any definite mitotic figure (see Fig. 10, p. 30). The chromatin is in the form of granules distributed throughout the nucleus, and surrounding a central, deeply staining, larger granule, the division centre. When the cell divides this granule first divides into two equal parts, about which the chromatin granules are equally massed, and it corresponds to the entire mitotic spindle of metazoan cells. This type of nucleus (the centronucleus) is quite common among the protozoa, and from it we can trace the evolution of the mitotic figure of higher animal cells through forms like *Noctiluca* and the heliozoa.

In some forms among the flagellates, and in some infusoria, there is no definite nucleus, but the chromatin granules are distributed throughout the cell unconfined by a nuclear membrane. This is the case with some forms of *tetramitus* and with some ciliates like *dileptus*. In the

FIG. 30

Nucleus of *Noctiluca miliaris* in division. The light streak through the middle is the groove in which the central spindle lies.

former, the chromatin granules collect about the division centre at the time of cell division, and the nucleus then divides like one of the centronucleus type. In the latter each of the separate granules divides, although this does not mean that each granule is represented in both daughter cells; on the contrary, only those granules pass into a daughter cell that lie in the half of the parent organism represented by that daughter cell. Division here is a means of keeping the quantity of chromatin material and the active surface up to a standard (Fig. 31).

Budding differs widely from simple division, in its external appearance, at least, for, in the majority of cases, the nucleus does not divide until the daughter individual is nearly formed. In many rhizopods, for example, the protoplasm swells out as a large protuberance from the surface of the cell until it is quite as large as the parent cell, and then the nucleus divides and the organisms move apart, each with a nucleus and an equal portion of the protoplasm. This is the case in

forms like arcella, difflugia, or euglypha, where the cell is enclosed in a test or shell. Here the protoplasm wells out of the mouth opening of the shell until it forms a counterpart of the parent organism, then the nucleus divides, as stated, and the two individuals separate. Such a method is complicated, and to a certain extent anticipated, by the organism, for long before the cell divides the shell plates of a euglypha are formed and stored up in the protoplasm about the nucleus of the parent organism, to be used only when the bud has reached a certain size. They then flow into the bud with the protoplasmic streaming, and arrange themselves on the outside of the bud protoplasm, where they form a tightly fitting shell (Fig. 5, see *A*, p. 23). In other cases the buds are much smaller than the cell which forms them, and

FIG. 31

Dileptus, sp., with distributed nucleus in process of division. Each of the chromatin granules is drawn out in the form of a rod and divides (see Fig. 2, p. 19).

they first appear as mere protuberances on the surface of the parent (Fig. 32, *E*). This is the case in forms like sphastrum, for example, and several buds may form at one time. These are frequently different from the parent and are often provided with motile organs of a different type. Thus, in the heliozoa the buds may have pseudopodia of the lobose type and move around like small amebæ, or they may have flagella and move around like flagellates. The former are called pseudopodiospores by Lang, and the latter flagellispores. In all cases, however, the bud soon loses its larval motile organs and develops into an organism similar to the parent (see Fig. 11, p. 31). In the case of acanthocystis, the buds require five days for their complete development, the characteristic centralkorn and the ray-like pseudopodia appearing on the sixth day (Schaudinn).

Budding, in cases like the last, is very similar to spore formation, and can scarcely be distinguished from it. Many instances of budding are presented by different groups of the protozoa, and in all of them the process is characterized by the fact that the parent organism continues to live as an individual after giving rise to these motile offspring. In spore formation, on the other hand, the substance of the parent in the majority of cases is used in the formation of the offspring, and it loses its life as an individual.

In noctiluca the buds are formed after the nuclei divide, and appear as minute swellings on the surface. The nuclei in these swellings divide repeatedly until about five hundred buds are formed; these

FIG. 32

Entameba histolytica. (After Craig.) A, organism showing rods and granules of chromatin in the nucleus, vacuole with some stained substance, and dense ectoplasm; B, the chromatin of the nucleus passing into the cell plasma, where it is distributed as chromidia, shown in C; D, aggregation of chromidia to form secondary nuclei (see Fig. 51, of *Ameba limax*); E, "spore formation" by budding; F, spores of *Entameba histolytica* as seen in feces.

develop two flagella similar to those of the dinoflagellata, and swim off. After a time one of the flagella turns into a tentacle, and the characteristic structures of the adult are then formed (Ishikawa).

Budding is the characteristic method of reproduction of the suctoria, and is interesting from the fact that it may be either on the surface, as in ephelota, or inside the body, as in acineta (Fig. 33). The latter condition is derived from the former by the bud-forming area sinking below the surface and being covered over by a membrane so that a small brood pouch is created within which the buds swim about by means of their embryonic cilia before making their escape (Fig. 34).

This so-called endogenous budding is perhaps the forerunner of the curious method of spore formation, or, better, budding, which occurs in one group of the sporozoa, the neosporidia. Here the individual continues to live while forming buds, as in acineta, within its protoplasm. Such buds, known as pansporoblasts, then form peculiar thread-bearing spores, the entire substance of the bud being used in

FIG. 33

Ephelota bütschliana, a budding individual with five daughter buds. *N*, macronucleus, which forms a branching organ connected throughout. (After Calkins.)

the formation of the spores, and these small bundles of spores are carried about by the grandmother organism until its protoplasm is loaded with them, and until it appears like a huge cyst filled with spores (see Fig. 61, p. 145). These organisms are frequent parasites on fish, where they may be the cause of costly epidemics.

Budding, furthermore, is frequently associated with the process of conjugation; the mother cell, loaded with chromatin granules in the

chromidia form, gives rise to numerous buds, each of which is provided with chromidia, but with no part of the vegetative nucleus. Such buds ultimately form the conjugating gametes in forms like arcella, difflugia, centropyxis, etc. In parasitic forms like *Entameba histolytica*, the cause of tropical dysentery, or *Neurorhynchus hydrophobiae*, the cause of rabies, there is a similar bud formation, the buds having the characteristic chromidia; their further fate, however, is unknown, the sexual processes of these organisms not having been made out (see p. 303).

A much more highly evolved method of division is found in some of the colony forms of protozoa, where, as in *Gonium pectorale* (Fig. 35), for example, each of the sixteen cells of the parent colony forms simultaneously a daughter colony of sixteen cells. Here

FIG. 34

Endogenous budding in Suctorians. (After Bütschli.) *A, B*, two stages in the formation of the bud in *Tokophrya quadripartita*, Cl. and Lach.; *c*, the bud liberated as a "swarmer;" *C*, buds (*e*) in *Acineta tuberosa*, Ehr.; *d*, a bud liberated.

simple division is followed by association of the daughter cells, and individuals result which have passed through an actual, although primitive, ontogeny.

In spore formation, finally, we find one of the most prolific methods of reproduction known. Here the organism breaks down simultaneously into great numbers of daughter elements, each dissimilar to the parent in size if not in other characters. This process, involving as it does the cessation of normal vegetative life with its ordinary processes of digestion, assimilation, etc., usually takes place under the protection of an outer covering or cyst, such encystment being a common phenomenon among the protozoa, an outer covering of gelatinous material being thrown out on the surface of the organism whenever the conditions of the environment become unsuitable. This investment becomes

firm and membrane-like upon continued contact with the water, and, finally, if conditions continue unsuitable, it turns into chitin, which withstands drought or heat, and within it the reduced sphere of protoplasm is protected until conditions are again favorable. The chitin is then reduced or dissolved by enzymes from within the cell, or by external agents acting on it, and the organism creeps out and

FIG. 35

Gonium pectorale in reproduction. Each of the sixteen cells of the colony is dividing to form a daughter colony of sixteen cells. (After Calkins.)

resumes active life. Within such protecting cysts many different types of protozoa go through the often complicated processes of spore formation. In some cases the protection seems to be hardly necessary, and spores are formed and liberated before the membrane has had an opportunity to harden. This is the case in *colpidium* and in *Tillina magna*, for example; in *colpidium*, four or eight daughter cells

may be formed within the cyst, in *tillina*, only four, and these are all alike, and, except for the smaller size, similar to the parent organism. In many cases only two daughter individuals are formed within the cyst, a fact showing that it is not a long step from the process of simple division to that of such so-called spore formation, and *tillina* and *colpidium* are examples illustrating the transition from the one mode of reproduction into the other. *Tillina* rarely varies from the formation of four spores, and then only to revert to the apparently ancestral mode of simple division. *Colpidium*, on the other hand, has progressed farther toward obligatory spore formation, and not infrequently forms eight spores within the temporary cyst. Other forms of ciliate infusoria form a varying number of spores; in some, as in *Holophrya multifilius*, a great number of swarm spores are developed in the cyst, each similar to the parent. It is a question whether such reproductive elements are entitled to the name spore, for they are not formed by the simultaneous fragmentation of the mother organism, but by repeated division, the cleavages following one another in rapid succession; in some cases, indeed, as in *tillina*, the divisions follow so closely upon one another that the two planes of division are sometimes seen at the same time, and this activity is followed by a period of rest lasting for from twelve to twenty-four hours or longer, according to the vitality of the individual. If this is not simultaneous, it is very close to it, and the process in these ciliates must be due to the same, or at least to similar, physiological causes that bring about spore formation in other cases.

Spore formation, apart from the spores that are formed in preparation for fertilization, is uncommon among the protozoa and is found chiefly in the one group—sporozoa—which gets its name from this method of reproduction. In many of the flagellates, however, it seems to be a method of reproduction which follows conjugation. Thus, in *Tetramitus rostratus* and *Cercomonas longicauda* a cyst is formed immediately after conjugation of two similar cells, and within the cyst the protoplasm fragments into hundreds of minute flagellated organisms. In these cases the ordinary method of reproduction is by cell division, the spore formation appearing to be a special method that follows upon fertilization (Fig. 67, p. 155).

It is in the group of the sporozoa that we find the highest development of the spore-forming power, and here it has been found necessary to distinguish between the spores that are formed sexually, *i. e.*, after fertilization, and those that are formed asexually, for they differ both in structure and in function. The spores that are formed after fertilization are protected by firm and resisting coverings, and are able to live outside of the body of the animal in which they are definitive parasites; the other type of spores, formed asexually, have no such coverings and cannot live apart from the host. With these various

differences to take into account, the use of the term spore has been very ambiguous and misleading, and protozoologists have given it up for two other terms, sporozoite and merozoite, now generally adopted. The term sporozoite is used to designate those spores or germs that are

FIG. 36

Life cycle of *Coccidium schubergi*. (After Schaudinn.) 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 a merozoite (*c*). These enter new epithelial cells, and the cycle is repeated many times. After five or six days of incubation, the merozoites develop into sexually differentiated gametes; some are large and well stored with yolk material (*d*, *e*, *f*); others have nuclei which fragment into many smaller particles ("Chromidien"), each granule becoming the nucleus of a microgamete or male cell (*d*), *h*, *i*, *j*). The macrogamete is fertilized by one microgamete (*g*), and the copula immediately secretes a fertilization membrane which hardens into a cyst. The cleavage nucleus divides twice, and each of the four daughter nuclei forms a sporoblast (*k*) in which two sporozoites are produced (*l*).

produced after fertilization, while merozoite is used for the asexually produced germs. The protected sporozoites have the power to carry the disease from one host to another, while the merozoites, as a rule, carry the infection only from one part of the host to another part (Fig. 36). Sporozoites, therefore, have the full potential of vitality

of a new individual, while merozoites have a shorter life to run and a lessened vitality (see Chapter III).

Merozoite formation is best illustrated by the coccidia, a group of cell-infesting sporozoa, and the genus *adelea* is an interesting type, because it combines asexual reproduction with sexually differentiated organisms. A word here as to the significance of this fact. In the sporozoa, both in the gregarinida and the coccidiidia, the cycle ends with the formation of sexually differentiated reproductive bodies, one of which is larger, corresponding to an egg cell, the other very minute and similar to a spermatozoon; the former is called a macrogamete, the latter a microgamete. The mother cells of these gametes are not visibly different in many cases, and it is impossible to tell whether a given cell will produce one or the other. In some cases there is a slight difference either in size, or in possession or absence of granules, or in the make-up of the nucleus. These differences do not go far back, as a rule, and in the ordinary run, male and female cannot be distinguished. In *adelea* and a number of other forms, however, the sexual differences do go back almost to the fertilized cell, and it is possible to distinguish any given cell as female or male. The formation of asexual reproductive elements, or merozoites, in these different parents is the same, and begins with the division of the nucleus into as many parts as there will be merozoites, in *adelea* usually twelve to sixteen. After their formation they occupy a peculiar and characteristic position, being rolled together like staves of a barrel, or like the segments of an orange, a peculiar arrangement which has given rise to the name *corps en barillet*, while the term eimerian cyst is also used designate the parent membrane cyst where they are formed (Fig. 20, A).

The sporozoites differ but little from the merozoites when they are deprived of their protecting cases. After fertilization of the macrogamete, which will be described in a later chapter, the nucleus of an ordinary coccidian, such as *Coccidium schubergi*, for example, divides twice and the protoplasm surrounds them in equal masses; these are the sporoblasts. The nucleus of each sporoblast then divides again, while the protoplasm secretes a sporoblast membrane, one of the protecting coats of the sporozoites. The second division of the nucleus in each sporoblast provides the nuclei of the sporozoites, two developing in each sporoblast. The germs are then protected by the sporoblast membrane, and by a membrane which is secreted by the original cell, and with this double safeguard the germs of the organism are thrown to the outside, where no further development takes place until the sporocysts are swallowed by some new host (Fig. 36, I).

The variations in these processes of merozoite and sporozoite formation are legion, and they are of great importance economically, as well as interesting biologically, but their description belongs rather to the special chapters dealing with protozoan diseases.

The protozoa are, then, complete living organisms, in which no function found in the higher animals is lacking, and we have seen enough of their structures and functions to show how the scope of protozoölogy leads us into all fields of biological pursuits, from taxonomy, the description and classification of living things, through morphology, physiology, cytology, psychology, and theoretical biology. In the following chapters I wish to show how this scope widens out and leads us into some of the most difficult, but at the same time fascinating, problems of biology.

CHAPTER III.

PROTOPLASMIC AGE OF PROTOZOA.

UPON watching one of these simple organisms through the microscope there is a certain fascination in the idea that this minute bit of naked protoplasm has been continuously living since life appeared upon the earth. As a matter of fact, the same sensations might be experienced upon gazing at any of our fellow-beings, or, indeed, at any other living thing; but somehow we do not think of the latter in this way; we associate with them the ideas of age, of senile degeneration and natural death, concepts which do not seem to be associated with the free-living cell. It would appear, furthermore, that the ameba protoplasm which we see under the microscope, and which has lived continuously for all of these ages, might continue to live for an indefinite time in the future. It would seem that this perfectly balanced cell, with its powers of growth and reproduction, would be self-sufficient, containing within itself the potential of an endless existence. Such, however, is not the case, protozoa, like metazoa, may die of old age.

In every higher animal we recognize certain more or less definite periods of physiological activity, and according to these we roughly divide the span of life into three stages, which are in no way sharply outlined. These we call the stages of youth, adolescence, and old age. Youth, characterized by a high degree of vitality, is the period of rapid cell multiplication and growth; organs are formed and perfected, functions are unimpaired and active and the body is a perfect living thing. The second period is characterized by functional and sexual maturity; the multiplication of tissue cells is less rapid; the organs strengthen and their functions are more perfectly correlated; growth comes to an end. In the perfected animal it is the period for perpetuation of the race, and in conformity with this great function sexual differentiation is fully established. The third period, old age, brings a marked change, the potential of vitality wanes, cells atrophy, and functions weaken; degenerations of all kinds appear; and cumulative weakness ends in natural death.

These three periods are characteristic of all of the higher many-celled animals, the last period being rarely seen in nature, because in the wild animals a violent death follows the early functional weakening and inability to fight off enemies. Do we find the same sequence of

physiological changes in the unicellular animals, and can we distinguish periods of youth, maturity, and old age?

Since the fundamental biological laws are much the same, on a priori grounds alone we should expect to find the same series of changes in protozoa as in the metazoa. But while we do find them in protozoa, they are manifested in a way that we would not at first suspect. We have been accustomed to look upon the single-celled ameba, or paramecium, or other protozoön, as a complete individual in itself; but when we come to compare such an individual with a metazoön we do not find the analogous periods of vitality which in metazoa we recognize as youth, adolescence, and age. A protozoön is a free-living cell, a complete organism indeed, but as such it has no period of youth nor of sexual maturity, nor, by itself, old age. It is formed by division or some modification of division; it regenerates the normal form in a few hours, and then again divides; with division its individuality is lost, to be merged into that of two new individuals, these two into four and so on. Obviously such an individual cell presents nothing comparable with the sequence of stages so characteristic of the "individual" in higher forms of life.

Students of the protozoa and biologists generally (*e. g.*, Bütschli, Weismann, etc.) early called attention to the fact that not the single cell of a protozoön, but the entire succession of cells that may be formed from the period of one conjugation to that of the next, should be compared with the metazoön. In the latter, the fertilized egg cell gives rise to a multitude of body cells by repeated divisions; the cells are bound together to form a uniform and differentiated whole. In the former, the fertilized protozoön divides, but the cells do not remain bound together; they separate and live as independent units. If we could take such an entire succession of cells thus formed from the repeated divisions of a fertilized protozoön, and if at any given period could combine them in one mass of cells, we would have the analogue of a metazoön and would find that the protoplasm represented by the aggregate of cells would manifest the same successive periods of vitality as those of youth, adolescence, and old age in metazoa. We would find that the young cells divide more rapidly than they do later in the cycle; we would find that after a certain period they become sexually mature and able to conjugate and so to perpetuate the race; and we would find that, ultimately, evidences of weakened vitality and degeneration appear in the aggregate of cells, and that they would finally die of old age.

Not only would such an aggregate show the characteristic periods of vitality, but with the changes from one period to another there would be, in a great number of cases, accompanying changes in the form of the cell body; changes of so great a nature that a casual observer would never regard such cells as belonging to the same

species as those of the younger generations. It is for this reason, mainly, that in recent years a number of biologists have strongly advocated the use of the entire life cycle of a protozoön rather than the cell, or many cells in the same stage of vitality, for the basis of species.

While Bütschli ('76) was the first to note the differences in vitality in a race of protozoa, and Hertwig, Maupas, and a score of others added many observations on different periods, it was Schaudinn (1900) who first clearly perceived the importance of studying the complete life history of every species. It is because of this importance that the life cycle forms such a conspicuous part of the definition of protozoa as given at the beginning of Chapter I.

Before outlining a typical protozoön's life history, it will be necessary to understand clearly what is meant by age in protoplasm. It is quite evident, broadly speaking, that there is some protoplasm that does not die, the living things on the earth today testify to that, for they represent protoplasm that has been continuously living since the advent of life on the earth, and which, through posterity, will continue for an indefinite time in the future. Such protoplasm forms the substance of the germ cells, and they alone of all cells have the potential of an indefinite existence. But this capacity to live without finite end is bound up with a biological phenomenon as little understood as life itself, namely, fertilization. Without the union of two germ cells even this endowed protoplasm would die no less surely than do tissue cells. The protozoa are like both tissue cells and germ cells, and consist of protoplasm which is differentiated into somatic and germinal parts, and this protoplasm, like that in higher cells, will die of old age if fertilization or its equivalent is prevented. The problem of age in protozoa, then, has to do with vitality as apart from the union of germ cells and as manifested in the ordinary processes of vegetative activity.

I. A TYPICAL LIFE CYCLE.

The manifestations of protoplasmic activity which occur in all cells from monads to man, involving processes of digestion, growth, irritability, etc., are easily studied in *Paramecium aurelia*, a very common infusorian that may be found in any stagnant ditch or pool (Fig. 37). To a trained eye it may be seen without the aid of a lens as a minute white spot of protoplasm which moves from place to place in an irregular line of motion. When magnified it appears as an asymmetrical, cigar-shaped organism, with a somewhat spirally wound depression or "peristome" leading from one end toward the mouth near the centre of the body. Within the protoplasm is a large nucleus, macronucleus, usually ellipsoidal in form but subject to wide variations in size; and a smaller nucleus, known as the micronucleus, which is embedded in

the substance of the macronucleus. At each end of the infusorian is a bright spot which appears and disappears with considerable regularity; these are the contractile vacuoles, their function being to throw to the outside of the body the waste matters that are formed during the physiological activities of the cell. Each vacuole is supplied by a series of canals from various parts of the body, the waste matters in fluid form collecting in them to be emptied into the contractile vacuole and thence disposed of. The peripheral protoplasm of paramecium is filled with minute thread-like structures, the trichocysts, which are thrown out when the cell is irritated. On the outside of the body, finally, is a dense covering of minute lash-like whips which are constantly in action during life, and by means of which the organism moves about freely in the water, turning the while on its long axis. These are the cilia which are arranged in spirally wound lines around the body, while a somewhat more powerful set are located in the asymmetrical peristome and are used to direct a food current toward the mouth.

FIG. 37

Paramecium aurelia. Macronucleus normal; micronucleus abnormally large.

The food consists of any proteid matter small enough to pass through the mouth opening. The organism will take in bits of flesh, or parts of vegetable matter, or bacteria or lifeless matter, such as carmine or indigo granules, all with equal voracity. The process of ingestion is hastened by the activity of an undulating membrane situated in the small gullet, and the bacteria or other food matters are collected in a vacuole which forms at the base of the gullet. Considerable water is taken in with the food, and when the vacuole is large enough it is caught up in the protoplasmic flow and carried away from the mouth opening. Numerous gastric vacuoles are thus formed and the food is digested in them.

When the organism is fully grown it reproduces by dividing into two cells, each cell having the characters of the former one cell, which has disappeared, indeed, although it has not died. Its protoplasm is still living in the two daughter cells; these repeat the processes of digesting and growing, and finally, each of them reproduces by transverse division. The metabolic processes leading to reproduction by division are thus repeated generation after generation, and, having all that is necessary in the form of cellular organs for an indefinitely

continued existence, they apparently offer some justification for the older view that protozoa are practically deathless, so far as old age is concerned.

The matter of physical immortality can be easily tested, however. After a little practice, a single cell of paramecium can be isolated and fed on the bacteria which develop in a previously sterilized hay infusion made by boiling small pieces of hay in water. The organism is placed in a small chamber filled with the hay infusion and made by supporting a coverglass on pieces of glass. When it divides, which it will do within twenty-four hours, the daughter cells can be similarly isolated and fed on freshly made hay infusion, and in this way the vitality of that originally minute bit of protoplasm can be watched day after day and generation after generation of cell divisions, until natural death from old age ensues. The writer successfully followed the life history of such a culture of paramecium from an initial cell to protoplasmic death from old age, giving fresh food medium and isolating the single cells day after day and generation after generation for a period of twenty-three months and 742 generations. The observations made during such a study deal with living protoplasm that is growing old more rapidly than in nature, and with the ageing process in an organism endowed with an initial potential of vitality.

A paramecium which is thus followed from generation to generation shows surprisingly regular variations in vitality. Some of the more minute variations are due to temperature changes, a warm day, for example, increasing, a cold day diminishing, their vigor. In the laboratory, however, such variations may be overlooked, for the changes in temperature from day to day are of minor importance. After much experimenting, a measure of vitality was finally found which made it possible to compare the activity of the physiological processes from time to time. This measure was represented in the form of a curve, the points upon it being obtained by averaging the number of divisions made by all of the organisms under observation in periods of ten days, each average giving the ordinate for one period; the abscissas represent the arbitrary ten-day periods (see Fig. 38).

Such a curve, representing the vitality of the paramecium protoplasm, shows that in a period of six months under cultivation, if the organisms are fed upon the same diet of hay infusion, there is a gradual exhaustion of vitality, the curve falling from an average of about twelve divisions in ten days in February to an average of one division in ten days in July. As the curve shows, the average number of cell divisions sinks more or less regularly during the six months, but undergoes periodic rises and falls, until at the end of that time the organisms are unable to digest and assimilate the bacterial food and the cells begin to die, the minute cellular corpses being abundant at such a period.

FIG. 38

Diagram representing the life cycles of *Parametium caudatum*. The numbers of divisions per day for all individuals of the race under observation were averaged in ten-day periods, thus giving a convenient means of comparing the vitality at different times. The first cycle ended in July, 1901, the organisms being stimulated with beef extract. The second cycle, thus artificially started, ended in January, 1902. The line in the diagram carried to the base indicates that half of the organisms which were kept on the hay infusion as control, all died, while the rest, which were given beef extract or simple salts, were restimulated and started on the third cycle, which ended in June, 1902. Half were again stimulated artificially, this time by extracts of pancreas and of brain, but all died out in December, 1902, the last ones in the seven hundred and forty-second generation. In the second and third cycle, the periods of sexual activity were from November to January and from April until June, while in the last cycle the organisms would not conjugate at all.

This exhaustion of the power to digest and assimilate is an unmistakable phenomenon in the life history of a protozoön, and marks a somewhat indefinite phase of the life history, which was designated the "period of depression." Many other observers have noted it in connection with protozoa of different kinds; the first, Bütschli, in 1876, in relation to paramecium, without noting the sequence of stages leading to this depression period, observed that the organisms become reduced in size and sluggish in movement, and that while in such condition they conjugate, an observation which led him to his famous suggestion that conjugation is not an act of reproduction, but a means of renewing the vitality of the organisms, including the power to reproduce; in other words, a *Verjungung* of the protoplasm. Later observers, including Maupas and Hertwig, likewise studying the organisms en masse, noted a similar stage of lowered vitality, the former concluding that it indicates a senile degeneration of the nuclei, the latter, that it indicates a changed relation between the volume of the nucleus and that of the cell. Woodruff and Gregory, as graduate students in the Columbia laboratory, have followed out, generation by generation, the life history of different protozoa, the former in connection with *Oxytricha fallax*, one of the hypotrichous infusoria, which he followed for 860 generations of cell divisions, requiring twenty-one months, the latter in connection with *Tillina magna*, one of the holotrichous infusoria, which was followed for thirteen months, dying out in the 548th generation. Periods of depression were observed in these organisms as in paramecium, and the same physiological derangements were noted by both observers, the first period of depression carrying off all the cells of tillina.

What is the explanation of the depression period? The organisms have abundant food; they are able to take in food up to a certain time, but they appear abnormal in structure, and if left to themselves they would die. The protoplasm at this period is markedly different from that at other times; in paramecium the endoplasm lacks the characteristic vacuoles of the ordinary organism and appears dense and homogeneous (Fig. 39), an appearance due to the aggregation of granules. The lack of vacuoles signifies a concentration of the cell protoplasm and, therefore, a reduction in size of the organism; the macronucleus, in the meantime, retains its full size, and it thus appears that the volume of the latter is relatively greater than it is under normal conditions. This is perhaps one reason why Hertwig, Popoff, and others have concluded that the cause of depression is the change in relative volume of nucleus and cytoplasm, but such a change in relative volumes may be equally well an effect of the depression and not its cause. Woodruff noted the same reduction in size of the cell in oxytricha (his figures 1 and 9) during the period of depression and a corresponding change in nature of the cytoplasm, which, in oxytricha,

became vacuolated instead of granulated. There is no doubt, from these daily observations on the same organisms, that there is a change in physiological activity, which cannot be interpreted as due to the difference in the relative sizes of nucleus and cytoplasm, but must be traced to some more deeply lying cause.

After two similar periods of depression had been successfully offset by artificial means, a fourth and final period, in which the protoplasmic structures were quite different from previous conditions, carried off the last generation of the race, 742d generation (see p. 129).

FIG. 39

Paramecium aurelia at period of depression, showing (at left) the dense granular condition of the protoplasm, which, if not relieved artificially, invariably ends in death. The central and right hand figures show the effects of such artificial relief in the vicinity of the nucleus, while the extremities are still dense.

While these initial experiments would seem to indicate a certain normal length of life (approximately 200 to 800 generations), it does not follow that all paramecia have the same endowment. Different races of paramecium, like different human individuals, vary in the initial potential of vitality, and are capable of living for different lengths of time upon the same medium. Thus, other cultures of paramecium, carried on at the same time as those described, yielded 376 and 379 generations before evidences of depression set in. A constantly changing medium, furthermore, may tend to offset the cumulative physiological weakness and so to prolong the life of the race. Such an experiment on paramecium has recently been carried out by Woodruff ('08), who, instead of constant hay infusion, used infusions of leaves, grass, etc., from natural pond water, frequently changing

the source of such food material. Upon such a continually changed diet he carried on a race of *Paramecium aurelia* through several hundred generations without the advent of a period of depression. It appears, therefore, that in the constantly changing conditions of nature a race of protozoa may live much longer than under the conditions of laboratory experiments on a single diet. It is probable that the salt contents of the medium rather than the food are of importance in this connection, since the bacteria of the laboratory air, with which all food media were inoculated, were presumably the same.

II. MORE COMPLICATED LIFE CYCLES AND THE PERIODS OF "YOUTH," "MATURITY," AND "AGE."

With different types of protozoa the three periods of vitality may be recognized with quite the same facility as in any of the lower forms of metazoa. There is no sharply defined difference between them, but, as Maupas first pointed out, there is a fairly definite period of protoplasmic or "individual" maturity, which is preceded by a period that may be designated "youth," and is followed by a period that may be called "old age." The period of maturity is so frequently accompanied by well-marked cellular changes, which distinguish the organisms at that period from the ancestral cells which gave rise to them, that we are justified in the attempt to generalize, if only for descriptive purposes, and to speak of periods of youth, maturity, and age in protozoa.

In the life history of *Paramecium aurelia* the three periods, youth, maturity, and age, of the life cycle are not so clearly marked by structural and functional manifestations as in some other forms of protozoa. Nevertheless, there is a physiological difference which becomes apparent when one follows out the complete history. The period of youth is marked by a high rate of division energy and by the fact that conjugation does not occur if many of them are put together in a limited space. After some time in culture, however, usually when the rate of division has begun to decline, the protoplasm of the cell body changes slightly in physical and chemical make-up, so that two or more cells upon meeting fuse and conjugate. The entire race of paramecium in such a culture may become sexually mature at the same time, and "epidemics" of conjugations may be thus obtained. At the last period of depression, however, in the experiments cited, there were no conjugations, a fact indicating, possibly, the exhaustion of the germ plasm. Such a final period of old age may be easily identified, involving, as it does, the curious vacuolization and degeneration of the protoplasm and exhaustion of the physiological energies.

A. The Period of Youth.—As with the fertilized egg of a metazoön, this first period of vitality of the copula or fertilized cell of a protozoön is characterized by the distinct excess of constructive over destructive metabolism, which indicates a high potential of vitality and great powers of cell reproduction, which may take the form of division, budding, or spore formation according to the difficulties successfully overcome by the type in the struggle for existence. These young forms show a well-marked conformity to type, and this feature, occurring when the greatest numbers of representatives of the species are in evidence, undoubtedly has given a false impression of the stability of form of the protozoan species. The protoplasm, as a rule, is transparent and without reserve matters, metaplast products, and the like, and the nucleus is often without the characteristic structures of the later forms.

It is along physiological lines that the young forms are most prominently marked. This is the period, for example, of the greatest resistance to adverse conditions in the surrounding medium, and in pathogenic forms it is the period of greatest malignancy. It is a well-known fact that in many parasitic forms of protozoa attempts to inoculate from animal to animal are either failures altogether or result in a weakened infection, the failures being due, presumably, to the inability of the organisms in a more or less weakened condition to withstand the natural immunity of a new host. The matter of malignancy is so intimately connected with restored vitality that in yellow fever, for example, it is almost sufficient to indicate that fertilization processes and renewal of vitality must have taken place in the body of the intermediate mosquito host.

At this period, also, is the greatest power of self-preservation in other ways than by resistance of a chemical nature; thus, the firm protective cysts are formed at this period within which the fertilized cell may resist heat, cold, and drought, as in many of the free forms of protozoa when the organisms live thus through the winter, or in parasitic forms like the sporozoa, when the organisms are protected in the interval of changing hosts.

The difficulties in determining which are young and which older cells of a life cycle are great, and much must be left for inference. It may be accomplished, however, in one of several ways: (1) By culture experiments for which cells are isolated immediately after conjugation, a method that may be easily employed for the larger free protozoa. (2) By inoculation of uninfected hosts with the spores of the form to be studied, a method which may be employed with sporozoa or with encysted amebæ. (3) By natural inoculation through the operation of intermediate hosts, such as insects, ticks, or leeches. Few observations, however, have been made upon the young forms, probably because the morphological characteristics of the mature cells are much more apparent than those of the young.

The high grade of vitality of the young protozoan is undoubtedly due to the perfection of the cellular structures and to their harmonious working. This was very well illustrated in some observations on so-called *Paramecium caudatum* (Calkins, 1906). This species has been generally regarded as distinct from another very similar form, *Paramecium aurelia*, which is regarded as much more rare than the former. The main difference between the supposed two species is the presence

FIG. 40

Diagram to show the relative vitality of the caudatum and aurelia forms of paramecium. The dotted line represents the division rate (average for ten-day periods) of an ex-conjugant from the same culture which reorganized normally, *i. e.*, as a *Paramecium* "caudatum." The solid line represents an ex-conjugant that reorganized abnormally, *i. e.*, as *Paramecium* "aurelia," but which changed into a normal form during the month of June. Note the rise in division rate with the assumption of the normal condition. (After Calkins.)

in the latter of two micronuclei as against one in the former, while certain physiological differences, as indicated by the rate of division and the rate of movement, were noted by Maupas ('89) and Simpson ('01). The observations mentioned were made upon some ex-conjugants from a culture of the more common "caudatum" form. The two cells derived from such a union were isolated, and one of them was maintained for months in culture, the other dying shortly after

isolation. In the reorganization of the cell following separation two micronuclei instead of one were left in the cell. This abnormality for the "caudatum" form was the "normal" condition for the "aurelia" form, and was maintained for more than three months, the animals showing every characteristic of form and function that have been ascribed to *Paramecium aurelia*. The movement was sluggish and the rate of division much lower than in the case of "caudatum" forms which had been isolated at the same time and carried along as a control (see Fig. 40). At the expiration of three months in culture the cells here and there showed the loss of one of the micronuclei, and ultimately all of the so-called "aurelia" forms had become "caudatum" forms and with the typical characteristics which mark this species. The rate of division rose to a much higher average than before, and the cells became much more animated and larger in size. The average number of divisions in ten-day periods rose from 11.3 from March 1 to June 10, to 19.3 in the time from June 10 to September 1, that is, during the time when the nuclear relations were normal. It is evident, therefore, that *Paramecium caudatum* and *Paramecium aurelia* are not distinct species but merely variants of the same species, and that the abnormal condition of the cell organs resulted in strongly marked physiological derangement.

B. The Period of Maturity.—There is no definite limit to the period of youth in protozoa, the changes which characterize the period of maturity coming on slowly and imperceptibly as they do in higher forms. The morphological characteristics of this period, when arrived, are clearly marked, however, and unmistakable. Such changes affect both the cell body and the nucleus, and may accompany either vegetative or germinal activities, or both.

1. Protoplasmic Changes at Maturity.—While the most important characteristic of the period of maturity is a general decrease in functional activity, with decline in the rate of multiplication, these physiological activities are accompanied by well-marked morphological changes which may be of a sexual character. In a single cell or specimen of a protozoan species there may be no clue to its position in the life cycle unless it is in some phase of sexual activity, and where sexual dimorphism does not exist it is quite impossible to tell from morphology alone. Thus, in the mature paramecium the sexual differences are so minute that unless one is following out the life history in culture the period of maturity passes unobserved. Nevertheless, the cells of paramecium do undergo a physical change at this period; the peripheral protoplasm becomes sticky and highly miscible, so that, in some cultures, two organisms upon meeting will adhere at any point, and groups of from six to nine cells may be seen whirling about in aimless movement among the normally conjugating pairs. This miscible state indicates a well-marked difference in the physical

make-up of the protoplasm, for in the early periods of activity the body wall, while plastic, always retains its firm contour and cortical density. Pearl ('07), furthermore, has shown by biometric analysis that conjugating paramecia are markedly smaller and less variable than non-conjugating forms.

Similar changes in density mark this period in other kinds of protozoa. Thus, among the flagellated forms like tetramitus or cercomonas the ordinarily firm contour of the cell becomes plastic and highly changeable in form, and two of them upon meeting fuse in conjugation. Here again a physical change is well illustrated.

FIG. 41

Polystomella crispa. Liberation of pseudopodiospores from the microspheric individual. (Photo by J. J. Lister.)

Still more remarkable is the change in form which some types of sarcodina undergo at this time. The rhizopods are especially noteworthy in this connection, Schlumberger ('83) noting for the first time a peculiar dimorphism in the shells of foraminifera (Fig. 42), a difference which Schaudinn ('03) and Lister ('05) were the first to explain. These observers found that the young forms, immediately after fertilization, give rise to what Schlumberger termed the "microspheric" type of shell. Upon reproduction, such a cell ultimately gives rise to pseudopodiospores which leave the old shell and secrete new ones of a different type, termed the "megalospheric" type (Fig. 41). The latter generation, when fully grown, gives rise to flagellispores which conjugate and thus complete the cycle (see Fig. 52, p. 123).

Even more marked is the change in trichospherium where the chemical composition of the skeleton parts changes with advancing age. The young forms resulting from conjugation grow into an adult characterized by a gelatinous membrane and radial spicules of magnesium carbonate. This adult reproduces by the formation of pseudopodiospores, which grow into organisms similar to the parent, or after advanced age (presumably) to a second adult type characterized by a firm membrane and entire absence of radial spicules. This second type, as in the foraminifera, finally gives rise to flagellispores, the progeny from different parents uniting and thus

FIG. 42

Megalospheric (A) and microspheric (B) shells of *Biloculina depressa*, Lam. (After Schlumberger.) Dimorphism is shown by the central chamber *c*.

completing the cycle. Such secondary types are morphological evidences of changed metabolic conditions characteristic of the second period of vitality. The possibilities of similar alternations in the life history of parasitic and pathogenic forms have hardly yet been realized.

2. **Nuclear Changes at Maturity.**—"Chromidia."—While changes in the body form are often characteristic of the second period of vitality, there are great numbers of protozoa in which the external structure gives no clue to the state of affairs within. The nucleus, however, undergoes changes at this period which are not only more widespread throughout the phylum, but are of far more theoretical and practical importance. These changes have to do with the formation of so-called "chromidia" and with the maturation phenomena of the cell (Fig. 43).

The first definite observations upon chromidia formation were made by Hertwig ('99) in connection with the minute structure of the shelled rhizopod *Arcella vulgaris*. Previous observers had noted that chromatin-like granules are distributed throughout the cell body in many of these types, but Hertwig was the first to describe the origin of this material from the nucleus in arcella and to show that it forms a dense zone of granules in the protoplasm (Fig. 44). At that time Hertwig described this material under the name of "chromatin net," but later, in 1902, he called it the "chromidialnetz," because of the reticulate structure assumed by the granules en masse. The function of this extranuclear chromatin was not made out, however, until the following year, when Schaudinn ('03) worked out the origin and fate of similar masses of granules in several different kinds of sarcodina

FIG. 43

"Chromidia" in rhizopods, *Arcella vulgaris* (on left) and *Ameba proteus* (on right). The dark granules are the idiochromidia distributed throughout the cytoplasm.

(*Polystomella crispa*, *Centropyxis aculeata*, *Chlamydomphrys stercorea*, and *Entameba coli*) and found that the nuclei of the conjugating gametes were developed solely from this extranuclear chromatin. He thus interpreted the material of the chromatin net of arcella and its allies as sexual or racial chromatin and correctly compared it with the micronuclei of the infusoria.

In the meantime the subject became more complicated by Hertwig's further observations upon extranuclear chromatin in the heliozoön *Actinospherium eichhornii*. These observations, first noted in 1897, were confirmed and extended in 1904, when it was shown that in starving forms and as well in forms that had been overfed, the nuclei all disintegrate and the chromatin contents becomes distributed throughout the cell body (Fig. 45). The distributed chromatin thus

formed was named by Hertwig, in 1902, "chromidien," from which the term chromidia is derived, a term now universally employed by protozoölogists. According to Hertwig this latter material in actinospherium cytoplasm is prophetic of the death of the animal, for when it is thus formed the renovation of the cell is impossible (1904).

FIG. 44

Arcella vulgaris. (After Calkins.) Three individuals in plastogamic union. P, idiochromidia; N, nuclei.

FIG. 45

Chromidia formation in *Actinospherium eichhornii*. (After Hertwig.) A, primary nuclei and chromidia; B, complete transformation into chromidia.

It thus appears that we have to do with two kinds of chromatin masses in the cell body and no little confusion has arisen in consequence of the mixed terminology applied to this material, which is alike in origin from the nucleus but very different in function. Chromidia, in Hertwig's sense, is functionless extranuclear chromatin, but Schaudinn and others have used the term to designate the sexual chromatin which is equivalent to the chromidialnetz in Hertwig's terminology.

Subsequent observers have tried to straighten the tangle by giving new terms for the different kinds of extranuclear material. Calkins ('04) proposed the term protogonoplasm for the gamete-forming substance; Goldschmidt ('04) proposed the terms "chromidia" and "sporetia" for chromidia and "chromidial net" respectively, and Mesnil ('05), the terms "trophochromidia" and "idiochromidia." Goldschmidt's suggestion is a good one, but the term sporetia is not indicative of the function, while Mesnil's term idiochromidia expresses the fate exactly and will undoubtedly supplant the other names. In the present instance the terms "chromidia" and "idiochromidia" will be used, the former on grounds of priority, the latter on expediency.

FIG. 46

Arcella vulgaris. Secondary (gametic) nuclei (*n*) forming from the idiochromidia *ch*; *o*, mouth opening of shell. (After Hertwig.)

(a) IDIOCHROMIDIA FORMATION.—As might be expected, the method of formation of the idiochromidia differs widely in the different types of protozoa, and frequently in the same type. Although all methods, in their final analysis, may be traced back to the same physiological causes arising during this period of maturity, the different types may be separated for purposes of description into three groups, as follows: (a) Idiochromidia formation by nuclear transfusion; (b) by dissolution of nuclear parts; and (c) by nuclear fragmentation.

Nuclear Transfusion.—This method of idiochromidia formation is most characteristic of the rhizopods, and has been worked out mainly in connection with arcella, centropyxis, diffugia, and other mono-

thalamous forms. In arcella it has been described by Hertwig ('99) and Elpetiewsky ('08), and the process here may serve as a type for all.

The normal vegetative cell of arcella contains two nuclei which at an early period begin to secrete chromatin materials, which collect in masses about the nuclear periphery (Fig. 44). With continued

FIG. 47

Gametes and copulation of *Arcella vulgaris*. C, copula. (After Elpetiewsky).

FIG. 48

Stages in development of *Mastigella vitrea* and *Mastigina setosa*. (After Goldschmidt. $\times 1270$. A, flagellate stage of *M. vitrea*; B, same, somewhat older and before chromidia formation; C, same during chromidia formation; a, entire cell; b, nucleus only, showing transfusion of chromatin to form chromidia; D, young flagella stage of *M. setosa*, with heap of chromidia; E, same, older form with pseudopodia, compact chromidia, and food vacuole; F, same, young form with peripheral "bristles;" G, same, formation of gametic nuclei a, from idiochromidia, b.

growth, and at maturity of the cycle, these masses become distributed throughout the cell body in the form of deeply staining chromatin granules (Fig. 43). When fully mature the protoplasm breaks down into a number of pseudopodiospores, each with distributed chromatin, and these form new arcella shells in which the protoplasm ultimately

breaks up into ameboid gametes, in which the nuclei are formed, as in centropxyxis, by fusion of the idiochromidia granules (Figs. 46 and 47).

Not only in rhizopods, but in flagellated protozoa as well, the idiochromidia arise in this manner. Thus, in the case of *Mastigina setosa*, Goldschmidt ('07) has shown that the idiochromidia accumulate in heaps about the nuclear membrane, as in arcella or centropxyxis, before being scattered throughout the cytoplasm, where they ultimately form the nuclei of gametes (Fig. 48).

Nuclear Dissolution.—There is probably no great difference between the above-described method of idiochromidia formation by transfusion, whereby the chromatin materials percolate through the nuclear membrane in fluid form, and that by nuclear dissolution, whereby the peripheral portion of the nucleus becomes scattered in granular form throughout the cell body. Nor is this second method

FIG. 49

Amoeba limax (group of five on left) and *Chilomonas paramecium* to show alveolar structure of protoplasm prior to idiochromidia formation. Two of the amoebæ are in process of division.

different, save in degree, from the third, which I have called nuclear fragmentation. The distinctions have, at best, only a descriptive value.

Nuclear dissolution, in substance, was described more than thirty years ago by Hertwig ('76) in connection with the radiolarian acanthometra. In this form there is a great increase in the thickness of the chromatin at the periphery of the nucleus and at the expense of the karyosome, and this cortex ultimately breaks down to form quantities of minute secondary nuclei of the macro- and microgametes (see Hertwig, 1907). Here, then, the peripheral rind of chromatin is little more than a condensed zone of idiochromidia, and is closely associated with the karyosome. In *Amoeba limax* (Fig. 49) there is no

such condensation, but the idiochromidia granules collect in a loose shell or rind about the karyosome, and from it granules of chromatin are discharged into the surrounding protoplasm prior to encystment (Fig. 50). During encystment these distributed granules are abundant in the cell while the karyosome becomes indistinct and ultimately degenerates. Under proper environmental conditions (which may be brought about artificially by changes in temperature) the idiochromidia fuse into sixteen groups of secondary nuclei (Fig. 51). A similar method of idiochromatin formation was described by Schaudinn ('03), and more recently by Craig ('08), in the case of *Entameba histolytica*.

Not only idiochromidia, but chromidia as well, may be formed by this method of nuclear dissolution. Thus, in some coccidia and gregarines according to the observations of Siedlecki ('07) and Lèger ('07), on caryotropha and ophryocystis, respectively, a similar disposal of the peripheral rind of chromatin gives rise to degenerating granules which, possibly, according to both observers, may have some vegeta-

FIG. 50

Ameba limax. Chromidia forming from nucleus and collecting in the cytoplasm prior to encystment.

tive function in cell metabolism. The latter, therefore, apparently agree with Hertwig's chromidia in actinospherium.

Nuclear Fragmentation.—Idiochromidia formation by fragmentation is widely scattered among protozoa, and has been described by numerous observers, first by Schaudinn ('94), and by many others since, in connection with various forms of foraminifera, rhizopods, flagellates, and sporozoa. The most widely recognized example of this mode of idiochromidia formation is the case of *Polystomella crista*, one of the foraminifera. Here, according to the independent observations of Schaudinn ('03) and Lister ('05), the nuclei of the microspherical generation increase by division until a large number are formed. The older ones then disintegrate, or fragment, into minute chromatin granules, which are ultimately distributed throughout the protoplasm. Later aggregations of these idiochromidial granules give rise to the nuclei of the conjugating gametes (Fig. 52). Similarly in the coccidian *Klossia octopiana*, according to the researches of Siedlecki the nuclei of the microgametes, and in *Gregarina cuneata*, according

to Kuschakewitsch ('07), the gametic nuclei, are formed by nuclear fragmentation.

A slight modification of this method of idiochromidia formation is found in *Ameba proteus*, where, according to Calkins ('07), the primary nucleus divides repeatedly until about seventy nuclei are present in the cell. These primary nuclei then give rise to secondary nuclei, which form from the chromatin granules inside of the primary nuclei. The chromatin substance of the primary nuclei is thus metamorphosed into secondary gametic nuclei, and these conjugate two by two. Here the process may be interpreted as a precocious development of the gametic nuclei, a development taking place before the primary ones are completely fragmented (Fig. 53).

FIG. 51

Ameba limax. Aggregations of idiochromidia to form sixteen secondary nuclei, which then unite to form eight.

The vegetative distributed chromatin granules or true chromidia, as seen in *Actinospherium eichhornii* are formed by similar nuclear fragmentation; it is quite obvious, therefore, that the method of formation of these distributed chromatin granules has little or nothing to do with the subsequent function.

(b) THE SIGNIFICANCE OF IDIOCHROMIDIA.—It is quite apparent from even the few cases cited above that we cannot generalize as to the function of the deeply staining granules of nuclear origin in the cytoplasm of protozoa. In some cases (*e. g.*, *actinospherium*, *ophryocystis*, *caryotropha*, etc.), whatever may be their significance in the

cell, they certainly are not connected with the formation of the gametic nuclei. On the other hand, there can be no doubt of the propagative nature of such distributed granules in the great majority of protozoa, and in such cases we may, with reason, speak of a definitive germ

FIG. 52

Life cycle of *Polystomella crispa* S. (Lang and Schaudinn). A young form derived from the union of two flagellated gametes (A) develops into an organism with microspheric type of shell. The nucleus increases by mitosis until many nuclei are present when they break up into granules of chromatin (B). The protoplasm fragments into reproductive bodies, equivalent to merozoites (C), each having several granules of the distributed chromatin ("Chromidien"). Each reproductive body (D) develops into an adult with a macrospheric type of shell, and with nuclei in the form of small chromatin granules (E). When mature these forms fragment into hundreds of flagellate gametes (F) which conjugate, and so complete the cycle. (See, also, Fig. 41, p. 114.)

plasm as contrasted with the somatic plasm. With such an assumption we are brought in touch with a problem of high theoretical interest in general cytology, and with the protozoa, as with the metazoa, we have this question to consider: Are there two kinds of substances in

the nucleus, the one superintending exclusively the processes having to do with germinal life, heredity, and the race, the other having to do only with the metabolic processes of the individual?

In connection with higher animals and plants we meet with conflicting answers to such a question. Weismann, Roux, and their followers maintain—and their contention is strengthened by the constantly increasing evidence as to individuality of the chromosomes and their connection with specific characteristics of the adult organism—that a specific inheritable substance—idioplasm—is always present in the cell from the start, and is gradually sifted out with growth as the various organs are formed. Others, notably O. Hertwig, take the view that nuclear materials are fundamentally the same, and that as growth advances, environmental changes affect and alter the original homogeneous stuff. It is in connection with the latter point of view that R. Hertwig approaches the problem of dualism in the protozoan nucleus (1907). He believes that “functional degeneration” becomes localized in certain substances of the cell nucleus, so that a dualism is gradually brought about through such degenerative changes, and indicated, morphologically, by the different chromatin elements scattered throughout the cell. Chromidia, therefore, according to this point of view, would be the same as idiochromidia save for a difference in potential, the latter having the possibilities of continued existence, the former not.

Neglecting, for the present, the question of original dualism in nuclear substances in protozoa, we must accept the fact that there are, at times, specific germ substances within the cell and localized in the chromatin of the cell. In the higher animals the analogous germ plasm becomes segregated and separated from the somatic plasm in the form of germ cells or germinal epithelia. Differentiated from somatic plasm during ontogeny, this racial protoplasm becomes functional only after the period of maturity is reached. Similarly with protozoa, there is, at periods of maturity, a definite germ plasm distinct and separate from the somatic plasm. In some cases, like the germ cells of higher animals, this specific racial substance is early differentiated from the vegetative, functional, or somatic plasm. Such is the case in infusoria, where, in *Paramecium aurelia*, for example, germ nuclei and functional vegetative nuclei are differentiated as micronuclei and macronuclei, respectively, after the third division following conjugation; and such is the case in arcella and allied forms where the germ plasm is not aggregated in a compact micronucleus, but as idiochromidia is scattered throughout the cell.

In other cases the germinal and somatic parts are not separated until later in the life history, or in some cases not until full maturity, when for the first time chromatin of conjugation and of vegetative function can be distinguished. Such is the case in *Ameba proteus*, in

FIG. 53

Idiochromidia formation in *Amoeba proteus*. (After Calkins.)

Polystomella crispa, in gregarines, and coccidia, where the residual primary nucleus, or the Restkörperchen, may be interpreted as the now functionless somatic chromatin.

Idiochromidia, or germ plasm, therefore, must be interpreted, in some cases at least (infusoria), as a definite and distinct substance of the cell. In other cases its segregation and separation from somatic chromatin occurs only during the second period of the life cycle, and its formation is the index of advancing age (sarcodina). It is, in point of fact, the chief morphological feature characteristic of the period of maturity in protozoa.

3. Sex Differentiation.—At the present time the hypothesis first advanced by Montgomery ('01) is widely accepted, that during maturation of the germ cells the reduced number of chromosomes is brought about by union, two by two, of chromosomes representing the same characteristics of the adult in maternal and paternal ancestors. Of such characteristics, none are more marked than those primary and secondary characters which distinguish the sexes. Wilson's observations, following and enlarging upon those of McClung, Stevens, and others, on the structure of the germ nuclei in insects, have practically demonstrated that sex here, like other adult characteristics, is a matter of inheritance.

In protozoa, sex differentiation, when present, is, apparently, the final expression of the period of maturity. We have seen that, with advancing age, the structure of the protozoan cell may become materially altered, and that these alterations may give rise to similar conjugating gametes, or, directed possibly by inheritance, may give rise to male or female germ cells. In the former case (isogamy), conjugating elements may be similar in size to normal cells or only slightly reduced, as in paramecium, didinium, and the majority of infusoria; or both may be reduced to small-sized equal cells (isomicrogametes), as in many gregarines and rhizopods. In the latter case (anisogamy) one cell, macrogamete, may be similar to the ordinary vegetative cells (as in vorticella, coccidium, etc.), or only slightly changed, while the other cell (microgamete) may be relatively minute (vorticellidæ, coccidiidia, etc.); or both cells may be reduced and of dissimilar size (as in polytoma, centropyxis, schaudinnella, stylorhynchus, and other gregarines).

In sexually dimorphic gametes there is no difference between the early cells in the majority of cases, differentiation coming only as a last step in maturity (hemosporidia, coccidium, and coccidiidia generally); in some cases, however, notably in adelea (Siedlecki, 1899) and cyclospora (Schaudinn, 1902) among coccidia, and in trypanosoma (Schaudinn, 1904) among flagellates, the sex differences are said to extend as far back as the schizont stage immediately after fertilization; hence, if this is true, it is possible to speak in some cases of male

and female protozoan individuals. The evidence for this conclusion is in every case somewhat inconclusive; the differences seemingly are not beyond the range of individual variation.

In the majority of free forms, gamete formation, with their liberation, is accomplished in the ordinary medium in which the organisms live, although these processes may be hastened or influenced by artificial changes in the environment. Thus, Hertwig ('98) noted that the quantity of food had much to do with these phenomena in the case of actinospherium, and Klebs, Dangeard, Greeley, and others have found that changes in temperature or in density of the medium may induce gamete formation in different kinds of flagellates. Similar changes in environment seem to be a *sine qua non* for sex differentiation in many parasitic forms, the most notable and best-established case being the malaria organisms where microgametes are formed only in room temperature, in the mosquito's gut, or, in general, in a colder (denser?) medium than the blood.

In the great majority of cases where gametic differentiation obtains, if the gametes do not conjugate they die. This is invariably true of the microgametes, and their fate is probably due to the extreme specialization which they have undergone. In the female forms this is not the invariable fate, for in some cases the cells undergo parthenogenesis, a process of renewal which is accompanied by nuclear activities of a special kind. (See Chapter IV.)

C. The Period of Old Age.—Protozoa quickly die after the period of maturity is passed, and old age, the final period of a life cycle, is rarely seen or recognized. Maupas ('89), however, using the culture method, gave a very graphic description of old age in certain forms of infusoria. Thus, in *Onychodromus grandis* the body of the cell becomes much reduced in size, loses cilia and cirri, while other organs both external and internal, atrophy, and the organisms die of senile exhaustion. In *Paramecium aurelia* the circumstances accompanying old age have been described above, but in this case the metabolic processes had been restimulated, and apparently the cell organs were suitable for a continued activity, but something was wrong and the race died. This "something" had to do with the germ plasm, for, as stated, the micronucleus was hypertrophied and divisions were abnormal.

"The first clearly marked period of depression came in July, about six months after the cultures were started. It was characterized by a well-defined reduction in size (down to 109 microns) and by vacuolization of the endoplasm, while the ectoplasm did not appear to be much involved. Many of the individuals were characterized by great vacuoles similar to those in starved forms, which distorted the body almost out of recognition; in others the nuclei were fragmented into two or three parts, and in all there was a marked

absence of the larger food granules and gastric vacuoles which characterize the normal animals, and this, notwithstanding the fact that bacterial food was present in abundance (see Studies I). As stated in these Studies (III), the organisms under these conditions still take food, and in some cases the endoplasm appears opaque with the undigested food balls, but the decrease in size continues and the endoplasmic vacuolization is not prevented by the presence of the food. It is the digestive function, apparently, which becomes ineffective at such periods, and if this is a correct assumption, this function can be stimulated, as I have shown by the experiments.

"Identical results were obtained in the period of depression in December, 1901, a depression which was again overcome by the use of beef extract, while the individuals of the series which had been continued on the hay diet all died. These became smaller and smaller, and again gave morphological indications of starvation, notwithstanding the fact that the individuals which had been stimulated with the beef extract were living and reproducing normally in the same food medium. They became much reduced in size, the endoplasm became distorted with vacuoles, and they died with absolutely no indication of disease through parasites.

"These observations show, therefore, that starvation effects may be produced, even though the animals are living in a medium rich in food. It is trite to say that to prevent starvation we must have not only food, but the ability to digest and assimilate it, yet common as this observation is, it is important in the present connection, and involves a factor which cannot be overlooked in any discussion on old age.

"In the June period, as stated previously, the same conditions were not observed, for the organisms, in part at least, had been treated with the beef extract every week during the first three months, since the previous period of depression. The division rate began to run down in the case of the B series in April, in the A series in May, and in all of the material that had been continued on the beef the characteristic structure was a densely granular endoplasm (Fig. 26, p. 82). In the specimens that had not been treated with the beef since the preceding December this character of the endoplasm was not noted. These unstimulated individuals died out in about the 508th generation (B series) after becoming much emaciated and reduced in size, and with reduced nuclei. . . . The unstimulated A series did not die out until about two weeks later. At the time when the B individual described above died (May 12) the unstimulated A series was characterized by somewhat reduced size, a declining division rate, and absence of the dense protoplasmic granules. In the stimulated A series, on the other hand, (A1 and A2) of about the 560th generation, the structures were normal, gastric vacuoles were numerous, and

divisions were frequent. Toward the end of June, however, when the A series nearly died out in the 620th generation, the conditions were very different. Fig. 26, left, is from a specimen in the 615th generation; its size is below the normal; its endoplasm is choked up with granules, and there is no trace of vacuoles save the contractile vacuole near one end. The macronucleus is definitely granular, and its contour is irregular, as though devoid of nuclear membrane. The micronucleus is elongate and spindle-formed. The ectoplasm is not deformed, and save for the absence of trichocysts it appears to be normal. This was the condition of the protoplasm when the usual large number of culture individuals was reduced to 6 A's and no B's, and a condition from which the A series was rescued only with the greatest difficulty by the use of pancreas extract.

"From this time until the race died out the division rate was sluggish. The conditions of the protoplasm in the latter individuals was decidedly characteristic. Throughout the fall individuals would appear with densely granular protoplasm, which is invariably the

FIG. 54

Paramecium aurelia from culture in 741st generation. The macronucleus and endoplasm are normal, the micronucleus is abnormal, and the cortical plasm is filled with vacuoles. (After Calkins.)

sign of death, unless the animals are stimulated in some way. In such forms the macronucleus may or may not be normal, whereas the micronucleus, as a rule, becomes hypertrophied and the ectoplasm full of great vacuoles. Fig. 54 is a good representation of the conditions at this time. The endoplasm is apparently normal; there are food vacuoles and endoplasmic granules and vesicular structure, but the micronucleus is spherical and vesicular, has lost its usual place in a niche in the macronucleus, and shows evidence of granular modification of the previously homogeneous chromatin.

"One of the two oldest of the A series (742 generations) showed the following points while alive: 'A12 was alive this morning and was picked out for examination. It had two contractile vacuoles situated dorsally and close together. The astral canals were absent; in their place was a row of dorsal feeding canals, such as those characteristic of the more generalized holotrichida (*e. g.*, *Chlamydomontidæ*). The rest of the body contained eight or ten large vacuoles not contractile. The macronucleus was slightly hypertrophied and visible, indicating

the approach of disintegration. The papillæ of the cuticle were plainly visible, and what I have taken to be apertures of the trichocysts were more or less numerous. (This is shown in the preserved cell, Fig. 54.) A few trichocysts remained in the cortical plasm, but there were many vacuoles in this layer, indicating that when the trichocysts were discharged they were not reformed. The peristome was normal and the mouth had a vigorous oral membrane. The size was large, fully as great as any of the preparations that had been made at any time during the 742 generations. Movements vigorous to slow, with a tendency on the part of the animal to remain stationary.¹

"It was while the organisms were in this structural condition that the many attempts to rejuvenate the race were made as described in the previous pages, and it was in this condition of the protoplasm that the race finally died out from exhaustion. Before dying, however, the individuals, as indicated in the above paragraph from my notes, were of full size and were filled with gastric vacuoles and partly digested food, while the body form was normal.

"It must be admitted that these forms were capable of individual growth at this period, and since the macronucleus was normal in the last individuals, while the micronucleus was considerably changed, it must be further admitted that the vegetative metabolic processes were presumably re-invigorated; on the other hand, the functions of reproduction, that is, of division, were degenerated possibly, if not probably, because of the apparent degeneration of the micronucleus and of the cortical plasm, whose functions were not reinvigorated by the artificial means which were tried."

We are not in a position yet to demonstrate the nature of the cause of the depression periods. It is probably to be sought in the chemical make-up of the constituents of the cell, the chemical changes necessary for the functions of digestion, such as the formation of proteolytic ferments, oxidizing ferments, and the like, being no longer possible with the same food. We may compare a paramecium or oxytricha with a storage battery, the one having, at the outset, a certain potential of physiological activity, comparable with the initial electric charge of the battery. With the same food for a period of six months the initial charge of vitality is drawn upon, as work done draws upon the initial potential of the battery, until in a period of depression the resources of the cell are exhausted and the organism dies by what Hertwig calls "physiological" death.

The battery, however, to continue our analogy, can be recharged and is good for another period of work. So can the paramecium protoplasm. The six months of culture does not exhaust the germinal possibilities of that protoplasm; in the cultures referred to, the organ-

¹ From my notebook.

isms, or rather the race, were in the 200th generation at the time of the first depression, but the vitality of the protoplasm was not exhausted until the 742d. Woodruff's race of oxytricha protoplasm was in the 235th generation at the first depression period, but lived through 860 generations. There is no doubt whatsoever that all of the cells of paramecium would have died in the first period of depression had nothing been done to revive them. Joukowsky, in 1898, followed paramecium through 170 generations, when they all died during a period of depression; Simpson, in 1901, noted the gradual loss of vitality and death in his three to four months' cultures of paramecium. My cultures would have disappeared in a similar manner had it not been for a change of diet, by which it was found that beef extract, if given to paramecium for several days during this depression period, would restore the vitality and start the organisms off on another cycle of cell generations. In this way the few surviving organisms of the original culture were stimulated to new activity, or, to carry out the analogy with the battery, were given a new potential of vitality and a potential which again lasted through a period of six months, and through approximately the same number of generations (actually, 198) (see Fig. 38, period, August, 1901).

How can the renewal be interpreted? Obviously, the change in diet gave the cells an entirely different assortment of chemical substances, and it is to this fact that we may attribute the artificial rejuvenescence. Woodruff found that the same expedient renewed the vitality of his race of oxytricha, the effect being slower than in the case of paramecium. It was also found by Calkins that a change in the salt content of the usual food media would produce a similar stimulating effect, and dilute solutions of potassium phosphate were used, the organisms experimented with being allowed to swim in the solutions for half an hour (a longer period being followed by death in a few days). This simple salt, like the beef extract, was enough to renew the vitality, and the stimulus thus given was sufficient to enable the organisms to live again in the same medium for another cycle of 193 generations.

The effect of the change on the organism's structure is of interest, and is represented by Fig. 39. The cell in a depressed condition is shown on the left; a cell twenty-four hours after treatment is shown in the centre, where a lighter area in the vicinity of the nucleus will be noted, the ends meanwhile showing the same densely granular structure as that of the depressed condition, thus indicating that the organism is recovering from the disease, if we may so designate its trouble. It is important, in this connection, to note that the reestablishing of the normal structure occurs first in the neighborhood of the nucleus, a fact that indicates that here is the region of greatest chemical activity in the cell. A cell forty-eight hours after successful stimulation is shown on the right. These show that the "labile" condition of the

protoplasm is now extended nearly throughout the cell, the extremities alone retaining the granular structure of the depressed condition.

After such successful stimulation the digestive processes recommence, the organisms divide, and the division rate, as indicated by the curve, rises to an average of more than one division per day (see Fig. 38).

Three times in the history of this paramecium culture were the cells stimulated to new activity by this artificial means. The first time, as stated, was after the 200th generation, the stimulant being beef extract; the second time was after 198 generations more (398th of the race), the stimulant being beef extract and potassium phosphate; the third was after about 193 generations more (about the 600th of the race). This third period of depression was most interesting, for it was found that the same stimulants that had been previously used with success were now without effect; beef and potassium salts of various kinds were tried in vain, and the final extinction of the race was threatened; indeed, one race, which was called the B series, died out entirely in the 540th generation. Only six cells were left, finally, for experimentation, but some of these were successfully stimulated by treatment with an extract of pancreas, which contains many different salts in solution. The effect of this last stimulation was a renewal of the vitality, but the potential given to the protoplasm was not so great nor so clearly defined as in the previous periods of depression, and after another six months, in which the organisms showed great sluggishness, the race died in the 742d generation. This fourth cycle is the most important for our present purpose, since it represents the period of old age in the protoplasm under observation. The cells divided only 123 times, and toward the end manifested curious and hitherto unobserved degenerative phenomena, which deserve special attention.

The protoplasm of the cells in this final period of depression had at first the same appearance as the protoplasm of the organisms at previous periods of exhaustion; the cell body became granular, the size decreased, and the general appearance was similar to that which had been successfully met at previous periods. The same stimulants were used; the diet was changed for short periods as before; and, singularly enough, the same effect on the structures of the cell was produced. The granules disappeared, the nucleus and cytoplasm appeared perfectly normal, and the organisms were able to take in food, digest, and assimilate it. The normal size was restored, and it seemed, from morphological grounds, that the depression period had been successfully overcome for a fourth time. Still, the cell divisions were very infrequent and irregular, while the few that did take place were mostly of a pathological nature, complete fission not taking place, the result being monsters of different size and form (Fig. 55). The macronucleus was perfectly normal in the last cells of the race, but the

micronucleus, which has but little part to play apparently in the ordinary functions of vegetative life, now appeared enlarged and vesicular, and entirely different in structure and size from the micronucleus of the ordinary paramecium. The protoplasm was not granular nor chemically stable, and was apparently as active as ever. Still the organisms died, and death was not due to infection or disease. Something in the cells that had been operative before had given out, and the only part of the cell which had not responded to treatment was the micronucleus. Here, then, was a pathological condition which could not be met, and the organisms died.

FIG. 55

A "monster" formed by incomplete division of *Paramecium aurelia* as an indication of the exhaustion of division energy. (After Calkins.)

Was it death from old age that carried off the race under observation? There seems to be no other alternative to consider, and by old age we mean the wearing out of an organ and the cessation of a function. If old age may be thus defined in a simple organism like paramecium, it follows that three times previously had the race been weakened by old age, since the organisms were unable to digest and assimilate food. As soon as this power was restored by artificial means, old age was overcome and cell division was resumed. The cells would have died without any doubt had they not been stimulated, so that we are justified, as I believe, in speaking of this condition of paramecium as "physiological" old age, which leads to physiological death through the cessation of one or more of the vegetative functions. It is obviously death from a different cause that carried off the last cells of the race, and since the ordinary vegetative functions were apparently in perfect working condition at this final period, it follows that the cause of death must be looked for in the cessation of some other than the ordinary vegetative activities. The history of the micronucleus in conjugation (see next chapter) shows that this is the organ of the paramecium cell endowed with the characteristics of the race; in other words, that it alone of all the structures of the cell, must contain the

germinal elements. It is to be compared with the germ plasm contained in the germ glands of the many-celled animals, while the macronucleus and the cytoplasm are to be compared with the relatively much more voluminous somatic tissue of the higher animals. Its degeneration, therefore, indicates an exhaustion of the potential of activity of the germinal functions, including the power to divide, and with this exhaustion comes the death of the race, but death due to "germinal" rather than physiological exhaustion. While physiological death may be averted by stimulants of different kinds, germinal death, at least in the experience of all investigators up to the present time, cannot be offset, and with this comes the inevitable death of the race of protoplasm or death from germinal old age. Still paramecium are plentiful in ditches and ponds, a fact indicating that there is some natural way in which germinal death can be averted. Here is where the process of fertilization comes into play, and with fertilization the protoplasm of an exhausted paramecium is made over into a new "individual," in the same way that the protoplasm of a germ cell of a bird, mammal, or man is made over into a new individual.

These various experiments indicate, therefore, that natural death from old age under the conditions of the laboratory is actually inherent in protoplasm as little differentiated as in these single-celled animals, and they fail to confirm Weismann's claim that natural death is a penalty which higher animals must pay for the privileges of differentiation. They likewise fail to show that natural death by old age is due to any malevolent action on the part of certain structures of the body, as Metchnikoff would have us believe is one cause of old age in man. It is a natural condition of all protoplasm to grow old, and if we find the phenomenon in the generalized cells of the infusoria, how much more probable is it in the highly specialized somatic cells of the body. Each paramecium has a certain allotment of natural life and division. I have called it the potential of vitality. When this is exhausted under given conditions the protoplasm dies. It also has a certain allotment of germ plasm, so that by exhaustion of the physiological potential it still may retain a certain capacity for cell division, the germinal potential not being exhausted. It may, therefore, be stimulated by artificial means. In different kinds of animals and in different individuals of the same species it is probable that the initial potential varies, in some representing a longer, in others a shorter, life. In paramecium and the protozoa generally we find the greatest relative germinal potential, but as we go higher in the animal scale the tendency is for the germinal plasm to concentrate in a definite tissue of cells, the germinal epithelium, while the somatic cells have a correspondingly low degree of germinal plasm. To illustrate, while in all probability every cell of the paramecium race is capable of becoming or of giving rise to a germ cell, the same is not true of the animals next

higher in the scale of animal forms, such as the hydroids and jelly fish. A very small fragment, indeed, of a hydra will reproduce the entire animal, but one cell of the hydra will not do so; each of the two germ layers must be represented in the small piece. In worms and in still higher forms of the invertebrated animals this power to regenerate the entire animal decreases *pari passu* with the differentiation of the animal, and although not absolutely true, it may be stated in general that the higher the differentiation the less is the power to regenerate lost parts. In other words, something is lost from the highly differentiated somatic cells, something which is segregated in the germ cells and something which we find in each cell of the lower forms of invertebrates, but most widespread in the unicellular protozoa. It has to do with the racial characters of the organism, that is, with the germ plasm. In hydra and in some of the worms the cells retain enough of this germ plasm to reproduce the entire organism, but in the mammals the somatic cells have so nearly lost this germinal power that regeneration of an organ or limb is no longer possible, and is limited to the mere repair of an injury. In this sense, therefore, Weismann's claim that natural death is the penalty higher animals must pay for differentiation is justified.

The so-called "noble" cells (Metchnikoff) of the body, that is, the cells of brain, liver, kidney, and other important centres of physiological activity, are somatic cells in which this regenerative power is reduced to a minimum; the potential of germinal activity in them is less than in connective-tissue cells, and after an injury their power of repair is less than that in connective-tissue cells. This is seen in the fact that a wounded epithelium is repaired less by the proliferation of the neighboring epithelial cells than by the adjacent connective tissue, and the "scar" tissue which results is composed of these "baser" cells.

Like the physiological activities of paramecium, all somatic cells of the body are endowed with a certain potential of physiological activity, and like paramecium, when exhausted the particular function of those cells ceases; they have reached the limit of their activity, and when enough of them are so worn out a general impairment of the body functions results. This condition of the exhausted cells may be relieved by stimulants which, we imagine, may come from the general body itself, or from artificial treatment, as in the case of paramecium. But we have no reason to believe that in the human somatic cells this stimulation can be repeated indefinitely. If in the generalized protozoön there comes a time in which the potential of germinal activity of the cell gives out, how much more probable would it be that the somatic cells, with their low potential of germinal activity, likewise fail to respond to the stimulants. Unable to reproduce by division, with their potential of physiological activity reduced to a minimum,

these "noble" cells atrophy, their positions being taken by the connective-tissue cells.

Here, then, is the condition of old age; the somatic cells lose what germinal power they possess through physiological usury; their potential of physiological activity is greatly reduced; the function of the organ is impaired and the entire organization correspondingly weakened; the useless cells are attacked by phagocytes (?) (Metchnikoff), and they are replaced by the non-functional connective tissue.

Old age, therefore, is a biological condition of protoplasm, characteristic alike of the lowest protozoön and the highest mammal. Its progress is inexorable, its advent inevitable, while the only permanent plasm is that which has the highest power of germinal activity, and this is contained in the germ cells. Here, however, that other unfathomable mystery of life—fertilization, or its equivalent—is essential for the proper stimulation of the latent developmental activity and the distribution of the somatic and germinal cells in a new individual organism. How this occurs in paramecium and other protozoa will be shown in the following chapter.

While the experiments on the lowest animals show that old age is a necessary condition of vitality and inherent in all protoplasm, it does not follow that man or any other animal has made the best possible use of the vital endowments. It may very well be, as Metchnikoff maintains, that the traditional three score and ten is not an adequate allowance for man, and it is conceivable that the normal length of life may be increased by careful living to four or five score of years or more. If there is a certain amount of vitality upon which one can draw, it is obvious that the faster it is drawn the shorter will it last, and conversely, the more saving one is by careful living, the longer will it endure. Only one thing are we sure of, and this is that somatic vitality, whether in protozoön or man, is a *peau de chagrin* which constantly diminishes with use until finally nought is left.

CHAPTER IV.

CONJUGATION, MATURATION, AND FERTILIZATION.

IN the preceding chapter it was shown that the protoplasm of which a protozoön is composed, as demonstrated by continual observation, gives evidence of advancing age no less surely than does a many-celled organism. It was shown further that the advance from youth to age in such protoplasm is indicated by more or less well-marked physiological and structural changes, the former being characterized by the onset of a noticeable "period of depression," the latter by morphological changes, of which the most important is the development of a well-defined germ plasm. Experimental work on free-living protozoa has shown that the cells die a natural death during such periods of depression, but also, in some cases, that these periods may be overcome by artificial stimulation. They show, also, that a final depression, distinguished from ordinary physiological or metabolic weakness, and characterized by loss of the germinal protoplasm, could not be thus overcome. Apart from death by violence, therefore, the free-living protozoön may lose its life by what Hertwig calls "physiological death" at some period of physiological depression, or by "germinal death" occurring with the exhaustion of the division energy and degeneration of the germ plasm.

Notwithstanding the many natural enemies which a paramecium or other protozoön has, and in spite of the fact that if it escapes such enemies it may die from physiological or germinal "old age," it still exists in more or less abundance in natural waters, and will probably continue to exist in the future. In natural waters, salts, changes in the local environment, and other external causes undoubtedly tend to stimulate lagging physiological activities and to do on a large scale what we have done in the laboratory; but in nature, as in the laboratory, such means of rejuvenation probably have their limits, and we must turn to other vital activities for an explanation of the continued existence of these living cells.

There is little reason to doubt that the explanation lies in the secrets of the same mysterious and at present unfathomed phenomena which underlie the newborn infant; which are repeated in all living things with the creation of a new individual; and which are universally regarded as among the subtlest of vital activities. These secrets are deeply hidden in the phenomena of fertilization, and philosophers today, like the ancients, have only speculations to offer

in explanation. The phenomena of conjugation and maturation of the germ plasm which accompany fertilization are more easily interpreted, for they are largely matters of observation and deduction. In protozoa we have a particularly rich field for investigation of these problems, for the union of germ plasms is accompanied by phenomena of such relative simplicity that they are more easily observed, controlled, and interpreted than with metazoa.

In interpreting the phenomena of fertilization of protozoa we are in accord with those naturalists who, since the time of Harvey, have advocated some "dynamic" theory or other. (See Wilson, *The Cell*, p. 178.) In recent times this explanation is usually based upon the facts of decreasing vitality with advancing age, and, as expressed by Hertwig, fertilization is the means of restoring to a labile condition the protoplasm which, with continued physiological activity, has become stable in physical and chemical equilibrium. It is, therefore, essentially a process of rejuvenation.

Opposed to this point of view are those who, with Weismann and his followers, maintain that protozoa do not die of old age, and that conjugation with fertilization is an incidental occurrence in the life of a race. Fertilization, in higher forms, is a means of bringing about variation within the species, and at the same time a means of keeping the species true to its structural type.

Weismann still maintained his contention in regard to the immortality of infusoria after Maupas' classical experiments had demonstrated old age, and held that conjugation does not alter the individuality of the cells, since that individuality is retained after conjugation. Such a point of view would seem to be, however, merely an expedient to save the argument, for the essential part of the fertilized protozoön, like the metazoön, results from the union of two germ plasms, the protoplasm resulting from this union being a new individual in both cases. Like the metazoön, the protozoön is physically immortal only in the same sense of continuity of the germ plasm, for, with each fertilization there is a re-organization of the protoplasm, new chemical and physical combinations, and new individuality. There is no difference in kind in protozoa and metazoa, only a difference in degree.

The essential feature of fertilization appears to be the union of two masses of chromatin. We can only conjecture as to the significance of such union, but whatever hypotheses are framed to explain it, they must take into consideration a great variety of conditions under which the phenomenon is manifested. It is quite evident that complicated processes in metazoa are the highest and last steps, so to speak, in the elaboration of this universal biological phenomenon, and it is probable that they differ only in degree from the lowest and most primitive steps shown by the simple syngamic processes in protozoa.

In this lowest group of animal forms we find every grade in complexity in the sequence of syngamic processes, from those of undoubtedly primitive character to processes quite as complicated as in many metazoa. We may pass from cases where only the one cell is involved, fertilization taking place by union of two chromatin masses derived from the same primary nucleus (autogamy); through cases where the chromatin has had the same ancestry but is derived from different cells (endogamy); to cases where sex differentiation and maturation processes are quite as complicated as in higher animals and plants (exogamy). With our present incomplete knowledge of the life history of lower forms, no great value is to be attached to such a classification, but its main purpose is served in providing a convenient frame for attaching the manifold variations presented by the phenomena of syngamy in protozoa.

A. FERTILIZATION BY AUTOGAMY (AUTOMYXIS, HARTMANN).

In the primitive forms of protozoa, as in those of plants, this method of fertilization is widespread, and whatever may be the significance, its wide distribution among the most diverse of these lower forms and under the most varied conditions of life, indicates a natural and simple, if not primitive, fertilization phenomenon. Even in these more primitive cases, however, grades in complexity of the processes involved are to be observed, and the transition from autogamy to endogamy

FIG. 56

Ameba limax budding, division, and idiochromidia forming stages.

may occur in the same group. So far as the protozoa are concerned, the most primitive methods are to be found among the free and parasitic amebæ, but even here there are indications of a more advanced process.

The main element that enters into the complexity of these more primitive cases of autogamy is the formation of so-called secondary nuclei from idiochromidia and the differentiation of somatic and

germ nuclei. But in the simplest form such complication is not apparent, for the idiochromidia becomes segregated in masses without nuclear walls, and these masses fuse. This is the case in *Ameba limax*, a small free-living ameba common in ponds or decaying matter. It may be easily cultivated on artificial culture media, such as agar, in connection with various types of bacteria serving as food. Under normal conditions of temperature, salt contents, etc., the amebæ reproduce by simple division and by budding (Figs. 56 and 57). Under certain conditions of the cultures, conditions which have not been thoroughly investigated, the organisms encyst and remain so until transplanted to new culture media. Occasionally, and again under conditions unknown, they form sexually mature cells, but this latter condition may also be brought about by suitable temperature changes.

FIG. 57

Ameba limax. Nucleus in upper cell in full mitosis; in lower cell (right) in anaphase of the mitosis.

Syngamic nuclear union is always preceded by idiochromidia formation within the cyst, but the formation of this material does not necessarily imply sexual maturity. In all cultures, after a time, the nucleus, which consists of a central karyosome and peripheral chromatin, gives rise to idiochromidia by dissolution of the peripheral portion. The idiochromidia become scattered throughout the cell, and, under ordinary conditions of the culture, are evenly diffused. If the cultures be subjected to rapid changes of temperature, the idiochromidia may be caused to accumulate in masses about the periphery (Fig. 51, p. 122). Sixteen of these masses are usually formed, and then by fusion two by two the number is reduced to eight. This fusion possibly represents a sexual union, or, more strictly speaking, takes the place of sexual union, being the fusion not of secondary

nuclei, but of masses of idiochromidia which in other protozoa become differentiated into such nuclei. The karyosome and some of the peripheral chromatin form a degenerating "somatic" nucleus which takes no part in the later processes.

The further fate of the encysted form thus brought about has not been followed, but in *Entameba histolytica*, according to the observations of Schaudinn and, later, of Craig ('08), such a stage is followed by spore formation. Schaudinn ('03) observed, and his observations have been confirmed in every detail by Craig ('08) upon living and fixed material, that in this ameba the nucleus fragments into idio-

FIG. 58

Entameba histolytica. (After Craig.) A, organism showing rods and granules of chromatin in the nucleus, vacuole with some stained substance, and dense ectoplasm; B, the chromatin of the nucleus passing into the cell plasma, where it is distributed as chromidia, shown in C; D, aggregation of chromidia to form secondary nuclei (see Fig. 51, of *Ameba limax*); E, "spore formation" by budding; F, spores of *Entameba histolytica* as seen in feces.

chromidia (chromidia) which collect in masses at the periphery, and these masses, with some cytoplasm, are protruded from the surface as buds. The buds become covered with a hard and resistant membrane which is so deeply colored by the intestinal fluids that further internal processes could not be followed (Fig. 58). Neither Schaudinn nor Craig observed union of these idiochromidia masses, and the resemblance to *Ameba limax* can only be inferred from the similarity of preliminary processes.

In the closely allied forms, *Entameba coli*, *Entameba muris*, and *Ameba proteus*, the process of autogamy is somewhat more compli-

cated because of the formation of definite nuclei from idiochromidia, and because of so-called maturation divisions of these nuclei before union (*coli* and *muris*).

Here, again, the early observations of Schaudinn ('03) upon *Entameba coli* have been fully confirmed by Craig ('08) and their conclusions have been fully supported by Wenyon ('07) in connection with *E. muris*, a closely allied intestinal parasite of the mouse, and by Hartmann ('07) upon *Entameba tetragena* in man. Schaudinn's excellent description was not accompanied by illustrations, but the

FIG. 59

Autogamy in *Entameba* (ameba) *muris*. (After Wenyon.) A, ordinary ameboid form with nucleus in process of division; B, ordinary individual encysted and with one nucleus; C, nucleus divided; D, chromatin has passed into cytoplasm, leaving no definite nuclei in the cyst; E, two small nuclei reformed from the scattered chromatin, other chromatin residue and food remains are being voided; F, two nuclei and so-called "reduction" bodies remaining in cyst; G, a cyst with two spindles, food remains, and some waste chromatin; H, the two spindles give rise to four nuclei which conjugate two and two; I, cyst with two recently conjugated nuclei which next divide to form four and finally eight (J) spore nuclei.

corresponding stages may be illustrated by Wenyon's figures of *E. muris*. Here and in *E. coli* the organisms encyst after a period in the intestine; the nucleus of the encysted cell divides (Fig. 59, A, B, C) and the cell body indicates a corresponding division into two parts, but the connections between these parts is never lost, and we are thus dealing at the beginning of fertilization with a binucleated cell. The nuclei next fragment, forming idiochromidia, from which two much smaller nuclei (D, E) are formed by segregation of the scattered granules. Each nucleus then divides twice, one-half of each division forming nuclei which degenerate in the cell (reduction nuclei) and two

fertilization nuclei finally result, each of which divides again, this time with the long axes of the spindle parallel with one another; the final daughter nuclei which are formed fuse two by two, the cleft in the cell disappears, and an encysted ameba results with two fertilized nuclei. Each of these nuclei divides twice, and eight spores are formed about the eight resulting nuclei. Hartmann ('07) mentions a similar process of autogamy in the case of an ameba from the frog and in one of the free-living *limax* forms, but describes a quite dissimilar process in *Entameba tetragenä*.

In these cases, therefore, there is a concentration of the idiochromidia in secondary nuclei which then undergo so-called maturation processes. A still greater complexity is shown by *Ameba proteus*, where, according to the observations of Calkins ('07), there is no formation of diffused idiochromidia, but the secondary conjugating nuclei are formed directly from chromatin granules within the primary nuclei, which, prior to this stage, had divided repeatedly until about 70 are present. These secondary nuclei next fuse two by two in the cytoplasm and give rise to spore-mother cells (sporoblasts), of which there may be as many as 250 within one parent organism (Fig. 60), while at least one of the primary nuclei remains unused and finally degenerates in the cell. In *Ameba proteus*, therefore, the organism forms not one spore-mother cell, as in the parasitic amebæ, but many such spore-forming centres.

In all of the above cases of autogamy, we have to do with the fusion of chromatin materials which at one time or another were parts of the same nucleus of the same cell. In all of them, with the exception of the free-living *Ameba limax* and the parasitic *Entameba histolytica*, where further observations are much to be desired, the union of the "gametic" nuclei does not take place until after two or more divisions of the primary or secondary nuclei; that this fact has some significance cannot be doubted, but there is no inkling as to what the significance is, unless, indeed, it is evidence of an earlier gamete-forming stage, autogamy thus being, as Hartmann ('09) suggests, a degenerative rather than a primitive phenomenon.

With the myxosporidia the process is much more complicated, involving the formation of vegetative and germinal nuclei. It is well described by Schröder ('07) for the case of a parasite of the seahorse, *Spheromyxa labrazesi*, where the multinucleate ameboid body of the parasite appears to contain two kinds of nuclei distinguished by size and structure. Within this protoplasmic body small areas become differentiated from the surrounding matrix. These areas characteristic of the myxosporidia, termed pansporoblasts (Gurley), contain two nuclei, one of each kind (Fig. 61, K, Q). With development of the pansporoblast each nucleus divides in such order that seven daughter nuclei finally result from each, the fourteen nuclei being

characterized as follows: Two are destined to degenerate as "reduction nuclei," four become the centres of shell formation of the spores, four become centres of pole capsule formation, and four remain as

FIG. 60

Autogamy in *Ameba proteus*. In upper figure secondary (gametic) nuclei are shown emerging from the primary nuclei. In central figure is pictured the union of gametic nuclei together with some undeveloped ones in a primary nucleus. In lower figure is shown the mass of sporoblasts which develop from the fertilized gametes. (After Calkins.)

Conjugation in myxosporidia. *A* to *I*, *Myxobolus pfeifferi*, Th. (after Keysseltz); *K* to *Q*, *Spheromyxa labrazezi*, Lav. and Mes. (after Schröder); *A*, *B*, formation of gametoblasts; *C* to *G*, union of sporocysts and multiplication of nuclei; *H*, young sporoblast with polar capsules forming and gametic nuclei not yet united; *I*, spore with capsules (not filled in) and gametic nuclei united; *K*, young pansporoblast of *spheromyxa*, with dimorphic nuclei; *L*, pansporoblast with fourteen nuclei; *M*, pansporoblast divided into sporoblasts, each with two pole capsules (*p*), four globules present (*x*) and with two central reduction nuclei; *N*, sporoblasts having two shell nuclei (*s*), two polar capsules, each with a nucleus and two germ nuclei; *O*, young spore, shell nuclei disappeared, capsule (*p*) and germ nuclei (*gg*) compact and lying in a row; *P*, same, with union of gametic nuclei in the sporoplasm; *Q*, same, ripe spore with polar capsules and sporoplasm.

germinal nuclei. The sporoplasm of the pansporoblast divides into two parts (*M*), the sporoblasts each containing six of the fourteen nuclei, while the reduction nuclei remain outside. The six nuclei in each sporoblast are thus differentiated into somatic and germinal nuclei, four in each case going into somatic modifications of the spores (shells, pole capsules, and threads), and two, presumably one of each of the original two kinds, remaining as pronuclei (*N*, *O*, *P*). After the spores are mature and only traces of the somatic nuclei remain, these germinal nuclei fuse, so that the spores, when taken into a new host, are uninucleate (*P*, *Q*). If, as Schröder suspects, the multinucleate ameboid adult is formed by fusion of two or more cells, then such a process would be like that of the mycetozoa and exogamic rather than autogamic (p. 150). Observations, however, are wanting to confirm this supposition, the many obstacles in the way of observations to this end making confirmation extremely difficult, but the other matters relating to number of nuclei formed, their fate, etc., are well corroborated (see actinomyxidæ, and *Myxobolus pfeifferi*, Fig. 61, *A*, *I*).

B. FERTILIZATION BY ENDOGAMY (PEDOGAMY, PROWAZEK).

The transition from autogamy into endogamy, whereby the sexual union is between descendants of the same original cell, is marked by numerous intermediate stages which are sometimes described as autogamous. The difference is largely one of degree only, and among these intermediate forms, at least, to include them under one or the other heading is mainly a matter of expediency. The principle underlying the distinction is, however, of considerable theoretical importance, and the difference which exists between the partially divided cell in *Entameba coli* (see above) and the union of separated parts within the same parent cell (see myxobolus and other cases below) is a difference which becomes magnified in higher types into all of the differential characteristics which distinguish exogamic processes.

The transition from autogamy to endogamy is well shown in mycetozoa and myxosporidia, where, as may be seen, the difference is only one of degree. There are numerous examples of the phenomenon, from which we select a few showing different grades in complexity, and it should be noted that the same arguments as to the possible exogamic nature of the processes apply here among the mycetozoa and myxosporidia as well as in the cases cited above.

Keysselitz ('08) has quite recently described the process of pansporoblast formation in a myxospore (*Myxobolus pfeifferi*) which differs in one important respect from the process in spheromyxa. Here the pansporoblasts which Keysselitz names the "propagation

cells" arise in the plasm of the adult organisms in the same way as in other myxosporidia, but the nuclei and with them the cell body of the germinal area divide (Fig. 61, *A, B, C*). These propagative cells later unite two by two, and are separated only by a thin cell wall, which later disappears. Within this united mass the nuclei divide until there are fourteen, as in spheromyxa; their formation differs in some unessential details, but their fate is the same in both cases, two germinal nuclei finally resulting which conjugate in the mature spore (Fig. 61, *D, I*).

Caullery and Mesnil ('05) have carefully described the process of spore formation in spheractinomyxon, one of the actinomyxidæ, an aberrant group of myxosporidia named by Stolž ('90). Here the process is a little more complex than in the case cited above, but it agrees in essence with that described by Keysselitz. The youngest stages are found as intestinal parasites of the tubificid worm clitellio, and are either uninucleated or binucleated. The observers are inclined to believe that the uninucleated stage comes first and that it represents, possibly, the youngest form, or sporozoite, and that the binucleated stage represents the first division of this nucleus. If this possibility is not well founded the fertilization process here must be taken out of the present category. Whatever may be the origin of these nuclei in the binucleated stage, they divide, and two of the first four nuclei formed become somatic nuclei and are connected with the formation of the cyst wall, within which the further processes take place. With the division of the nuclei the cell body also divides until there are sixteen independent, nucleated subdivisions. These unite two by two, the process of fertilization being thus affected, and eight spores ultimately result. The interpretation of this interesting case, as Caullery and Mesnil point out, depends entirely upon the mode of origin of the early binucleated stage. If these two nuclei represent a plastogamic union of gametes, as Lèger ('04) believed to be the case in an allied form triactinomyxon, then the process might be one of exogamy, but, as Caullery and Mesnil contend, this would involve two sexual processes in the life cycle, which seems improbable. The subject certainly needs further study.

The endogamous process in the mycetozoön *Plasmodiophora brassicæ* is somewhat less complex than in the forms just described. Here, as Prowazek ('05) has shown, the protoplasm breaks down into many centres, each containing a sexual nucleus, and these centres—gametes—fuse two by two, a spore wall being formed about each copula (Fig. 62).

In the majority of parasites the probability of endogamous fertilization is readily apparent, and the fusion of gregarines, for example, two by two, may be a union of cells from the same sporocyst or different sporocysts. In such cases it is impossible to state definitely,

therefore, whether the process is endogamous or exogamous, and the same obscurity obtains in the union of free flagellated or ciliated gametes. In some cases, on the other hand, there is no doubt about the union of nearly related cells. Schaudinn ('94) described the union of gametes of the same brood in *Hyalopus dujardinii*, and it is proved in the case of *Basidiobolus lacertæ* by Loewenthal ('03); in *Actinospherium eichhornii* by Hertwig ('98); in yeasts by Guilliermond ('02), and in cultures of free-living infusoria (*Paramecium aurelia*) by Calkins ('02).

FIG. 62

Endogamy in *Plasmodiophora brassicæ*. (After Prowazek.) A, portion of plasmodium showing ordinary vegetative nuclei; B, reconstruction of the gametic nuclei; C, division of same; D, union of gametes formed about gametic nuclei; E, F, stages in fusion of nuclei and formation of the spore.

In *basidiobolus*, an intestinal fungoid parasite of the turtle, the organism forms straight or branched hyphæ composed of sister cells lying end to end, and at maturity two adjacent sister cells conjugate, a process recalling conjugation among the lower plants (*conjugatæ*, diatoms, etc.). In *actinospherium* the phenomena of fertilization are much more complex and have been made the subject of careful study by Hertwig ('98). The first evidence of the process is the encystment of the adult organism and excretion of waste matters contained in the protoplasm. The many nuclei of the ordinary forms are here reduced to about 5 per cent. of the total by a process of fusion and absorption in the protoplasm, and after this has occurred the mother animal fragments into as many daughter cysts (cytospores No. 1) as there are

nuclei remaining (from one to twenty). Each of these daughter cysts secretes a gelatinous envelope about itself, and the nucleus of each divides by mitosis. This mitotic division is followed by division of the cytopore into two daughter cells (cytospores No. 2), and in these there are two successive nuclear divisions resulting in four nuclei. Three of these nuclei degenerate ("polar bodies") and one remains as a pronucleus. The cytospores of the second order next unite again, reforming the cytospores No. 1, and the fertilization is completed by

FIG. 63

Endogamy in *Actinospherium eichhornii*. (After Hertwig.) A, two gametes (cytospores No. 2), resulting from the division of cytosporc No. 1; B, both polar bodies are formed in the right gamete, the second one forming in the left gamete; C, later fusion of the gametes, the nuclei now uniting and the polar bodies being absorbed at p; D, young actinospherium leaving cyst.

fusion of the pronuclei. Thus, by a process of union of sister cells (endogamy) fertilization is brought about after complicated maturation processes (Fig. 63).

Finally, in *Paramecium aurelia*, Calkins ('02) found that cells removed by not more than eight or nine divisions from a common ancestral cell would conjugate normally, and that such fertilized cells were able to live through an entire cycle of cell generations (379 actually). Conjugation between closely related forms, therefore, is quite as potent as between those of diverse ancestry.

C. FERTILIZATION BY EXOGAMY.

It is not at all improbable that some of the cases that have been described as autogamous may be in reality exogamous. In the multinucleate forms, in order to decide such a matter it is necessary not only to observe the union two by two of such nuclei, but their mode of origin must also be known. Thus, in the mycetozoa the plasmodium from which the sexual nuclei are generated is formed by the fusion of two or more ameboid cells at an early period of development, hence the nuclei which later fuse may be derived from different ancestral cells, and such fusions would not be examples of autogamy, but of exogamy. In some cases of sexual reproduction among myxosporidia (notably in the actinomyxidæ and possibly in *Spheromyxa labralesi*) a similar derivation of the conjugating nuclei has been suspected. Such cases of possible exogamy are well illustrated in almost any of the higher types of mycetozoa, and one such has been well described by Kränzlin ('07) for *Arcyria cinerea* and *Trichia fallax*, and by Olive ('07) and Jahn ('07) for *Ceratomyxa hydroides*. Without going into the details the process may be summarized shortly as follows: The young ameboid or flagellated spores, after assumption of the ameboid state, fuse into plasmodia of considerable size. Cell boundaries are entirely absent and the nuclei have an opportunity to become thoroughly mixed in the protoplasmic streaming. Fructification ensues after a longer or shorter vegetative life and in these fruiting bodies, or before their formation, the nuclei unite in pairs, the union being followed by synapsis and double divisions and formation of the ripe spores.

A somewhat similar union has been described by Hartmann and Nägler in the case of *Ameba diploidea*, H. and N., where the organism is binucleated throughout the ordinary vegetative stages and until the period of maturity, when two cells place themselves side by side within a common cyst. The two nuclei of each cell then unite, forming a single synkaryon in each cell. The two adjacent cells finally unite by dissolution of the cell walls that separate them, and the recently fertilized nuclei, after some very questionable so-called maturation processes, assume the characteristic position of the vegetative forms. Here, then, if this observation is accurate, there is an exogamic fertilization, but the end stage does not occur until the next following period of maturity (Fig. 64).

In the majority of protozoa the germ of the new individual, as in metazoa, is produced by the union of cells from different ancestors, and these cells, for the most part, show characteristic evidences of the period of maturity. In some cases there is but slight difference, if any, between the conjugating cells and the normal ones, the conditions

of maturity manifesting themselves in other ways than by size changes. In other cases the conjugating cells are reduced in size, but without differences of a sexual character, and in still other cases there is a

FIG. 64

Ameba diploidea, Hartmann and Nägler. *A* and *B*, ordinary individuals at early and mid-phases of division; *C*, *D*, *E*, union of two individuals within cyst, and fusion of the double nuclei in each cell; *F*, ameba after fusion of cell bodies, now with two nuclei, creeping out of cyst.

marked sexual dimorphism, the manifestations of maturity showing in greatly reduced size and relatively great kinetic energy on the one part, and increased nutritive potential and relative sluggishness on the other. For purposes of description these various conditions are usually grouped under the headings isogamy (fusion of equal gametes) and anisogamy (fusion of dissimilar gametes).

FIG. 65. Conjugation of *Actinophrys sol.* (After Schaudinn). A, union of two similar cells; B, C, formation of cyst and formation of first maturation spindle; D, E, degeneration of one nucleus in each cell (p) and union of pronuclei; F, first nuclear spindle after fertilization.

1. **Isogamy.**—Not only may isogamous conjugation occur between full-sized and reduced-sized individuals, but among the former there may be a further difference in that the conjugating cells do not fuse to form a zygote, but separate after a few hours (copulation). This process is particularly characteristic of the infusoria and is not met with elsewhere.

FIG. 66

Copromonas subtilis. (After Dobell.) A, normal adult cell before division, from life; B, cells in conjugation, one flagellum being withdrawn; C, fusion, first stage in "nuclear reduction;" D, heteropolar division of nuclei for second "reducing division;" E, fusion of nuclei and formation of cyst; F, fertilized cell in permanent cyst.

(a) **The Union of Full-sized Cells.**—With the exception of the lower flagellates, there are few instances of conjugation among full-sized individuals. It has been described by Schaudinn ('96) in the case of the heliozoön *Actinophrys sol* (Fig. 65), where the two cells fuse after a preliminary process of maturation. Here there is little change in the normal aspect of the two conjugating cells beyond the withdrawal of the pseudopodia and secretion of a protective cyst. So, too, among some of the flagellates there is little difference in the gametes from the normal. In *Bodo saltans* (Dallinger) they are all alike, while in *Copromonas subtilis*, according to Dobell ('08), one of the two cells is absorbed in the other, and its flagellum is lost, while the flagellum of the other

serves for locomotion (Fig. 66). So, too, in *Dallingeria drysdali* one of the conjugating gametes has three flagella, while the other has only one.

Analogous processes occur in *Lambliu intestinalis* (see Schaudinn, 1903), *Hexamitus intestinalis*, and among many of the phytoflagellates, where size difference, however, appears to be facultative. In a number of other cases, however, the adult form is lost during the period of sexual maturity, the organisms becoming ameboid or losing their characteristic motile organs. Thus, in *Cercomonas dujardini* and in *Tetramitus rostratus* (Fig. 67) the ordinary firm contour of the body is lost and it becomes highly plastic and changeable in shape, although in the latter the anterior end with the four flagella does not materially change in character until fusion of two cells is well advanced. In *Trichomonas intestinalis*, on the other hand, the flagella are discarded and the body becomes ameboid before fusion (Schaudinn, 1903), a condition in which, as Schaudinn observes, it is often difficult to distinguish the flagellate from intestinal amebæ.

(b) **The Union of Diminutive Cells.**—There appears to be no hard and fast line between the phenomenon of union of adults and of smaller cells, for there are cases, especially among the phytoflagellates, where a larger cell may unite with one similar to itself, or with a smaller one, or two smaller ones may unite, and these, in turn, may be similar or dissimilar. Such facultative differences are rarely met with among the animal flagellates, and one consistent rule is usually followed. The union of reduced or diminutive cells is very rare among ciliates, but an interesting exception is the case of *Opalina ranarum*, where, according to Neresheimer ('07) the gametes are minute ciliated cells. On the other hand, it is quite common among the rhizopods and seems to be the rule among the foraminifera, but in many cases, as, for example, among the radiolaria, the diminutive cells are at the same time dissimilar, so that they do not properly come under the heading of isogamy. These differences, however, are often so minute that no great value can be placed upon such an artificial distinction.

Very frequently these diminutive gametes are totally different from the parent cell in mode of locomotion, the rhizopods often forming flagellated gametes which conjugate, the copula developing into the ordinary form. This is the case in *Polystomella crista* (see Fig. 52, p. 123), and in *Trichospermium sieboldi*, Schaudinn ('03); in *Pseudospora volvocis*, Robertson ('05), and in other sarcodina. In other cases an ordinarily motionless form like *Gregarina ovata* (Schnitzler, '05) and some species of monocystis produce ameboid isogametes.

A very interesting case of isogamy has been recently described by Lèger ('07) in *Ophryocystis mesnili*, one of the schizogregarines. Here two cells unite in *accouplement*, as Lèger terms it, a characteristic preliminary union of two gregarines (pseudoconjugation) before

formation of the gametes. So-called processes of reduction occur in each of the nuclei, and a mature nucleus is formed in each cell which becomes surrounded by protoplasm very much as in the case of a myxospore pansporoblast (Fig. 80, p. 190). These two gametic areas then fuse, forming a zygote or copula inside of the joined gregarines, and within this copula the sporozoites are developed, while the surrounding parent cells degenerate and die.

FIG. 67

Different stages of the flagellate *Tetramitus rostratus*, Perty (Stein). Ordinary vegetative individuals (*A*, *B*, from side and front) reproduce asexually by longitudinal division. They ultimately become plastic (*C*) and miscible, and two individuals upon meeting (*D*) fuse. The copula secretes a membrane, and its protoplasm fragments into hundreds of spores, (*E*) which quickly grow into the parent type (*F*, *G*, *H*).

Such a condition is perhaps to be traced back to the process of gamete formation in other types of gregarines, where, as in *Monocystis ascidia*, the two organisms unite in couples and give rise to numerous minute gametes which move by amoeboid movements through the liquid

of the common parental cyst, the gametes from one cell ultimately meeting and fusing with those of the other (Fig. 75, p. 181).

If, in cases like the preceding, the coupled cells should separate, the process would be analogous to that characteristic of the infusoria, and such processes may give a clue to the explanation of the highly enigmatical processes in the latter group, where copulation, including mutual fertilization, takes the place of gamete formation. A typical example of this type of isogamy is that of *Paramecium aurelia* (*caudatum*), which may be briefly outlined as follows:

A culture of *Paramecium aurelia* can be easily prepared in the laboratory by seeding a hay infusion with a dozen or more cells from pond water. After some weeks they will have accumulated in great numbers, and quantities of conjugating forms may be obtained by removing them to watch glasses. Pearl ('07) has shown biometrically that the "conjugating population" consists of individuals of measurably smaller size than those of the usual pond water. There is also a difference in the physical and chemical make-up of the cells, by which the protoplasm becomes much more sticky, so that two individuals upon meeting frequently fuse at any point, but this extremely miscible condition is probably evidence of physiological weakness indicative of old age, and represents an excess of the conditions under which conjugation is possible.

The union of the two paramecium cells is apparently the signal for the beginning of the maturation processes of the nucleus (Fig. 68). In many egg cells of metazoa, and in all spermatic cells, these processes precede union, showing that they are more generally phenomena of the ripening or maturity of a cell than phenomena induced by cell union, as in paramecium. At the outset the two organisms are more loosely attached, so that forceful ejection from a pipette is sufficient to separate them. After twelve hours' union, however, the attachment is so firm that no amount of force will break them apart without killing one or both. Such forcibly separated conjugants are by no means without vitality, five out of twelve which were followed in cultures continuing to live and divide, one being followed through more than 158 generations before it was abandoned.

The normal course of conjugation requires from eighteen to thirty hours, according to the temperature, and during the process the micronucleus of each cell divides twice; one of the four cells in each case then divides again into dimorphic nuclei. One of these nuclei is smaller than the other and acts as a spermatic or wandering nucleus, while the other remains in the parent cell. Each cell receives a wandering smaller micronucleus from the other organism; this fuses with the larger micronucleus to form the fertilization nucleus of the new individuals. Each fertilization nucleus then divides three times in quick succession, and eight micronuclei are formed. Four of these

FIG. 68

Conjugation of *Paramecium aurelia*.

begin to swell and to metamorphose into four new macronuclei, while four remain as micronuclei. In the meantime, the two conjugating cells separate soon after the interchange of micronuclei, and the processes of reorganization are carried out independently. The old macronucleus begins to disintegrate by first forming a skein-like structure and then breaking down into granules which are finally absorbed in the cell protoplasm. The process of reorganization requires from one to three days before the first division of the fertilized cell, which, as we have seen, contains four micronuclei and four macronuclei. The daughter cells after the first division each contain two macronuclei and two micronuclei, and the normal nuclear relations are not reestablished until after the second division, when the resultant four cells have each one macronucleus and one micronucleus.

This phenomenon may be interpreted in terms of the conjugation in opalina, where minute ciliated cells conjugate, fuse, and form a zygote (Neresheimer), if we assume that each of the daughter micronuclei formed represents the nucleus of a microgamete in some phylogenetic ancestral stage, and if it is further assumed that in successive phylogenetic stages (1) coupling of the adults occurred, as in gregarines; then (2) formation of endoplasmic gametes, as in ophryocystis; and (3) interchange of micronuclei or gametic nuclei without the formality of endoplasmic gamete formation. The vorticellidæ show an aberrant development in such a hypothetical history, for here one of the conjugating cells is smaller than the other and fuses with it. But here as many as eight micronuclei (Maupas) may be formed in the preparatory stages, a number difficult to explain on any other hypothesis. Copulation, therefore, as seen in the infusoria, involving temporary union of two similar cells, may be interpreted as a regression of the gametes or a reminiscence of gamete formation in ancestral cells, and as entirely different in its essential character from processes of coition of the higher animals.

2. **Anisogamy.**—Under this term the greatest number of heterogeneous phenomena are usually collected, and in all probability there is a wide physiological difference between them, involving in some of the higher types all of the characteristics of sex differentiation. In those cases where size differences are not obligatory, as in polytoma, for example, it is hardly justifiable to speak of sex differentiation, by which is usually meant the formation of definite somatic characters in individuals destined to form either eggs or spermatozoa. So far as the ultimate products are concerned, the protozoa give evidences of a gradual evolution toward complete dimorphism of the conjugating gametes. This is particularly well shown in the gregarinida, where a series of forms shows the gradual development into gametes that might well be interpreted as eggs and spermatozoa (Fig. 69). In coccidia and in hemosporidia there are similar varieties of forms,

but not as complete as in the gregarines; one case, *Adelea ovata*, is interesting in that one of the conjugants is a large form similar to the ordinary vegetative individuals, while the other is much smaller and is derived from an individual which forms four gametes while attached to the other cell, one of these gametes penetrating the larger cell, while the other three degenerate and disappear. In this form also we have

FIG. 69

Different forms of gametes in gregarines and coccidiidia. (After Shellack.) A, *Stylorhynchus longicollis* (after Lèger); B, a species of monocystis from *Lumbricus* (Cuenot); C, spermatozoid of *Echinomera hispida*, to the left the two gametes of *Pterocephalus nobilis*; D, the two gametes of *Urospora lagidis* (Brasil); E, the same of *Gregarina ovata* (after Schnitzler); F, the same of *Schaudinnella henleæ* (after Nusbaum); G, the same of *Coccidium schubergi* (after Schaudinn).

what may be regarded as complete sex differentiation, since the protoplasm of the race forms individuals of male or female character, never both. Schaudinn and others have shown that the difference between the two conjugating forms is present in potential throughout the entire series of forms, the first division of the fertilized egg giving rise to individuals which can be identified as male or female. In this case,

and among the flagellates as well, this primitive sex differentiation can be traced throughout the entire series, or the "individual" in the sense used in the preceding chapter. In *Coccidium schubergi* (Fig. 74, p. 179) a similar difference is demonstrable for a considerable number of generations, but is not so marked apparently as in adelea. Here fertilization is accomplished by union of a flagellated microgamete or spermatozoid, and a food-stored macrogamete.

The flagellates, also, present wide variations in anisogamic conjugation, some of them, like *Trypanosoma noctuæ*, being sexually differentiated, according to Schaudinn, from the time of the first division of the fertilized cell. In this form of trypanosome, and in other species as well, Schaudinn and different observers have described three distinct types of the organism, females, males, and "indifferent" forms, the latter, under appropriate circumstances, becoming either one or the other sex.¹

The female trypanosome of *Trypanosoma noctuæ* is of relatively large bulk, nearly spherical when mature, and somewhat inactive during vegetative life. These are the most hardy of all forms of the parasite, because of the reserve store of nutriment which they contain, and these are the forms which, under certain conditions, may undergo parthenogenesis (see p. 163). In order to undergo their full sexual development, the parasites must be taken into the body of a mosquito of the genus *Culex*, and here the male individuals are transformed into microgametocytes and the females directly into macrogametes. In the male gametocytes the kinetonucleus fuses with the vegetative nucleus and the pigment granules are eliminated. The fused nucleus next divides by a heteropolar mitosis into two nuclei, one large, the other small. The larger nucleus degenerates, while the smaller one divides repeatedly until eight nuclei are present. Each of these divides still again to form a larger vegetative and a smaller kinetonucleus of the future microgamete. The periphery of the cell then draws out into eight projections, each containing one pair of the recently formed nuclei, and these projections are finally pinched off the parent cell as microgametes, each of which, in the meantime, has formed its definite locomotor apparatus of the typical character. The macrogamete, on the other hand, does not form a locomotor apparatus, but after undergoing maturation processes is sought out and fertilized by one of the microgametes.

Similar processes have been described by Prowazek, Keysselitz, and

¹ Schaudinn's observations have been severely criticised and his conclusions denied by numerous investigators, in particular by Novy and his collaborators; but while these criticisms are of undoubted value, the fact remains that Schaudinn's description of the life history of this parasite of the owl is entirely consistent and the most plausible of all that have been presented in connection with trypanosomes, and I give it here as a type of fertilization in trypanosomes in general.

others for different kinds of trypanosomes and for trypanoplasma, a closely allied form; none of the descriptions, however, are sufficiently convincing to establish a life cycle, while numerous contradictory accounts indicate the need of further careful and unprejudiced research.

With the exception, therefore, of the case of *Trypanosoma noctuæ*, the flagellates present few well-defined instances of sex differentiation, but other examples might be cited in which fertilization is accomplished by the union of anisogametes. In *Mastigella vitrea*, Goldschmidt ('07) has shown that a small non-motile gamete unites with a larger flagellated gamete (Fig. 48, p. 119), a condition which reverses the ordinary process, where the resting cell is usually larger and possesses the attributes of an egg cell. Anisogamous conjugation occurs also in *Bodo caudatus*, *Bodo lacertæ*, and *Monas dallingeri*, and among many of the phytoflagellates, where in *Pandorina morum* and *Eudorina elegans* sex differentiation is well established, but in other forms, as chlamydomonas, size differences are quite facultative.

Among the rhizopods the formation of anisogametes appears to be widespread, especially among the fresh-water types. Schaudinn ('03) and Elpetiewsky ('08) showed that minute but anisogamous gametes are formed in centropixis and arcella, the gametes in all cases having nuclei derived from the idiochromidia (Fig. 47, p. 119).

Fertilization by exogamy appears to be, therefore, the most widespread and the most complicated of all methods of fertilization among the protozoa, while in the higher types the process is accompanied by well-marked maturation phases, approaching in complexity very close to the reducing divisions and polar body formation of the higher animals and plants.

D. PARTHENOGENESIS.

The processes of autogamy, as outlined above (p. 139), seem to have many points in common with parthenogenesis or development of egg cells without fertilization. While the end result is undoubtedly the same in both, a difference is implied from the fact that differentiated egg cells, which normally develop after fertilization by a spermatozoön, in parthenogenesis develop without such union. Parthenogenetic eggs, therefore, are, in a sense, abnormal and may be interpreted as presenting a phenomenon of cenogenesis whereby the egg returns to a primitive condition. Boveri ('87) suggested and Brauer ('93) confirmed the suggestion in connection with the parthenogenetic eggs of *Artemia*, that parthenogenesis is a result of the fertilization of the egg nucleus by a polar body (Wilson, *The Cell*, p. 281). Such fertilization, as in the case of autogamy, is brought about by the union of sister nuclei. In

autogamy, however, we have to do, probably, with a much more advanced cenogenetic process, and the cells at such periods of activity cannot be regarded as egg cells, since there is no trace of sexual differentiation.

Not only in different kinds of metazoa, but among some of the protozoa as well, the so-called "females," or egg cells, under certain conditions, may develop by parthenogenesis, thus showing a first step in degeneration leading to the method of fertilization by autogamy. Such a possibility seems to have been first suggested by Grassi ('01) in connection with the organisms of malaria, for he stated "the macrospores (macrogametes) and possibly the microspores (microgametocytes) can increase by parthenogenesis," but the process was first described for the malaria organisms by Schaudinn ('02) in connection with *Plasmodium vivax*, the cause of tertian fever. Here the macrogametocytes (but not the microgametocytes) return to the condition of an ordinary

FIG. 70

Regression and merozoite formation (parthenogenesis) in *Plasmodium vivax*. (After Schaudinn.) A, macrogametocyte in blood with nucleus differentiating into a denser and a lighter part; B, the denser part of the nucleus now divides preparatory to schizogony, C, D, while the paler portion with a part of the original cell degenerates; D, numerous merozoites formed about the divided nucleus.

schizont after nuclear changes involving loss of a portion of the chromatin. The cell partly divides, one portion containing a faintly staining nucleus, and the majority of the pigment finally is cast off and degenerates. The other portion, containing more intensely staining chromatin, undergoes schizogony in the manner characteristic of an ordinary blood parasite (Fig. 70).

A still more remarkable process of parthenogenesis was described by the same author in the case of the flagellate *Trypanosoma noctuæ* (1904), where, as stated above, three kinds of cells were identified as male, female, and indifferent. While the ordinary course is fertilization of the female by a much more minute male cell, the macrogamete, or female, may, under certain conditions, undergo parthenogenesis. The conditions of the environment at such times are such as to bring about marked changes in the organisms. The male cells, or microgametocytes, are too delicate to withstand the changed conditions

and are killed off; so, too, are the indifferent forms, but the female cells, being much hardier, continue to live apparently upon the stored up products of a nutritive character. The protoplasm finally becomes vacuolar and the kinetonucleus migrates to a position alongside of the trophonucleus (Fig. 71). Each nucleus then divides, the latter equally, the former by a heteropolar mitosis, which gives rise to a much smaller nucleus and a larger one (*A, B*). This smaller nucleus, like the kinetonucleus, then divides, equally, one of the daughter nuclei of this division degenerating while the other divides again. The result of this second division is the formation of two nuclei, one of which becomes attached to the larger trophonucleus, while the other degenerates. The same history is repeated by the products of the kinetonucleus. One degenerates, while the other divides a second time to furnish a nucleus which similarly unites with the trophonucleus, and one

FIG. 71

Parthenogenesis in *Trypanosoma noctuae*, (After Schaudinn.) *A, B*, approach of the kinetonucleus and division of both nuclei; *C, D*, division of the kinetonucleus and of the "male" nucleus, degeneration of one-half of each, and union of one-quarter of each with the trophonucleus; *E, F*, fusion of the two smaller nuclei in the trophonucleus to form the karyosome of the fertilized cell.

which degenerates (*C, D*). The two smaller nuclei ("polar bodies") then migrate into the trophonucleus and unite to form a new karyosome (*E, F*). With this fertilization the cell is again ready to form other individuals of either male or female type.

In other trypanosomes similar but not identical processes of parthenogenesis have been described by different observers. Moore and Breinl ('07) describe the union of a portion of the kinetonucleus in *Trypanosoma gambiense* with the trophonucleus, but without any of the divisions, as described by Schaudinn. The kinetonucleus (their "centrosome") grows out into a long rod which reaches the trophonucleus, where a small part is taken into the trophonucleus, uniting with the karyosome. A similar long rod was observed by Prowazek ('05) in *Trypanosoma lewisi*, but it was described as arising from the trophonucleus and not from the kineto-

nucleus, and interpreted as a characteristic of the male individual. In this species, also, Prowazek described a union of portions of the two nuclei, the process being much the same as that described by Schaudinn. Phenomena which may be interpreted as parthenogenesis seem to be, therefore, quite widespread among these parasitic flagellates, and not only in species of this genus but in allied genera as well. (See Keysselitz, 1906, for parthenogenesis in *Trypanoplasma borreli*).

In view of the possibility of confusing normal parthenogenetic processes in these various forms of parasites, with involution and degeneration phases of the vegetative individuals, the various, and usually conflicting, observations on parthenogenesis cannot be accepted as established. On purely theoretical grounds, however, and in view of the processes of autogamy in primitive protozoa and of parthenogenesis in metazoa, it is not improbable that such methods of fertilization may be found among the parasitic protozoa, where every adaptation for preventing extinction of the species has apparently been evolved.

E. THE PHENOMENA OF MATURATION IN PROTOZOA.

As Boveri ('90) long since pointed out, the numerical reduction of chromosomes during the maturation of germ cells, first observed by Van Beneden ('83), is no theory, but an accepted fact. Upon this fact, however, a great superstructure of theories has been erected, and around it some of the most fascinating and successful of modern biological researches have been conceived and executed. In connection with the higher animals and plants, the early view of Van Beneden, that reduction is simply a process of eliminating one-half of the chromosomes so that the number characteristic of the species may be kept constant when the germ cells unite, has been given up. Subsequent research has shown that, in the maturation period of both eggs and spermatozoa, after elimination in some cases of fully nine-tenths of the nuclear material, the chromatin substance is redistributed in such a way as to warrant the assumption of some deep-seated purpose. In recent years biologists are coming more and more to accept the hypothesis that this purpose has to do essentially with the phenomena of inheritance, and that the orderly rearrangement of chromatin with the ensuing maturation divisions is evidence of the cellular mechanism by which the physical representatives of hereditary characters are minutely halved and distributed.

While reducing divisions in highly differentiated forms of life, according to this view, have their *raison d'être* in the fact that the great multiplicity of characters of an individual must have their physical representatives concentrated at some time in a single cell, reducing

divisions in protozoa, particularly in the simpler forms, bring such an explanation almost to the limits of *reductio ad absurdum*. It is highly probable that many of the so-called "reducing divisions" which different observers have noted in protozoa are not to be interpreted in the same way as in metazoa. Indeed, there are but few instances where chromosomes, using the term as applied to metazoan cells in division, are formed, and too frequently suspicions are aroused that the observer is influenced by what should be found according to metazoan standards. The granules of chromatin, for example, apparent after the technical processes which appear to be necessary in using the Giemsa stain or any of its modifications, have been generally, but erroneously, interpreted as chromosomes. Not only is there an entire absence of the preliminary processes which characterize chromosome formation in higher animals and plants, but these definite granules cannot be demonstrated after use of the careful cytological methods of fixing and staining that are used for tissue cells. Such "chromosomes," appearing only after use of what Moore and Breinl have characterized as a "barbarous technique," can only be regarded as artifacts, and the various descriptions of reduction in number of such granules cannot be accepted until verified in every detail after the use of methods whose reactions have been fully tested. On the other hand, there is sufficient *a priori* reason for the belief, and numerous observations to prove, that some process akin to reduction of chromosomes of higher types of germ cells occurs in protozoa, and these must be taken into consideration in any attempt to explain the biological significance of the phenomenon.

In higher animals and plants the number of fully formed chromosomes is primarily reduced to one-half, not by division of the nucleus, but by fusion of the chromosomes two by two. Tetrads are then usually formed by transverse division of the double chromosomes. Separation of the tetrads and distribution of their four parts is then accomplished by two divisions of the cell, resulting in four functional spermatozoa in case of the male, and in three polar bodies and one egg in case of the female. Two maturation divisions are thus characteristic of all higher types.

It is quite remarkable, and not without significance, that two rapidly following divisions of the nuclei characterize the preliminary phases of fertilization in many different kinds of protozoa. They are not necessarily connected with the two kinds of chromatin and do not bring about an elimination of the chromidia from the idiochromidia of the cell, for the double division not infrequently occurs after such elimination has taken place. Thus, in cases of autogamy cited on page 141 the nuclei formed from the idiochromidia in *Entameba coli* and *Entameba muris* divide twice, one-half degenerating each time, before the fertilization nuclei are mature (p. 142). In *Acti-*

nophrys sol and *Actinospherium eichhornii* (see Figs. 63, 65) the former exogamic, the latter endogamic, similar divisions may occur, two degenerating nuclei being formed in actinospherium, but only one in actinophrys, a result which led Hertwig ('98) to believe that Schaudinn ('96) had overlooked one of the division stages. In gregarines there is evidence to indicate that the preliminary divisions are not of the nature of reducing divisions, but are qualitative, whereby idiochromidia or germinal chromatin is separated from vegetative. Thus, in Lèger's beautiful work on ophryocystis ('07), the nuclei divide twice before the internal bud or gamete is formed, one of the products of this division becoming a somatic or nutritive nucleus of the parent cell, the other a "reduction" nucleus (Fig. 80).

In foraminifera and in fresh-water rhizopods reducing divisions do not occur, but a "primary" vegetative nucleus remains unused and degenerates in the residual body. Other instances of the elimination of chromatin from all subdivisions of the protozoa might be cited, but among them there are but few cases where the characteristic metazoan conditions prevail. Certainly, the so-called reducing divisions of the mxyosporidia are not analogous, for here, according to Schröder ('07) and Keysseltz ('08), fourteen nuclei are formed, ten of which are "somatic," two of them degenerate, while two only remain to conjugate (Fig. 61); nor are they in the actinomyxidæ, where Caullery and Mesnil ('05) found eighteen nuclei arising from the single primary nucleus, two of them somatic and sixteen germinal, the latter conjugating two by two.

Such a list might be further enlarged by the addition of case after case of so-called reducing divisions, scarcely a paper being published on the reproduction of protozoa that does not describe some such process. But in none of them is there sufficient evidence of the formation and division of chromosomes, and until such evidence is forthcoming we cannot draw accurate comparisons between the processes of maturation in protozoa and in metazoa. In a few cases, however, notably among the infusoria, definite maturation chromosomes are formed and divided, and here we find the nearest approach to the conditions in metazoa. They were first seen and correctly interpreted by Bütschli ('76), while numerous observers (Balbiani, Maupas, Hertwig, Hoyer, Hamburger, Prandtl, Popoff, and others) have since added little by little, until, in some cases, notably in *Paramecium aurelia* (*caudatum*), the phenomena may be brought directly in line with those of the metazoa.

In paramecium, as in other ciliates, the idiochromatin is separated at an early stage from the vegetative chromatin, occurring with the third division of the fertilized micronucleus when macronuclei and micronuclei are differentiated.

The macronucleus of the cell plays absolutely no part in the conjuga-

tion process. Its work is done, and, like the somatic cells of the metazoa, it dies. The micronucleus, on the other hand, after lying dormant so far as the vegetative functions of the cell are concerned, now begins its germinal activity. It moves away from the macronucleus, where it usually lies in a cleft in the substance of the macronucleus, and begins to swell. It contains two substances: one, located at one pole of the nucleus, is the substance of the division centre, and gives rise to the fibers of the spindle figure, so that in it rests the potential energy which is later converted into the kinetic energy of division. The other substance is chromatin, which is concentrated at this time in a number of granules closely packed against the division centre. The nucleus then elongates by fragmentation of the chromatin granules, the fragments arranging themselves in lines radiating out from the division centre. They correspond to the idiochromidia of the rhizopod cell, but are now assuming definite form, the irregular and distributed idiochromidia of the more primitive organisms being replaced here by the more definite chromosomes. The elongation of these lines of chromatin continues until the nucleus is an enlarged, narrow structure many times longer than the resting nucleus. The intranuclear division centre, which is concentrated at one end of the nucleus, likewise increases in size (Fig. 72).

The micronucleus next becomes curved in such a way that the two ends are brought close together, forming a distinct crescent, with the long lines of chromatin uniting to make a branched network extending from tip to tip, while the division centre, now much enlarged, moves toward the centre of the crescent. The chromosomes of the first division figure are formed by the transverse division of the elongated lines of chromatin granules, but, owing to the net formation and association side by side, these short fragments are each double, a longitudinal split appearing in each. All of the chromatin is thus utilized and an uncountable number of chromosomes are thus formed. The substance of the division centre then diffuses through the nucleus in a kind of flowing division and the two poles of the first maturation spindle are formed by the accumulation of this material at the opposite sides of the nucleus. With this flow the chromosomes are divided, so that when the spindle is entirely formed the daughter halves of the chromosomes are separated and now lie end to end in the so-called anaphase stage of division (Fig. 72, C). (See Calkins and Cull ('08) for the details of this spindle formation.)

The nucleus then divides by constriction through the middle and the first two maturation nuclei are the result. Each of these next divides again, the process of division being identical with that described above and four maturation nuclei are formed. Two of these immediately begin to degenerate, while a third follows suit shortly after, the fourth alone dividing a third time. Here the chromatin is not divided

by longitudinal but by transverse division, and this division is heteropolar, so that the resulting nuclei are of different sizes. The smaller

FIG. 72

The micronucleus of *Paramecium aurelia* during conjugation. *A*, concentration of chromosomes after the crescent phase. Accumulation of kinoplasm at upper pole; *B*, early anaphase of first maturation division; *C*, late anaphase of first maturation division; *D*, prophase of second maturation division. (After Calkins and Cull.)

is the migratory nucleus, the larger the stationary. Each migratory nucleus wanders through the connecting bridge of protoplasm and fuses with the opposite stationary nucleus, the fusion beginning at one

pole of the nuclei. The first division of the fertilization nucleus takes place before the chromatin of the two nuclei is completely united. The other two divisions of the fertilized nucleus follow in quick succession and the processes of reorganization bring the phenomena of conjugation to an end (see Fig. 68, p. 157).

The mere statement of the consecutive acts in maturation and fertilization gives no clue to the significance of the processes whereby the cell is reëndowed with a potential of vitality which will again carry it through the periods of a life cycle. We see that fully three-quarters of the chromatin of the resting nucleus is eliminated to disintegrate in the protoplasm of the cell, while still more is lost in the material of the connecting strands of the daughter nuclei; we see that there is a union of this reduced ("purified") chromatin when the pronuclei come together, and we see that the new macronucleus of the early generations of cells is derived from part of the fertilization nucleus.

It thus appears that the nuclear parts of the fertilized cell of paramecium are distinctly new creations, for they consist of the union of chromatin material from two distinct organisms; the one, the macronucleus, has from this period the essentials of vegetative activity, while the micronucleus apparently enters into a "resting period," dividing, and possibly controlling, cell division, until the next period of sexual activity. Not only is the nuclear apparatus new in the fertilized cell, but the cytoplasm is also new, for it receives and changes over into its own substance not only the remains of the old macronucleus, but more than three-fourths of the entire quantity of chromatin possessed by the maturation nuclei. There is no reason to doubt that this addition makes over the protoplasm of the cell in a manner analogous to the reorganization of the nuclei, and presumably provides a physical basis for the reinvigoration of the activities peculiar to the cytoplasm. Except for the absence of a cellular corpse, therefore, there is no support for Weismann's contention that the old individual still persists; it is a new individual, nucleus and cytoplasm, no less surely than a fertilized ovum, or its progeny of cells, is a new individual.

The secret of development lies in this fertilization act, and if we could work it out in paramecium and its allies, we would have a basis, at least, for its discovery in the higher animals. The protozoön offers a much more suitable organism for the study of this problem than any many-celled animal, for the conditions under which successful conjugation is brought about may be experimentally studied and controlled. Not much has been done, as yet, in this direction, but numerous observers are at work on the problem, and it thus presents one of the most fascinating aspects of protozoölogy.

It is mainly in connection with such complicated phenomena of chromosome formation and reduction that theories of inheritance and of fertilization have been formulated.

In simple division by mitosis each of the chromosomes divides longitudinally, so that the daughter cells obtain the same number, and an equal part of each chromosome. In 1883 Roux pointed out that this wonderful mechanism in the cell, and the extreme care with which each of the chromosomes is equally divided, must be connected in some way with the phenomena of inheritance. Van Beneden, in the same year, first showed that the number of chromosomes in the uniting nuclei is just one-half that of ordinary tissue cells of the body, the number characteristic of the species being restored by the union of the two halves, half from one parent, half from the other. Weismann, in his brilliant essays on heredity, suggested that each chromosome is composed of a number of units, which he called biophores, each unit representing some characteristic or group of characteristics to be manifested in the prospective individual. These units are divided in ordinary mitosis in such a way that each daughter cell would receive a portion of each biophore, a result that could be reached only by longitudinal division of the chromosome. To account for the differences in characteristics of different offspring, he prophesied that in the formation of the germ cells the ordinary longitudinal division of the chromosomes would be replaced by a transverse division, and thus daughter cells would result with different biophores. The apparent confirmation of this prophecy a few years later was one of the great events in the history of biology and a vast literature has accumulated since 1891 on this subject of "reduction." Today it is generally admitted by cytologists that the reduced number of chromosomes is brought about by association of the ordinary chromosomes in pairs (synapsis), the union taking place by end to end or side to side association (telosynapsis and parasynapsis).¹ In the preparation for fertilization such double chromosomes are divided twice, so that four germinal elements are produced from each primordial germinal cell. Furthermore, it was suggested by Montgomery that the chromosomes representing the same groups of characters are present in duplicate in the nucleus, half coming from the male parent, half from the female, while synapsis is the association of chromosomes representing the same groups of characters but from different parents. This suggestion was rendered more probable by the observations of Sutton, McClung, and others, who showed that the chromosomes in insects have different forms and that the different forms are present in pairs, and, further, that these pairs unite in synapsis.

There is reason to believe, therefore, that Weismann's original hypothesis of the make-up of the germinal chromosomes is as close as

¹ These excellent terms were first used by Professor Wilson in lectures at Columbia University, and were used by the present author and Miss Cull with the mistaken impression that Professor Wilson had already published them. For this breach I offer my tardy but sincere apologies.—G. N. C.

we can come at the present time to an explanation of the physical basis of inheritance. The theoretical conclusions have been strengthened and supported by morphological evidence upon the most widely separated groups of animals and plants, and by experimental evidence in connection with the principle of Mendelian inheritance.

Such, in brief, is the statement of the modern problem of inheritance from the cytological standpoint. Now, what connection has this problem with the protozoa? The chromosome in a metazoön must be a wonderfully complex element of the cell if there is anything in this physical conception of its organization, for we find that the number of chromosomes in the cells of metazoa does not increase with the high grade of differentiation which we find in the higher animals, and if there is a physical basis for adult characteristics, the few chromosomes of a man must be wonderfully more complex individually, than those of invertebrates like a sea urchin or an earthworm which have approximately the same number. In protozoa the chromosomes, when present, are of enormous numbers, in paramecium at maturation more than 200, and the only interpretation, on a purely physical basis, is that each chromosome must represent a simple character, or, at least, a simpler group of characters, than the chromosomes of higher animals. In the more primitive protozoa the physical basis of inheritance (idiochromidia) is not moulded into definite chromosomes, but is uniformly halved while in granule form. In other words, a study of protozoa chromosomes leads to the theory that chromosomes, the characteristic structures of the nucleus in mitosis, have had an evolution no less surely than has the nervous system, digestive system, or supporting system of the higher animals, and that the chromosomes of the protozoa have the same relation to chromosomes of the metazoa that the organization of the protozoan body has to that of the metazoan, *i. e.*, a unit structure.

F. THE SIGNIFICANCE OF FERTILIZATION.

It is perfectly obvious that whatever view is taken of the significance of fertilization, it must be sufficiently general to account for the phenomena of parthenogenesis, autogamy, and endogamy, as well as for the more complicated processes of exogamy. Bütschli's ('76) early view that conjugation is a process involving rejuvenation (*Verjungung*) of the individual, while giving no idea as to what the nature of the rejuvenating process actually is, has been but little improved upon by the work of subsequent observers. Maupas' conclusion that nuclear rejuvenescence is alone involved is not wholly consistent with the facts, and his attempt to penetrate more deeply into the mysteries of the matter by defining the conditions of conjugation has been only partly

successful. The conditions, as he outlined them, are, briefly: (1) diverse ancestry of the conjugating cells; (2) scarcity of food; and (3) sexual maturity.

That diversity of ancestry has no great biological significance is borne out by the facts of parthenogenesis, autogamy, and endogamy, and on this ground alone might well be dismissed as a necessary condition of fruitful conjugation. Not only in these instances, but in exogamic fertilization as well, diverse ancestry is not essential. Thus, in *Paramecium aurelia* (*caudatum*), which was one of the examples cited by Maupas as an obligatory exogamous type, Calkins ('02) showed that two cells removed by not more than eight or nine divisions from the same ancestral cell, conjugated, and one of the exconjugants gave rise to descendants through 379 generations of divisions. In these experiments it was shown, furthermore, that fully as many conjugations between related forms were fruitful as between forms of diverse ancestry.

The second of Maupas' conditions, scarcity of food, seems to have some connection with the ability to conjugate, although in no case has it been proved that such a condition is a necessary factor. Certainly in cultures of paramecium, or of any other ciliate, dividing forms indicate the presence of food, and in such cultures conjugating and dividing individuals may be found side by side, and Maupas himself states that conjugating forms may still actively take in food. It is not improbable that surplus of food, followed by starvation, may assist in bringing about the protoplasmic conditions where conjugation is possible. Changes in the density of the surrounding medium, and changes in temperature, certainly act to this end, but all of such conditions seem to be dependent upon a third condition, sexual maturity.

Maupas' third condition of conjugation, sexual maturity, seems to be quite probable, provided we mean by sexual maturity the appropriate chemical and physical condition of the protoplasm when conjugation is possible. The time element, which seems to be implied, is not a necessary factor, however, for the proper conditions may be induced by temperature and density changes in the surrounding medium.

Finally, it appears to be not improbable that the interpretation of fertilization rests in the obscure chemical relations and hypothetical enzymatic action of idiochromatin elements whose potency depends more or less upon the diversity of environment of the conjugating forms. Culture experiments upon some of the larger forms of protozoa, while not proving such a theory, nevertheless seem to point in this direction. Thus, Cull ('07) found that out of a total of 186 paramecium individuals from pond water, 70 per cent. continued to live after conjugation, *i. e.*, were fruitful. Calkins ('02), on the other

hand, found that out of 80 paramecium individuals that had been continuously on the same food for many months in culture, only 6 per cent. continued to live, and this low percentage was the same whether the conjugating forms were of the same or of diverse ancestry. It may be as Stevens ('03) pointed out, that such low percentages were due to the lowered vitality of the organisms in culture, but in all cases the food medium was the same, and the explanation may lie in the fact that the culture forms, having lived upon the same food material for many months, were too similar to give rise to appropriate chemical combinations upon fusing. The injurious effects of too close and too prolonged inbreeding of higher forms may have their explanation in such experiments, and similar experiments and observations on the unicellular animals under culture may ultimately furnish the key to the riddle of fertilization.

CHAPTER V.

PARASITISM.

It is a well-recognized biological principle that degeneration is the inevitable outcome of continued parasitism (Lankester Degeneration). A certain crustacean parasite begins life with the same number of appendages as other crustaceans, but when it becomes attached to a crab host, its appendages atrophy, evidences of other structures disappear, and it becomes a mere bag—sacculina—on the abdomen of its victim. *Ascaris*, *trichina*, and their allies similarly have lost most of the dermal musculature and the power to move as most worms do. *Tenia* and other tapeworms in like manner have lost not only the body musculature, but digestive organs as well. Such parasites, living in the digestive tracts of their hosts, are surrounded by digested and partly digested food which passes by osmosis through the body wall; mouth and digestive organs are unnecessary, and their disappearance is to be accounted for on the theory of disuse.

While degeneration of the usual vegetative organs is the inevitable outcome of parasitism, the restricted mode of life of the parasite may require certain accommodations which may lead to structural adaptations on its part. The internal parasites of the digestive tract, for example, might easily be dislodged and carried out of the intestine with the muscular contraction and currents of that organ, while external parasites would be readily detached and swept away, were they not provided with some means of holding on, hence sucking disks, hooks, and spines are characteristic of internal and external parasites. In addition to increased development of certain attaching organs and degeneration of vegetative organs of digestion, etc., there is an enormous increase in the power of reproduction. It is a biological fact that the number of offspring of an animal is in inverse proportion to the chances of reaching maturity, and the number is always great enough to maintain the species. It is quite apparent that a parasite living in a certain portion of a given host would experience no little difficulty in reaching that spot, hence every parasite has acquired the power of reproducing immense numbers of progeny; a tapeworm, for example, produces many hundred thousands of eggs, and yet the frequency of infection by tapeworms is not great enough to cause any apprehension among people who live with ordinary decency.

With a ubiquitous group of organisms like the protozoa, it is to be

expected that some of them, at least, would have acquired the parasitic mode of life. The enormous literature which annually appears in connection with the protozoan parasites, perhaps better than anything else, shows that such an expectation is well founded (Lühe ('06) points out that in connection with the blood-dwelling protozoan parasites alone there are from 600 to 700 papers published every year), and every division of the protozoa numbers among its genera some that are wholly or in part parasitic.

I. STRUCTURAL MODIFICATIONS AND MODE OF LIFE OF PROTOZOAN PARASITES.

It is not a too sweeping generalization to state that every living thing, large enough to contain another living thing, is subject to invasion by parasites. The protozoa, themselves single cells, often play the part of host to smaller protozoan cells, and parasites often infect even the nucleus of ameba, paramecium, vorticella, and other types.

If the imagination were allowed full play, it would not be very difficult to work out a logical hypothesis as to the transition of different kinds of protozoa, from a free life in ponds and ditches to a parasitic life in the digestive tract or other organs of various animals. It is certainly true that representatives of all groups of protozoa have from time to time in the past become adapted to life within some other animal or plant, and it is equally true that in many cases their presence is harmful to the host and may become fatal. Frequently such parasites have become so modified by their changed mode of life that their structures furnish little or no hint as to the original or primary form. Such is the case in the majority of sporozoa, where every member is a parasite, the origin of the group, as a whole, whether from rhizopods or flagellates, being purely conjectural. In some cases the method of locomotion by pseudopodia formation, the presence of a contractile vacuole, and the mode of reproduction indicate rhizopod affinities; in other cases the evidence of degenerating structures, taking place before our eyes, as it were, at the present time, is unmistakable, and such forms write their own phylogenetic history. This is true of some members of the blood-dwelling parasites, where, as in *Herpetomonas* (*Leishmania*) *donovani*, the adult organism is a flagellated protozoön in the gut of its definitive host (bugs of the genus *Cimex*), but becomes an intra-cellular parasite without motile organs of any kind in the intermediate host man; or in *Trypanosoma noctuæ* (*Hemoproteus noctuæ*), where a highly differentiated free-swimming flagellate becomes an intra-cellular blood parasite of the bird (*Glaucidium* (*Athene*) *noctuæ*), and with a much simpler structure (see page 244). From such evidence

it is conceivable that the entire group of the hemosporidia may have been thus evolved from the flagellated protozoa, as the majority of protozoölogists now suspect, the evidence, as Schaudinn, Minchin, Lühe, Hartmann, and others admit, being supported by the casual formation of flagella-like structures in different species of the malaria organism and the peculiar thread or pseudopodium-like appendage of *Babesia canis* [Nuttall and Graham-Smith ('06), Patton ('07), Kinoshita ('07)]. This evidence, however, is not strong enough to justify far-reaching changes as yet in the well-established system of classification, and we cannot support Hartmann, Sambon, Manson, and other recent contributors in their attempts to do away with the old group of hemosporidia. Hartmann's ('07) group of "binucleata," including hemosporidia and the binucleated flagellates, is premature, misleading, and demoralizing, and on the present evidence would be no more justified than a zoölogist would be justified in classifying pisces and batrachia together in one group on the strength of the tadpole larva. In each case the vanishing structures show no more than a suggestion of a possible relationship.

In other cases of parasitic protozoa the cellular structures are practically identical with those of the nearest allied free-living forms. Balantidium, opalina, bütschlia, dasytricha, and other ciliated parasites show unmistakable resemblance to the infusoria; pyrsonympha, trichonympha, and some others a less perfect resemblance. Ameboid parasites like *Entamoeba histolytica*, *E. coli*, or *Chlamydomphrys stercorea* are similarly related to the rhizopods.

Like parasitic worms and mollusks, these parasitic forms may become highly modified by their parasitic mode of life, and suckers, hooks, spines, and other attaching organs may be well developed. Such changes in cell structure may be the outcome of the specific mode of life of the parasite and their methods of nutrition. Some of them, like the majority of motile forms in the fluids of the digestive or circulatory system, absorb their food as saprophytes do, by osmosis; others, like the gregarines, trichonympha, pyrsonympha, and others, have especially adapted attaching or feeding organs which may act as haustoria to absorb food from the fluids of the host (*e. g.*, pyxinia, Fig. 73).

The parasitic forms may be divided for descriptive purposes into unnatural groups, according to their modes of life. Some are purely *enterozoic*, spending the entire life in the lumen of the digestive tract (flagellates like copromonas, cercomonas, herpetomonas, crithidia, etc.); others are *coelozoic*, dwelling in the coelomic cavities of the body (many gregarines); others are *cytozoic*, living throughout the vegetative period of life as intracellular parasites (coccidiidia, in epithelial cells; myxosporidia, in muscle cells; and intracorpusecular hemosporidia); still others are *caryozoic*, passing into the cell body to find

lodgement in the cell nucleus; such *caryozoic* forms are only specially adapted cytozoic types, but their habitat is always the same (*Cyclospora caryolytica*, *Nucleophaga ameba*, and in part *Cytoryctes variolæ*, and others); others, finally, are *hematozoic*, living in the blood (trypanosoma, plasmodium, hemoproteus, etc.). In many cases there may be modifications of these modes of life, or combinations of two or more. Thus, plasmodium may be hematozoic, cytozoic, enterozoic, and coelozoic during some period of its life history in the mosquito or in the blood, and the terms are too indefinite to be employed in any way save for purposes of description. In many cases, as, for example, in gregarines, the young phases are cytozoic, the adult coelozoic or entero-

FIG. 73

Pyxinia möbiuszi, from Lühe. (After Lèger and Dubosq.)

zoic, and in such cases the young forms may have special organs serving for attachment or for feeding, and as they grow to maturity such processes may remain in the host cell, serving for attachment, or as haustoria for the absorption of nutriment. Sometimes these are great prolongations at one end of the cell, as in *Pyxinia möbiuszi* (Fig. 73); again, many such processes may be present, as in *Pteroccephalus giardi*, or in ophryocystis (Fig. 80). When the organism is sexually mature or ready for reproduction the attaching processes are discarded and left behind in the epithelial cell of the host, while the freed parasite lies in the lumen of the organ. Such attached gregarines are known as *cephalonts*, and the detached forms as *sporonts*. The cephalonts may be variously ornamented, according as the attaching

organ is produced into hooks, etc., the attaching portion being known as the *epimerite*. The portion suspended from the cell in the lumen of the organ may be further differentiated by septa of ectoplasmic origin into an anterior and a posterior part, the former called the *primate*, the latter, usually containing the nucleus, the *deutomerite* (Fig. 1, D, p. 17).

Other special adaptive structures brought about in the protozoan cell, as a result of parasitism, are undoubtedly the protective capsules which envelop the spores. When the parasite becomes sexually mature it fuses with another cell in conjugation, and fertilization is followed by spore formation. The spores thus formed do not reinfect the same host, but, contained usually in the lumen of the digestive tract or similar cavity of the body, they are finally carried to the outside in one way or another with the waste matters. Here, were it not for the protective coverings which they possess, they would soon be killed by exposure, but, protected by resistant chitinous membranes, such spores resist drying and retain their vitality until again taken into a new host, usually by way of the digestive tract. Animals of gregarious habits are particularly subject to protozoan infection, the spores usually contaminating the food. In the intestine the germs of the organisms are liberated from their coverings and make their way by one means or another to the definitive locality where growth is possible. The so-called "selection" of locality is a matter of mere passive resistance on the part of the parasite, that part being "selected" where they are not destroyed by the reactions of the host, and where conditions of life are most satisfactory for nourishment and security.

If the young organism is a gregarine or coccidian, it makes its way to the epithelial cells lining the digestive tract and grows to adult size. Some forms penetrate the walls of the gut and get into the celom where, as celozoic parasites, they grow to maturity. Coccidia remain in the first cell-host until it is destroyed, such destruction allowing the parasite to fall into the lumen of the organ, where fertilization occurs. Coccidian infection, for this reason, is much more severe than gregarine infection, and may give rise to acute enteritis (*e. g.*, *Cyclospora caryolytica* in moles).

II. REPRODUCTION AND THE LIFE CYCLE.

In common with the many-celled parasites, the protozoan forms have acquired varied and prolific means of multiplication, which may differ in type at different periods of the life cycle. In the majority of cases such multiplication may involve sexual processes, or it may be entirely asexual, the former occurring at the end of the vegetative life of the parasite, the latter, during the vegetative life, in the host.

Sexual reproduction is bound up with spore formation, whereby germs of the parasite are prepared to withstand various unsuitable conditions of the external environment, such reproduction being termed *sporogony*. Asexual reproduction, on the other hand, taking place within the host, is a means of spreading the infection among different cells

FIG. 74

Life cycle of *Coccidium schubergi*. (After Schaudinn.) 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 a merozoite (c). These enter new epithelial cells, and the cycle is repeated many times. After five or six days of incubation, the merozoites develop into sexually differentiated gametes; some are large and well stored with yolk material (d, e, f); others have nuclei which fragment into many smaller particles ("Chromidien"), each granule becoming the nucleus of a microgamete or male cell (d, h, i, j). The macrogamete is fertilized by one microgamete (g), and the copula immediately secretes a fertilization membrane which hardens into a cyst. The cleavage nucleus divides twice, and each of the four daughter nuclei forms a sporoblast (k) in which two sporozoites are produced (l).

and organs in the same host, or a means of *auto-infection*. This means of asexual increase is termed *schizogony*, although, as a rule, the term is restricted to multiple increase or asexual "spore" formation.

Similar alternations of sexual and asexual methods of reproduction are invariably present in free forms of protozoa, but asexual increase

is usually limited to simple division or budding, although spore formation is occasionally met with here (*e. g.*, *noctiluca*, *colpidium*, etc.), while after fertilization spore formation is quite common, especially among the free flagellates. In parasitic forms, on the other hand, and especially among sporozoa, simple division and budding are extremely rare, being replaced here by the more prolific multiple reproduction by asexual spore formation in response to the greater need of numbers in maintaining the species. Two kinds of "spores," therefore, may be present in these parasitic protozoa, the one giving rise to infection of new hosts (spores *s. str.*), the other to auto-infection of the same host. No little confusion has arisen because of this difference, and various writers have sought to avoid it by giving different terms to the "spores" of varied origin. Such efforts, instead of helping, have, in the main, made "confusion more confused," and students of the group have recognized the need of adopting some one standard and acceptable terminology. At the present time there is a tendency to eliminate the term "spore" as applying to any definite reproductive body, and to reserve it for a general designation of any reproductive body formed in brood. Specialists, however, especially those dealing with the sporozoa, have generally applied the term in a still more limited sense to the reproductive bodies in gregarinida and coccidiidia within the sporocysts which give rise to the *sporozoites* or final reproductive elements (Fig. 74). Such a young sporozoite as that of *Coccidium* (*Eimeria*) *schubergi* grows into a vegetative organism termed a *trophozoite*, which finally becomes a *schizont* and reproduces asexually, forming spores known as *merozoites* (Fig. 74, *c*). These reproductive bodies are naked and unable to withstand the unfavorable exigencies of an external life, but are capable of developing within the same host. They, too, grow into trophozoites, and the process of *schizogony* may be repeated many times; ultimately, however, vitality wanes and the organisms become sexually mature. The trophozoites, at this period, instead of forming schizonts, turn into *gametocytes* and give rise to conjugating *gametes*, which may or may not be sexually differentiated. The gametes conjugate and form a zygote or copula which becomes a *sporoblast* or by division gives rise to sporoblasts. The sporoblasts are enclosed in protective coatings termed *sporocysts*, and within these they multiply again to form from two to many germs, the *sporozoites*, or the sporoblast may, in some cases, become the sporozoite directly without further division. The various forms assumed by the sporozoan parasites and the many kinds of reproductive bodies bring about great complexity in the life cycle, and where only one phase of such a cycle is known, confusion is apt to follow attempts at classification.

There is no doubt that the group of sporozoa which furnishes some of the best and most complete examples of the life cycle of protozoa

is made up of heterogeneous and unrelated forms which may in time be resolved into more natural groups than those of our present-day classification. But notwithstanding the varieties in form, mode of life, and diverse origin of these organisms, all seem to agree in the possession of two distinct phases of activity, one, the *endogenous* cycle, the other, the *exogenous* cycle, the former within the same host, the latter outside of any definitive host and either free or temporarily

FIG. 75

Life cycle of *Lankesteria* (Monocystic ascidiæ, Siedlecki). The young sporozoites enter epithelial cells (A, B, C), and grow into adult gregarines, which leave the cells (D) and live as "sporonts" in the cavity of the intestine. Two sporonts unite (E), their nuclei divide repeatedly (F), until many daughter nuclei are formed (G). These become nuclei of ameboid gametes (H), which move about inside of the cyst and soon conjugate two by two (I), the nuclei fusing to form cleavage nuclei of the sporoblasts (J). The cleavage nuclei then divide thrice to form eight daughter nuclei (K, L, M, N), which ultimately become nuclei of the sporozoites (O). The sporoblasts, meanwhile, secrete firm cysts within which the sporozoites are protected.

parasitic in some other animal. The life history of *Coccidium schubergeri*, as outlined above, is neither the simplest nor the most complicated of these histories, and may well serve as a starting point for a description of the various modifications.

1. **Variations in the Endogenous Cycle.**—In some cases the life history of parasitic protozoa is simplified to such an extent that no reproductive processes take place during the endogenous cycle, the young sporozoites developing directly into trophozoites and these into

gametocytes (e. g., *Lankesteria* (*Monocystis*) *ascidiæ*, Sied. (Fig. 75) *Eucoccidium* (*Benedenia*) *octopiana*, and *E. eledone*).

FIG. 76

Intracellular schizogony in gregarines. A to D, *Eleutheroschizon dubosqui*, Brasil, intestinal parasite of *Scoloplos armiger*, showing multiplication of nuclei (A, B) and formation of merozoites (C, D). (After Brasil.) E to G, *Schizocystis sipunculi*, Dogiel; E, adult organism; F, merozoite formation; G, mature merozoites in brood cavity. (After Dogiel.)

In other cases, processes of schizogony in one form or other complicate the endogenous cycle. The simplest of these processes of

multiplication is binary division of the trophozoite, as found among the schizogregarines, where, as in ophryocystis, according to Lèger ('07), vegetative increase may be by simple division, as in ameba. In other cases, however, the nucleus of the organism divides repeatedly until many are present, when the cell divides into as many *schizozoites* or merozoites as there are nuclei (Fig. 76). Schizogony becomes more complicated in other genera of schizogregarines, where, as in *Eleutheroschizon* (Brasil, 1906) or in *Schizocystis* (Dogiel), a process of internal budding similar to that in suctoria (acineta, tokophrya, etc.) takes place. In the former, a parasite of the marine annelid *Scoloplos armiger*, Brasil ('06), has shown that the nucleus multiplies by mitosis until many are present, when each is surrounded by a small part of the protoplasm and all remain in the trophozoite, which acts as nurse (Fig. 76, A-D). Similarly, in *Schizocystis sipunculi*, a parasite of *Sipunculus nudus*, Dogiel ('07), described the formation of a brood pouch with many merozoites (Fig. 76, E-G), and, as in the preceding form, the nurse cell or parent trophozoite is finally discarded as an empty shell.

Processes like these would seem to be less primitive than simple division, more primitive than merozoite formation in the coccidiidia, where the entire cell is utilized in the formation of such asexual spores; a further stage leading to full schizogony is illustrated by another gregarine selenidium, in which, according to Brasil ('07), the entire protoplasmic contents of the cell are used in merozoite formation. These methods of increase have probably arisen from simple division in response to the environmental conditions, and the resulting germs, like sister cells from division, are produced by simultaneous division of the entire cell. Such asexual spores are never protected by chitinous coverings, and for this reason have been called *gymnosporos*, as the equivalent of merozoites and as distinctive from the covered spores or *chlamydosporos*, of the sexual generation. In some rare cases, *e. g.*, in *Lègerella nova* among the coccidiidia, the sporozoites, like merozoites, are naked.

In still other cases among the coccidiidia endogenous multiplication is further complicated by the division of the trophozoite into fragments ("cytomeres" of Siedlecki), each of which becomes the centre of merozoite formation. Such further complications are characteristic of *Klossiella muris* (parasite of the mouse) and *Caryotropha mesnili*, a parasite of the germinal cells of the annelid *Polymnia nebulosa*. The highest type undoubtedly occurs in those forms of coccidiidia, where merozoite formation accompanies the permanent differentiation of the sexes, where, as in *Cyclospora caryolytica* (parasite of the mole), *Adelea ovata* (parasite of the centipede), a series of male and female merozoites are produced, which give rise to male and female trophozoites, and these, finally, to sexually differentiated gametocytes.

While most of the examples cited above are to be found among the clearly defined forms of unquestioned systematic position, quite a variety of endogenous variations have been described in the lesser known parasites. Here, especially in the recently created group of haplosporidia and in the sarcosporidia, the former, including parasites mainly of annelids, crustacea, and fish, the latter, mainly of mammals, the method of asexual spore formation is much more primitive than in the better-known parasites, and, as in selenidium, all of the cell contents are used in the formation of the reproductive elements. Some of these forms are cytozoic (*Haplosporidium heterocirri*, *H. vejdoskii*); some are coelozoic (*H. marchouxii*), and some combine the

FIG. 77

Schewiakovella in the body cavity of cyclops. (From Minchin, after Schewiakoff.) *A*, free ameboid form; *B*, encysted ameba; *C*, sporulation of ameboid stage; *D*, plasmodial stage; *E*, sporulation stage of plasmodium.

intracellular with lumen dwelling life; but all agree, according to Caullery and Mesnil ('05), in having an endogenous and an exogenous cycle; although the full life history in no species is known. In all cases the trophozoite begins as a uninucleated cell, similar to a young form of *Plasmodiophora brassicae*, and develops into a multi-nucleated ameboid form which fragments into as many germs (merozoites?) as there are nuclei. Conjugation processes are quite unknown, although Caullery and Mesnil suspected a fusion of nuclei (autogamy) comparable with that in plasmodiophora or that in the more closely allied group of actinomyxidæ. The haplosporidia are forms of considerable theoretical interest, as indicating a possible close connection

with the mycetozoa, and through these a phylogenetic relationship between the neosporidia and the rhizopoda. This is particularly well illustrated in the case of *Schewiakovella schmeili*, a parasite of copepods where there is not only a multinucleate trophozoite stage, but the parasite differs from all other sporozoa in having a distinct rhizopod characteristic in its contractile vacuole, while it agrees with mycetozoa in that young forms come together and fuse to form plasmodia. A further peculiarity of this organism is the binary division of the spores (Fig. 77).

In this group of little-known forms, one case of human infection has been reported by Minchin and Fantham ('05). The connective tissue of nasal tumors in natives of India was found to contain quan-

FIG. 78

Rhinosporidium kinealyi, Minchin and Fantham. (After Minchin and Fantham.) A segment of a section through a cyst from a tumor of the human nasal septum. The ripe pansporoblasts are accumulated in the centre of the cyst and gradually encroach upon the peripheral plasm until all is utilized. One ripe "spore-morula" is shown on the right.

ties of haplosporidian parasites—*Rhinosporidium kinealyi*—in all stages of development, from young multinucleate organisms to adults filled with pansporoblasts (Fig. 78). The pansporoblasts give rise to sporoblasts (spores) which are formed successively until about a dozen are developed. (As in other myxosporidian pansporoblast formation, the possibility of sexual union of nuclei (autogamy) is not excluded by the authors.) When mature, the cysts appear to burst, the merozoites (?) thus being distributed to neighboring tissues, giving rise to new tumors by auto-infection.

In sarcosporidia, including muscle parasites of birds, lower mammals and man, the process of endogenous multiplication proceeds in a manner quite similar to that described above. In its earliest stages the parasite appears as a minute white body embedded in the material

of a muscle fiber (Fig. 79), in which condition it is known as *Miescher's tube*, a name applied to the vegetative forms of the mouse parasite *Sarcocystis muris*. As the young trophozoites grow, the nuclei increase in number, a definite sac-like membrane develops around the protoplasmic body, while in the centre groups of spores begin to form. The ripening spores (merozoites, gymnosporos) gradually encroach upon the more peripheral unused protoplasm of the tube until the ends only appear to be active, and capable of vegetative functioning, and even these, finally, are used in spore formation. In *Sarcocystis tenella* of sheep such cysts may grow to a length of two inches in the muscle bundles, where they ultimately burst, the spores being scattered or carried by blood to new regions, where development begins anew (auto-infection). In some cases the entire body may be over-run by such parasites, mice especially often being killed in this manner.

FIG. 79

Sarcocystis muris, a muscle parasite of the mouse. (After Minchin.)

In all cases there is every reason to believe that this method of endogenous multiplication cannot be continued indefinitely any more than a paramecium can continue to divide indefinitely, and there is reason to suppose that the potential of vitality gives out at the end of a more or less definite cycle of generations. In many cases, especially in the disease-causing forms in man, the organisms seem to have devised a means of counteracting this senile process and of being stimulated to renewed activity in much the same way that paramecium was stimulated by artificial means (see page 131). It is a recognized fact that many of the blood diseases are characterized by relapses in which the organisms reappear after having disappeared from the circulation.

It was shown in the last chapter that paramecium could be restimulated, even during a well-advanced period of depression, by means of salts of different kinds. Such stimulation, preventing natural physiological death of the organism, is analogous to the artificial parthenogenesis by use of salts in the case of eggs of sea urchins and star fish. The researches of Morgan, Loeb, Wilson, Delage, and others have shown that fertilization is not necessary for development of the egg of such forms. So in the case of paramecium and other ciliates in which the life history has been followed out, the use of a new medium with some appropriate salt effected the same reaction as salts do in artificial parthenogenesis. The observations upon the lower organisms went a step farther, however, by showing that in paramecium such stimulation could not be continued indefinitely, a time coming when the stimulants failed to produce the effect previously obtained.

So it may be with the blood parasites; some of them, like the malaria organisms, may be artificially stimulated by some minute change in the constitution of the blood, and so bring about a relapse (see Calkins, 1906). Parthenogenesis, effecting the same end, has been described by Schaudinn in the case of *Plasmodium vivax*, the cause of tertian fever in man, and in the case of *Trypanosoma noctuæ*, a blood parasite of the little owl (see p. 163).

Variations in the endogenous cycle of parasites thus have to do mainly with the methods of asexual increase. The more primitive forms of parasites, *i. e.*, those which have most recently adopted the parasitic mode of life, still reproduce as do the free-living or non-parasitic types. In other forms simple division is replaced by more or less prolific methods of brood formation, in response, probably, to the needs of the race, and methods which culminate in fully developed schizogony, usually serving as a means of auto-infection.

2. Variations in the Exogenous Cycle.—The exogenous cycle begins with the fertilization of the cell and formation of the external spore coverings within which the young organisms are protected from adverse conditions. There is reason to believe that such protective structures and adaptations of the exogenous cycle are distinctly characteristic of the period of youth in the life history, and due in large part to the high potential of vitality which distinguishes the fertilized cell from all others. The reproductive processes involved are certainly more complicated than those of the endogenous cycle, and are more definitely correlated with the perpetuation of the race.

In the simplest cases the fertilized cell forms a chitinous spore covering which, with desiccation, may become hard and resistant, while no internal nuclear or cytoplasmic processes take place. When taken again into a new host, where conditions are favorable for the dissolution of the cyst, a single, young, and uninucleate parasite emerges. Such is the condition in many of the parasitic flagellates

and rhizopods of the digestive tract of different animals, and is well illustrated by the case of *Copromonas subtilis*, a parasite of the frog (Fig. 66, p. 153). Here two complete individuals are fused into one, the copula forms a chitinous cyst and passes with the feces to the outside. No multiplication takes place within the cyst, and infection of a new host is brought about by feeding. A somewhat more complicated history is presented by the intestinal amebæ, where encystment and fertilization (in these cases autogamous) is followed by the formation of spores, usually in small numbers, which are not liberated until the definitive seat of parasitism is reached. Here, again, although several young may be formed at the period of fertilization, there is apparently little reason to imagine any great difficulties to be overcome by the parasites in finding a new host.

Since, in flagellates, amebæ, and sporozoa, encystment is thus bound up with fertilization, it would not be unreasonable to argue that where such cysts are found, previous fertilization may, at least, be suspected. Too much importance must not be attached to encystment, however, for in many forms, especially in the free flagellates, ciliates, and rhizopods, encystment may be brought about by the temporary adverse condition of the surrounding medium, or even for purposes of digestion. The encysted trypanosome, *Trypanosoma grayi*, which Minchin ('07) discovered in the rectum of the tsetse fly, *Glossina palpalis*, may be due to such change in the medium, or, which is less probable, may be interpreted as a result of fertilization. This is the only case among trypanosomes in which an encysted stage has been noted, although Moore and Breinl ('07) have described small reproductive bodies in *Trypanosoma gambiense*, which may have a like significance. In this case, however, they are found in the blood and belong obviously to the endogenous cycle (see p. 267). Metcalf ('07) has shown that encystment of *Opalina intestinalis* and *dimidiata* which occurs in the rectum of the frog, has nothing to do with conjugation. The cysts pass out with the feces and after a longer or shorter period may again be taken into a tadpole's digestive tract, where, after dissolution of the cyst, a larger macrogamete fuses with a smaller "tailed" gamete, so that fertilization in this case follows encystment. (Metcalf does not find conjugation between two "tailed" forms, as Neresheimer describes, see p. 158.)

It is among the sporozoa that the most remarkable and best-illustrated phenomena of exogenous sporulation are to be found, and here there is almost every conceivable grade of complexity. Owing to the heterogeneous nature of the sporozoa and the wide variations in the processes of sporogony, confusion must follow any attempt to describe them all in one category. Generalizations can be made only in connection with the more homogeneous groups of gregarinida and coccidiidia, while the hemosporidia and other parasitic forms will

be considered more appropriately in connection with the diseases due to them.

(a) **Sporulation in Gregarinida.**—As shown in the preceding chapter, the number of gametes formed by the conjugating gregarines varies within wide limits. In ophryocystis, according to Lèger ('07), there is but one gamete formed in each cell, while only one sporoblast results from the fusion of the gametes (see Fig. 80). In other gregarines there are many gametes, which, as previously shown, may be sexually differentiated. In most cases these gametes arise from the parent gametocytes, which are enclosed together within one common cyst wall (pseudoconjugation), but in the remarkable case of *Schaudinella henleae*, described by Nusbaum ('03), the organisms are sexually differentiated even before the gametocytes are formed, and pseudoconjugation of the gametocytes does not occur, each organism forming its microgametes or macrogametes, as the case may be, independently of one another, the gametes then meeting and fusing in the lumen of the digestive tract.

At the other extreme we may place the two species of diplocystis, where the organisms pair immediately after entering the celom of their hosts and continue to live in couples, while any individual remaining solitary dies without further growth (Cuènot, 1901). Here, therefore, pseudoconjugation appears to be a necessity for the organisms.

In all cases when the coupled gregarines are mature, the nucleus of each divides by mitosis to form a residual nucleus and a so-called "micronucleus" (Cuènot). The latter undergoes successive mitotic divisions, and the resulting nuclei finally reach the periphery of the cell, where the gametes are formed as buds.

Development of the fertilized egg is essentially the same in all of the gregarines. The fertilization nucleus (synkaryon) divides by a primitive mitosis three successive times, and the sporoplasm separates into eight parts, one around each of the nuclei. Eight sporozoites are thus formed in the typical case, only one exception, that of selenidium, where there are but four sporozoites, being known.

The arrangement of the sporozoites in the sporocysts presents the greatest variety, but has no importance from a systematic point of view (Fig. 20). More important are the surrounding envelopes of the bundle of sporozoites. In the majority of cases the sporocyst consists of one (monocystis forms) or two tough, resistant membranes which may become greatly hardened. When two are present, the inner or *endospore* is smooth and relatively thin, forming a closely investing sheath about the sporozoites. The second or outer covering, the *epispore*, is more resistant and may consist of several layers (ophryocystis), while it is frequently drawn out into spines, lateral processes, or long filaments (Fig. 20, *D*, *F*). Under the proper conditions the

FIG. 80

Gamete formation and sporulation in *Ophryocystis mesnili*, Lèger. (After Lèger.) $\times 2000$.
 A, two individuals attached by processes to ciliated epithelial cells of Malpighian tubule of *Tenebrio mollitor*; B, union of "gamonts;" C, D, E, first division of nuclei to form germinal and somatic (s) nuclei; F, division of germinal nuclei to form first reduction nucleus (r); G, segregation of protoplasm to form gametes (g); H, I, J, fusion of mature gametes; K, L, M, first division of zygote; N, normal sporoblast with eight sporozoites.

epispores open either by dehiscence (Fig. 20, *B*, *N*,) or by dissolution at certain points, and the sporozoites emerge by typical contractile movements. In the majority of cases there is a residual mass of sporoplasm, which has received various names (*reliquât sporal*, *sporenrest*, *sporal residuum*, etc.), and about which the sporozoites may be grouped in characteristic manner. In some cases this residual protoplasm is more than a mere degenerating mass, but is provided with special nuclei and plays a definite purpose in the reproductive process. Thus, in *Ophryocystis mesnili* it is nucleated, and functions as a nurse cell or cells for the developing sporoblast (Fig. 80). In *Monocystis* and other

FIG. 81

Cysts and sporoducts of *Gregarina cuneata*. (After Kuschakewitsch.) *A*, surface view of cyst with ripe spores (*s*) issuing from sporoducts (*e*); *B*, section with ripening spores and points on wall where sporoducts will form; *C*, section showing ingrowth of finger-like sporoduct (*t*), which finally evaginates to form the emission ducts (*e*).

gregarines the residual mass is gradually absorbed as food during the formation of the sporozoites.

In some cases the residual mass of protoplasm plays an important part in the dissemination of the mature sporozoites; in *Gregarina cuneata* and probably in allied forms, according to the recent observations of Kuschakewitsch ('07), the residuum takes the form of a hollow brood chamber (*Brutraum*, of Kuschakewitsch), and its protoplasm retains a quantity of the residual chromatin from which as "amphichromidia" the gametic nuclei had previously been formed. This residual "chromidial net" collects in rings at the periphery and around

the borders of the brood chamber, which is connected by broad spaces with the peripheral rings of chromatin. From the walls of these rings tubular ingrowths next develop and grow down into the brood chamber among the sporocysts (Fig. 81). When mature, and under proper environmental conditions, not as yet recognized, these tubular ingrowths are evaginated and the sporocysts ejected through them.

(b) **Sporulation in Coccidiidia.**—In coccidiidia the processes of conjugation and sporulation are involved with complex sex differences, pseudoconjugation, as observed in gregarines, being unknown. Here a spermatozoid and an egg cell are formed and fusion is complete. The fertilized cells, furthermore, have a somewhat different history from those of the gregarines, where the zygote becomes at once the sporoblast and secretes a single or double sporocyst. In the coccidian forms, on the other hand, the fertilization nucleus of the zygote or copula only rarely (*Lègerella*, Mesnil) divides to form sporozoites directly, but in the remaining genera the primary divisions give rise to nuclei of two or more independent sporozoite-forming centres. Thus, in *Coccidium schubergi* the zygote nucleus divides twice, forming four daughter nuclei, about which the protoplasm of the zygote forms four sporoblasts. Each sporoblast secretes its own covering or sporocyst, and each gives rise to two sporozoites (Fig. 74, p. 179). The final mature germs are thus inclosed within two membranes, their own sporocysts and the oöcyst which forms as a fertilization membrane, *Lègerella* alone being protected by the latter only. Classification of the coccidiidia is frequently based upon the number of sporocysts thus formed. *Crystallospora crystalloides*, like coccidium, has four such sporocysts, but the great majority of the tetrasporocyst forms belong to the latter genus. Others, notably cyclospora, diplospora, and isospora, have only two sporocysts; still others, and perhaps the most common forms, have more than four sporocysts, adelea, caryotropha, and klossiella belonging to this category.

In these forms, as in the gregarines, the number of sporozoites is independent of the number of sporocysts; in barrouisia, echinospora, and diaspora (Fig. 20, p. 64) the sporocysts are monozoic; in adelea and minchinia, dizoic; in benedenia, trizoic; in klossia, tetrazoic; and in caryotropha, polyzoic. It is significant that in the malaria organisms there are several centres of sporozoite formation, each of which, if covered by a membrane, would be homologous with the polysporocystid sporocyst. This may be merely a parallel development, or it may have some phylogenetic significance, showing descent from coccidium-like forms, with loss of the now useless protective sporoblast membranes.

(c) **Sporulation in Myxosporidia.**—Little more need be added to what has already been given in connection with spore formation in this group (see p. 143), for it is closely connected with the phenomena of fertiliza-

tion considered in the preceding chapter. The spores are usually protected by thick and tough membranes, and are distinguished from all other sporozoan spores by the presence of spirally wound threads contained in two to four polar capsules. They are often ornamented in some way and are always in the form of two valves, which meet in a suture representing the line of splitting when the spores germinate (Fig. 20, *G, K*, p. 64). The polar capsules are variously arranged in the spore, and the usual interpretation of the thread is that originally given by Thèlohan ('92), that they are for the purpose of anchoring the spore in the lumen of the digestive tract. The most curiously

FIG. 82

Spores of actinomyxidæ. (After Caullery and Mesnil.) *A*, Hexactinomyxon psamoryctis (after Stölč), $\times 450$; *B*, Spheractinomyxon stolci (after Caullery and Mesnil), $\times 900$; *C*, Triactinomyxon ignotum, Stölč, $\times 250$; *D*, Triactinomyxon ignotum, spore-bearing part of same enlarged (after Lèger), $\times 900$; *E*, Synactinomyxon tubificis, Stölč, $\times 900$. In *A*, *B*, *D*, and *E*, the evaginated spiral filaments are shown.

ornamented of all spores are those of the actinomyxidæ, where long processes and curiously placed polar capsules and sporozoites are characteristic (Fig. 82).

3. Exogenous Life of Protozoan Parasites.—By exogenous life of parasites is meant here the life outside of the usual host, whether this is the primary or "intermediate" host. It is the most critical period in the entire life history of a parasite, and a successful outcome is dependent upon several factors, the most important being: (*a*) dissemination of the spores, and (*b*) infection of new hosts, the latter factor in particular having given rise to the most diverse adaptations.

The environmental conditions which parasites have to meet and overcome are well stated in principle by Manson in the following excerpt: "The pathogenic protozoa are responsible probably for a very large number of diseases. Many appear to be able to pass directly from host to host, unaffected apparently by the atmospheric conditions they encounter on the passage; that of smallpox and of most of the exanthematous fevers probably belong to this category. Others, on the contrary, demand special climatic conditions. Such are the germ of scarlet fever, which does not spread in the tropics, and the germ of dengue which, conversely, does not spread in cold climates. That of the first is killed or paralyzed by heat; that of the latter by cold. Or, it may be, they do not find appropriate transmitters except in special climatic conditions. Many of the protozoa acquire the power of successfully invading the human body only after certain developmental changes, which take place after they leave their first host. Thus, according to Schaudinn, the germ of amebic dysentery has to pass through a sporulating stage before it becomes infective, and this stage is accomplished outside the body and in conditions of tropical heat. Hence, amebic dysentery is a tropical disease. Other protozoan disease germs, notably those of malaria, yellow fever, trypanosomiasis, and relapsing fever, require an animal intermediary to remove them from the body of their original host, foster them during a necessary stage of development, and reimplant them in the human host. These animal intermediaries being tropical, the diseases they disseminate are also necessarily tropical." (Introduction to Vol. II, Part II, of Allbutt and Rolleston's *System of Medicine*, 1907.)

The majority of facultative parasites (some species of entameba, cercomonas, copromonas, etc.), and many obligatory parasites, find their best environment for further development in the digestive tract of different animals, and the spores, when formed, are discharged with the feces. Protected by their tough sporocysts, they may resist drying for long periods or until taken again into some digestive tract, infection being due to the more or less gregarious mode of life of the hosts and to their indiscriminate feeding. An essentially similar result is obtained in the case of cannibalistic animals, where, as in centipedes, the weaker forms are eaten by the stronger and with them whatever parasites they happen to harbor; it is in large part for this reason probably that centipedes are rarely found without sporozoan parasites of some kind. In water-dwelling animals the spores of myxosporidia are usually disseminated through the water, so that infection is brought about in the same way through the digestive system. In land-dwelling or air-breathing animals of clean habits such sources of infection are rare, and comparatively few protozoan parasites occasionally found in them acquire a new host in this way. Other means, however, especially in the higher animals and man, are effective

in keeping up the various races of parasites, and infection of new hosts may be brought about by (a) breathing; (b) by direct transmission or contact; (c) by inheritance; or (d) by indirect transmission through the agency of intermediate hosts.

(a) **Air-borne Protozoan Parasites.**—So far as the protozoa are concerned, this method of infection plays but little part, and then only in cases of certain diseases, such as scarlet fever, smallpox, and a few others which are not yet accepted by all as due to protozoan parasites. The great majority of protozoa capable of withstanding the condition necessary for this mode of infection are too large and heavy to be conveyed as dust. In trachoma, smallpox, and scarlet fever, which no one would question as being germ diseases, the spores of the organism causing them are so minute as to be readily disseminated with cutaneous debris, or as Flügge ('97) has shown in experiments with bacteria of different kinds, they may be spread in minute droplets of mucus or sputum. So far as known, the seat of invasion of these spores or minute organisms is the respiratory tract, where the nasal lining may harbor the spores of trachoma, or the corrugated surface and imperfect epithelium of the tonsils may give lodgement for the spores of smallpox and scarlet fever. It is possible that the organism found by Minchin and Fantham ('05) in nasal tumors (*Rhinosporidium kinealyi*) is transmitted in this way, although nothing is known as to the exact method of its dissemination.

(b) **Transmission of Protozoan Parasites by Contact.**—A large number of protozoan diseases are due to the transmission of the parasites by direct transmission through contact which may be brought about in various ways. Wherever external lesions occur this means of infection is possible. In the case of rabies, where contact is brought about usually by the bite of some infected animal, the parasites are introduced with the saliva and gradually make their way into the central nervous system, although, as Pasteur first showed, the entire nervous system from periphery to centre may contain the virus. Not only by biting, but by other ways as well, may the organism of hydrophobia (*Neuroryctes hydrophobiæ*) get into the human organism; infection may follow from carelessness in the operating room, or, a particularly potent way, from the licking of infected animals on abraded or chapped surfaces of hands or face.

Usually the organisms thus transmitted by contact have the power of spontaneous motion, the passive sporozoa being rarely spread in this way. A possible exception, however, appears to be the case of the so-called *Coccidioides immitis*, described by Rixford and Gilchrist ('97), in Argentina and the Southern States. The disease first manifests itself in the human skin, and may pass by way of the lymphatics to liver, spleen, peritoneum, and other organs of the body, ultimately causing death. The organisms first form small granulation tumors in

the corium and give rise to minute papilla-like protuberances, which may run together, continually increasing by peripheral growth. Blanchard considers these parasites to be sporozoa, but doubts their affinity with the coccidiidia (Lühe, Minchin).

The genitalia are frequently the seat of infection for several kinds of protozoan parasites; *Trypanosoma equiperdum*, Dofl., for example, the cause of dourine in horses, is transmitted solely by coition, the flagellates getting into the blood by penetrating the epithelium. Similarly, with *Trypanosoma gambiense*, the cause of sleeping sickness in man, the organisms are said to pass from person to person in this way (Koch), while the organism of syphilis in man—*Treponema pallidum*—is readily transmitted from person to person by coition. Resting or encysted stages of the latter organism are unknown, but vitality is apparently retained for long periods, for infection may be brought about by contact with places contaminated by infected persons; abrasions and chapped surfaces are particularly dangerous.

(c) **Transmission by Inheritance.**—The transmission of protozoan parasites by inheritance is only a modified form of contact transmission, and might well be expected in the case of such parasites as are capable of independent motion. It is satisfactorily established at the present time that bacteria are not transmitted from mother to child and that bacterial infection in utero is practically nil. With protozoa, on the other hand, infection in utero by way of the placenta and umbilical cord is fully established in some cases, while in the lower animals, such as invertebrates and aplacentalia among vertebrates, inheritance of such disease-causing forms is much more common.

Pasteur ('58) early discovered that the only successful means of combating the silkworm disease, due to *Glugea* (*Nosema*) *bombyces*, was to carefully examine the eggs of the insect for cysts and to destroy all that were found to be infected. Careful prophylaxis of this kind, together with proper scrutiny of food, finally put an end to the inheritance of the disease from generation to generation and brought to a close a long-continued epidemic which had cost nearly one thousand millions of francs. The later observers have placed such inheritance among insects and arachnids upon a much safer basis, and in many cases the transmission of protozoa from parent to offspring is fully established. Smith and Kilbourne ('91) discovered that ticks belonging to the genus *Rhipicephalus* (*Boophilus*) draw blood from cattle infected with *Babesia bovis* (*Piroplasma bovis*), and convey the infection in time to some new host. Koch observed that the ova of ticks were actually infected, and that the young, in addition, feed upon the infected blood, so that the second generation transmits the disease, and Christophers ('07) showed that reproductive bodies of *Babesia* (*Piroplasma*) *canis* penetrate the ova, either in the ovary or during the

passage of the eggs down the oviduct, develop in the yolk of the egg, and become disseminated throughout the embryonic cells, reproducing the while, and finally lodging in the salivary glands of nymphs and imagos. Similarly, with ticks of the genus *Argas*, which are known to transmit spirochetes of different species infecting birds and fowls, Levaditi has shown that the spirochete of relapsing fever or spirillosis in chicks penetrates the ova of *Argas miniatus*, and in this way infects the young chickens. Relapsing fever in man due to *Spirocheta duttoni* is conveyed by ticks of the genus *ornithodoros*, in the eggs of which Carter ('06) and others have shown that the ova are frequently the seat of multiplication of the parasites derived from the infected parent.

FIG. 83

Section of lung infested by *Treponema pallidum*; congenital syphilis. $\times 800$.

A final stage in the development of this means of transmission is suggested by Ward ('08), in connection with the parasites of the intestine of the housefly, which, no longer drawing blood, transmit the parasites from generation to generation only through the embryos. This suggestion, however, loses weight from the fact made out by Patton ('08) that direct infection follows ingestion of encysted forms of the intestinal parasites.

With man and mammals transmission by inheritance is much more difficult, if for none but mechanical reasons. The parasites must penetrate the placenta and the solid tissue of the umbilical cord, and it is conceivable that only minute and highly motile forms can do so. It is a well-established fact, however, that certain kinds of parasites

belonging chiefly to the trypanosome and spirochete group are capable of passing through the finest filters, and such forms of protozoa, if any, might be expected to infect an embryo in utero. This is certainly true of the organism of human syphilis, congenital cases not infrequently occurring in which the parasites are transmitted either with the spermatozoa or with the egg, or through the placenta from the mother infected during pregnancy. Such congenital cases are often highly virulent; all organs and tissues of the unfortunate infant may be over-run with the malignant spirals (Fig. 83).

With transmission by contact or by inheritance, there is, strictly speaking, no free or external life of the parasite, the organisms passing directly from one living host into another, and this form of infection is often bound up with one of the most interesting and important of the protozoan vital phenomena, the transmission by intermediate hosts.

(d) **Transmission by Intermediate Hosts.**—Direct infection by way of the digestive tract by ingestion of spores of the parasites with food may become complicated by passive carriage through intermediate hosts often of a quondam character. While not proved, this appears to be a highly probable means of infection. Thus, as Minchin points out, in the case of the monocyctis parasites of the earthworm, where the organisms are parasitic in the seminal vesicles of the worm, there is but slight possibility of the parasite spores passing to the outside with the spermatozoa or through the dorsal pores of the worm, and there is little doubt that the animals are infected by way of the digestive tract. It is suggested by Minchin that the infected worms are eaten by birds, and that the spores of the gregarine, protected by their resistant coatings, pass undissolved through the avian digestive tract, to be disseminated with the bird's feces about the ground, where in time they may be again eaten by a worm. Similar conjectures might be made for other animals whose habits, life histories, and parasites are known.

A mode of transmission such as this would involve only a passive phase in the life history of the protozoan parasite; in the majority of cases where the relation of parasites to intermediate hosts are fully made out the period in such a host involves some of the most important activities in the life of the parasite. Here are to be found some of the most perfect adaptations of means to ends that are known in biology; those forms which are not protected by resistant coverings and where infection is brought about through the aid of an obligatory intermediate host are the most remarkable. The malaria organisms, for example, if sucked with the blood into the digestive tract of a mosquito of the genus *Anopheles*, are all digested save the conjugating forms, which are apparently endowed with some greater power of resistance than are the vegetative forms. But if the same parasites are taken into the stomach of a mosquito of the genus *Culex*, gametes, and

other stages as well are alike digested; hence the various species of culex cannot transmit malaria to man. Similarly with other forms of blood-dwelling parasites, each is apparently restricted to certain types of hosts, although in some cases a certain latitude in this direction is noted (*Trypanosoma brucei*, some species of *Babesia*, etc., may be carried by different hosts). The ultimate explanation of this resistance lies in the domain of physiological chemistry, and until this branch of biological science is more fully worked up the full significance of these adaptations will not be known.

The same powers of adaptation that underlie the transmission of malaria by mosquitoes apply to other cases of parasite transmission. Mosquitoes carry trypanosomes from owl to owl; others (*stegomyia*) carry the organism of yellow fever; tsetse flies (*glossina*) transmit sleeping sickness in man or *Nagana* in cattle; other insects and ticks carry different kinds of disease-causing organisms in lower domesticated and wild animals; bedbugs transmit kala azar and relapsing fever; while leeches are intermediate hosts for some parasites of fish and amphibia.

In many of these cases the parasites undergo a definite developmental cycle in the body of the intermediate host, although in relatively few cases have the happenings in such cases been fully determined. In the case of malaria organisms, of *Herpetomonas* (*Leishmania*) *donovani* and some trypanosomes, the most important phases in the life history of the parasites, sexual reproduction whereby the vitality is restored, are known to take place. In other cases, including the majority of trypanosomes and spirochetes, and most other protozoan disease-causing forms, little more than asexual multiplication within the intermediate hosts is known to occur.

It makes a very pretty subject for an academic debate whether anopheles first gave malaria to man, or whether man gave acute enteritis to the mosquito. There is some reason to believe that these blood parasites, or at least some of them, have descended from the coccidiidia, and that they have become specifically adapted for life in the blood instead of in the epithelial cells of intestine or coelom. The evidence for this is based partly upon the intracellular mode of life characteristic of the majority of the hemosporidia and partly upon Hintze's (questioned by Lühe on the ground of confusion with some form of coccidiidia) observations upon the life history of the common blood parasite of the frog, *Lankesterella*. While his observations have been questioned, they have not yet been refuted, and his conclusions are still possible, especially in consideration of the recent findings of Miller ('08) in the case of *Hepatozoön perniciosum* (see p. 269). Fertilization, according to Hintze, takes place in the intestine of the frog, and the zygote moves like a gregarine through the fluids of the digestive tract until it enters an epithelial cell, where it encysts. As

Minchin suggests, it is possible that the organism is taken into the digestive tract and the sporozoites liberated there to pass through the epithelial cells into the blood, where asexual reproduction occurs. If this questionable life history is true, it is conceivable that the ancestral forms of the blood-dwelling hemosporidia were similar to coccidiidia and made their way into the blood spaces from the digestive tract. On the same hypothesis it is further conceivable that the blood-sucking insects or leeches, while usually able to digest such forms taken in with the food, in some cases provided a suitable environment for their further development. Spore cases, characteristic of the supposed ancestral forms, would be unnecessary with the substitution of the insect-dwelling mode of life for the former exposed life, and, on the other hand, would be of marked disadvantage to the young forms upon reinoculation in the blood of a new host. According to such an hypothesis, the first or original primary host of such hemosporidia would be man or other vertebrate type, while the secondary or "intermediate" host would be the insect or leech. On such an hypothesis it might be further assumed that in earlier times the intermediate host acted as a mere carrier, the organisms remaining passive during the interim.

The above is the opinion concerning intermediate hosts held by Minchin ('07) and other protozoölogists whose dicta carry much weight, but opposed to them are other students of the group, including Laveran, Mesnil, Grassi, Lühe, and others whose conclusions, based upon the recent observations on the blood-dwelling forms, are more convincing. Such conclusions are based largely upon the fact that the most important phases in the parasite's life history occur in the digestive tract of the invertebrate host, and that sporozoites, not merozoites, are transmitted by them to man. Recent observations on blood-dwelling forms in man indicate that the ancestral forms were not coccidiidia but mastigophora. Schaudinn was the first to note the relation between a free-swimming *Trypanosoma noctuæ* in the blood of the little owl, *Glaucidium (Athene) noctuæ*, and the intracorporeal parasite of birds which had been known as halteridium (hemoproteus); also, he was the first to see the transformation of the intracellular into the flagellated form. Since then his observations have been confirmed by various observers, the brothers Sargent ('C5) finding most of the details as he had described them. In a number of other forms as well the relation of a flagellated type to intracellular types has been established. Rogers, Christophers, Leishman, Patton, and others have noted the transformation of the intracellular Leishman-Donovan bodies into flagellated parasites similar to the genus *herpetomonas*, such transformation taking place both in the digestive tract of the invertebrate host (*Cimex rotundatus*) and in artificial culture media. From these observations there is reason for

the belief of Lühe, Mesnil, and others, that the original forms of some at least of these organisms were flagellated protozoa which have lost their motile organs and assumed an intracorpuseular or cytozoic mode of life with the accession of parasitism in man. Also, it appears from such cases that the original hosts were insects and not man, so that here at least man would appear to play the part of intermediate or secondary host.

The further deductions which some recent observers have made (notably Hartmann and Kisskalt, and others), that all hemosporidia are to be traced to flagellated ancestral forms, and that the group as a division of the sporozoa should, therefore, be abandoned, does not follow from the evidence and cannot be sustained at the present time (see p. 269).

III. EFFECTS OF PROTOZOAN PARASITES UPON THEIR HOSTS.

The malevolent effects of various kinds of protozoan parasites on their hosts may be either chemical or physical in nature, and due to products of their own metabolism, or to mechanical destruction of cells and tissues. The majority of the former type give rise to antibodies which may persist for varying periods, thus setting up an active or a passive immunity.

Beyond the fact that they differ in different cases, little is known about the chemical effects produced by protozoan parasites. Nor is the definite action known in many instances. In the case of malaria the pyrexial attacks are supposed, by the majority of authorities, to be due to the liberation of a toxin contained in the pigment melanin which is elaborated by the parasites. The sudden precipitation of this pigment in the blood upon dissociation of the merozoites causes intoxication and convulsions. Celli, Gualdo, Montesano, and others have produced similar convulsions by inoculation with the serum of malarial blood without the organisms, while, as Thayer points out, the coincidence of the convulsions with the schizogony of the parasites and the liberation of these pigmented substances, when taken together with the degenerative changes often found in the brain, nerves, liver, and kidney, all point to the conclusion that some toxic substance or substances are present.

A widespread effect of protozoa is the lysis set up by their presence in cells and tissues. This was clearly worked out by Councilman and Laffleur ('91) in the case of amebic dysentery, where the parasites penetrate the submucosa, where they cause the cells to jellyfy and degenerate. Similar destructive changes are brought about by the organisms of trachoma, of rabies, and of smallpox. *Neurocytes*

hydrophobia, presumably by the secretion of some toxic substance, causes the destruction of brain and nerve cells, while *Cytoryctes variola* produces a like destruction of the generative cells of the skin.

A much more subtle action is shown by those parasites which cause hypertrophy or multiplication of the infected cells. The great tumors often found in the cruciferæ arising from the root cells owe their origin to some chemical effect produced by the intracellular parasites *Plasmodiophora brassicæ*, and numerous observers have sought to explain human cancer and other tumors in like manner.

FIG. 84

Caryotropha mesnili, Sied. A, coccidian parasite of spermatogonium cell which is much hypertrophied while the remaining spermatogonia of the bundle form an epitheliod layer about it. An intracellular canal in the parasite connects the nucleus (*n*) of the host cell and the nucleus of the parasite while a stream of foodstuff proceeds from the former to the latter. (After Siedlecki, combination of drawing and photograph.) $\times 760$.

The demoralizing effect which an intracellular parasite has upon an animal cell is well shown by Siedlecki in the case of the sporozoön *Caryotropha mesnili*. The organism is a parasite in the spermatogonia of the annelid *Polymnia 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 a merozoite or sporozoite, one only of the bundle, 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 germinal structure, becoming hypertrophied and distorted (Fig. 84). Not only the infected cell, but all of the other cells of the spermatogonia bundle are affected, and none of them continue the normal development, but become arranged like epithelial cells about the hypertrophied infected cell.

Here, then, is a change which, as Siedlecki points out, recalls the condition which Hertwig ('04) shows is characteristic of degenerating cells, the simplification of the cell type from a more complex "organo-type" into a simple "cytotype," or a return to the embryonic condition.

The specific effect of the young caryotropha on the infected cell consists not only in the enlargement of that cell, but of a definite feeding mechanism by which the parasite is supplied with food. That the nucleus is the seat of constructive metabolic changes is well assured at the present day, and the conditions in these parasites suggest the peculiar relation which Shibata ('02) 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. 84). In the cytoplasm of the cell an intracellular 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.

Not only hypertrophy of the cell, but of the nucleus as well, may be caused by the presence of protozoan parasites. Doflein ('07) has shown that the nucleus of *Ameba vespertilio* becomes greatly enlarged through the action of intranuclear parasites, and similar enlargement is characteristic of the skin cells in smallpox lesions. Lèger and Duboscq ('04) noted that gregarines may cause the formation of multinucleated cells, while in some forms (*Stylorhynchus oblongatus* and *St. longicollis*) the epimerite penetrates the cell and rests in the vicinity of the host nucleus. In these cases the French observers state that the parasites attached to the epithelial cells prevent the normal nourishment of the latter and also prevent the cells from secreting properly, so that they do not develop but remain of embryonic type, and may even divide. Where the parasites are abundant in an organ the destruction of cells is too rapid for regenerative processes to keep up. Thus, Schaudinn ('02) has shown that *Cyclospora caryolytica* may be so abundant as to cause acute enteritis and death of the mole, its host, within a few days. Here the effects are purely mechanical, and in this category belong the great majority of protozoan parasites, especially those forms which are intracellular during part or the whole of their life. Liver cells, muscle cells, even heart cells, may all be destroyed by some form or other of protozoan parasite, usually a sporozoan, and these cytozoic forms rarely confer immunity on the host organism.

IV. PROTOZOA AND THE CANCER PROBLEM.

Before describing, in the following chapters, the well-defined and accepted pathogenic protozoa, it may be well to consider first some pseudoprotozoa that have been brought forward from time to time as the cause of cancer. This disease, more than any other human ailment, has been a fruitful field for such forms, and the many structures that have been described as protozoa must be regarded only as monuments to innumerable well-meant but immature efforts to discover the cause of this subtle malady.

Of the many varieties of tumor occurring in man, carcinoma, or "cancer," is the one offering the most striking biological phenomena, although there is reason to believe that other tumors, especially sarcomata and epitheliomata, are manifestations of the same or of similar causes. In all cases, whether benign tumor or malignant growth, the one common characteristic is the power of cell proliferation, and the "cancer problem" which today engages the best effort of many pathologists, chemists, biologists, and medical men in general in every civilized country is to ascertain the cause or causes underlying such proliferation. Many believe that the secret is bound up with the problem of life itself, and will be solved only when the latter is an open secret; but the great majority of investigators fortunately take the more hopeful view that cancer, being an abnormal growth, has some specific and demonstrable cause.

In every type of animal, including even the protozoa, there is a more or less well-defined power or "potential" of division energy of its cells, a power which gradually diminishes with advancing age and ultimately gives out (see p. 134). The individual cells then cease to multiply, and in the higher animals their activities are directed toward the one physiological object for which they are specialized, and division is resumed only when some external and unforeseen cause, such as a wound, starts up the inhibited development. Even this power of regeneration is lost to some types of physiologically unbalanced tissue cells.

In the higher animals the cells of the epithelial group retain the physiologically balanced condition longer than any other type. This is the group to which the germ cells and the endothelial and secreting cells belong, the so-called "noble" cells of the body, some of them, like the skin cells, retaining their division energy throughout life, while others, the germ cells, possess the potential of endless existence. Even among the cells of the epithelial type the potential of division energy varies, and in the highly specialized and physiologically unbalanced tissue cells it is early exhausted. It is in connection with these cells that we must look for the cause of carcinoma; in their vital

manifestations and in the reanimation of their latent division energy lies the cause of from five to six deaths from cancer in every hundred deaths from all causes.

The carcinoma cell biologically is a perfect vital mechanism endowed with far greater power of resistance than normal cells, a resistance which enables it to withstand long exposure to liquid air, or long periods apart from the sources of nourishment. In reality it is no longer an epithelial cell; something has changed it from such a physiologically unbalanced unit, subject to the coördinating control and regulation of the organism, into a physiologically balanced cell, uncontrolled and unregulated. Functionally, it is a more perfect type than its orderly associates of the epithelium from whence it springs; it takes in and assimilates abundance of food, grows rapidly, especially when near the source of food, and reproduces its like by means of the same complicated processes of mitosis that characterize normal cells, although it does not become differentiated into organs, as do embryonic cells. "In short, it is a complete organism in itself, simulating in many ways the parasitic protozoön, but differing in some of the most important respects connected with the continued life of the latter." (Calkins, 1908, p. 286.)

By this continued cell division masses of tissue are formed which grow out into lymph channels, pressing into spaces wherever found, mechanically obstructing the normal activities of surrounding tissues and organs, or breaking through such tissues, and ever giving off small groups of free cells which may be carried by the blood to various parts of the body, there to set up independent growths (metastases) and to become new centres of malignant activity. With the local disturbances caused by such abnormal growths, many normal cells are killed for lack of nourishment, or by poisonous degenerative matters of one kind or other, while the cancer cells themselves undergo hyperplasia and hypertrophy through lack of food, pressure, or natural resistance of the victim. The march of cancer, therefore, is invariably accompanied by multitudes of degenerating cells, leukocytes of all kinds, blood platelets, and the like, and these different structures are the things which, in various stages of involution and degeneration, have been interpreted as "coccidia," "amebæ," "X-bodies," or, more specifically, as "strombodes" (Sjöbring), "Rhopalocephalus carcinomatosus" (Korotneff, '93), "Cancrionameba macroglossa" (Eisen, '00), "Histosporidium carcinomatosum" (Feinberg, '03), or as other "organisms" with resounding names, the "cause" of cancer.

Little interest is excited at the present time by description of such cell inclusions in cancer, and investigators, on the whole, are content to regard all such structures as degenerations or products of the disease rather than its cause, and with this change in attitude the problem of cancer has passed from the descriptive into the much more fruitful stage of experimental research.

The early history of animal cancer has a certain historical interest in medical circles, but the present-day activity dates back only to 1902, when Jensen, of Copenhagen, discovered that mouse cancer (adenocarcinoma) can be transplanted from one mouse to another. With more than usual breadth of view and scientific generosity, Jensen distributed his cancer material to all who wished it, and the result is that the "Jensen strain" of mouse cancer is being studied and transplanted in all parts of the civilized world, while special laboratories for the exclusive study of cancer have been established in Buffalo, in London, Heidelberg, and other places. Investigation has brought out the fact that this mouse tumor differs but little from human carcinoma, while similar primary tumors are now known to occur in one mouse in every 2500 (Bashford). Hundreds of such primary cancers have been transplantable, so that today many in addition to the Jensen strain are being studied. Malignant growths in other animals (rats and dogs especially) have been discovered, and are all contributing data for the ultimate control of human cancer. This dreaded disease, therefore, which is still impossible to control and the cause of which is still unknown, is at present in the full swing of experimental study.

It was early shown by Jensen and his followers that a tumor induced in a normal animal by inoculation is derived not by the abnormal division of cells of the normal animal, but by proliferation of the transplanted cancer cells of the diseased mouse. The induced tumor, therefore, is not equivalent to a primary tumor, but may be regarded as equivalent to a metastasis from such a primary growth. Furthermore, it was early shown that human cancer when similarly transplanted in mice, or any other lower animal, will not grow; nor will the mouse or rat tumor grow in any other animal than the definitive species. Cancer in lower animals, therefore, need not cause apprehension, although it is always possible that the unknown cause or causes may be the same or similar in all cases.

The Jensen tumor, to take only one example, has now been transplanted through nearly 100 generations, or possibly more, counting as a generation the successive tumors produced by inoculation. The average length of time required by the Jensen strain to develop into a cancer fatal to the inoculated mouse varies from three to four weeks, but it may be reduced to ten days or two weeks, or increased to three or four months or longer.

This long-continued transplantation and the fact that each new transplantation results in the formation of a mass of cancer cells derived from the transplanted cells, yielding a growth which, up to the present time, amounts to a small mountain of mouse tissue, indicates that the cancer cells are somehow endowed with the possibility of an indefinitely continued division energy. The cancer cell, therefore, is different from any animal organism that we know, for in all

cases indefinitely continued protoplasmic existence is bound up with the phenomena of fertilization and inheritance. The cancer cell, so far as we know, undergoes no process analogous to fertilization. Farmer, Moore, and Walker ('03) have described "heterotypical" mitosis in cancer cells, and claim that, as in germ cells, this is evidence of the preparation for fertilization, but numberless critics have shown that it indicates only the degenerative changes which the majority of the cancer cells that are formed must undergo, since all that are formed cannot find nourishment, or escape the protective reactions of the host organism. Cytologists, also, are constantly demonstrating that heterotypical mitosis is a form which the mitotic figure may assume under almost any abnormal condition; Haecker ('04) obtained them in embryonic cells treated with ether and other poisons, while Bonnevie ('07) has shown that they are common enough in normally developing cells of different animals and plants. The further observations of the English observers as to a reduced number of chromosomes in cancer cells are more safely explained upon the lines early laid down by Hansemann ('93), as due to abnormalities brought about by deranged mitotic figures in degenerating cells.

It is beyond the scope of the present volume to discuss the various theories that have been advanced to explain the source of the stimulus to cancer-cell proliferation. Ewing ('08), in an excellent summary of the present status of the cancer problems, broadly divides all theories into two categories, which he designates the parasitic theory and the cell-autonomy theory. The former, held by von Leyden, Behla, Borrel, Gaylord, and a host of others, interprets cancer as due to the action of some foreign living organism stimulating the cell to divide, and so to produce the primary tumor, and by its continued presence maintaining the stimulus to proliferation. The other theory, held by the great majority of pathologists and medical men in some form or other, and taking concrete form in the theories of Cohnheim, Ribbert, Ehrlich, Ewing, and others, interprets cancer as due to the breaking loose of some cell or cells from the regulating control of the organism and starting off on an independent career of lawless development.

Against the former theory must be charged the fact that no specific parasite has been continuously found in human or animal cancer, nor does the clinical history of the disease furnish anything similar to that of known infectious diseases. Against the latter must be raised the important objection that in no form which the theory assumes is there a satisfactory explanation either of the cause of cancer or of the power of continued proliferation. It is true that normal vital processes are not yet sufficiently known to enable us to predict what might happen under abnormal conditions, and with those who are pessimistic enough to believe that the problems of cancer and of life itself are all one, we may assume that only in time will further knowledge show

how the power of regulation may be lost to these specialized tissue cells, and the power of endless proliferation gained. To say, as Adami ('01) does, that in cancer cells the "habit of growth" has replaced the "habit of work," or to admit with Oertel ('07) that if a gland cell can be induced to excessive secretion we might with equal right expect it to be induced to divide excessively, is simply to say with Hertwig ('04) that the cells of carcinoma have changed from an "organotype" into a "cytotype." Such statements, forming the real substance of many polemical writings on cancer, merely state the problem and are perfectly true, for cancer, or malignant growth of cells, does exist. These truths do not furnish any clue to the cause which underlies the abnormal growth, nor do they in any way explain the apparent power of endless growth which the cancer cell, unlike any other mammalian cell, possesses. The phenomena of normal regeneration cannot be invoked; a begonia plant or hydra animal may be cut into small pieces and each will grow into a perfect organism, but here in these generalized forms, apparently, the all-important germ plasm is present in all cells, and they are widely different from the highly specialized, physiologically unbalanced, tissue cells of mammals, and are always subject to the coördination and regulation of the organism, as a whole.

On the other hand, the parasitic theory of cancer in its naked form is altogether too simple an explanation, and the clinical symptoms of the disease differ so widely from those of different germ diseases, as to weigh heavily against it. Nevertheless, there is some positive evidence, as shown by the frequently localized distribution of cancer, by cancer à deux, by the facts of cancer immunity (Gaylord, Clowes, and Baeslack, Ehrlich, "athreptic" immunity), by cage infection (Gaylord, Borrel, Lignières, etc.), and by the "infectivity" of cancer cells, as contrasted with cells of benign or embryonic tumors, of vegetable galls, or with normal transplanted tissue cells.

While there is little doubt that the morbid symptoms of cancer are due to the autonomous activity of these malignant growths, the problem is deeper than mere descriptions of the symptoms caused by the onrush of the anarchistic cells, and is resolved into the biological inquiry as to what was the initial cause of the loss of organic regulation and what underlies the secret of their inexhaustible division energy. The advocates of the cell-autonomy theory have no satisfactory explanation for the first, but throw the burden of proof upon the biologist and look for enlightenment to the school of experimental embryology and zoölogy. Nor are their explanations of the continued power of proliferation more successful, for they call upon the mysteries of fertilization, finding, with Klebs ('89), Farmer, Moore, and Walker ('03), that epithelial cells conjugate with leukocytes, or with Recklinghausen ('96), that they are "fertilized" by fibroblasts, or with Waldeyer ('87), that

vitality is renewed by parthenogenesis, and they fail for the most part to see that their supposed applications of this biological phenomenon are far more improbable than the parasitic theory which they deride.

Many advocates of the theory of cell autonomy go so far into the other camp as to believe that the cancer cell is itself a parasite. This parasitism is shown by the fact that when placed in a suitable medium it reproduces cells similar to itself and continues to multiply in this way, without showing signs of differentiation into organs, a phenomenon which has given rise to the term "infectivity" of cancer cells, and it certainly is an attribute which parasites possess. Bashford, Murray, and Bowen ('06), confirmed later by Hertwig and Poll ('07), made the observation, based upon statistical data, that the growth energy in these cancer cells in mice undergoes rhythmical variations in vigor and depression. Calkins ('08) found similar rhythms, based upon the records of the New York State Cancer Laboratory, but showed that the rhythmical variations were not in the growth energy of the cancer cells, but in the infectivity of these cells, the growth energy and infectivity showing no relationship after the tumor is established in transplantation.

The advocates of the parasite theory believe that the cancer cell became a parasite in the above sense, not from any derangement of metabolic processes, nor from any vague, hypothetical, inherent tendency to cellular anarchy, but because of the susceptibility to the poisonous stimulus of some parasite. In this they are supported by the facts of gall formation in plants, where a known poison, secreted by insects, stimulates the latent division energy of the plant cells, and a tumor is produced. The counter argument, so often made, that such abnormal growths are nothing like cancer, is certainly true; the analogy, however, is not with the form which the growth assumes, but with the cell which is stimulated to divide by the activity of a parasite. Among other things, the gall differs from the cancer cell in having no infectivity, the stimulus not being continuous.

Another analogy is drawn from the great tumor-like growths in certain vegetables (cruciferae), due to the presence in the root cells of a protozoön parasite, *Plasmodiophora brassicae*. These growths, known as club root, hanburies, fingers and toes, etc., are highly infectious and are frequently a serious menace to market gardens. The organism causing the tumors penetrates the root hairs of the cabbage or other allied vegetables, in the form of a minute ameboid flagellate (Woronin, 1878, Prowazek, 1905). Two or more may enter the same cell, where, immersed in the fluid cytoplasm, they lose their flagella and grow into larger ameboid organisms (Fig. 62, p. 148). Later, these ameboid cells fuse, forming, as in all myxomycetes, a syncytium or plasmodium. The infected cells are caused to divide by the presence of the parasite, the infected cells thus carrying the

disease-causing germ, which apparently has no power of migrating from cell to cell (Prowazek, '05). After a number of such divisions the infected cells undergo hyperplasia and hypertrophy; the pressure and possibly the toxins from the organism cause neighboring cells to proliferate until large abnormal growths result. The parasites, in the meanwhile, having exhausted the nutriment of the host cells, form permanent spores, the spore formation being preceded by endogamous fertilization processes, as described on p. 147. These spores are stored up in the plant cells until the latter decompose and disintegrate in the soil.

In club root, therefore, we find an analogy not in the form or type of the tumor produced, but in the renewed division energy of tissue cells through the presence of an intracellular parasite. Here, again, infectivity is entirely independent of growth energy of the tissue cell, and dependent upon the parasite alone. The vegetable cell cannot long withstand the inroads of the relatively large parasites, and ultimately dies because of them. It is conceivable that a cancer parasite may exist within a cancer cell and serve as a source of continued stimulus to the division energy without causing more harm to the cell than anaplasia or hyperplasia. Such an aspect of the cancer problem was stated as follows in an earlier publication: "It is certainly conceivable that a parasite of cancer may be too minute to be seen with the technique at our disposal. At the present time we know a great deal about the yellow fever organism; we know the period of incubation it requires in the human blood; we know that it requires from twelve to fourteen days to develop in the body of the mosquito before the latter is able to transmit the disease; we know that the disease (apart from blood inoculation) cannot be transmitted in any other way, and yet, knowing all these things, the organism of yellow fever has never been seen. It will pass through the finest filters, and belongs, therefore, to a group which, until they are actually seen, we must perforce consider as ultramicroscopic organisms. Such parasites might be adapted to life within the epithelial cell as well as the organisms of club root are, and there in the protoplasm might easily be overlooked. It has been suggested that a species of spirocheta is responsible for yellow fever, and spirochetes have actually been found in the kidney of yellow fever victims. But they apparently do not exist as such in the blood or in the mosquito. We know nothing about the life history of the spirochetes as a group; if it is analogous to the life history of most protozoa, we might well look for stages in which the organism is of ultramicroscopic size."¹

Many so-called parasites from human tumors have been described. Protozoa representing all groups of these unicellular animals have

¹ Calkins, The So-called Rhythms of Growth-energy in Mouse Cancer, Jour. of Exper. Med., 1908, vol. x, No. 3, p. 304.

been held responsible by one or more investigators, but in no case have the claims been made good. Not only protozoa, but yeasts and bacteria, and still other forms of living things, have been drawn into the vortex of a discussion over the parasite theory when that discussion was more spirited than it is today. Many of the structures thus interpreted as organisms are characterized by surrounding shells or capsules which some investigators have interpreted as parts of an invading organism (Fig. 85 2, 3). Cell invasions, however, are common in cancer tissue, leukocytes, or even cancer cells themselves, invading other cells and there degenerating or causing degeneration, while the capsules are only condensations of the invaded protoplasm. This is the view adopted by Sjöbring ('02), Sawtschenko ('95), Ruffer and Walker ('93), and many others, while numerous observers have described the successive changes in the degeneration of the contained leukocytes and interpreted the various "organisms" that had been described as merely one form or other of such degenerating cells (Fig. 85). One type of these inclusions, on account of its minute size, characteristic structure, and occurrence, was designated the "X-body" by Behla ('03), and was regarded as different from other cell inclusions which were due to degeneration. This "body" occurs under many different forms and has been variously interpreted (Fig. 85, 12, 14, 15, 18). It is known in literature as the "Plimmer body," as the "bird's-eye inclusion," as the astrosphere or centrosphere of Borrel ('01), as the "cancer parasite" of Bosc ('98), the "plasmodiophora-like bodies" of Gaylord, as "*Histosporidium carcinomatosum*" of Feinberg ('03), as the "intracellular secretions" of Nösske ('02) and Greenough ('01), as "chytridiæ" of Behla ('03), as the "yeast cells" of San Felice ('98) and others. Pianese ('96), Sawtschenko ('95), Soudakewitsch ('92), Ruffer ('92), and others observed similar bodies inside the nuclei of cancer cells, and interpreted both these and the cytoplasmic forms as colloidal degenerations of the chromatin and cytoplasm, Sawtschenko regarding them as masses of food material for the real parasite. Calkins ('05) described stages leading to the conclusion that all of such bodies are derived from the degenerating nucleoli of the cancer cells, these nucleoli first becoming clathrate, irregular in outline, and surrounded by local thickenings of chromatin or cytoplasm. Other forms, however, might better be interpreted as blood platelets or portions of leukocytes having the power to move from cell to cell (Fig. 85, 13, 17), but in no case is there evidence to regard them as specific organisms.

While these cell inclusions in human cancer cannot be interpreted as organisms, it does not follow that real organisms are not present. Later stages of the disease are particularly suitable for secondary infection, and exposed surface lesions form a suitable medium for the growth of bacteria, yeasts, or protozoa, while in one case of epithe-

lioma spores of the fern lycopodium, which were probably introduced with a face powder, were found. All such organisms, finding a favor-

FIG. 85

Types of the cell inclusions found in human cancer. (After Calkins.)

able medium for growth in the degenerating masses accompanying cancer, cannot be regarded as the causes of the disease, and as such saprophytic organisms we must include the ameba *Leydenia gemmipara* of Schaudinn ('96), which was found by E. von Leyden ('96) in the peritoneal fluids of ascitic dropsy and associated with cancer. This organism is a definite ameboid rhizopod measuring about $25\ \mu$ in diameter. It moves rapidly in body temperatures, by forming flat and lamellose pseudopodia. Structurally it differs from most parasitic rhizopods in having a pulsatile vacuole which contracts ordinarily every fifteen minutes. It reproduces by simple binary division and also by bud formation, the buds often being very minute ($3\ \mu$ to $4\ \mu$; cf. intestinal amebæ). Schaudinn considered it possible that these organisms may have been the cause of the cancers in the two patients in which they were found, and even compared the buds with the small cell inclusions described by Sawtschenko ('95). He was never inclined to push the suggestion in subsequent work, how-

FIG. 86

Spirocheta microgyrata (Lw.) var. *gaylordi*, in cancer tissue of mice. (After Calkins.)

ever, and later (1903) regarded *Leydenia gemmipara* as only a phase in the life history of an intestinal rhizopod *Chlamydomphrys stercorea* (see p. 294). The general belief now is that they had nothing to do with the cause of the disease.

The organisms of epithelioma contagiosum of fowls and of moluscum contagiosum of man are not to be included with such saprophytic forms, nor with these degeneration products, but are protozoa directly connected with the disease (see p. 312).

Similar degenerative products have not been found in mouse cancer, and there is less chance here for secondary infection. One organism, however, discovered by Gaylord ('07), *Spirocheta microgyrata gaylordi*, occasionally found in the blood of mice, is invariably found in the stroma of mouse cancer, both in primary and transplanted tumors, and is present in enormous numbers in the more malignant strains

(Fig. 86). It is sometimes found inside the cancer cells and very often in the detritus of degenerating centres. The dimensions and general character of this spirochete agree with the one which Löwenthal ('06) described from ulcerating human cancer, dog tumors, and in feces, and which he named *Spirocheta microgyrata*, because of the minute size of the nodes and abruptness of the turns (Fig. 86, left). The ends of the organism are blunt and rounded and there is no evidence of undulating membrane or flagellum (as to the nature of spirocheta flagella, see p. 223). Reproduction is evidently by transverse division, but nothing is known in regard to the life history. Similar but not the same species of spirochetes have been found by Borrel ('05) and by Wenyon ('06) in the blood and tissues of mice, and Tyzzer ('07) has found it in tissues of so-called normal mice. It can hardly be claimed that these spirochetes are the cause of mouse cancer, at least not in the form as ordinarily seen. Gaylord and Clowes have found that they are much reduced in number in the tissues after the material for inoculation had been treated with potassium cyanide, although they reappear later. There is reason to believe that, as with *Trypanosoma gambiense*, under treatment with atoxyl, the ordinary form of the organism may be lost, and that the poison does not kill, but causes them to encyst. The absence of all evidence of similar organisms in human cancer, however, makes it probable that these mouse spirochetes, like *Leydenia gemmipara*, are only commensals finding here a suitable soil for life and multiplication. On the other hand, the possibility that they are inciting or aggravating agents must not be overlooked.

The cancer problem or problems, finally, must be regarded as still in the stage of working hypotheses, of which no one points out with unmistakable clearness the path for future research. That the field of parasites thus far has been harrowed in vain is no reason for abandoning this particular working hypothesis, at least not until we know more about the still invisible organisms of yellow fever, or those of foot and mouth disease, or until we know more about the minute forms of the organisms of "fixed virus" of rabies, or the stages which pass the filters in clavelée, molluscum contagiosum, dengue, and similar diseases.

CHAPTER VI.

THE PATHOGENIC FLAGELLATES.

It is a well-recognized zoölogical principle that some groups of animals, families, orders, classes, or even phyla, may be stationary, so far as evolution is concerned, and not easily adapted to new environmental conditions. Other groups, on the other hand, are remarkable for the variety of structures, for ready adaptability to new conditions, and, in general, for their high "potential of evolution."

Similarly with the protozoa we meet with the same variations; the infusoria, for example, both ciliates and suctoria, are highly differentiated, and, as shown by the well-defined orders and families, are fairly stable in evolution, while the mastigophora, on the contrary, possess a remarkable power of variation and a high potential of evolution. It is among these latter forms that we meet with all methods of nutrition and with all grades of organization connecting animals with plants, while it is here, also, that we look, especially among the colony forms, for cellular division of labor or developmental processes, that may throw light on the origin of multicellular from unicellular animals.

With their great power of adaptation combined with the variety of available modes of life, it is to be expected that many types of flagellated unicellular parasites should be known, and among them, that we should find numerous cases of incomplete adaptation. This is particularly probable in organisms like the hematozoic flagellates, where the uncertain conditions of the definitive invertebrate and secondary vertebrate hosts make stability of form and life cycle difficult to work out. There is reason to believe, with R. Koch, that certain types of trypanosomes are established, or are "good" species (*e. g.*, *Trypanosoma lewisi*, *T. theileri*), while others are undoubtedly in that phase of adaptability which De Vries calls the period of mutation. While such an hypothesis probably contains an element of truth, it is just as well to keep it for the present as a generality, and not to apply it as the famous bacteriologist does, to specific cases until after the life histories of such cases are known. "Good" or "bad" species of protozoa, especially in this group, have no scientific standing until the life cycle is accurately established, and "degrees of virulence" or "promiscuity of secondary (vertebrate) hosts" have no more to do with establishing a protozoan species than the salt- or fresh-water habitat has to do with actinophrys, chilodon, or colpoda, and whether there is

one species of trypanosoma with many varieties, or seventy different ones, cannot be determined on the basis of physiological effects alone, or by the nature of the habitat.

The uncertainties and the many contradictions which characterize our present knowledge of the parasitic flagellates make the group very difficult to handle from a zoological point of view, and deductions and generalizations made upon the strength of slender lines of evidence are not only premature but very confusing to those who are seriously concerned with protozoology, and distracting to medical men whose energies are directed toward the cure and extinction of diseases due to these organisms. The attempt to classify hemosporidia and flagellates in one group, as certain recent writers have done (Hartmann, Sambon, etc.), rests upon a very shaky foundation of fact, and until that foundation is better built, we would do much better to adhere to the older system, which, even if not entirely accurate, at least has the advantage of established familiarity and of accepted limits, while those forms in which the life history is now known can be safely placed. To illustrate, the Donovan-Leishman bodies were first seen as intracellular parasites, and were classified as aberrant forms of hemosporidia similar to babesia. But with the discovery of the flagellated phase in culture and in the definitive host cimex, the enigmatical "bodies" were found to be only intracellular phases of a flagellated protozoön similar to herpetomonas, and, under the name *Herpetomonas (Leishmania) donovani* (Mesnil), are today classified as flagellates. Similarly the hematozoic parasite of the little owl, halteridium, was found to be a phase of the life cycle of *Trypanosoma noctuæ*, and should be removed from the hemosporidia and placed with the flagellates.

These two instances, while safely established, do not justify a zoologist or a medical man in jumping to the conclusion that all hemosporidia have a flagellate stage, and should, therefore, be classed with the mastigophora (Hartmann), or that all trypanosomes have an intracellular stage, or that the hemosporidia, as a group, should be abandoned (Hartmann). An intracellular stage of herpetomonas or of trypanosoma does not make a sporozoön of either one; nor does a flagellated stage of *Plasmodium vivax* (if such a stage exists, which is extremely doubtful) or of proteosoma, make flagellates of these any more than the tailed tadpole makes a fish of a frog. The old group hemosporidia should not be given up until each species it now contains is proved to be only a phase of some flagellate. To give it up, or to classify these protozoa under the caption of "blood-dwelling forms" (Sambon, Manson), save for purely physiological or therapeutic reasons, is misleading and unnecessary.

With these parasitic flagellates the condition of affairs at present is analogous to that in the group hydrozoa among celenterates. Here many species are characterized by two distinct phases: one, the

sexual generation, is a free-swimming medusa or jelly fish, the other, an attached and often branched asexual hydroid. The greatest confusion grew out of the fact that each of these generations received a distinct name and were supposed to be different forms of animal life. The medusa phialidium, for example, was regarded as independent at first, but later was shown to be only the sexual generation of the hydroid clytia; the genus ecope also was proved to be only the medusa of the hydroid obelia. With the increased knowledge of the life history of these forms of coelenterates the confusion was gradually cleared, and the group is now well understood. It was found that some medusæ have no hydroid generation, and that some hydroids have no medusæ, and such forms were classified in appropriate subdivisions. So it will be, probably, with the hemosporidia; some others, like the Leishman-Donovan bodies, may be found to have a flagellated stage; babesia, for example, is said to have such a stage by some observers (Kinoshita), while certain others have labored hard to make out a flagellum in one form of plasmodium. Others, like *Plasmodium malariae* and *P. vivax*, are certainly obligatory cytozoic forms.

Some forms of parasitic flagellates are of such doubtful structure that the taxonomic position must be left in abeyance. The much-discussed spirochetes, for example, when all is said, cannot be distinguished from certain spiral forms usually classed with the bacteria, and transitional forms bridge the gap between the protozoön *Spirocheta balbianii* and *Spirocheta plicatilis*, and the bacterial form *Spirillum gigantea* and *Spirillum recurrentis*. It is possible that some morphological or developmental feature may be found ultimately which will permit of a definite limitation of the two types, but it is equally possible that future research will demonstrate the close affinity of the supposedly different types, and to my mind the present conditions of facts indicate the latter and not the former alternative, and justify the non-committal term spirillochetidæ as a family name for the contested forms. Certainly, the spirochetes are so close to the spirillæ that hard and fast lines cannot now be drawn, and, like the phytoflagellates and the lowest plants, the questionable forms indicate once more the high mutability of the group.

THE GENUS SPIROCHETA AND ALLIES.

C. G. Ehrenberg, in his masterly treatise on the *Infusionsthier-schen*, published in 1838, described spirocheta and spirillum as follows:

28th Genus. *Spirocheta*: Animal e familia Vibrioniorum, divisione spontanea imperfecta in catenam tortuosam S. cochleam filiformen *flexibilem* elongatum.

29th Genus. *Spirillum*: Animal e familia Vibrioniorum divisione spontanea imperfecta (et obliqua?) in catenam tortuosam *S. cochleam rigidam* et in cylindri formam extensam abicns.¹

This first description of the organism which Ehrenberg named spirocheta is certainly very meagre and not much more enlightening for present-day purposes than the spirilliform figures of Köhler, published in 1777, or the crude descriptions and figures of similar forms by O. F. Müller, in 1786. The essential point of difference between the genus spirocheta and the genus spirillum was the rigidity or inflexibility of the latter as against the flexibility of the former. Schaudinn, in 1905, added another point to the diagnostic characterization of the genus by describing a definite undulating membrane.

Spirocheta thus characterized as an organism with flexible, spirally twisted body with laterally placed undulating membrane, would seem to be definitely distinguished from the genus spirillum with rigid corkscrew-like body and no membrane; but, unfortunately, the problem is not so simple, for we have to do with exquisitely minute things which offer extreme difficulties in technical treatment and require carefully trained eyes. Statements as to structure and activities of certain species, even though made by equally eminent authorities, are frequently directly contradictory, and only too often the individual prejudices are so strong as to weaken the scientific value of the observations.

Schaudinn's discovery, in 1905, of the organism of syphilis, *Treponema (Spirocheta) pallidum*, was the direct inspiration to thousands of investigators to study anew the old forms and to penetrate unknown fields of pathology in the hope of finding and describing new forms. As a consequence of this activity, the systematist today is confronted with a most heterogeneous collection of spirilliform organisms, and is forced to wade through a most conflicting tangle of observations and deductions. The descriptions of organisms which have been classified as spirocheta are often obviously far from the original type of Ehrenberg, so far, indeed, as to justify new generic names. Some of them differ in having flagella (of the spirilla type) at one or at both ends; others have multiple flagella so called; and still others have neither membrane nor flagella. These discrepancies have been widely recognized and new generic names have been proposed and, in some cases, accepted. Some observers, on the other hand, have made the mistake of basing genera on physiological lines alone, and these, like the genus *spirochaudinna* of Sambon, based upon the fact of change of hosts, will not be accepted.

Observations are too incomplete and too often contradictory to justify a safe grouping at the present time, and in making groups of

¹ Ehrenberg, *Die Infusionsthierschen*, etc., 1838, p. 83, 84

spirochetes a given species will be placed in one division or another, according to the discretion of the present author in following one or another authority. With this preliminary caution the following table of the different kinds of spirochetes, classified according to the presence or absence of so-called flagella and undulating membrane, is based.

A. TYPE GENUS SPIROCHETA.

With undulating membrane; without flagella.

- Spirocheta plicatilis*. Ehrenberg, 1838. Free living. Length up to 200 μ .
Sp. balbianii. Certes, 1882. In oysters, clams, etc. Length up to 150 μ .
Sp. anodontæ. Keysselitz, 1906. Mussell (anodon). Length up to 60 μ .
Sp. vincenti. Blanchard, 1906. Human ulcers.
Sp. pyogenes. Mezincescu, 1904. Tuberculous cattle.
Sp. refringens. Schaudinn, 1905. Human syphilitic lesions (external).
Sp. pseudopallida. Kiolemenoglou and von Cube. Ulcerating carcinoma.
Sp. eberthi. Kent, 1880. Bird intestine.
Sp. gigantea. Warming, 1874.
Sp. buccalis. Steinberg, 1862. Probably same as dentium. Same habitat.

B. GENUS TREPONEMA.

Without undulating membrane; with flagella.

- Treponema pallidum*. Schaudinn, 1905. In human and ape syphilitic lesions.
Tr. pertenuis. Castellani, 1905. In lesions of frambesia or yaws.
Tr. anserinum. Sacharoff, 1890. Blood of geese.
Tr. gallinarum. March. and Salimbeni, 1903. Blood of chickens.
Tr. theileri. Laver. and Vallée, 1904. Blood of cattle.
Tr. muris. Wenyon (*Tr. Laverani*, Breinl and Kinghorn). Blood of mice.

C. UNDETERMINED FORMS REFERRED TO GENERA SPIROCHETA AND SPIRILLUM.

- Spirocheta dentium*. Koch, 1877. Human mouth and teeth.
Sp. vaccinæ. Bonhof, 1905. Pustules of calf.
Sp. recurrentis (*Sp. obermeieri*). Lebert, 1874. Cause of relapsing fever.
Sp. duttoni. Novy and Knapp, 1906. Cause of tick fever in man.
Sp. microgyrata. Löwenthal, 1906. Ulcerating human carcinoma.
Sp. microgyrata. Löw. var. *Gaylordi*. In non-ulcerating mouse tumors.
Sp. of dysentery. Le Dantec.
Sp. ovis. Novy and Knapp. Blood of sheep.
Sp. equi. Novy and Knapp, 1906. Blood of horses.
Sp. vespertilionis. Novy and Knapp, 1906. Blood of bat.
Sp. muris, variety *Virginiana*. MacNeal, 1907. Blood of rat.

So far as the morphology is concerned, the best known of these forms are the giant spirochetes *Sp. balbianii* and *anodontæ*, which have been described by Certes, Laveran and Mesnil, Perrin, Swellengrebel, Keysselitz, and Fantham (Fig. 88). The large size and definite struc-

tures make them relatively easy to study, and the conclusions that have been drawn are comparatively free from imaginative diversions, and for this reason they are the best representatives of the group for descriptive purposes.

FIG. 87

Spirocheta anodontæ. $\times 1500$. (After Fantham.) The membrane winds around the body in right-handed spiral; chromatin rodlets and basal granules shown.

A. Structures of *Spirocheta Balbianii*, Certes, 1882.—This organism, first studied by Certes as a trypanosome, may be found in the anterior part of the oyster's digestive tract, where, if present at all, it is usually in the crystalline style. Both Perrin ('06) and Fantham ('08) note that the organisms soon disappear after the oysters are removed from sea water.

The spirochete is a spirally wound thread from 50 to 150 μ long and about 2 to 3 μ wide. The inner protoplasm contains a number of transverse bands of chromatin, about 60 in all, which Perrin, erroneously, calls "chromosomes," and which constitute the sole nuclear apparatus of the organism. Sometimes these bands run together to form a more or less complete helix of chromatin; again, they are completely divided in preparation for longitudinal division of the cell; but at no time do they come together to form a definite

nucleus like that of most protozoa and higher types of cell. Nor do the granules collect in spore aggregates, such as Schaudinn ('02) described in *Bacillus bütschlii* and Guilliermond ('08) in different endosporous bacteria. The nuclear apparatus is of the "diffuse" type, therefore, and represents an intermediate condition between the "distributed nucleus" of bacteria and the morphological nucleus of higher cells.

The protoplasmic body is covered by a distinct sheath or periplast, which is twisted in a characteristic manner and which gives rise to a lateral undulating membrane likewise spirally wound and running from end to end of the organism (Figs. 87, 88). Laveran and Mesnil regard this membrane as a mere fold of the periplast (*gaine*) and of an accidental nature, but both Perrin and Fantham give sufficient evidence to show that it is a definite organoid of the cell, while Fantham has demonstrated the presence of numerous fibrils which he describes as myonemes and correctly interprets as the seat of movement of the cell (Fig. 88, *A, C*). Under abnormal conditions, the membrane, like that of the ciliated infusoria, may disintegrate, and the several myonemes then may assume the appearance of numerous flagella, a phenomenon which may account for the presence of many flagella occasionally found on *Spirocheta gallinarum* and *Spirocheta duttoni*. The movements brought about by this membrane are characteristic of spirochetes in general, and consist of rotation about the long axis, forward or backward translation, and bending movements at different levels of the body, all of which may occur simultaneously or independently.

Reproduction occurs by either longitudinal or transverse division. There is some difference of opinion in regard to the mode of division, however. Laveran, Mesnil, and Swellengrebel maintain that it is always transverse; Perrin, that it is always longitudinal; while Certes, Lustrac, and especially Fantham, whose account is the most convincing, found both types, cross-division more rarely than lengthwise. Transverse division, according to Swellengrebel, occurs, as in bacteria, by the preliminary division of internal granules and by the formation of a "cloison transversal," but he also figures and describes the double chromatin granules which can be interpreted only as a preparation for longitudinal division. Longitudinal division, according to Fantham, begins with division of the membrane, being first noted in the division of what he terms the basal granules (Fig. 88, *E*). The granules at one end separate while the others remain together, and with the separation the membrane, chromatin granules, and cell divide, the daughter cells remaining attached at the one end for a considerable time; ultimately a vacuole appears in the common terminal protoplasm and final separation takes place.

Perrin describes a number of different types of *Spirocheta balbi*-

anii as representing "male," "female," and "indifferent" forms of the organism; but there is little that is convincing in his descriptions, and

FIG. 88

Spirocheta balbianii. (After Fantham.) *A*, parasite showing myonemes in membrane, rounded ends and transverse bars of chromatin, $\times 3000$; *B*, a so-called "flagellated" form, the apparent flagella being myonemes from the dissociated undulating membrane, $\times 2000$; *C*, beginning of division, the undulating membrane being entirely divided and the chromatin arranged in characteristic spiral form; basal granules also divided, $\times 1500$; *D*, separation of longitudinally divided form, basal granules divided, $\times 1000$; *E*, daughter cells attached at one end, $\times 1000$.

he himself is not altogether certain of his ground in some cases. Fantham was unable to confirm these observations, while Swellengrebel interprets these structures, probably correctly, as involution or degeneration forms. All evidence of so-called conjugation described by Perrin is unconvincing, and the sexual processes of these interesting forms, as with all other spirochetes, remain undetermined.

While *Spirocheta balbianii* is the best known of the spirochetes, it is quite evident, from the accounts of the various observers; that much yet remains to be done before its life history is known. But we know still less about the other forms of the group, especially those which appear to be the causes of specific diseases. Nevertheless, some problems connected with them have been solved, many careful experiments have been planned and successfully executed, and many

FIG. 89

Types of flagellum insertion in bacteria. (After Bütschli.)

structures and functions faithfully described. The literature is enormous, and in the limited space of this chapter only the general trend of observations and experiments can be given.

B. The So-called Flagella of Spirochetes.—As stated on page 45, there is good reason to doubt the specific flagellum nature of the attenuated ends of many of the spirochetes, and, owing to the extremely small size of most of these organisms, it is hardly probable that the question will be definitely settled one way or another very soon. Several factors, however, combine to show that these organoids lack the specific kinetic accompaniments characterizing flagellated protozoa. In the latter, wherever carefully studied, and in plant and animal flagellates alike, the flagella are deeply inserted in the protoplasm and arise as outgrowths from the nucleus or from special basal bodies (Fig. 100, p. 249). In spirillum the so-called flagella are of an entirely

different type, Bütschli ('02) finding only one case, and this not wholly satisfactory, where the flagellum appeared to be prolonged into the cell body of *Spirillum giganteum* (volutans) (Fig. 89). Swellengrebel ('07) described an occasional thickening at the lower end of the flagellum of this same species which he regarded as a basal granule, but as it lies outside of the protoplasmic body it is more probably a local thickening or condensation rather than a kinetic body similar to those of animal flagella. Furthermore, numerous observers (Fischer, Kutscher, Ellis, and others) affirm that the flagellum is not single, but consists, at times at least, of a bundle or tuft of "cilia." Zettnow, Fischer, and Bütschli give evidence to show that the flagellum arises as a prolongation of the periplast, the latter, with Ellis and Swellengrebel ('07), showing that it comes from an apical thickening (calotte) of the periplast.

In the spirillum group the flagellum thus appears to arise from the enveloping periplast, and is not, as in protozoa, of endoplasmic origin. In spirocheta the conditions have recently been carefully studied by Siebert ('08), who finds that the so-called flagellum of different forms arises in the same manner as in the spirillaceæ, and is morphologically different from the flagella of mastigophora. As processes of the periplast arising as the attenuated ends after division of the cells, *e. g.*, in *Sp. recurrentis* (*Sp. obermeieri*), the flagella have an entirely different significance from those of the monads and other mastigophora. Furthermore, the rare occurrence of "ciliated" forms—sometimes double (Schaudinn), sometimes single and variously placed (Levaditi)—of *Treponema pallidum*, or of *Sp. microgyrata*, may be interpreted, as Krzysztalowicz and Siedlecki ('05-'08) assert, as the attenuated ends which remain after division.

The myonemes characteristic of the undulating membrane of *Sp. balbianii*, indicate, however, a higher development of kinetoplasm than is to be found among the bacteria, and it is reasonable to assume that all spirochetes with undulating membranes have similar contractile fibrils. Furthermore, the energetic movements of spirochetes without flagella may be accounted for upon the hypothesis that the periplast or membrane is similarly provided with muscular elements. Siebert has shown that under the action of certain digestive fluids spirochetes break up into fibrillæ similar to those which have been described in peritrichous forms. Borrel ('06), Zettnow ('06), the former for *Tr. gallinarum*, the latter for *Sp. duttoni*, and Levaditi and McIntosh ('07), for a species of treponema similar to, if not identical with, *Treponema pallidum*, have described so-called diffuse flagella appearing at various parts of the cell, sometimes terminal, sometimes lateral, while oftentimes they are multiple and irregularly placed. Whatever these chance peritrichous appendages may be, they are certainly not flagella in any strict morphological sense, and

Siebert's conclusion that they are products of periplastic dissociation, or Prowazek's ('06), that they are dissociated myonemes, appears to be the more probable explanation.

C. The Spirochete Nucleus.—As already shown for *Spirocheta balbianii* and *Sp. anodontæ*, there is no definite morphological nucleus in these forms, and the distribution of chromatin granules recalls the condition of bacteria. Nevertheless, the occasional aggregation of these granules into a heliform cord or the permanent rod form, as in *Sp. plicatilis* (Schaudinn), indicates a higher organization than in bacteria and a step toward the condition in protozoa, where, as in tetramitus, there may be only granules which come together at periods of division to form a loose but nucleus-like aggregate (Calkins, 1898). The view expressed by MacWeeney, that spirochetes are all nucleus, or chromatin only, brings back the controversy over the nature of bacteria which has now been definitely settled, and it is unnecessary to go over the matter again for these spirilliform types.

In the great majority of spirochetes that have been described more or less minutely, no nucleus of any kind has been mentioned. In the better-known forms, however, chromatin granules of one form or another have been described somewhat fully. Bonhoff describes a single brightly staining central granule in his *Sp. vaccinæ*. In *Sp. recurrentis*, the cause of relapsing fever, Novy and Knapp ('06) made out no internal structures; the organisms "invariably gave a solid stain, exactly as in the case of ordinary spirilla or bacilli" (p. 300). But ordinary bacilli and spirilla do show internal structures, many of them analogous to chromatin and interpreted as such by different observers (Bütschli, Schaudinn, etc.). So, too, the organism of relapsing fever possesses granules which may be chromatin and may correspond with the chromatin granules of *Sp. balbianii*. In the closely allied *Trep. gallinarum* Prowazek ('06) finds local condensations which stain like chromatin and which he interprets as such (his Fig. 6). Similar but more numerous granules were observed by Dutton, Todd, and Tobey ('06) in *Spirillum (Spirocheta) duttoni*, and by Carter ('06) in the same species from the eggs of *Ornithodoros moubata*. Finally, in *Treponema pallidum*, Krzysztalowicz and Siedlecki ('05-'08) have observed small deeply staining granules which they regard as condensed chromatin surrounding a clear space of "achromatin." (It might be pointed out, however, that this observation might be used equally well in support of Swellengrebel's view of transverse division through the medium of a *cloison transversal*.) Weichselmann and Löwenthal (1900) have observed similar granules by aid of the ultraviolet light. Summing up the evidence as to nuclei of spirochetes, it may be safely affirmed that these primitive types of organisms possess nuclei in the form of scattered chromatin granules which may come together at times to form rod-like or sphere-like aggregates, a condition duplicated by the

bacteria on the one hand, and by unquestioned flagellates on the other.

D. Division of Spirochetes.—In regard to the mode of division of spirochetes the greatest diversity of opinion prevails, and every species whose reproduction is known is interpreted by some as dividing transversely, by others longitudinally. As in the case of *Spirocheta balbianii*, it is possible that both methods occur. The greatest number of

FIG. 90

Different forms assumed by *Treponema pallidum*, the organism of syphilis. (After Krzysztalowicz and Siedlecki.) *A*, three ordinary forms with "nuclear space" from primary lesion; *B*, six contracted and ring forms from initial lesion; *C*, *D*, *E*, late stages in condensation of organism from papule; *F*, minute forms from initial lesion; *G* to *M*, successive stages in longitudinal division; *N*, "enigmatical" bodies from an eruptive papule (similar to "cytotyetes luis").

observers and the liveliest disputes on this point have been in connection with *Treponema pallidum*, the organism of syphilis (Fig. 90). Without entering into an extensive review of the literature, it may be stated that Krzysztalowicz and Siedlecki ('05) were among the first to describe longitudinal division, which Schaudinn in the same year confirmed by observations on the living organisms. Herxheimer, Hoffman, Siebert, and others agree with this view. Many others, on the other

hand, are equally positive that division here is transverse, Borrel, Laveran, Zettnow, Koch, Novy and Knapp, Levaditi, Goldhorn, and many others taking this view. Schaudinn and the Hungarian observers note that the greater part of the organism divides with great rapidity, and that, as in *Spirocheta balbianii*, the partly separated daughter cells remain attached for a long period, and finally pull apart as though dividing transversely (Fig. 90, *G, H, I, J, M*). The advocates of transverse division, on the other hand, explain the apparent longitudinal splitting as an illusion caused by the dividing cells turning and twisting upon one another. No final decision can be made at present; it is certainly difficult, on the basis of longitudinal division only, to account for the strings of cells that are often found with thinned regions, and skepticism regarding the schematic course of events as given by Krzyształowicz and Siedlecki cannot be wholly

FIG. 91

Spirocheta duttoni (Novy and Knapp). *A, B, C*, after Breinl, $\times 4500$; *D*, after Carter; *A, B*, spirochetes reproducing by transverse division; *C*, by longitudinal division; *D*, parasites from egg of *Ornithodoros moubata* with chromatoid granules divided equally and cell bodies partly split.

dispelled by their explanation of these strings as "colonies." If, like *Spirocheta balbianii*, the organism of syphilis divides both longitudinally and transversely, the catenoid colonies are easily interpreted.

Similarly with *Treponema gallinarum*, *Sp. recurrentis*, and *Sp. duttoni*, equally competent observers take diametrically opposite sides regarding the plane of division. It is highly probable that *Sp. recurrentis* of relapsing fever divides usually by cross-division, but Carter's and Prowazek's observations on *Sp. duttoni* and *Sp. gallinarum* certainly show that lengthwise division occurs in these forms, Carter ('07) especially showing that the granules of chromatoid matter within the cell are placed opposite one another in the divided daughter halves (Fig. 91, *D*).

E. Form Changes and Life History.—Stability of form, due to the firm body wall, is one of the characteristics of bacteria, while polymorphism is equally distinctive of protozoa (p. 19). With the spirochetes, some appear to be remarkably stable in form (*e. g.*, *Sp. microgyrata*, *Sp. recurrentis*, etc.), while others are highly variable (*e. g.*, *Tr. pallidum*). All seem to have a greater or less power of agglomeration comparable with the agglutination of bacteria, and indicating some physical change in the cell analogous, perhaps, with the "miscible state" at certain periods of the life history of infusoria.

Another matter of considerable importance in the structure of the spirochetes is colony formation and the question as to the "unit" individual. The number of nodes often varies within such wide limits that the problem as to what constitutes a single spirochete cell has a more than theoretical interest. Migula ('00) and Fischer ('03) suggested that spirochetes may be composed of many units, a point of view supported by the effect of abnormal conditions upon the spirochete strings. Warming ('75) and Zopf ('82) described the fragmentation of the spirochete body after death in the cases of *Sp. plicatilis* and *Sp. giganteum*, while Laptschinsky ('80) claimed to have made out such segmentation in the living cells of the former. These early observers may have been misled by the segmented appearance due to the bands of chromatin in these forms. Similar observations, however, have been made upon other forms, and under such different conditions by competent observers that there is some justification for the view that the "unit" consists of one node. Wechselmann and Löwenthal ('05) showed that long forms of *Tr. pallidum*, upon treatment with mercury, break up into short forms with from one to four nodes. Karlinsky ('90) found very short forms of *Sp. recurrentis* in the blood of patients having previously had malaria, and these short forms, when placed in normal blood, developed into normal spirals. In connection with the same organism Afanassiew ('99) observed comma- and S-shaped forms in addition to the usual spirals, while Novy and Knapp ('06) described the fragmentation of the long forms into such comma- and S-shaped types under the action of phagocytes. Löwenthal ('05), Krzyształowicz and Siedlecki ('05), and others described minute types of *Tr. pallidum* somewhat similar to those of the organism of relapsing fever.

In view of these facts, and in connection with the apparent disappearance of spirochetes from the blood and organs of the body, the possibility of the unit organism being much more minute than that usually seen should not be overlooked. The actual life history, furthermore, of no form has been satisfactorily worked out, and it is quite within the bounds of probability that excessively minute stages occur. Fertilization and the sexual phenomena, if they exist, are unknown at the present time, and most of the attempts to formulate a

sexual cycle have been too fantastic for belief. Prowazek ('06) observed curious local swellings in *Treponema gallinarum* and *Sp. buccalis*, which he regarded as similar to those seen by Heydenreich in *Sp. recurrentis*, by Perrin in *Sp. balbianii*, and by Keysselsitz in *Sp. anodontæ*, all of which he interpreted as possibly indicating a sexual process. Swellengrebel's and Fantham's observations on *Sp. balbianii* leave little reason to doubt that in this form, at least, the structures in question are the results of abnormal or degenerative processes. Krzysztalowicz and Siedlecki ('05) described a complex cycle of *Treponema pallidum*, involving many form changes, including a so-called trypanosome stage, and sexually differentiated gametes. In their more extended and very valuable paper of 1908 they express doubt as to this earlier interpretation,¹ but give most convincing evidence of the manifold form changes which these organisms may assume under normal conditions. Muhlen's ('07) and many others have noted the same polymorphism, enough, indeed, to show that no one standard of form or size can be depended upon in identifying *Tr. pallidum*. The most marked and characteristic of these varieties are the short and thick forms with from two to four nodes (noted also by Muhlen's and Hartmann in *Sp. dentium* and *buccalis*). The other variations shown in Fig. 90 are sufficient to indicate the difficulty in distinguishing this spirochete from other harmless ones and the danger of basing diagnosis upon structures alone. Krzysztalowicz and Siedlecki, who have studied this species for years, admit that they cannot distinguish some stages in its life history from other spirochetes. They conclude that the ring forms (Fig. 90, B) are resting stages, the baguette forms stages during the "period of depression," while the oblong or granular forms are involution or degeneration types. The curious and interesting structures called *Cytoryctes luis* by Siegel ('05) may well be stages of unknown significance in the life history of *Tr. pallidum*; they certainly have no resemblance to the bodies described by Guarnieri ('92) under the generic name of cytoryctes (see p. 307), but do recall the "spindle-formed bacilli" found by Seitz and interpreted by Silberschmidt, Wechselsmann, Löwenthal, and others as stages in the life history of *Spirocheta vincenti*.

So-called encysted forms of spirochetes have been mentioned from time to time. Breinl and Kinghorn ('06) suggest that *Sp. duttoni*, which they found occasionally coiled up within a definite membrane, represent the encysted state of this organism, while "resting stages" have been noted by many different observers in different species of spirochetes without, however, their significance being known.

F. Mode of Life and Change of Hosts.—Many of the spirochetes are undoubtedly intracellular parasites, although differences of opinion

¹ A vrai dire, nos études ultérieures nous ont inspiré beaucoup de doutes à cet égard, p. 221.

exist in regard to this. Many are lymph or blood-dwelling forms, while some are neither parasitic nor commensal in their mode of life.

Some forms may be both cœlozoic and cytozoic. *Tr. pallidum*, for example, is considered by some observers (*e. g.*, Bandi and Simonella, 1905) to be a typical intracellular parasite, although usually found in the lymph. *Treponema gallinarum* frequently leaves the blood serum and penetrates the blood cells of chicks (Prowazek, Marchoux, and Salimbeni). *Sp. duttoni* penetrates the egg of the tick *Ornithodoros moubata* and multiplies there (Koch, Carter), while *Sp. microgyrata* var. *Gaylordi* is frequently found in the cancer cells of mice (Fig. 86, p. 213).

Closely connected with their habitat and mode of life in the host is the possibility of transmission by insects, which, according to Lühe ('06), are the definitive hosts of these forms. It is generally believed, upon the basis of experiments made by Nuttall, that bedbugs convey *Sp. recurrentis* from man to man, while Schaudinn found that the organisms multiply within the body of this insect. Similarly, the closely allied spirochete *Sp. duttoni* of tick fever was found by Dutton and Todd ('07) to be conveyed by the bite of a tick *Ornithodoros moubata*; they also showed that the larvæ were capable of transmitting the disease with the first feeding operation, while Koch ('05) described spirochetes on the surfaces of ovaries and eggs of the insect and gave strong evidence to indicate that they multiply there. This evidence was fully confirmed by Carter ('07), who found the organism dividing rapidly in the protoplasm and yolk of the egg (Fig. 91). Here, therefore, is a case of direct inheritance, in insects, of disease-causing organisms. *Treponema gallinarum* and *Sp. theileri* are similarly transmitted by ticks, the former by *Argas miniatis*, the latter by *Rhipicephalus decoloratus*. Borrel and Marchoux, for the former, and Theiler, for the latter, showed that multiplication likewise occurs here in the bodies of the insects, and that the eggs may be infected and may carry the organisms.

Beyond simple division there seems to be no important life phase in the bodies of insects; but this fact of multiplication is of considerable importance, as showing that the insect hosts are not merely passive carriers, but are active agents in the transmission and distribution of the parasites, and therefore are important agents in spreading these spirochete diseases among vertebrates. Further research will probably bring to light some conjugation process, but as yet nothing of the kind is known.

Schaudinn ('04), on the strength of his observations on the reduction in size until almost invisible of *Leukocytozoön ziemannii*, after repeated divisions, suggested that yellow fever might well be a disease due to spirochetes. The now well-known agent of transmission, *Stegomyia fasciata*, requires a period of twelve days before it is capable of giving

the disease to man; after infection, the human victim is first prostrated in from three to five days; after the onset, the blood is capable of infecting a mosquito again only for a period of three days. These facts indicate that the organism undergoes some cycle of activity in the mosquito; that it has a period of incubation in man, and that it disappears from the blood after three days (see *Reports of Yellow Fever Commission*, 1900, 1901; also Goldberger, 1900). In spite of all that is known about yellow fever, the organism causing it has never been seen; it passes readily through the finest filters, and must, therefore, be extremely minute, possibly justifying a position in Borrel's group of the ultramicroscopic or invisible organisms. It may be pointed out, however, as Schaudinn does, that known forms of spirochetes become progressively smaller with successive divisions, and it is conceivable that spirochetes consisting of a single unmeasurable node may exist and multiply without forming catenoid colonies in the blood, and, because so minute, remain unseen. Stimpson's ('06) discovery of spirochetes in the kidney of a yellow fever victim is interesting and suggestive in this connection, but they must be found more often before much importance can be attached to them.

G. Are Spirochetes Protozoa or Bacteria?—From the foregoing review of the structures and life histories of the spirochetes there is little that is definite to determine the natural affinities of these spirilli-form organisms. The plastic nature of the body and polymorphism are protozoan characters. The structure of the so-called flagellum is a point in favor of the bacterial nature, but the highly kinetic membrane is an equally strong point in favor of the protozoa. The nucleus or its equivalent is more like that of the bacteria than like the morphological nucleus of the protozoa; but there are protozoa with distributed nuclei (p. 29), so that this character is not distinctive. The physiological characteristics are quite as typical of protozoa as they are of bacteria; division, so often a subject of acrimonious and contradictory statements, is not decisive, for many protozoa divide transversely (all ciliates and *Oxyrrhis* and *Polykrikos* among flagellates), while some bacteria are said to divide longitudinally. Cultivation on artificial media, thus far unsuccessful with spirochetes, is now, thanks to the excellent work of Novy and MacNeal and their followers, no longer a distinctive feature, for trypanosomes, like most bacteria, may be so cultivated. The results of plasmolysis, urged by Novy and Knapp ('06) as an argument in favor of the bacterial nature of spirochetes, have but little value, for the time factor necessary to plasmolyse is a purely relative matter dependent upon the nature and resistance of the cell membrane. Differences among the bacteria themselves, in this respect, as Pröwazek, Siebert, and many others have pointed out, are quite as marked as the differences between undoubted protozoa and spirochetes. The periodicity of symptoms in the hosts of

disease-causing forms is more characteristic of protozoa than of bacteria, but the formation of toxins and the installation of immunity give no light on either side. So, too, the passive carriage or active multiplication within the insect host, which Stiles ('06) regarded as a sufficient test of the plant or animal nature of spirochetes, only pushes the problem a step farther back, for some spirochetes, at least, multiply in the insect host and some trypanosomes are apparently carried and transmitted in a passive state.

On the whole, therefore, while again repeating that the controversy now has only an academic importance, the weight of evidence favors the view that spirochetes as a group are structurally (ectoplasmic) more complex and more plastic and variable in form than bacteria, while functionally they have a more complicated life history. On the other hand, their structures (endoplasmic especially) are much less complex than in protozoa, and their life history, so far as it is known, more simple than that of the known protozoa. Until further observations on the life histories of different species are made we are justified in doing no more than to place the spirochetes as an intermediate group between the bacteria and the protozoa, but leaning more toward the latter, and in this sense they are included under the name *spirochetida* in our classification.

CHAPTER VII.

THE PATHOGENIC FLAGELLATES—(CONTINUED).

THE GENERA HERPETOMONAS (INCLUDING "LEISHMANIA") AND CRITHIDIA.

WITH these genera belonging to some of the more primitive forms of the mastigophora, there is no question as to the animal nature, and from the biological standpoint they form an extremely interesting series of protozoa. Among them may be found all of the stages leading from a free, flagellated, and celozoic mode of life to a non-motile, intracellular, or cytozoic life, while some of them (*H. donovani*) during the latter phase may give rise to fatal diseases in man. Again, they are interesting in a zoölogical sense, in that here (crithidia) may be found variations in cellular structure pointing toward that complicated kinetic structure of the trypanosomes, the undulating membrane. On the other hand, they show, through herpetomonas, a close relation to free-living forms in stagnant water and belonging to the family cercomonadidæ. Undulating membranes are uncommon among flagellated protozoa, but are frequently found among ciliated forms. Here, however, they represent quite different morphological structures (Fig. 92).

Novy, MacNeal, and Torrey ('07) hold that all forms of herpetomonas and crithidia are in reality trypanosomes, basing their conclusion upon the fact that cultural forms of trypanosoma lack the undulating membrane and appear in no wise different from these ordinary flagellates of the insects' digestive tracts. Such a conclusion cannot be allowed in any zoölogical sense, for at no time in the life history of any species of herpetomonas or crithidia are stages present with characteristic structures specific to the genus trypanosoma.¹

The point of view held by Lèger, Caullery and Mesnil, and some others is quite different. According to this the trypanosomes are

¹ If species admittedly do not conform to a generic diagnosis, there is no possible reason for enrolling them in such a genus where they obviously do not belong. What would a zoölogist say to a naturalist who claims that neoturus and other perennibranchiate amphibia are only species of amblystoma, on the ground that the larval form of the latter has gills? And yet it is exactly this, in effect, that Novy, MacNeal, and Torrey claim for herpetomonas and crithidia, and the high position which these investigators occupy in medical circles makes an error like this particularly unfortunate. The group of trypanosomes is quite complicated enough as it is, without the added difficulties of other genera.

regarded as developed herpetomonas forms which have become specially adapted for life in the blood, the undulating membrane being a special reaction on the part of the organism to the conditions in the blood.

FIG. 92

Types of undulating membranes. *M*, membrane. (After Calkins.)

It is quite otherwise with the supposed genus *leishmania* in regard to which every new observation tends to strengthen Rogers' ('05) view that this organism of kala azar agrees with *herpetomonas* in all of its generic diagnostic characters. *Crithidia* also rests upon differences of a very slight nature, but the primitive type of membrane at the base of the flagellum is of positive diagnostic value and in most cases it is sufficient to distinguish this genus from *herpetomonas*.

In all forms the flagellum is well defined and of the characteristic

flagellate type (Fig. 93), arising from a distinct kinetic body, the blepharoplast. The nucleus is not of the diffuse type so characteristic of the bacteria and spirochetes, but is compact and cytologically similar to the nucleus of tissue or of typical protozoa cells, while in primitive mitosis it passes through more or less complicated form changes.

All are parasites, and all are apparently typical intestinal forms of definitive insect hosts. *Herpetomonas* is found chiefly in the stomach

FIG. 93

A, B, C, *Herpetomonas muscae domesticae*; A, ordinary form with double flagellum; B, dividing form; C, form encysted in slime coat; D to F, *Crithidia subulata* Lèger, from gut of *Tabanus glaucopsis* Meig; D, free monad form; E, gregarine-like resting forms showing withdrawal of flagellum; F, the same fixed to an epithelial cell in great numbers; k, kinetodonucleus; t, trophonucleus; d, diplosome; A to B, after Prowazek, D to F, after Lèger. $\times 1800$.

and intestine of various kinds of insects, *H. donovani* in the digestive tract of the bedbug *Cimex rotundatus*, while crithidia has a wide range among diptera and hemiptera. With development of the blood-sucking habit these various insects have furnished the opportunity for their parasites to adapt themselves to man and other intermediate hosts.

Non-flagellated, quiescent, and encysted stages are known in all cases, the quiescent forms remaining passive in the digestive tract (*herpetomonas*), or actively migrating ("gregarine" forms) to the

epithelial cells, to which they attach themselves often in large numbers (crithidia), or they may migrate into the cells, multiply there, and cause serious trouble (*Herpetomonas donovani*). Because of these dual motile and quiescent phases, they have quite upset the taxonomic balance of many recent writers and have caused some of the latter to sacrifice the well-known group, hemosporidia, while some have gone, prematurely, to the length of entirely giving up the established subphylum sporozoa as a group, although, indeed, even the most conservative of systematists must admit that this group is not a natural one.

A. The Genus *Herpetomonas*.—The most primitive and the least changed from the free-living forms of cercomonadine flagellates is the genus which Kent, in 1881, named *herpetomonas*, characterizing it as follows:

"Animalcules free-swimming, elongate or vermicular, highly flexible; the posterior extremity often the most attenuate, but not constituting a distinct caudal appendage; flagellum single, terminal; contractile vesicle conspicuous." To this he added the following note: "This new genus is instituted for the reception of the form figured by Stein, 'Infusionsthier,' Abth. III, 1878, under the title of *Cercomonas muscæ domesticæ*, and identified by that authority with the *Bodo muscæ domesticæ* of Burnett, and the *Cercomonas muscarum* of Leidy. The entire absence of a distinct caudal filament serves, however, at once to distinguish it from the typical representatives of either of the two last-named genera and approximates it the more nearly to *leptomonas* or *ophidomonas*. A second minute form recently discovered by Mr. T. R. Lewis in the blood of rats (*Trypanosoma lewisi*) is provisionally referred to this generic group." Kent *Manual*, p. 245.

The contractile vacuole seems to have been more or less imaginative, certainly subsequent observers have not described it and it is quite possible that Kent and others mistook the vacuole about the blepharoplast for a contractile organ. Among the species that are now recognized are the following:

H. muscæ domesticæ, found in the intestine of the housefly.

H. sarcophagæ, Prow. Intestine of meat flies.

H. lesnei, Lèger. Malpighian tubules of *Dasyphora pratorum*.

H. gracilis, Lèger. Malpighian tubules of the sucking fly *tanypus* sp.

H. campanulata, Lèger. Intestine of larva of a sucking fly.

H. jaculum, Lèger. Intestine of the water bug *Napa cinerea*.

H. donovani, Lav. and Mes. Intestine of cimex and cause of kala azar.

H. lygei, Patton. Intestine of the water bug *lygæus*.

Herpetomonas of *culex* sp., Patton.

The most primitive and least changed from the free-living forms of

flagellated intestinal parasites is the genus which Kent named *herpetomonas*. It is a widely distributed parasite of flies; that of the common housefly, *Herpetomonas muscæ domesticæ*, Burnett, is among the best known of these species, largely through the observations of Prowazek ('04). This organism is elongate and somewhat flattened at one end, which gives rise to the single, long, vibratile flagellum (Fig. 93). Apart from the nucleus and blepharoplast, the inner protoplasm has no characteristic structures and the nucleus is of the characteristic mastigophora type, with chromatin granules (often erroneously called chromosomes) of more or less definite number. The blepharoplast (*k*) lies between the nucleus and the flagellum, and is frequently of large size, while from it the base of the flagellum (rhizoblast) takes its origin. Prowazek describes the flagellum as double, the two parts being connected by a delicate membrane. If this were true, then, as Minchin ('07) remarks, this organism would have to be enrolled in some other genus than *herpetomonas*, but it is more than probable that Prowazek described an early phase of division in which the flagellum is precociously divided, as the typical form of the adult, an interpretation supported by his own figure (*B*) of a dividing form. Patton ('08), furthermore, has been unable to confirm Prowazek's observation, and finds that the flagellum is single both in *H. muscæ domesticæ* and *H. sarcophagæ*, but that it with the blepharoplast divides first in reproduction. At the base of the flagellum, just outside of the body, is a small basal granule (*d*), which in the cells with a double flagellum was called the diplosome by Prowazek.

Reproduction occurs by longitudinal division (Fig. 93, *B*). The nucleus divides by a primitive process of mitosis, the granules being equally distributed. This nuclear division is preceded by division of the blepharoplast and of the flagellum, which in this case appears to divide throughout its entire length instead of one being formed, as in some trypanosomes, by outgrowth from the blepharoplast.

Conjugation has been described by Prowazek as taking place between forms which are not sexually differentiated beyond the fact that one appears to be denser and larger than the other. During conjugation the flagella are withdrawn and the nuclei undergo so-called reducing divisions, similar in character to those occurring in *Trypanosoma noctuæ* (see p. 255). After conjugation a permanent resting cyst is formed by the fertilized cell, and in this condition the parasite passes from the intestine with the feces of the host.

According to Prowazek, infection of new hosts takes place usually by ingestion of these permanent cysts with the food; but he also finds that 5 per cent. of the flies examined and known to contain the allied form *H. sarcophagæ* had parasites in the body cavity and in the ovaries as well as in the intestine. It is probable, therefore, that the organism may be transmitted by inheritance. In *H. lygei*, on the

other hand, there is no evidence, according to Patton, of parasites in the body cavity, nor in the nymphs and larvæ reared from the egg. In this form, therefore, inheritance appears to be out of the question, the insects becoming infected solely by the ingestion of encysted forms of the parasite.

As in trypanosoma, the various species of herpetomonas are characterized by the habit of forming rosettes or agglomerations through the union of individuals by the flagellated ends. Also, in common with trypanosomes and with the merozoites of malaria organisms, they manifest a well-marked rheotropism or reaction against a current, a property, especially in the latter case, which enables the organism to make headway against a blood flow or intestinal current.

In all forms of herpetomonas there are free-moving monadiform parasites, or motile gregariform parasites, which move with a worm-like motion and finally aggregate about the epithelial cells, where they often form masses of considerable size. In both of these conditions the organisms may reproduce by longitudinal division. The gregariform phase may also encyst by secreting a slimy covering, which becomes more or less hardened, and in this cyst the organisms pass out of the digestive tract with the feces, thus serving to spread the infection.

The history of *Herpetomonas donovani*, Lav. and Mes., is particularly interesting from both the medical and the biological points of view, and shows the devious paths which an organism may follow before reaching its definitive place in a zoölogical system. The etiology of a number of peculiar diseases of India, characterized by well-marked splenomegaly (dum dum fever, kala azar "black sickness"), by irregularly recurrent fevers, anemia, and emaciation, resulting finally in profound cachexia and usually in death, has been only recently established. Leishman ('03) found peculiar bodies in cells obtained in films from a postmortem, and considered them degenerated forms of trypanosomes; from this they were given the name of "Leishman bodies." Donovan ('03) found peculiar bodies in the peripheral blood of cases of kala azar, and sent his preparations to Laveran and Mesnil, who, in November, 1903, described the peculiar bodies as similar to the blood parasites of Texas fever (babesia "piroplasma") and named the organism accordingly *Piroplasma donovani*. From this the bodies became known as the "Leishman-Donovan bodies," although considerable difference of opinion existed as to the identity of the forms in the spleen and in the blood. In December, 1903, Wright described peculiar structures, which he interpreted as organisms belonging to the microsporidia, in a case of tropical ulcer, and named the organism *Helcosoma tropicum*. Having a well-marked resemblance to the bodies found in kala azar, these new structures added a third term to the series, and they became known as the "Leishman-Donovan-Wright bodies" (Woodcock). In the meantime,

however, R. Ross, examining the Leishman-Donovan bodies, came to the conclusion (November 14, 1903) that they were distinct forms of protozoa, and named the organism causing kala azar *Leishmania donovani*, but Rogers ('05), on the basis of culture experiments, found no perceptible difference between the flagellated phase and herpetomonas, while Patton ('08) has demonstrated that the non-flagellated phases are likewise identical. The genus leishmania, therefore, cannot hold. If the organisms discovered by Wright are found to belong to the same genus, but are specifically different, then the name for Wright's organism must be *Herpetomonas tropica*, Wr.

Rogers' discovery of the flagellated stage was quickly confirmed by Christophers and by Leishman, the latter finding in this discovery a confirmation of his earlier belief that the organisms were trypanosomes, basing his view on the fact that some trypanosomes under culture have no undulating membrane. Rogers gave many reasons for considering the bedbug the means of transmitting the disease from individual to individual, and his surmise was not only confirmed, but the transformation of the intracellular bodies into flagellates within the intestine of *Cimex rotundatus* was fully worked out by Patton in 1907. With this discovery Leishman's conclusions regarding the trypanosome relation cannot hold, the organism finding its nearest relative, as stated above, in the genus herpetomonas.

The Leishman-Donovan bodies, as the intracellular forms have been called, are present in large numbers in the cells of liver, spleen, and bone marrow, while, according to Christophers, leukocytes and great macrophages of endothelial origin may become crowded with them, 100 to 200 in a single cell (Leishman). They are taken into the stomach of the bedbug still as intracellular forms, and are liberated there by degeneration and digestion of the human cells. When first liberated, and during the early changes in the gut, the parasites measure from 4 to 7 μ (Patton); they may be oval or spherical in shape, but they soon divide and may form small "rosettes" of six to eight cells.

No sexual differences and no conjugation processes have been made out, although Leishman described the formation of very slender forms from larger ones (Fig. 94) in organisms under culture; such conjugation processes are to be sought in the intestine of the bedbug, and it may be predicted that within a very short time they will be found there.

Herpetomonas donovani, in its quiescent phase, is undoubtedly an endothelial cell parasite which multiplies in human tissue cells until the normal histological relations of such cells are broken down and the cells are liberated as macrophages in the general circulation. Here many of the parasites become free, only to be captured and ingested by leukocytes, so that toward the end of the disease the peripheral blood contains great numbers of parasite-filled leukocytes and endo-

thelial cells. When such blood is sucked into the digestive tract of a bedbug the cell bodies of leukocytes and macrophages are broken down and their contained parasites liberated. Patton found that the parasites thus introduced into male or female bugs could remain in the mid-gut for at least five days before beginning to develop, although the majority of them are well under process of development by the second or third day.

Development of the parasite begins with a well-marked increase in volume, and the cell nucleus (trophonucleus) early divides. This process of division is not described in great detail by Patton, but it is evidently similar to the process of mitosis of the euglena type. The cell then rapidly undergoes flagellation, a pink staining (with Giemsa) area being the seat of flagellum formation. This area was noted by other observers and called the "flagellar vacuole" (Leishman, Patton), the "vacuole-like area" (Christophers), and the "eosin body"

FIG. 94

Herpetomonas donovani, unequal division to form slender flagellated individuals.
(After Leishman.)

(Rogers), and is probably the same organoid of the cell that Kent ('81) described as the contractile vacuole in his characterization of the genus *herpetomonas*. This enlarged flagellar vacuole passes to the cell periphery, where it bursts and a small "brush" of pink-staining fibers protrudes from the cell, and these, later, by coalescence, form the definitive flagellum. In other cases the parasites do not undergo division in this manner, but the nucleus divides and the blepharoplast divides two or three times, and eight flagella are formed at various points on the cell periphery. These so-called "rosettes" divide to form elongated flagellates as many in number as there are flagella and blepharoplasts. The size of the flagellates varies considerably from relatively long ones (up to 20 microns), by continued division, to minute spirilla-like forms.

Patton finds no evidence of encystment and no evidence of infection of the bedbugs other than from human victims. Nor is there any evidence to support the idea of direct inheritance from female bugs to their offspring, but Patton suggests in a later paper (1908) that nymphs of blood-sucking forms of such bugs may take in the infection with their food. The method of reëntry into a human host is likewise unknown.

B. The Genus *Crithidia*, Lèger, 1902.—The genus *crithidia*, by reason of its non-kinetic prolongation of protoplasm at the base of the flagellum, forms an interesting link in the evolution of the trypanosomes. It is quite true, as Novy, MacNeal, and Torrey ('07) point out, that the distinctions between these several genera are extremely "fragile," and that the points of difference are so minute as not to

FIG. 95

Crithidia melophagia, Flu, from the gut of *Melophagus ovinus*. (After Flu.) A, fully developed parasite with myonemes; B, individual with degenerated trophonucleus; C, encysted form (see *herpetomonas*); D, division form.

count for much. It must not be overlooked, however, that minute differences must be utilized in connection with organisms that are themselves minute, and a definite structural feature which Lühe points out as the most characteristic of the genus *crithidia*, since it exists in all of the parasites regardless of their size, is a perfectly satisfactory differential characteristic, and unlike Lèger's original basis of distinction (smaller size of *crithidia* and truncated ends), has morphological value.

The type species is *Crithidia subulata*, Lèger, a parasite of the intestinal tract of a tabanid fly. The body is elongate and slender and drawn out upon the base of the flagellum in a typical manner (Fig. 93, p. 235). The nucleus and blepharoplast are distinct and persistent after

withdrawal of the flagellum. This gradually shortens and disappears, a rhizoblast remaining for some time; but this too is ultimately absorbed, and as a "gregarine" form the minute organism makes its way to epithelial cells, where it becomes attached (Fig. 93).

Since Lèger's original observations several others have worked upon different species of crithidia, the most recent results being obtained by Patton ('08) in connection with a species (*Cr. gerridis*, Patton) from a water bug, *Gerris fossarum*, and by Flu ('08) in connection with a species (*Cr. melophagia*, Flu) from *Melophagus ovinus*, an ectoparasite of sheep. In each of these there are well-defined, non-flagellated conditions of the organisms similar to those of *H. donovani*. A nucleus and blepharoplast are present, and the flagellum develops from the latter by the apparent outgrowth of its substance (Fig. 95). In

FIG. 96

Stages in the development of *Crithidia gerridis*, Patton. (After Patton.) A, group of young forms from mid-gut of nymph of *Gerris fossarum*, Fabr.; blepharoplast and origin of flagellum; B, development of the flagellum inside the periphery of parasite; C, further development and division of flagellum; D, E, adult forms, flagellum dividing in E; F, two stages in withdrawal of flagellum to form resting stages; G, cyst.

Cr. gerridis the flagellum forms as a ridge upon the surface, and often divides as it grows, the basal bodies first dividing into two. By continued division rosettes of many individuals may be formed before the fully developed flagellated adults break away. Division occurs as in *herpetomonas* (Fig. 96).

Encysted forms similar to those described by Prowazek for *herpetomonas* were observed by Flu in the case of *Cr. melophagia*, but not in *Cr. gerridis*. *Cr. melophagia* further differs from other forms in possessing definite myonemes which run the length of the body, uniting in the anterior end with the rhizoblast of the flagellum (Fig. 95).

Neither conjugation nor mode of infection has been observed in connection with these parasites, and the caution which Novy, MacNeal, and Torrey express in regard to the possible confusion of such flagellates of insects, with developmental stages of human or other vertebrate blood parasites, is certainly well grounded, but we cannot indorse their view that all such parasites are to be looked upon as developmental stages in the life history of trypanosomes.

CHAPTER VIII.

THE PATHOGENIC FLAGELLATES—(CONTINUED).

THE GENUS *TRYPANOSOMA*, GRUBY.

At the present day more than sixty species of trypanosoma have been described from different types of vertebrates, and although the greatest difference of opinion exists here, as with spirochetes, it is not in connection with the animal or plant characteristics, but rather with the relationships and life history. Various students of the group, beginning with Lèger ('04), have attempted to separate all of the different varieties known into distinct groups, according to the morphologically "anterior" end. In some the flagellum issues from the cell at the supposedly posterior end, in others at the supposedly anterior end. The former, including all of the piscine trypanosomes, are grouped by Lühe ('06) in a distinct genus, to which he applies Mitrophanow's name, *hematomonas*; the latter includes all of the mammalian trypanosomes to which Lühe gives the distinct generic name *trypanozoön*, while a third generic name, *hemoproteus*, is given for the trypanosome of the owl, having a dual life in the serum and in the blood cells, as described by Schaudinn. Woodcock ('06) likewise separates the latter from all other trypanosomes, under the generic name of *trypanomorpha*.

The scientific value of these divisions of the trypanosomes stands or falls with their phylogeny and with the terminal homologies of the different species. A typical trypanosome, for example, *T. theileri*, Bruce, found exclusively in the blood of cattle, consists of an elongate, more or less serpentine cell body, from one end of which projects a vibratile flagellum (Fig. 97). The flagellum is continued toward the opposite end of the cell as a well-marked marginal cord, and takes its origin from a minute granule (blepharoplast) not shown in Lühe's figure. Near this terminal granule lies a large, deeply staining body of chromatin (*k*), which in some species is larger than the nucleus, and in others has a typical reticulate nucleus character. In agreement with the views of Schaudinn, Woodcock, Lühe, Minchin, and others, this chromatin or nucleus-like body will be designated the "kinetonucleus," a term suggested by Woodcock ('06) because of its close connection with the motile elements of the cell (see p. 33). Between the attached part of the flagellum and the body is a delicate protoplasmic mem-

brane, which, as in *Spirocheta balbianii*, is frequently, if not always, provided with contractile myonemes. The non-flagellated end of the cell may be pointed, as in *T. theileri*, or rounded or blunt. The endoplasm frequently contains granules of chromatoid material, and may have a vacuolated appearance; little importance, however, has been attached to these structural details of the endoplasm. The nucleus of the cell, the element, that is, which superintends the vegetative processes and sometimes called the "trophonucleus," is a clearly defined morphological nucleus in which a nuclear membrane may be made out in some cases, again not. The chromatin is usually in the form of granules (miscalled chromosomes) of usually a definite number; but there is reason to believe that under satisfactory cytological methods the chromatin is finely granular, surrounding a central division centre, as in the majority of free flagellates (see p. 30). Reproduction of the cell is by longitudinal division preceded by division of the blepharoplast, kinetonucleus, and vegetative nucleus.

FIG. 97

Trypanosoma "Trypanozoön" *theileri* (Bruce), blood of cattle Transcaucasia. $\times 3000$.
(After Lühe.) *k*, kinetonucleus; *t*, trophonucleus; *u*, undulating membrane.

There are two different theories as to the phylogenetic history of this well-marked and highly characteristic type of organism: one deriving it from heteromonad forms like *bodo* or *anisonema* (Fig. 15 p. 43), the other from forms like *herpetomonas* and *crithidia*. According to the first hypothesis, the trypanosome condition is brought about by the union of the trailing runner, flagellum, or *Schleppgeissel* with the cell body. If this were the case, then the flagellum end of the organism would be posterior. A certain amount of evidence in favor of this point of view is given by two interesting types of blood-dwelling parasites of fishes, *trypanoplasma* and *trypanophis*, in both of which there are two flagella, one directed in advance at the anterior end, the other attached to the body throughout its length and terminating as a

free flagellum at the posterior end (Fig. 98). Such forms may be readily conceived as coming from bodo-like types in which the posterior or trailer flagellum becomes attached to the cell, while the trypanosome type may arise from such forms by the suppression of the

FIG. 98

Trypanoplasma borreli. (After Keysselitz.) *A, B*, old, developmental stages; *C*, a so-called "male" form; *D, E*, so-called "female" forms; *G, H*, the "copula."

anterior flagellum and elaboration of the lateral protoplasm into an undulating membrane. According to such a derivation, the flagellated end of a trypanosome would be posterior, and this is the view taken by a number of authorities. As Minchin ('08) points out, however, the developmental history of no trypanosome points to this mode of origin, but tends rather to support the second hypothesis of the origin of trypanosomes from herpetomonas and crithidia-like forms by the posterior migration of the kinetonucleus and blepharoplast, whereby these structures become secondarily posterior, while the flagellum

FIG. 99

Trypanosoma noctuæ. (After Schaudinn.) Schematic representation of the metamorphosis of a fertilized cell into an "indifferent" type of *Trypanosoma*. F, G, H, formation of the undulating membrane and flagellum from kinetoplasmic material.

would be attached to the cell, as in herpetomonas, at the anterior end. Schaudinn has shown that the flagellum in *Trypanosoma noctuæ* has this mode of origin, and grows out from the anterior end, while the kinetonucleus and blepharoplast (Fig. 99) remain anterior to the nucleus. In other species, however, the developmental history shows that young forms and culture forms are similar to crithidia with rudimentary membrane and anterior blepharoplast and kinetonucleus. This is well described in the case of a trypanosome of the ray, *Trypanosome raia* (?), by Robertson ('07). Here in young forms, after division in the gut of the leech *Pontobdella muricata*, the kinetonucleus

is anterior to the nucleus, but becomes posterior to the nucleus as development progresses (Fig. 100) until the adult posterior position is attained. Novy has laid great stress upon the fact that in trypanosomes in culture the form is similar to that of herpetomonas and crithidia, and for this reason regards the species of these genera as true trypanosomes. It will hardly be allowed by anyone familiar with the morphological changes of protozoa that trypanosomes under culture in artificial media are in any way normal, either structurally or physiologically, and his purely hypothetical conclusion that herpetomonas and crithidia "really represent cultural forms of true trypanosomes" (1907), zoologically speaking, is far-fetched. The herpetomonad form assumed by some types may be evidence of a phylogenetic ancestral state, but it certainly cannot be accepted as evidence that the more primitive, ancestral organisms are themselves trypanosomes.

In the present state of knowledge of trypanosomes it is extremely uncertain as to where lines should be drawn between species; morphology is no aid in this, for the same species in the same animal may present so many form changes that were they found in different animals they would be assigned to different species without hesitation. No safe limitations can be established until the life histories are known, and as these have been worked out in only a few cases the difficulties are not much relieved. Physiological grounds, culture relations, etc., are equally unsatisfactory, but there is reason to believe that differences in such respects are indications of different specific relationships. For the present, therefore, it is expedient to consider each new form described in a new host as a distinct species until its affinities are established by the full life history, and until then, furthermore, it seems better not to break the genus *trypanosoma* into other genera as Lühe ('06) has done on the basis of supposed different ancestry. This supposition is purely hypothetical, and it is quite possible that we have not yet found the true explanation of the anterior and posterior ends of trypanosomes.

Trypanosomes are present in all kinds of vertebrates, where they are normally parasites of the blood system; they are also found in the intestines of different blood-sucking insects. Lühe, with Lèger, believes the latter to be the definitive hosts, the trypanosomes coming from ancestors like herpetomonas and crithidia, which are typical intestinal parasites. Novy also takes this point of view, holding, with Lèger, that the trypanosome structures are special adaptations which the organisms have developed as a response to conditions in the blood. Minchin ('08) regards the trypanosomes as originally parasitic in the vertebrate intestine, basing his conclusion largely upon the observations of Hintze ('02) and upon theoretical considerations of the fact that trypanosomes may be transmitted by leeches as well as by insects. There is much to be said in favor of his point of view, and Lèger's

criticism that no sexual phases have as yet been found in the blood of vertebrates is not wholly unanswerable, since we do not yet know

FIG. 100

Trypanosoma raia. (After Robertson.) Forms observed in the digestive tract of the leech, *Pontobdella muricata*. *A*, mature specimen from blood of skate; *B* to *F*, stages in the development of the flagellum from the kinetonucleus, and change in position of the latter in relation to the nucleus.

much about the conjugation processes in any species, while Hintze's view is conceivable, viz., that the organisms migrate from the blood back into the intestine, where they conjugate, while in blood-sucking

forms the stomach and intestine of the invertebrate is substituted for that of the vertebrate. Minchin holds that trypanosomes are never found in the alimentary tract of insects which do not draw blood, and finds in this a further support for his hypothesis.

All such speculations, while interesting and stimulating to further research, are, however, unsubstantial, and generalizations cannot yet be drawn with any safety. The following list of species, founded in large part upon the species enumerated in Lühe's excellent paper on these forms, shows what a large field for research this group presents, and that "material" is at hand for investigators everywhere.

The most plausible hypothesis concerning the origin of the trypanosomes interprets them as more highly evolved organisms of the herpetomonas or crithidia type. Like the latter they are characteristically fluid-dwelling parasites either in the denser fluids of the digestive tract of invertebrates (*Trypanoplasma borreli* in the leech) or in the less dense fluids of the blood. As crithidia and herpetomonas may lose their motile organs and pass into a quiescent phase, or in the case of *H. donovani* into a cell invading phase, so trypanosomes may assume resting or encysted phases (*e. g.*, *T. grayi* in the rectum of the tsetse) or even the cell-invading phase (*T. noctuæ*) in the blood.

LIST OF SPECIES OF TRYPANOSOMA.

Name of species.	Vertebrate host.	Serum or cell parasite.	Invertebrate host known or suspected.	Size.
<i>T. remaki</i> , Lav. and Mes.	<i>Esox lucius</i> , L.	Serum		
<i>T. danilewskyi</i> , Lav. and Mes.	<i>Cyprinus carpio</i>	"		
<i>T. tinca</i> , Lav. and Mes.	<i>Tinca tinca</i> , L.	"		
<i>T. carassii</i> , Mitrop.	<i>Carassius carassius</i> , L.	"		
<i>T. abramis</i> , Lav. and Mes.	<i>Abramis abramis</i>	"		
<i>T. granulorum</i> , Lav. and Mes.	<i>Anguilla anguilla</i>	"		
<i>T. cobitidis</i> , Mitr.	<i>Cobitis fossilis</i>	"		
<i>T. barbatulæ</i> , Læg.	<i>Cobitis barbatula</i> , L.	"		
<i>T. rhamdiæ</i> , Botello	Brazilian fish	"		
<i>T. macrodonis</i> , Botello.	Brazilian fish	"		
<i>T. soleæ</i> , Lav. and Mes.	<i>Solea solea</i> , L.	"		
<i>T. platessæ</i> , Leb.	<i>Platessa platessa</i>	"		
<i>T. flesi</i> , Lebailliy	<i>Flesus flesus</i>	"		
<i>T. laternæ</i> , Leb.	<i>Arnoglossus later-nus</i> , Walb.	"		
<i>T. limandæ</i> , Br. and Leb.	<i>Limanda limanda</i>	"		
<i>T. gobii</i> , Brump. and Leb.	<i>Gobius niger</i>	"		
<i>T. callionymi</i> , Br. and Leb.	<i>Callionymus dra-cunculus</i> , L.	"		

Name of species.	Vertebrate host.	Serum or cell parasite.	Invertebrate host known or suspected	Size.
<i>T. cotti</i> , Br. & Leb.	<i>Cottus bubalis</i> , Eu.	Serum		
<i>T. delagei</i> , Br. and Leb.	<i>Blennius pholis</i>	"		
<i>T. scyllii</i> , Lav. and Mes.	<i>Scyllium canicula</i> and <i>S. stellare</i>	"		
<i>T. raia</i> , Lav. and Mes.	<i>Raja punctata</i>	"		
	<i>R. macrorhyncha</i>	"		
	<i>R. mosaica</i>	"		
	<i>R. clavata</i>	"		
<i>T. rotatorium</i> , Mayer	<i>Rana esculenta</i>	"		
	<i>R. temporaria</i>	"		
	<i>Hyla arborea</i>	"		
<i>T. mega</i> , Dutton and Todd	<i>Rana</i> sp. (Africa)	"		
<i>T. karyozeukton</i> , Dutt. and Todd	<i>Rana</i> sp. (Africa)	"		40-80 μ
<i>T. inopinatum</i> , Sergent	<i>Rana esculenta</i>	"		60-72 μ
<i>T. nelsprutense</i> , Lav.	<i>Rana</i> sp.	"		82.4 μ
<i>T. borrelli</i> , March. and Salimbeni	<i>Hyla</i> sp.	"	Leech <i>Helobdella al-gira</i> , Moq.	25-30 μ
<i>T. clamata</i> , Steb.	<i>Rana clamata</i>	"		24-35 μ ,
<i>T. damonia</i> , Lav. and Mes.	<i>Damonia reevesi</i>	"		with flagellum
<i>T. boueti</i> , Martin	Lizard	"		
<i>T. noctua</i> , Celli and San Felice	<i>Glaucidium noctua</i>	Both	Leech (probable)	32 μ , with flagellum
<i>T. danilewsky</i> , Kruse	<i>Corvus cornix</i>			
<i>T. columba</i> , Celli and San Felice	<i>Columba livia</i>		<i>Culex pipiens</i>	
<i>T. passeris</i> , Celli and San Felice	<i>Passer</i> (many sp.)			
<i>T. alauda</i> , Celli and San Felice	<i>Alauda arvensis</i>			
<i>T. fringilla</i> , Lab.	<i>Fringilla celebs</i>			
<i>T. aluco</i> , Celli and San Felice	<i>Syrnium aluco</i>			
<i>T. bubonis</i> , Celli and San Felice	<i>Bubo bubo</i>			
<i>T. maccallumi</i> , Novy & MacNeal	<i>Zenaidura carolinensis</i>			
<i>T. sacharovi</i> , Novy and MacNeal	<i>Passer domesticus</i>			
<i>T. rouxii</i> , Novy and MacNeal	<i>Syrnium aluco</i>			
<i>T. avium</i> , Lav.	<i>Syrnium aluco</i>			
<i>T. confusum</i> , Lühe (T. avium, Novy)	Common American birds			
<i>T. laverani</i> , Novy and MacNeal	<i>Astragalinis tris-tis</i>			
<i>T. mesnili</i> , Novy and MacNeal	<i>Buteo lineatus</i>			
<i>T. padda</i> , Lav. and Mes.	<i>Padda orizzivora</i>			
<i>T. johnstoni</i> , Dutton and Todd	<i>Estrilda astrild</i>			
<i>T. mathisi</i> , Serg.	Common swallow			

Name of species.	Vertebrate host.	Serum or cell parasite.	Invertebrate host known or suspected.	Size Includ'g flagellum
<i>T. lewisi</i> , Kent	Blood of rats	Serum	Louse <i>Hæmatopinus spinulosus</i> Bur.	7-30 μ
<i>T. criceti</i> , Lühe	<i>Cricetus cricetus</i>	"	Flea? <i>Pulex fasciatus</i> Bur.	?
<i>T. cuniculi</i> , R. Bl.	<i>Lepus cuniculus</i>	"	<i>Hematopinus ventricosus</i> . Denny? or <i>Pulex</i> sp?	?
<i>T. duttoni</i> , Thiroux	Mice in Senegal	"	?	25-30 μ
<i>T. indicum</i> , Lühe.	<i>Funambulus palmarum</i> (Madras)	"	?	18-20 μ
<i>T. blanchardi</i> , Br.	<i>Myoxus glis</i> , L.	"	?	
<i>T. vespertilionis</i> , Battaglia	<i>Vespertilio noctula</i>	"	?	12-15 μ
<i>T. nicolleurum</i> , Sergeant	<i>Myotis myotis</i> , <i>Vespertilio kuhli</i>	"	?	20-24 μ
<i>T. gambiense</i> , Dut.	Man	Serum, sleeping sickness	<i>Glossina palpalis</i>	17-28 μ
<i>T. brucei</i> , Plimmer and Bradford	Horse and other domestic animals	Serum, nagana	Tsetse flies, especially <i>Glossina morsitans</i> , W.	25-35 μ
<i>T. equiperdum</i> , Dofl.	Horses, asses	Serum, dourine	(Transmitted by coitus)	25-28 μ
<i>T. dimorphon</i> , Lav. and Mes.	Horses	Serum	?	13-30 μ
<i>T. nanum</i> , Lav.	Cattle	"	?	10-14 μ
<i>T. vivax</i> , Ziemann.	Sheep and deer	"	Tabanid flies?	18-26 μ
<i>T. congolense</i> , Broden	Sheep	"	?	10.5-15.5 μ
<i>T. suis</i> , Ochmann	Swine	"	?	?
<i>T. evansi</i> , Steel	Horse, cattle, buffalo, camel, etc.	Serum, surra	<i>Stomoxys calcitrans</i> ? <i>Tabanus lineola</i> ?	22-30 μ
<i>T. equinum</i> , Voges	Horse, cattle	Mal de Caderas	?	22-24 μ
<i>T. theileri</i> , ¹ Bruce	Cattle	Gall sickness	<i>Hippobosca rufipes</i>	60-70 μ
<i>T. mustesari</i> , Ling	Cattle	Serum	?	
<i>T. pecaui</i> , Lav.	Sheep	"	?	
<i>T. soudanense</i> , Lav.	Dromedary	"	?	

So perfectly have trypanosomes become adapted to mammalian blood and mammalian temperature, that in the majority of species removal from the circulation, even if the blood be kept sterile, results in loss of virulence or activity, and in death. In some cases, *e. g.*, *T. lewisi*, different observers have kept infected blood for considerable periods (Francis, eighty-one days), but in the majority of cases the organisms do not remain alive for so long a time (*T. brucei* or *T. evansi* only two to three days). Even when successful such experiments involve no multiplication processes, the organisms being merely preserved alive, and with a few exceptions such appears to be the case

¹ Includes *T. transvaaliensis*, Lav., and *T. lingardi*, R. Bl.

when mammalian blood and organisms are taken into the digestive tracts of different insects (*T. brucei* disappears from the tsetse in from two to three days).

It is quite otherwise with cultivation on artificial media first successfully accomplished by Novy and MacNeal, in 1903, with *T. lewisi*. These keen investigators opened a new era by this application of bacteriological culture methods with pathogenic protozoa, the method, as we have seen (p. 239), giving excellent results with seemingly obligatory cytozoic forms (Leishman-Donovan bodies). The culture medium is made up of nutrient agar and defibrinated rabbit's blood. When desired for use the agar is melted and cooled to about 50° C., the blood added and thoroughly mixed. The organisms collect and multiply in the water of condensation or even on the agar directly. It was found that the organisms gradually lose their virulence and die as a result of the exhaustion of the food medium, but that renewed virulence and vitality could be established by transplanting to fresh culture tubes. In this way Novy and his associates have maintained trypanosomes in pure culture for several years. While *T. lewisi* appears to be an especially favorable subject for this method of research, other forms as well have been studied in this way, Novy and MacNeal being successful with *T. brucei*, *T. evansi*, and with several bird trypanosomes, while Laveran and Mesnil have succeeded with *T. brucei*, *dimorphon*, *T. gambiense*, and others.

A. The Motile Apparatus of Trypanosomes.—In fresh blood the presence of trypanosomes, when abundant, may be easily noted by the agitation of the blood corpuscles, which are whipped about by the lashings of the ever-active flagellum. This movement of the trypanosomes may be analyzed as a combination of snake-like undulations, active bending, rotation, and translation. In some, notably in *T. vivax*, the peculiar writhing movements without progression, which are characteristic of a great many species, are replaced by an active, business-like forward movement in straight lines across the field of the microscope. In such movement the flagellum, as with free-living flagellates, is always in advance.

As shown in Chapter I, the flagellum of a typical mastigophoran is formed by the outgrowth of substance from the kinetic centre, which may be in the form of a basal granule or blepharoplast, or in the kinetic material within the nucleus. Such kinetic centres have the appearance and often the functions of centrosomes, so that the term centrosome sometimes used for the basal granule has some significance.

In trypanosomes, the flagellum has the same mode of origin as in other flagellates, coming from a basal granule or blepharoplast which may or may not be included in the kinetonucleus. In some cases, during division of the cell, it appears to divide longitudinally as it does

in herpetomonas, but in other cases, and apparently in the best authenticated cases, the flagella are always formed by newgrowth from the basal body.

The flagella are always accompanied by a protoplasmic membrane, to which they are attached as a lateral cord. This membrane, if drawn out straight, is often longer than the body whence it is attached in folds or undulations, while by its movements, directed by the attached flagellum, the organism moves through a liquid medium with a peculiar auger-like movement, and gave the reason for Gruby's name, trypanosoma. In the majority of forms the flagellum is continued beyond this membrane as a free "whip" in the surrounding medium, but in other cases, as in *Tryp. dimorphon*, it terminates with the membrane.

As to the minutiae of flagellum and membrane formation the best and most complete account has been given by Schaudinn in the case of *Tryp. noctuæ*, the blood parasite of the little owl *Glaucidium noctuæ*. The kinetonucleus divides by heteropolar mitosis, the smaller part becoming the blepharoplast, the larger remaining as the kinetonucleus. The smaller then divides again and a spindle figure is formed which, except that it is heteropolar, resembles that of free flagellates, having a central spindle formed by the division centre, and eight "mantle fibers" corresponding to the chromosomes. The central spindle forms the flagellum at the edge of the undulating membrane which now grows out from the anterior end of the organism, while the eight fibers form the myonemes of this membrane (Fig. 99, p. 247). There is reason to believe that if this account of the formation of the membrane is accurate, the so-called chromatin of the kinetonucleus is in reality kinetic substance. Schaudinn's figures were acknowledged by himself to be schematic, and it is quite probable that the formation of flagellum and membrane does not follow such a clean-cut scheme; it illustrates the fact of widespread occurrence, however, that the flagellum does not emerge from the kinetonucleus direct. A similar granule is formed from the division centre of the kinetonucleus (Prowazek, 1905) of *Tryp. lewisi*, and the flagellum is held by Prowazek to arise in the same way as in *Tryp. noctuæ*, while the mantle fibers become eight longitudinal but ill-defined lines running the length of the cell. Similar myonemes were observed by Prowazek in *Tryp. brucei*, while Dutton, Todd, and Tobey ('07) found striations (myonemes) in every trypanosome examined by them in Africa; neither Moore and Breiul nor Minchin could find myonemes in *Tryp. gambiense*, although the basal granules (which Moore and Breiul laboriously call the "bead," in order to save their very strained nomenclature) are found. Eight myonemes, furthermore, were found by Keysseltz ('06) in *Trypanoplasma borreli*. It is quite probable, therefore, that the ectoplasm of a trypanosome cell is provided with myonemes or elementary muscular fibers of kinetoplasm.

B. The Trypanosome Nuclei.—The terms micronucleus and macronucleus are frequently used to designate the trophonuclei and kintonuclei of these flagellates, but this use of the term micronucleus is greatly to be deplored, since the kintonucleus has absolutely no analogy with the micronucleus of infusoria, and the binucleate condition of the trypanosomes is to be explained upon other grounds than that of the ciliates.

The nucleus of an ordinary trypanosome is constructed upon the same plan as that of simpler flagellates, and consists of a spherical body of chromatin with a more or less well-defined nuclear membrane, and a central division centre similar to that originally described by Keuten in euglena. The nucleus, therefore, belongs to the category of centronuclei, as described by Boveri ('01). Many observers have been careless in describing the chromatin in such nuclei under the term "chromosomes," the custom originating with Schaudinn's description of the structure of *Tryp. noctuæ*. Cytologists have repeatedly pointed out the impossibility of getting accurate cytological demonstrations from poorly fixed material, and the ordinary technique recommended in connection with the Giemsa staining fluid gives unreliable preparations. The nucleus in particular undergoes modifications of a well-marked character; the chromatin here appears to be a fluid substance which when dried, as in a smear, coagulates in irregular masses without definite structure. Moore and Breinl have made similar criticisms of the so-called chromosomes of various authors, and in *Tryp. gambiense*, *Tryp. lewisi*, and other forms have observed nuclei of the same type as those pictured in Fig. 102, p. 260. The descriptions of "chromosomes" in different accounts, therefore, must be taken with reserve.

Nearly all of the subsequent observers have followed Schaudinn's description of the happenings in *Trypanosoma noctuæ*, and there is a certain ground for the suspicion that the multiple and confusing forms assumed by these nuclei, especially when the usual methods are employed, are more easy to interpret along the lines of a path already made than to be described as involution or degeneration types. Hence we find in the literature all kinds of nuclei arranged in definite series as illustrating "reducing divisions," or "karyogamy," or "parthenogenesis," where it is more than likely that the structures thus interpreted are artefacts, or evidences of hyperplasy and degeneration. The schematic figures and categorical descriptions of Schaudinn's original contribution are still the most convincing of all such attempts to describe the nuclear changes, and may well serve as a type, although the terminology employed by this gifted and careful observer, borrowed from the nomenclature of animal cytology, cannot be employed in the same sense for these flagellates.

The nuclear structures of *Trypanosoma noctuæ* is shown in Fig.

99, p. 247, of a so-called indifferent form. Here, after elimination of waste material from the fertilized cell (*A*) the nucleus divides by heteropolar mitosis to form a trophonucleus and a kintonucleus. The former consists of a central division centre (karyosome) and chromatin which is arranged in eight groups; the latter, as described above, divides to form the substance of the motile apparatus and the permanent kintonucleus, in which, again, Schaudinn finds eight chromatin masses and a central division centre. The nucleus of the "female" type of organism differs from that of the "indifferent" form in that a large part of the "achromatic" portion of the nucleus is eliminated before the first division of the copula. This eliminated part divides

FIG. 101

Trypanosoma noctuæ. (After Schaudinn.) *A*, elimination of the "male" part of the nuclear material; *B*, division of the so-called "male" part; *C*, heteropolar division of the female nucleus and degeneration of the daughter nuclei of the "male" part; *D*, formation of adult female cell.

three times, forming eight minute nuclear masses, which finally degenerate and disappear (Fig. 101), while the nucleus now divides by heteropolar mitosis, as in the previous case. The nucleus of the "male," on the other hand, eliminates the larger part of the nuclear material which ordinarily goes to form the nucleus (trophonucleus) of the cell, and this degenerates, while the smaller denser nucleus resulting from the first division now divides three times to form the nuclei of the eight microgametes and a fourth time to form the trophonucleus and kintonucleus of these gametes.

The nuclei are thus sexually differentiated, according to Schaudinn, a statement which, if true, gives the first complete confirmation of

the early hypothesis of Balfour and Minot that the nucleus of the primordial egg or sperm cell contains both kinds of sex chromatin, the opposite kind being eliminated by the reducing divisions of each sex. It may be noted in this connection that while modern cytology has brilliantly confirmed the essence of this theory, it is not at all in the way supposed by the early speculators, nor at all in the way outlined by Schaudinn in this trypanosome (see Wilson, Stevens, and others on sex chromosomes in insects).

The kinetonucleus varies greatly in size, from a mere granule, as in *Tryp. gambiense*, to a large body equal to, or larger than, the nucleus (as in *Trypanoplasma borreli*); the great majority of forms present no such structures as described by Schaudinn, the kinetonucleus usually being homogeneous and dense in appearance; Robertson ('07), however, finds "chromatic" thickenings in *Tryp. raiæ* which she interprets as equivalent to the chromatin of Schaudinn's form.

The relative positions of kinetonucleus and nucleus are used by many observers as of sufficient importance to justify specific distinctions; this was considered of more importance formerly than it is today; indeed, at the present time no conclusions as to taxonomy can be drawn from such relations. Novy, Minchin, Robertson, and a host of others have shown that in the same species the kinetonucleus may be anterior, lateral, or posterior to the nucleus (Fig. 100, p. 249).

C. Form Changes of Trypanosomes.—The variations in the relative position and sizes of the nuclei accompany the greatest variety of form changes in the body as a whole and next to the ameboid forms, which after all have a certain constancy in their form changes, these trypanosomes are perhaps the most variable of protozoa. They seem to be highly susceptible to the conditions surrounding them. "I am convinced," says Minchin, "that the appearance, and even the structure, of trypanosomes may be greatly affected by the condition of their hosts" (1908, p. 178). If slight changes in the blood of vertebrates can bring about such marked changes in structure of the parasites, it is obvious that the much greater change in external conditions, when transferred from the vascular system, especially of mammals, to the relatively cold environment of an insect's digestive tract, should be the cause of even greater changes. The modifications brought about by these several different conditions have been variously interpreted as sexual differences, as resting phases, degeneration phases, and the like, while so-called latent bodies and encysted forms have been found in some cases.

Size differences were first brought into prominence by Schaudinn in connection with the rapid multiplication of *Tryp. ziemannii* of the owl, where, he states, "as a result of the rapid multiplication the indifferent spirochetes (trypanosomes) become remarkably small; indeed, I have found forms which are so unmeasurably fine that they can be

recognized only when agglomerated or when in motion" (1904, p. 432). The majority of observers have confirmed this observation, although in no form are the extremes so far apart as in this case. Minchin ('08) finds the greatest variety of form changes in *Tryp. gambiense* in the body of the tsetse fly, *Glossina palpalis*. Here, during the first twenty-four hours, the trypanosomes multiply by division in the fly's digestive tract, two distinct types being formed, one stout, the other slender. During the next twenty-four hours the two types are connected by all kinds of intermediate forms, which in the third day become thinned out and presenting some degeneration forms, and many trypanosomes of great length, both stout and slender; while after the fourth day no organisms were found at all. Similarly *Tryp. grayi* was found in the digestive tract of the same fly to manifest the most "bewildering variety of forms and sizes," while in different flies the run of organisms might be much larger than in others. Division, also, is responsible for variation in size, Minchin finding that smaller daughter trypanosomes are formed by unequal division of the parent cell.

Following Schaudinn, many, indeed the majority of, observers have attempted to distinguish these manifold form changes as male, female, and indifferent types. While some of their descriptions are manifestly labored and far-fetched, others are supported by more or less convincing evidence. In the type form *Tryp. noctuæ* the chief differences are found in the nuclei, where, as described above, the male and female organisms are freed from female and male chromatin respectively (Figs. 99 and 101). In addition to this difference, Schaudinn noted that the male cells were hyaline and more free from granules of one kind or another than the female, while the indifferent forms were distinguished from both of the other types by the complete nucleus and by minor cytoplasmic differences. It must be confessed that, despite the scientific acumen of this observer, one's credulity is greatly stretched by these findings, and in view of the fact that so much of the subsequent work has been interpreted in terms of these descriptions, it is much to be regretted that Schaudinn's figures were wholly schematic. Prowazek ('05) found only a slight difference between the sexes in *Tryp. lewisi* while in the gut of the louse, the male being smaller and more fragile than the female and much more liable to degenerate, while the nucleus assumes an elongate band form or rod form in the male. These might be identified as degeneration forms were one inclined to be skeptical, especially as fertilization stages were rarely seen; the "rod" form of nucleus, as Doflein ('09) points out, may be interpreted as an abnormally developed flagellum.

Moore and Breinl ('07) question the advisability of designating arbitrarily chosen extremes in a series of varying forms as male and

female, while Minchin ('08) states, in connection with *Tryp. gambiense*, that only the extremes remain after twenty-four hours in the digestive tract of the fly, thus indicating that such extremes are physiologically adapted to resist unfavorable conditions, while the intermediate forms are killed off. It is intimated that such resistance may be interpreted as indicating two physiological grades, which may be identified as male and female. This conclusion, however, is weakened by the fact that intermediate forms reappear during the second day. Doflein's ('09) criticism that such size differences may represent young and old individuals is certainly to be considered. Moore and Breinl describe very remarkable forms of *Tryp. gambiense*, in which the kinetonucleus grows out into a long rod reaching to the nucleus. Such forms recall Prowazek's "male" of *Tryp. lewisi*, but the English observers hold that it indicates the preparation for union of a part of the rod with the nucleus, *i. e.*, a type of autogamy.

While it is quite obvious that the last word has not yet been written in regard to such trimorphism in trypanosomes, there is no doubt at all of the form changes, and it is highly probable that some of them, at least, are characteristic of different periods in the life history and that some, at least, are gametes. Further than this, the evidence at the present time does not warrant generalizations.

The encysted stages of trypanosomes are particularly interesting as an important phase in the life history whereby the organisms are able to withstand unfavorable conditions. The first observations were made by Minchin ('07) in connection with *Tryp. grayi* in the posterior region of the gut of *Glossina palpalis*. The flagellum is retracted and a slime cyst similar to that described by Prowazek in *Herpetomonas muscæ domesticæ* secreted. The last trace of the flagellum disappears and the nucleus fragments into chromidia, while the kinetonucleus is no longer demonstrable. The cyst wall becomes more definite and resistant, changing the while from an ellipsoidal to a spherical form. Internal changes were not seen beyond evidences of division observed in a few cases. It may be suggested here that chromidia formation and disappearance of nuclei and subsequent division of a nucleus in the cyst may indicate a method of autogamous fertilization similar to that occurring in entameba.

The "latent bodies" described by Moore and Breinl ('07) are entirely different from encysted forms such as Minchin describes, and different from the encysted forms of *Tryp. gambiense* which they themselves describe as being formed after the action of atoxyl in the blood. These cysts are much larger than the latent bodies and similar to ordinary cysts which free flagellates secrete under abnormal conditions. These "latent bodies," which Moore and Breinl regard as the same things seen by Rodet and Vallet, Plimmer and Bradford in infections with *Tryp. brucei*, and by Lingard in the blood of cattle

infected with *Tryp. indicum*, or Holmes in connection with *Tryp. evansi*, are regarded as normal stages in the life history of the organism. From the meagre account of the English observers these appear to be nothing more than the nucleus of the cell with a very small layer of protoplasm about it; in rats they become stored up in the spleen and bone marrow, and the authors believe that they ultimately give rise to adult organisms in much the same way that crithidia or herpetomonas is metamorphosed from the resting stage into a flagellate. Without further evidence such phases may be interpreted as special reactions to abnormal conditions rather than stages in the ordinary life history.

FIG. 102

Trypanosoma gambiense; stages in longitudinal division. Original from a preparation by F. W. Baeslack.

D. Reproduction.—Reproduction by division is easily observed in all types of trypanosomes, and seems to follow a similar method throughout, the details varying in some cases. As in herpetomonas, it is inaugurated by the division of the kinetic elements of the cell, the flagellum dividing first, according to some observers (*e. g.*, *T. gambiense*, according to Minchin), the kinetoplast dividing before the nucleus, the latter dividing as does the centronucleus of free flagellates. Abnormal division figures are frequently observed, due to the division of nuclei and the formation of new flagella before the cell body splits. As in spirochetes, the daughter cells in the last stage of division are connected only at one end—in this case the anterior or kinetoplast

end—and, seen alone, such a stage might be wrongly interpreted as transverse division (Fig. 102). Very often the cells divide without becoming entirely separated, repeated divisions following one another until rosettes are formed. A very remarkable process of multiple division was described by Dutton, Todd, and Tobey ('07) in *Tryp. loricatum*, a parasite of African toads and frogs; here the organism, by repeated binary division, gave rise to more than forty cells, "all apparently inside the outer covering of the original trypanosome" (op. cit., p. 312). Such a process of multiplication is quite novel for trypanosomes, and needs confirmation.

E. Agglomeration.—Rosettes due to incomplete division are quite different from the aggregations of trypanosomes known as agglomerations, which are due to abnormal conditions of the environment, or, as Laveran and Mesnil first observed, may be phenomena due to decreasing vitality. It may be brought about in the blood by mixing immune serum with the normal infected blood, by addition of weak chemicals (*e. g.*, acetic acid), by lowering the temperature, or by conditions arising in artificial culture media, Novy and MacNeal finding agglomerations of more than a thousand cells at times.

F. The Invertebrate Hosts and Life Cycle of Trypanosomes.—At the present time nothing can be farther from settled than the happenings within the bodies of invertebrate hosts of trypanosomes, and much unfortunate controversy of an entirely unnecessary character has been filling the pages of medical and scientific journals.

Although mammalian trypanosomes were first observed and described by Lewis, in 1877, for *Tryp. lewisi* of the rat and in 1880 for *Tryp. evansi*, the cause of surra in horses, little importance was attached to them as the causes of disease until Bruce, in 1894, demonstrated the connection between the disease nagana of horses in Africa of unknown etiology, and the tsetse fly diseases of horses. The history of this discovery is best given in his own modest account, while at the same time it reveals the *modus operandi* in establishing the connection between invertebrate host and protozoan parasite. "In October, 1894," says Bruce, "when serving in Natal, South Africa, the governor of that colony, the Hon. Sir Walter Hely-Hutchinson, G.C.M.G., asked me to go to Zululand to report on a disease which was causing a severe loss among the native cattle. The native name of the disease was nagana. At this time no suspicion that nagana and the tsetse fly disease were identical was entertained. The writer at once proceeded to Zululand, and after a month's travelling by ox wagon from Eshowe, the capital of the country, arrived in the infected area. A small laboratory having been set up and some of the affected cattle obtained from the surrounding natives, examination by the ordinary bacteriological methods was begun. The animals were emaciated, with staring hair, some fever, and sometimes edema of the subcutaneous tissues of

the neck. Examination of the blood and organs for bacteria by microscopic and cultural methods produced no result. At this time it was my custom, when starting on a study of a new disease, to make a careful daily examination of the blood of the living animal, enumerating the number of the red and white blood corpuscles and estimating the percentage of the various varieties of leukocytes. After a few days of this blood examination it was noted that there were sometimes to be seen a peculiar stained body, having something of the appearance of an artistic dolphin, lying among the red blood corpuscles. It must be remembered that the trypanosomes are usually found in very small numbers in cattle, so that it is only after a long search that a single one can be found. It was thought at first that this small, peculiarly shaped object was an accidental appearance due to the stain, but thinking that if the body was a parasite, it might show motion, several specimens of fresh blood were examined. A long search was rewarded by finding a very active body wriggling and twisting about with great energy and dashing in and out among the red blood corpuscles. It was the first time the writer had seen a trypanosome, and, as then there was little or no literature on the subject of these parasites, it was difficult to know how to place it. It seemed it must be a filaria, but having compared the description and drawing of the rat trypanosome in Lewis' book with my parasite, it was concluded it was a trypanosome. But there was no proof that the parasite was the cause of nagana; it occurred only in small numbers in the blood of the cattle, and the rat trypanosome lives as a harmless guest in healthy animals. Therefore the blood of infected cattle was inoculated into horses and dogs. The disease in the horse and dog is much more acute than in the ox.

“In a few days the blood, especially of the dog, was found to be teeming with thousands of trypanosomes. It therefore began to appear probable that this parasite might be the cause of nagana. At that time there was no suspicion that this disease among the native cattle, occurring in kraals situated many miles from the ‘fly country,’ was the same disease as that known to travellers as the tsetse-fly disease. The work at this time was being done on the summit of a mountain called Ubombo, some 2000 feet above the surrounding low country. The low country to the east of the mountain was known to be infected with the tsetse fly, and having often read, in Livingstone's and other books of travel and hunting, about this disease, it was determined to take a few animals into this ‘fly country’ and see what the disease was like. Two young oxen, a horse, and several dogs were taken into the heart of the ‘fly country.’ After being there a fortnight the animals were brought back to the top of the mountain and examined in the usual way—their temperature taken, their blood examined, and any symptoms that might occur noted. It was found that the blood of these

animals affected with the tsetse-fly disease contained the same parasite as that found in nagana. In this way, after many experiments and many observations, it was forced upon me that the two diseases, nagana and tsetse fly, were one and the same. It is a characteristic of this species of tsetse fly, *Glossina morsitans*, that at rare intervals, probably due to long-continued drought, it overspreads its usual bounds to a distance sometimes fifty or sixty miles, and so sets up an epidemic among the native cattle in a previously healthy district. This was the case in 1894; the disease had overspread its natural bounds and given rise to a widespread epidemic among the cattle to a distance of sixty miles.

"When it was once established that the two diseases were the same, experiments were made to find out how the animals became infected, whether the fly was the carrier or the mere concomitant of the low-lying, unhealthy district, and, if a carrier, if it was the only carrier of the disease from sick to healthy animals. Horses taken down into the 'fly country,' and not allowed to feed or drink there, took the disease. Bundles of grass and supplies of water, brought from the most deadly parts of the 'fly country' to the top of Ubombo and there used for fodder for healthy horses failed to convey the disease. Tsetse flies caught in the low country and kept in cages on top of the mountain, when fed on affected animals, were capable of giving rise to the disease in healthy animals up to forty-eight hours after feeding. Tsetse flies brought up from the low country and placed straightway upon healthy animals were also found to give rise to the disease. The flies were never found to retain the power of infection for more than forty-eight hours after they had fed upon a sick animal, so that if wild tsetse flies were brought up from the low country, kept without food for three days, and then fed on a healthy dog, they never gave rise to the disease. In this way it was proved that the tsetse fly, and it alone, was the carrier of nagana. Then the question arose as to where the tsetse flies obtained the trypanosomes. The flies lived among the wild animals, such as buffaloes, koodoos, and other species of antelopes, and, naturally, fed on them. It seemed that, in all probability, the reservoir of the disease was to be found in the wild animals. Therefore, all the different species of wild animals obtainable were examined both by the injection of their blood into healthy susceptible animals, and also by direct microscopic examination of the blood itself. In this way it was discovered that many of the wild animals harbored this trypanosome in their blood. The parasites were never numerous, so that it was only after a long search that they could be discovered by the microscope alone. The wild animals did not seem to be affected by the trypanosomes in any way; they showed no signs or symptoms of the disease, and it, therefore, appeared probable that the trypanosomes

lived in their blood as harmless guests, just as the trypanosome of the rat lives in the blood of that animal."¹

In a very similar way the cause of human trypanosomiasis, *Trypanosoma gambiense*, was shown to be transmitted by another tsetse fly, *Glossina palpalis* (Fig. 103). Dutton, whose own life was the first to be martyred in the cause of sleeping sickness, gave the name to this trypanosome, which was first seen by Forde, in 1891, in the blood of victims of gambia fever. Castellani ('03), later, found trypanosomes in five cases of sleeping sickness in the cerebrospinal fluid, and in one of these cases, also, in the blood. This organism was regarded by

FIG. 103

Glossina palpalis, Rob. $\times 3\frac{1}{4}$.

Castellani as different from all others and named by him *Tryp. ugandense*. Bruce, in the same year, confirmed these observations of Castellani, and also those of Dutton and Todd on gambia fever, and succeeded in demonstrating that the latter is only the first phase of sleeping sickness, and that the trypanosome is conveyed to man by only one agent, a species of tsetse fly.

Confirmatory observations followed rapidly, English, German, French investigators risking their lives in scientific rivalry to get at the life history of this protozoan pest and its insect carrier. Tulloch's

¹ Bruce, Trypanosomiasis, Osler's Modern Medicine, pp. 462 to 464.

life was a second English sacrifice to this end, and his own observations, together with those of Todd, Koch, Brumpt, Greig, Gray, Minchin, Nabarro, and a host of others, have made *Trypanosoma gambiense* one of the best known of all mammalian trypanosomes.

In the meantime other students of the protozoa were showing the connections between different species of vertebrate trypanosomes and invertebrate transmitting forms, so that today not only biting flies, but mosquitoes, lice, and leeches are known to carry trypanosomes from one vertebrate host to another, while only one case of direct transmission from animal to animal has been demonstrated. This is of considerable interest, as showing the power of trypanosomes to penetrate membranes, the organism *Trypanosoma equiperdum* being trans-

FIG. 104

A tsetse fly (*Glossina longipennis*, Corti, from Somaliland) in resting attitude, showing position of wings. ($\times 3\frac{1}{2}$.)

mitted by coitus, and thus giving rise to the disease dourine or mal de coit. Koch and Doflein ('09) suggest that sleeping sickness may be transmitted in the same way.

Very great importance attaches to the happenings within the body of the blood-sucking host, and here the matter is still in the whirl of controversy. Bruce states that in the hundreds of tsetse flies examined by him he has never found different stages of the parasite in the digestive tract and no indication whatsoever of migration into the body cavity of the fly. He regards the fly as a mere passive carrier of the protozoön, transmitting the disease during a limited period, by inoculating the victim with trypanosomes adhering to the proboscis either inside or out. In this he is supported by Koch, Moore and Breinl, Novy, Roubaud, and a host of others, who note that the organisms

disappear from the digestive tract of the fly within three or four days after feeding. Others, on the other hand, notably Gray, Minchin, Tulloch, have found abundant multiplicative forms in the anterior part of the digestive tract, and encysted forms in the posterior part (proctodeum). These observers hold, and many others on a priori grounds alone support them, that important developmental stages of *Tryp. gambiense* will yet be found outside of the human body. That such an external life is obligatory for trypanosomes in general is disproved by the fact of direct transmission in the case of *Tryp. equiperdum*, where all of the developmental phases must take place in the mammal.

A very strong argument in favor of the advocates of an external cycle are the observations, by different investigators, of the life history of trypanosomes infecting other than mammalian hosts. Keysseltz ('06), for example, found both multiplicative and propagative (terms used in Doflein's sense) development of *Trypanoplasma borreli* in the digestive tract of the leech *Piscicola geometra*; Prowazek ('05) found similar phases of *Tryp. lewisi* in the gut of the louse *Hematopinus spinulosus*; but these, and all of the subsequent observers, go back to the classical work of Schaudinn ('04) upon *Tryp. noctuæ* of the owl for their models, a work fully confirmed by the brothers, Et. and Ed. Sergent ('05).

The mosquitoes used by Schaudinn and by the Sergeants were raised from eggs and larvæ, so that previous infection was thereby excluded, the chances of their being infected by inheritance, which Novy, MacNeal, and Torrey ('07) claim in criticism, being so remote that the results are by no means vitiated by this possibility.¹

Mosquitoes which are allowed to feed upon owls (*Glaucidium noctuæ*) infected with *Tryp. noctuæ* take male and female trypanosomes into the gut with the blood. Here fertilization takes place in the manner described by MacCallum ('99), in connection with the parasite *Hemoproteus* (*Halteridium*) of the American crow. The so-called halteridium, therefore, of the owl is only a stage in the life history of a trypanosome, the microgametes being formed in response, apparently, to the changed conditions of temperature and chemical composition in the new environment. The fertilized gamete, called oökinete, or copula, by Schaudinn, develops into a trypanosome which may be male, female, or indifferent, according to the changes undergone by

¹ Schaudinn (loc. cit., p. 390) states: Die Zucht der Mücken, die Art der Infection, die Blutuntersuchungen usw. erfolgte in derselben Weise wie bei meinen Malariastudien. For the latter work he made use of carefully watched mosquitoes bred from the egg. Knowing from personal experience Schaudinn's keen zoölogical sense, quickness of vision, and remarkable talent in handling protozoa of various kinds, I personally do not share in the skepticism which has grown up in regard to his observations, and, although not always agreeing with his interpretations, I find much more reason for accepting his conclusions than those of his many critics which are based mainly on a priori arguments or upon negative results with artificial culture methods, which, at best, are unnatural media for protozoa.

the nucleus and cytoplasm (see above p. 256). The males and females appear to lose the power of division, but, like indifferent forms, have the power of penetrating epithelial cells of the gut and making their way to various parts of the insect's body, including even the ovaries. Under conditions of extreme cold and starvation of the insect, all stages of the trypanosome die save these females, which appear to have a remarkable power of resistance, and Schaudinn suggests that they may be retained in the ovaries of the hibernating mosquitoes until spring, when they may develop and infect the new generation. It is in these forms that parthenogenesis occurs (see p. 168). The power of changing, as crithidia does, from a free, flagellated, into a quiescent parasite, not only in the gut of the mosquito, but also in the blood of the bird, is a feature known to occur in no other trypanosome. According to Schaudinn and Sergent the intracellular parasite is the typical form of the organism during the day, while it leaves the blood cell, changes into a typical trypanosome, and grows during the night, the change being induced, as Schaudinn believed, by the lowered temperature of the bird at night.

Although "latent bodies," encysted forms, and other non-flagellated stages of trypanosomes have been observed by Moore and Breinl, Minchin, Robertson, Laveran and Mesnil, and others, this is the only case of trypanosomes known where, as in *Herpetomonas donovani*, the flagellated organism becomes an intracellular parasite. The phenomenon must be interpreted zoologically, as an indication of the more evolved phylogenetic state of *Tryp. noctuæ* and leading to the group hemosporidia of permanently intracellular blood parasites. In our opinion these facts do not justify the use of a different generic name for *Trypanosoma noctuæ* as Lühe proposes, but are only further evidence of the tendency to polymorphism exhibited by the group as a whole.

The Effects of Trypanosomes on Vertebrate Hosts.—The great majority of trypanosomes, especially the parasites of cold-blooded forms, have no evident effect upon their hosts. But among warm-blooded animals they rank with the most deadly parasites known. The horse, mule, and dog always succumb to infections of *Tryp. evansi*, the cause of surra, while cattle, camels, etc., are less affected (Lühe). The organism of nagana, *Tryp. brucei*, is fatal to horses, dogs, and cattle, and that of mal de caderas (hip sickness) is fatal to horses, rats, and mice. On the other hand, the rat trypanosome, *Tryp. lewisi*, and the cause of galzielte (gall-sickness) in cattle, *Tryp. theileri*, are relatively harmless. Immunity, in some cases, is set up by one invasion of the parasites, wild animals, as Bruce has shown, being immune to *Tryp. brucei*, which quickly kills imported animals. Laveran and Mesnil ('02) showed that immunity was conferred on rats by one infection with *Tryp. lewisi*.

Human trypanosomiasis is particularly malignant, having a fatality

of 100 per cent. According to Bruce ('05) the disease is rapidly spreading, now that Africa is being opened up. In regard to this he says: "This sleeping sickness, which occurs on the west coast of Africa, particularly in the basin of the Congo, has within the last few years spread eastward into Uganda, has already swept off some hundreds of thousands of victims, is spreading down the Nile, has spread all around the shores of Lake Victoria, and is still spreading southward around lakes Albert and Albert Edward." (*Science*, 1905, vol. xxii, p. 298.)

Just how the ill effects are produced is not known. There is evidence, supported by the facts of acquired immunity in other forms, that a toxin is produced causing more or less chronic inflammation, or rapid destruction of erythrocytes. In man it produces a gradually increasing lethargy, with mental and physical degeneration, rapid pulse, increasing emaciation, all finally resulting in marked drowsiness, which passes into a state of coma ending with death (Fig. 105). Mott ('99)

FIG. 105

Sleeping sickness; shortly before death.

explained the lethargy as due to the action of some toxin, probably of microorganism derivation, in the cerebrospinal fluid and acting on the neurons. Bruce regards the disease as essentially "a disease of the lymphatic system, and the irritation and proliferation of the lymphocytes is probably due to a toxin secreted by, or contained in, the bodies of the trypanosomes. The characteristic symptoms of the disease are, no doubt, due to the accumulations of these lymphocytes in the perivascular spaces of the brain, compressing the arteries and so interfering with the normal nutrition of the brain cells." (Bruce, 1907, p. 483.)

It is still too early to speak of a cure for human trypanosomiasis, and it is outside the limits of this work to enter into a discussion of the various attempts that have been made to cure. The preliminary success with atoxyl, alone or in combination with other salts, gives reason to expect an ultimate control over the disease.

CHAPTER IX.

THE PATHOGENIC HEMOSPORIDIA.

MANY recent students of the protozoa (*e. g.*, Hartmann, Lühe) are inclined to place the group of parasites which Danilewsky ('85) named hemosporidia with the mastigophora rather than with the sporozoa. It is possible that future research will justify this step, and that the large, relatively immobile blood parasites, like *Lankesterella* of the frog, hemogregarina of turtles and tortoises, karyolysis of lizards, hemoproteus of birds, and plasmodium of man, are, like the Leishman-Donovan bodies, only passing phases of some flagellated protozoan, but at the present time the evidence is not weighty enough to warrant such a step even as a working hypothesis. The weakness of the evidence, apparent as soon as reviewed, may be briefly summarized as follows: *Trypanosoma noctuæ* has an intracorpuseular cytozoic phase; *Herpetomonas donovani* has an intracorpuseular cytozoic phase; babesia (*Piroplasma*) a genus whose several species infect erythrocytes of various mammals, at certain periods possesses a blepharoplast (?) and gives rise to so-called "flagella;" merozoites of *Plasmodium vivax* and of hemoproteus are said to show at times rudimentary flagella (Hartmann).

Evidence is constantly accumulating, on the other hand, to show that the full life history of hemosporidia may be completed without any sign of a flagellated stage. Such is the case, for example, in Hintze's account of the life history of *Lankesterella ranarum*, while the incomplete accounts in cases of other hemosporidia give no ground for assuming the occurrence of such a stage. The carefully studied life history of a new genus and species of hemogregarinidæ, *Hepatozoön perniciosum*, Miller, of the rat, gives the best evidence of the independent position in classification of these forms. This organism, discovered by W. W. Miller,¹ somewhat resembles *Leukocytozoön canis*, Bentley, of Indian dogs. In the majority of cases it causes death of the infected rat, the disease being normally transmitted by mites of the species *Lelaps echidninus*. The sporocysts are taken into the digestive tract of the rat together with its mite host, and the sporozoites (16 μ long) are liberated by the action of the digestive juices (Fig. 106). The young forms penetrate the intestinal walls and enter

¹ The premature death of this gifted young observer, his life a sacrifice to duty, was a sad blow to the cause of protozoölogy in America.

the blood stream, which conveys them to the liver (Fig. 106, *a* to *d*). Here they enter the liver cells and undergo schizogony, about 16 merozoites being formed. These may enter other liver cells or pass into the blood stream, where they are taken up by large mononuclear leukocytes in which they remain protected by a distinct membrane or cyst (Fig. 106, *f*). If such infected blood is taken by a mite, the encysted parasite is set free in the insect's digestive tract. Two similar ones conjugate in the lumen of the gut (Fig. 106, *g* to *k*) and a motile ookinete penetrates the stomach wall and gets into the body cavity. In the body tissues the fertilized cell rapidly increases in size, the fertilization nucleus divides a number of times, and the daughter nuclei migrate to the periphery of the cell, where they lie in minute papillæ on the surface. The papillæ enlarge and grow into sporoblasts, each of which ultimately gives rise to about sixteen sporozoites (Fig. 106, *o*, *p*). Mature parent cysts contain from 50 to 100 of such sporocysts. When such an infected mite is swallowed by a rat the sporozoites are liberated and the cycle completed.

Trypanosoma and Leishman-Donovan bodies (*herpetomonas donovani*) are acknowledged flagellates, but babesia and a fortiori plasmodium, and other hemosporidia, stand very far removed from such more primitive forms, and although there is good reason to believe that hemosporidia and, through them, coccidiidia have been derived from mastigophora, to classify them as such would be unwarranted. The so-called "flagella" of babesia have little in common with this characteristic motile organ of the flagellates, and Doflein's, Nuttall and Graham-Smith's, and Kinoshita's view that they may be microgametes, although not demonstrated in any case, seems much more plausible and will remain so until the process of fertilization is fully known. The method of microgamete formation in plasmodium gives rise to reproductive bodies which are strikingly similar to the so-called flagella of *Babesia canis*, as described by Bowhill and Le Doux ('04), Nuttall and Graham-Smith ('04-'07), and especially by Breinl and Hindle ('08), who find two "flagella" appearing successively. The long history of the "polymitus" form of plasmodium should be a warning against premature conclusions regarding these structures. The process of sporulation in plasmodium and in *Babesia canis*, according to Christophers ('04), in the bodies of the invertebrate hosts is entirely different from reproduction in pathogenic flagellates, while save for the absence of spore cases, it conforms exactly with the sporozoan type. For these reasons, therefore, I believe it premature to separate the hemosporidia from sporozoa, but recognize the phylogenetic possibilities indicated by such a series as *herpetomonas*, *crithidia*, *trypanosoma*, *babesia*, *hemoproteus*, and *plasmodium*.

A. The Genus Babesia.—Smith and Kilborne ('93) found peculiar minute parasites in the red blood corpuscles of cattle sick

FIG. 106

Hepatozoön perniciosum, Miller, a hemosporidian parasite of the rat. (After Miller.) *a* to *d*, development of the schizont in the liver cells of the rat; *e*, free parasites in the blood; *f*, encysted parasites in lymphocytes; *g* to *k*, stages in conjugation of isogametes; *l*, *m*, *n*, growth of the ookinet into sporont; *o*, sporocyst derived from the ookinet, with sporoblast buds covering the surface; *p*, section of same; *q*, older sporoblast with sporozoites; *r*, a single sporozoite. Stages *g* to *r* are formed in the tissues of the intermediate host, a mite, *Lelaps echidninus*.

with "Texas fever." These were so often found in pairs that the specific name *bigeminum* was given to them, while the new genus was named pyrosoma. The latter name, however, having been long used for a genus of tunicates, was changed to piroplasma by Patton ('95), and is still widely used. Starcovici, however, in 1893, gave the name babesia to a blood parasite of European cattle which Babes first described in 1888 under the name of *Hematococcus bovis*. This organism appears to be the same as that found by Smith and Kilborne, and if proved so by the full life history the organism of Texas fever must have the specific name *bovis*, while, since hematococcus is the generic name of a phytoflagellate, Starcovici's name babesia must supplant Patton's piroplasma.

FIG. 107

Stages in the development of *Babesia canis*. (After Kinoshita.) A, round discoid parasite in a blood corpuscle; B, ameboid form with long processes; C, a pair of "mature gametes"; D, a mature "female" gamete; E, a mature "male" gamete; F, a budding form in blood corpuscle; G, a group of sixteen young "gametes."

Subsequent observers have found babesia in many different animals. R. Koch ('03) was sent by the German Government to investigate a cattle disease which he called East Coast fever, in German East Africa, and the organism causing it was named (piroplasma) *Babesia parvum* by Theiler, in 1904. Babes ('92) discovered a blood parasite in Roumanian sheep which he named *Babesia ovis*; Piana and Galli Valerio ('95) discovered a similar parasite in the blood of dogs, naming it (piroplasma) *Babesia canis*; Gugliemi ('99) found a blood parasite in horses, Laveran ('01) naming it (piroplasma) *Babesia equi*; Fantam ('05) discovered one in the blood of rats and called it (piroplasma) *Babesia muris*. Similar parasites have been found in monkeys

(Ross, 1905), in goats, horses, and asses (Ziemann), and in man (Wilson and Chowning, 1901; Anderson, 1903).

In all cases the medium of transmission, where known, is some species of tick, and with their discovery of this important function of tracheates, Smith and Kilborne ('93) opened up a new era in the history of preventive medicine, a discovery followed by the brilliant work of Bruce with trypanosomes and flies; of Ross and Grassi with malaria organisms and mosquitoes; and of a host of other investigators upon blood parasites in all kinds of animals.

Structural Characteristics.—Unlike *Herpetomonas donovani* an endothelial parasite, or unlike the serum-dwelling forms of flagellates generally, the various species of babesia are intracorpuseular parasites, although at periods they may become free in the serum. The general form is spherical or pear-like (whence the names piroplasma and piroplasmosis), the size varying from 0.5μ (Smith and Kilborne for *B. bovis*) to 5μ (occasionally in *B. canis*, according to Nuttall and Graham-Smith). As a rule, they are single in the blood corpuscles in peripheral blood (50 to 76 per cent., according to Graham-Smith, in dogs with *B. canis*), although double infection, arising usually by division of the parasite, occurs in from 20 to 30 per cent. Such double ones were regarded as characteristic by Smith and Kilborne, who suggested the name "bigemina" for the organism of Texas fever (*Babesia bovis*).

The parasite of dogs, *Babesia canis*, has been more thoroughly studied than any other form, and furnishes a good object for general description. It has been monographed by Nuttall and Graham-Smith ('05-'07), by Kinoshita ('07), by Bowhill and Le Doux ('04), by Christophers ('07), and by Breinl and Hindle ('08), so that, although the various observers are not always in agreement, nor the life history in any case complete, there is a good basis of facts for others to work on.

According to all observers, the living parasite is very active, throwing out processes of pseudopodial nature at various points of the periphery, and with such vigor "as sometimes to move the corpuscle in which the parasite is situated" (Christophers). Sometimes these protoplasmic processes are drawn out into long filaments resembling flagella (Fig. 107), while crescents, ring forms, triangles, etc., are forms assumed at one time or another, the greatest activity being shown during the febrile state (Nocard and Motas).

The nucleus of the cell, like that of plasmodium, is of indefinite shape, consisting of chromatin granules arranged in rod, ring, or semi-circular form, the size and form of the aggregate giving an indication of the developmental period (Kinoshita). The nucleus is usually excentric in position, becoming flattened at times against the periphery of the cell, but in free forms it usually lies in the centre of the parasite (Christophers).

In addition to the nucleus there appears to be a second brightly staining structure in the cell, which Schaudinn ('04) first drew attention to from blood smears made by Kossel and Weber from cattle with Texas fever, and regarded as a blepharoplast by Lühe, Nuttall and Graham-Smith, Christophers, and others who have worked with these forms. Nuttall and Graham-Smith, in addition, described a third chromatin structure as a reticulate and faintly staining mass of chromatin lying close to the nucleus, but Christophers and Kinoshita give evidence to show that this is but a part of the nucleus (Fig. 108).

FIG. 108

Babesia (*Piroplasma*) *canis*. (After Christophers.) Different stages in the erythrocyte culture media, and, I, in embryonic salivary cell of tick *Rhipicephalus sanguineus*.

A number of investigators have attempted to cultivate babesia on artificial culture media, a limited success only being obtained, the chief result being merely the prolongation of life of the parasites in the infected blood kept under proper conditions. In this way Christophers and others obtained various morphological changes in the organisms, but no developmental processes. Kleine ('06) and Miyajima ('07), on the other hand, claim to have produced many developmental stages in vitro, the former with *Babesia canis* in young dog's

blood, the latter with *B. parvum* in blood bouillon. In each of these experimental cultures the forms assumed were highly different from those in the blood. Kleine noted long protoplasmic filaments similar to those seen by Koch ('06) in developmental stages in the tick, while Miyajima gives descriptions and photographs of crithidia-like flagellates, "five times the diameter of an erythrocyte" in length, which he found in cultures and subcultures of *Babesia parvum*. Schaudinn ('04) apparently found similar flagellated forms in smears of fresh blood from cattle infected with Texas fever. The significance of these flagellates, which, according to Miyajima, reproduce by longitudinal division, cannot be interpreted until further observations and confirmations are made.

The parasites reproduce by division while in the erythrocytes, and thus form the typical twin forms characteristic of babesia, or larger groups of from four to eight cells. Kinoshita describes an irregular division or budding process which he regards as equivalent to schizogony and merozoite formation in plasmodium (Fig. 107, *F*). Christophers, however, does not confirm these findings, but describes the nucleus as dividing by a modified mitosis (Fig. 108, *D*), or in some cases the blepharoplasts divide and the chromatin flows about the daughter halves, which then push out from the periphery of the cell as buds. The buds thus formed are not pinched off, as Kinoshita describes, but the protoplasm of the cell flows into them in equal parts and the cell divides by fission. In some cases further division of the daughter cells begins before complete separation. The relative infrequency of multiple forms in erythrocytes¹ is an argument against Kinoshita's view that this represents schizogony.

B. Transmission by Ticks and the Life Cycle of Babesia.—

Since Smith and Kilborne's epoch-making discovery of the tick as the sole agent in the transmission of babesia, observations have accumulated in regard to intermediate hosts and developmental changes of the parasites in them, but, notwithstanding the number of observations made, the life history of no form is yet known.

The mechanism of transmission by ticks is often very complicated; according to Smith and Kilborne and Curtice, the insect becomes sexually mature at its last moult while hanging to the skin of the ox. In this condition the females are fertilized, gorge themselves with infected blood, and drop to the ground, where they lay an enormous number of eggs (up to 2000). Each egg case is supplied with a small quantity of ox blood, which serves as food for the larva. The latter, very much undeveloped, crawls upon a blade of grass, and if it manages to attach itself to the hair of another ox it will live; if not, it dies of starvation.

¹ Erythrocytes with four parasites number from 2 to 5 per cent. of all infected corpuscles; with eight, less than 0.05 per cent., and with sixteen, from 0.004 to 0.01 per cent., according to Graham-Smith and Christophers (1907).

The larva changes into the adult form on the ox and transmits the disease with its first feeding.

Lounsbury ('04), after three years of experimentation and observation in South Africa, proved that *Babesia canis* of the dog is conveyed by the tick *Hemophysalis leachi*, and not, as in Texas fever, by the larva from infected ticks, but only by adult ticks reared from the eggs of infected ticks. Later, Christophers ('05-'07) demonstrated that another tick, *Rhipicephalus sanguineus*, Latr., is also capable of transmitting the disease, and he believes this to be the primary agent in transmitting European dog piroplasmosis. According to Christophers, the larvæ or the nymphs, as well as eggs, may become infected directly from the dog, and so may carry the disease into the later developmental stages of the insect. The latter means of transmission seems to be the only one in the case of East Coast fever, where, according to Lounsbury ('04) and Theiler ('05), the parasite (*Babesia parvum*) can be conveyed only by the larva which becomes infected, the infection being carried to and transmitted by the nymph, while infected nymphs convey the infection to adults. The variations in regard to the mechanism of transmission, especially the time factor, indicate that obligatory changes in the life history take place in the insect's body. What these changes are must be very difficult to ascertain, because of the minute size of the parasite. The first observations to this end were made by Koch ('05) upon the organism of East Coast fever in the digestive tracts of different ticks (*Rhipicephalus australis*, *R. evertsi*, and *Hemaphysalis egyptium*). Here they become stellate in form and often appear in couples, a circumstance leading Koch to surmise some type of conjugation. Globular and peculiar club-shaped forms were also observed, but their significance was not made out (Fig. 108, E).

Evidence is accumulating to show that, as in the case of *Plasmodium malariae*, a sexual cycle takes place in the tick, and it is not oversanguine to state that the various conflicting observations on "flagella" and other structures formed by the parasite at different stages will shortly be straightened out in a consistent life history.

First, as to the so-called "flagella." Leaving out of account Miyajima's unconfirmed observations on a crithidia-like stage of *Babesia parvum*, there are repeated references to flagella formation, especially in the case of the dog parasite *Babesia canis*. Here the descriptions by Bowhill and Le Doux ('04), Nuttall and Graham-Smith ('06), Kinoshita ('07), and Breinl and Hindle ('08) are in agreement with the observations of Lignières ('03) on *Babesia parvum*, and with Fantham ('05) on *Babesia muris*. According to Kinoshita, the flagellum which he, with Doflein and many others, interprets as a microgamete invariably takes its origin from the blepharoplast (Fig. 107, C, E). It is not smooth and uniform, like a flagellum, but possesses granular

thickenings. He did not find more than one of these processes from the same cell, but Breinl and Hindle ('08) describe typical flagella, which appear to have no definite or constant place in reference to one another or with the cell. The latter observers, while stating that these "flagella" are formed only during a very transient phase in the life history, do not offer any interpretation in regard to them. Christophers ('07) failed to find flagellated forms either in vivo or in vitro.

Second, as to the so-called "club-shaped bodies" first observed by Nuttall and Graham-Smith ('06) and recently followed out by Christophers in *Babesia canis* in the tick *Rhipicephalus sanguineus*. These characteristic bodies have been found only in the insect's body where they give rise by direct metamorphosis to what Christophers does not hesitate to call "zygotes" or fertilized cells, although nothing in the nature of fertilization and nothing resembling gametes were described by him. Two varieties of this club-shaped body are described, one being "rigid, thorn-like," and relatively inactive; the other more "leech-like" and active. Curious disks, with or without short spines, and with the appearance of boring organs, are present at one end. Christophers states that these bodies may reproduce by longitudinal division, the daughter cells remaining attached so as to give the appearance of conjugation. They are found not only in the gut of infected ticks, but also in oviducts, ovaries, and ova of the adult, while in nymphs they may be spread throughout the tissues of the body. The "zygotes" formed by metamorphosis of these club-shaped bodies are intracellular parasites of oval or spherical form, and may grow to the size of 25 μ . The chromatin becomes diffused throughout the cell prior to the formation of reproductive centres which Christophers regards as sporoblasts, and the zygote ultimately gives rise to "sporozoites" similar to the intracorporeal forms in dog's blood.

Nuttall and Graham-Smith interpreted these club-shaped bodies as gametocytes, a view confirmed by Christophers, whose account certainly suggests a sexual cycle in the tick. If this account is confirmed, the life history of *Babesia canis* is very similar to that of plasmodium. The rarity of "flagellated" stages and their occurrence only at late stages of infection certainly point toward Dofflein's original view that the "flagella" are microgametes, a view which the majority of subsequent investigators have accepted. Dofflein and, later, Kinoshita maintained that there is a cyclical difference between the ameboid forms and the pyriform bodies, the former representing the schizogonous cycle, the latter the sexual. The prevalence of the pyriform bodies at the end of the disease in infected animals, and the formation of "flagella" from them, lends support to this hypothesis.

Babesia in man gives rise to an acute disease variously designated as "blue fever," "black fever," "tick fever," "spotted fever," "piroplasmiasis hominis," and the like. It appears to be local in distribu-

tion, occurring during the spring and early summer in the high valleys in the mountains of Montana and Idaho. The disease is conveyed to man by the bite of ticks (*Dermacentor reticulatus occidentalis*), and may be transmitted by them to rabbits, guinea-pigs, and monkeys as well (King, 1906; Ricketts, 1906), while the experimental animals show a high degree of immunity after one attack of the disease.

Unfortunately, authorities do not agree as to the cause of the disease. The transmission and general course of the disease, enlargement of the spleen, immunity, etc., are not against the facts of piroplasmosis, and this was the view taken by Wilson and Chowning ('02), who discovered minute bodies in the erythrocytes of infected blood, both fresh and stained. They named the organism *Pyroplasma* (*Piroplasma*) *Babesia hominis*, and were the first to suggest that ticks were the agents of transmission, while the gopher (*Spermophilus columbianus*) was regarded as the natural host or reservoir of the parasite. Anderson ('03) confirmed the observations of Wilson and Chowning, and noted with them the characteristic ameboid movements of the parasites within the erythrocytes, and the frequent occurrence of twins so characteristic of babesia. Their observations, descriptions, and figures were not convincing, however, and others, notably Stiles ('05), Ricketts ('06), and King ('06), failed completely to find the bodies either in fresh or postmortem blood. Boggs ('07) states that some of Wilson's and Chowning's descriptions and figures resemble blood platelets, while others appear like the "navicular body of Arnold," and like endothelial degenerations of various kinds.

None of Wilson and Chowning's critics have been able to demonstrate any other disease-causing organism, either by bacteriological, pathological, or cytological methods, and their negations or comparisons with previously known bodies, or with structures from that unlimited field of ill-defined possibilities, degeneration forms, cannot offset Wilson and Chowning's positive findings and the collateral evidence, and their "organism" must receive the benefit of the doubt until more definite observations on the cause of Rocky Mountain spotted fever are made. It is certainly interesting, in this connection, that Gotschlich ('03) and other investigators have noted the presence of protozoa in the blood of victims of Egyptian typhus fever, the former describing an "apiosoma" (babesia) in the erythrocytes.

Darling ('08) has recently described similar structures, under the name *Histoplasma capsulatum*, in the blood of natives of tropical America, and in endothelial cells lining blood and lymph vessels, spleen, liver, lungs, and bone-marrow. The symptoms are splenomegaly, emaciation, and irregular remittent temperature. The organisms are characterized by irregular masses of chromatin and an occasional small deeply staining dot which may be a blepharoplast. If the author's surmise is correct, that the organism has a flagellated

phase, his evidence for which is scarcely convincing, then it should be classed with the organism of kala azar (*Herpetomonas donovani*) rather than with babesia.

C. The Organisms of Malaria.—As late as 1896 the cause of malaria and of its mode of transmission were equally little known, while the idea of bad air, from which malaria gets its name, has a long traditional history reaching back to the time of Morton, in 1692 (Craig). The irregularity of infection, the curious sporadic nature of new cases, and the general history of the disease in damp and swampy localities, made malaria, in its several forms, a most uncertain and puzzling disease, the actual cause of which was entirely unknown until 1881, when a French military doctor in Algiers, Dr. Laveran, discovered a new and curious organism in the blood of malaria victims, which he characterized at once and without any misgivings as the cause of the disease. At that time the blood-infesting sporozoa were very little known, Lankester, indeed, having discovered, ten years before, in 1871, a sporozoön in the blood of frogs and a form which he named in 1882, calling it *Drepanidium* (*Lankesterella*) *ranarum*. Laveran did not recognize the possible relationship between the blood parasites of the frog and man, parasites which, in 1885, Danilewsky grouped together under the general name of hematozoa, which finally took the form of the name hemosporidia, but regarded it as a plant organism belonging to the genus *oscillaria*, and he named it *Oscillaria malariae*. The curious interpretation of the organism as a vegetable possibly owed its origin to the fact that the bacteria were being vigorously studied at this period, for we find not only Laveran, but Metchnikoff and Marchiafava and Celli, likewise giving to it a plant name. The latter, in 1885, from its supposed resemblance to some of the plasmodia-forming fungi, gave the malaria organisms the name of *Plasmodium malariae*, while the former, two years later, named them hematophyllum. Laveran's name being untenable, on the grounds of mistaken genus, the next name suggested in chronological order had to be accepted in conformity with the rules of zoölogical nomenclature, and thus it happens that a name which should be used only to designate a condition assumed by certain kinds of organisms (fungi and mycetozoa) has become a generic name.

Laveran's discovery did not attract much attention; indeed, the new organism as the cause of the disease was scarcely accepted by pathologists, and it was not until after 1896 that the real nature of the disease was recognized. Laveran ('91) in France, and Manson ('94) in England, quite independently suggested that the organism is transmitted from man to man by some blood-sucking insect, suggestions which were brilliantly proved, from 1897 to 1899, by Major Ross, an English army surgeon in the India service, and by Prof. Grassi, in 1899, who showed that mosquitoes belonging to the genera *culex*

(for bird malaria) and anopheles (for human malaria) are alone capable of transmitting the disease from host to host. Little by little new facts and discoveries were added, until by 1901 malaria was as thoroughly understood as perhaps any other germ disease, Grassi, in Italy, working out the complete life history of the pernicious type, and Schaudinn, in 1901, the life history of the parasite causing the tertian form of the disease; the latter adding the last link in the chain of evidence by watching the penetration of the sporozoite fresh from a mosquito's

FIG. 109

Temperature variations in tertian malarial fever.

FIG. 110

Temperature variations in quartan malarial fever.

proboscis in his own red blood corpuscles. Malaria was thus the first of the human diseases in which it was proved that a protozoön is the direct cause.

Authorities differ as to the number of kinds of these protozoan parasites responsible for malaria. On the clinical side, also, there seems to be some difficulty in the classification of the fevers due to the different kinds of parasites. Grassi and Laveran have reduced the large number of species that have been described to three, and they believe,

also, that the cause of the pernicious type belongs to a different genus from the others. There seems to be little justification for this increase in number of genera, and I am inclined to follow Schaudinn, Craig, and others in grouping all of the malarial parasites under the one generic name of plasmodium. Associated with these different forms of the organism there are three well-marked types of malaria, while some observers, notably Craig and the majority of the Italian authori-

FIG. 111

Temperature variations in tertian estivo-autumnal fever.

FIG. 112

Temperature variations in quotidian estivo-autumnal fever.

ties, distinguish four such types. The essential features by which they are distinguished are the differences in the rate of development as measured by the time between successive pyrexial attacks. Tertian fever, caused by *Plasmodium vivax*, is characterized by an attack every forty-eight hours; quartan fever, by an attack every seventy-two hours; estivo-autumnal or pernicious fever, by daily or more or less constant fever. The significance of these attacks, first made out by Golgi in 1886, is that they coincide and are caused, therefore, by the sporulation

(schizogony) of the parasites in the blood. Coming from the same original brood, the parasites in the blood all sporulate at the same time; this results in a constantly increasing number of reproductive bodies being liberated at stated intervals. At first the young forms are too few to cause any serious trouble, and there is no reaction on the part of the host. This is the period of incubation (ten to twelve days) of the disease, but with the increase in numbers of merozoites there is a continuous army of invaders, increasing in geometrical progression and entering the blood corpuscles until finally the numbers are incredibly large. With each invasion occurring every day or every three days or every four days, according to the nature of the parasite, there is a marked anemia and poisoning, which tend to produce cachexia and sometimes death. Fever coincides with the liberation of new swarms of merozoites, as shown in the accompanying charts or fever curves (Figs. 109 to 112). At the time of merozoite formation waste matters that have accrued as products of the parasite's metabolism and kept stored up in the body of the parasite are liberated and help in the general intoxication of the victim. These are modified products of hemoglobin digestion on the part of the parasite, and, known as the melanin granules, they are collected from the blood and stored in the liver, kidney, or spleen, or even in the lungs and brain, leading to pigmentation of these organs and frequently to hypertrophy, more especially of the spleen and liver.

The essential differences between the parasites of tertian, quartan, and estivo-autumnal fevers may be briefly summarized as follows:

1. **Tertian Parasite (*Plasmodium vivax*)** (Plate I, Fig. 1).

Young schizonts from 1 to 3 μ up to the size of normal blood corpuscles.

Melanin granules distributed throughout protoplasm or (at schizogony) collected at one point on periphery.

Merozoites, 12 to 24, formed every forty-eight hours. Peripheral circulation (see also Fig. 109).

Ameboid activity very pronounced.

Macrogametes spherical.

Effects slight enlargement of corpuscle.

Incubation period about fourteen days.

2. **Quartan Parasite (*Plasmodium malarix*)** (Plate I, Fig. 2).

Size as above, but never as large as normal corpuscle.

Melanin granules not distributed; collected in zone on periphery.

Merozoites, 6 to 12, formed every seventy-two hours. Frequent in circulation (see Fig. 110).

Relatively quiescent in the corpuscle.

Macrogametes spherical; less numerous than in *vivax*.

Effects no enlargement, frequently shrinkage of corpuscle.

Incubation period about three weeks.

FIG. 1

FIG. 2

Fig. 1. Tertian Malarial Plasmodium. Stained by Oliver's Modification of Wright's Stain. (After Craig.)

1 to 4. Ring forms of tertian parasite.	15 to 17. Segmenting forms within red corpuscle.
5. Ring form. (Conjugation form of Ewing.)	18. Segmenting forms after destruction of red corpuscle.
6 to 10. Pigmented organisms.	19. Microgamete.
11 to 14. Nearly full-grown forms, showing diffusion of the chromatin.	20. Sporozoite.

Fig. 2.—Quartan Malarial Plasmodium. Stained by Oliver's Modification of Wright's Stain. (After Craig.)

1 to 4. Ring forms of quartan parasite.	10 to 12. Segmenting forms of quartan parasite.
5, 6, 7, 8, 9. Pigmented parasites.	13. Segmenting stage after destruction of red corpuscle.

NOTE.—Chromatin of nucleus stained red; protoplasm stained blue; vesicular portion of nucleus unstained.

3. Estivo-autumnal Parasite (*Plasmodium falciparum*) (Plate II).

Young forms alone found in peripheral circulation; very small, occupying from one-fourth to one-half the corpuscle.

Melanin scarce, a few (2 to 3) granules usually central in position.

Merozoites, 6 to 15, formed at twenty-four to forty-eight hour intervals (see Figs. 111 and 112).

Ameboid activity marked, but less than that of vivax.

Macrogamete at first crescentic in form.

Effects slight shrinkage and often crenulation of corpuscles.

Incubation period usually from ten to twelve days.

As an example of the asexual reproduction of the malaria organisms we may select the cause of tertian fever, *Plasmodium vivax*, which has been carefully worked out and described by Schaudinn. The young sporozoite from the mosquito was studied in the living state and every stage confirmed in preparations. With characteristic ingenuity he succeeded in getting his own blood in sufficiently dilute condition to follow the movements of the young sporozoite in life. This he did by raising a blister on his hand and then teasing the contents of an infected mosquito's salivary gland into the fluid obtained from the blister; the blood corpuscles were thus relatively few in number, and with a warm stage he was able to follow the history of the parasites for hours. The young forms grow into a large organism which may nearly fill the erythrocyte. In the course of its growth a vacuole appears in the vicinity of the nucleus, probably due, as Schaudinn believed, to the active processes going on in the vicinity of the parasite's nucleus. In this way the ring-forms of the parasite are formed, the vacuole increasing relatively in size. Ewing ('98) interpreted these ring-forms as due to the coalescence of two horn-like pseudopodia, the vacuole thus arising in a purely fortuitous manner, and Argutinsky interpreted them as artefacts. Schaudinn's observations on the living organisms and his seeing this vacuole appear and disappear indicate that the vacuole and the ring forms are only evidences of physiological stages of the parasite, the vacuole serving only to increase the surface of absorption in relation to volume. It is not without significance, either, that he did not observe the formation of the vacuole in the sexual cycle. The nucleus of the young form consists of a relatively large karyosome and a minute vesicular part, the karyosome finally becoming granular and then dividing, the division being of a very primitive type of mitosis. At this period, which marks the full growth of the schizont, the organism becomes extremely motile within the blood corpuscle (Plate I, Fig. 1). Schaudinn graphically describes it as follows: "This period of the highest development of its vegetative activity is characterized by an important increase of its ameboid motion. It assumes the most unusual forms and is not for a moment at rest. The pigment becomes distributed throughout the body, long pseudopodia are

thrown out from all sides of the body and again drawn in, great vacuoles appear and disappear, deep incisions cut into the periphery, to be filled in immediately with the restless protoplasm. In short, this living organism is a most changing and fascinating spectacle to watch, and leaves the impression that the parasite is well-named 'vivax.'"¹

Shortly after this period of activity the organism becomes quiet, spherical, and rapidly undergoes the changes preparatory to merozoite formation. The nucleus divides, as stated, by a primitive method of mitosis, but with the continued division all traces of a mitotic process are lost, and at the end of the second division the process is little more than multiple fragmentation, division being so irregular that a definite plan is excluded. The end result is a number of daughter nuclei, each a small spherical granule of chromatin about which the protoplasm of the parasite divides to form a small reproductive element—the merozoite—while an unused residue containing the pigment and crystals remains behind to be dissolved in the blood plasma and carried to all parts of the system. The many merozoites thus liberated make their way to fresh corpuscles and the simultaneous attack leads to the characteristic symptoms of the disease.

The young quartan parasite cannot safely be distinguished from that causing tertian fever, save, perhaps, in regard to its relative inactivity, a function which decreases with growth of the merozoite. Its form, therefore, is more regularly spherical than that of *Plasmodium vivax* (Plate I, Fig. 2). After about ten hours of growth (Ziemann, 1906) it contains fine dark brown granules of pigment. At the sixteenth hour it occupies about one-quarter of the volume of the corpuscle, the pigment granules being unevenly distributed about the periphery, while the chromatin is less readily stained than that of the tertian parasite. At the end of two days the containing blood corpuscle remains only as a rim of material about the enlarged parasite, and this shortly afterward disappears, the freed organism being the size of the corpuscle. Characteristic merozoite formation follows, giving rise to what Golgi described as the "marguerite form," due to the regular segmentation of the cell body into from six to twelve merozoites (Plate I, Fig. 2, 12).

The merozoite of the organism of pernicious malaria is a very minute (1.5 to 2 microns) ring-formed parasite, the rings, according to Nocht, being optical illusions, due to discoid bodies with thickened rims. The chromatin is in the form of a small spherical granule which not infrequently elongates to a rod form and then fragments to form two or three similar chromatin granules (Plate II). Double or multiple infection of blood corpuscles is not infrequent, but union of these separate individuals never takes place, according to Ziemann (how-

¹ Schaudinn *Plasmodium vivax* G. and F. der Erreger des Tertianfiebers beim Menschen. Arb. a. d. Kais. Gesundh., 19: 1902: 216.

PLATE II

Tertian Estivo-autumnal Malarial Plasmodium. Oliver's Modification of Wright's Stain. (After Craig.)

- 1, 3, 4, 5, 6, 7, 8, 9, 10, and 15. Ring forms of tertian estivo-autumnal plasmodium.
2. Intracellular form.
- 11, 13, 14, 16, and 17. Pigmented ring forms.
12. Red corpuscle, showing infection with two "ring forms."
- 18 and 19. Pigmented forms, just prior to segmentation.
- 20, 21, 23, and 24. Round and ovoid forms developed from crescents.
22. Macrogamete.
- 25 to 36. Crescentic forms of estivo-autumnal plasmodium (tertian).
29. Ovoid form.
37. Segmenting form.
38. Sporozoites.
- a. Segmenting form of quotidian estivo-autumnal plasmodium.

ever, see p. 287). After some twenty-four hours the plasma of the ring form collects at one point, giving the effect of a signet ring, and the pigment granules first appear in the thickened portion. After about thirty hours the majority of the parasites have disappeared from the peripheral circulation, although a few may be found, especially in the Italian forms of the disease. In such cases the parasites, after thirty-six hours, appear round or oval and very sharply contoured, occupying from one-fifth to one-fourth of the whole volume of the corpuscle, which now begins to shrink. The chromatin divides (Plate II, 17 to 20, a) and the body of the parasite breaks up into from 12 to 16 merozoites, although the number of these may vary anywhere from 8 to 24 (Ziemann).

By analogy with other parasitic protozoa this process of asexual multiplication may continue for a longer or shorter time, or until the vitality is exhausted. A period finally ensues, the conditions being unknown, in which the merozoites develop into the sexual phases of the organism. These are the macrogametocytes and microgametocytes, the former female organisms, the latter mother cells of the male organisms. The stages in this development in the case of *Plasmodium vivax* are shown in Plate III, Fig. 1. The female organism is a large cell with reserve granules and a well-developed nucleus. The male mother cell is less granular and its nucleus divides by a multiple division into a number of daughter nuclei which migrate to the periphery and there become the long-drawn-out nuclei of the flagelliform microgametes. The female nucleus, before fertilization, divides to form a small nucleus, which is extruded from the cell, this corresponding to the polar body equivalent of other protozoa and metazoa (Schaudinn).

The processes thus briefly outlined do not all occur in the human blood. The early stages of gametocyte formation occur there while the remaining stages, viz., gamete formation and maturation processes, occur in the gut of a mosquito. Schaudinn had reason to believe that these sexual reproductive stages, especially of the microgametocytes, degenerate in the blood and come to nothing unless stimulated to development by the action of a cooler medium, such as room temperature or the cool medium of an insect's body. The organisms ready for this further development are constantly in the blood after the first few paroxysms, and when sucked up by the mosquito, the further changes take place rapidly in the latter's stomach and fertilization is brought about by the penetration of one of the slender microgametes into a macrogamete. The fertilized cell, called by Schaudinn the oökinet, now makes its way by a peculiar vermiform movement (giving rise to the name vermicule) to the epithelial cells lining the gut; it penetrates the mucous membrane and comes to rest in the submucosa. Here it rapidly grows into an organism of the size of a coccidium, the nucleus divides, and the cell body, at about the third or fourth day, forms

a permeable outer membrane and by the sixth day divides into as many portions as there are nuclei. These are special reproductive centres corresponding to the sporoblasts of the coccidia, and, as in the coccidia, each sporoblast forms by division a number of germs, the sporozoites. Unlike the sporoblasts of the coccidia, however, there is no protecting membrane or capsule about these plasmodium sporoblasts; the sporozoites are naked and unfitted by this very fact for a free existence outside the body of some host. When mature, after a period of about fourteen days in the mosquito, they are liberated from the sporoblasts into the body cavity of the insect where, by the circulation of the body fluids, they are carried to all parts of the body, collecting, however, in the region of the head. Here they make their way into the salivary glands in the thorax and pass into the proboscis of the insect and thence into the human blood at the time of the first meal subsequent to their maturity.

There is, perhaps, no better instance in the realm of biology of the delicate relationship existing between these intestinal parasites and the infected host. If the human blood of a malaria victim is taken by a mosquito belonging to the genus *Culex*, the blood and its parasites are alike digested by this mosquito's digestive fluids; no stage of the organism remains alive. But it is quite different with the species of mosquito belonging to the genus *Anopheles*. Here the digestive fluids kill the ordinary asexual forms of the parasite, but the gametocytes have in some manner acquired immunity to the digestive ferments of these mosquitoes and continue to live in the gut and to reproduce in the tissues lining it. Ross, in India, showed that this very phenomenon occurs in the case of bird malaria, in which the organism *Plasmodium precox* is digested by the fluids of *Anopheles*, but immune to those of *Culex* or *Stegomyia* (Newmann), so that species of *Culex* and *Stegomyia* are the carriers of bird malaria, but harmless to man, for the organisms of bird malaria do not live in human blood. It is generally supposed, also, that mosquitoes may become immune to all kinds of blood parasites, that is, capable of digesting all of the organisms, gametocytes and schizonts alike, and thus become quite harmless to man. This is the interpretation given to the fact that, although *Anopheles* is common in England, there is no malaria.

The phenomena of sporogony in connection with other forms of malaria are not essentially different from those of the tertian organism (Plate III, Figs. 2, 3, 4). The macrogamete of pernicious malaria is, however, distinguishable from those of other forms of malaria by its sausage or crescent form (Plate III, Fig. 3). A number of observers (Grassi and Felletti, Mannaberg, Ziemann, et al.) have observed the binary division of such macrogametes, a method of reproduction which recalls the multiplication of the female organisms in trypanosomes.

Schizogony and sporogony in the case of *Plasmodium precox*, the

EXPLANATIONS OF FIGURES IN PLATE III.

Fig. 1.—Tertian Malarial Plasmodium. (After Craig.)

1. Hyaline form.	8. Flagellate form. (Microgametocyte.)
2. Pigmented ring form.	9. Non-flagellate form. (Macrogamete.)
3 to 6. Pigmented forms.	10. Segmenting form after destruction of red corpuscle.
7. Segmenting forms.	

Fig. 2.—Quartan Malarial Plasmodium. (After Craig.)

1. Hyaline forms.	8. Segmenting forms after the destruction of the red corpuscle.
2 to 5. Pigmented forms.	9. Flagellate form. (Microgametocyte.)
6 and 7. Segmenting forms.	10. Non-flagellate form. (Macrogamete.)

Fig. 3.—Tertian Estivo-autumnal Malarial Plasmodium. (After Craig.)

1 and 4. Hyaline ring form.	9. Segmenting forms.
2, 3, and 7. Pigmented ring form.	10. Flagellate form. (Microgametocyte.)
5 and 6. Pigmented forms.	11 to 14. Crescentic forms.
8. Young intracorpuseular crescent.	

Fig. 4.—Quotidian Estivo-autumnal Malarial Plasmodium. (After Craig.)

1 to 4. Hyaline ring forms. Some cells show infection with more than one organism.	9. Flagellate form. (Microgametocyte.)
5 to 7. Pigmented forms. In 6 one hyaline form.	10, 11, 13, and 15. Crescentic forms.
8. Segmenting forms. Segmentation complete within infected red blood corpuscle.	12. Ovoid form.
	14. Non-flagellate forms. (Macrogamete.)

PLATE III

FIG. 1

FIG. 2

FIG. 3

FIG. 4

cause of bird malaria, are not different in essentials from similar phenomena in human parasitic forms.

Of great importance in the malaria problem is the fact of latent and recurrent malaria. In many cases, months after the first attack and apparent cure, the victim suffers anew from the parasites, and this without new infection. The matter has been studied carefully by many observers, among others by Craig and by Schaudinn, and it has been found that parasites, even after apparent cure, are stored up in the spleen and the bone marrow, where they live a comparatively passive existence, getting into the peripheral blood when the conditions for their further development are favorable. What these conditions are is the one remaining obscure point in our knowledge of the malaria organisms. Schaudinn claims that certain of the forms of *Plasmodium vivax*, which under ordinary conditions would form the macrogameto-

FIG. 113

Regression and merozoite formation (parthenogenesis) in *Plasmodium vivax*. (After Schaudinn.) A, macrogametocyte in blood with nucleus differentiating into a denser and a lighter part; B, the denser part of the nucleus now divides preparatory to schizogony, C, D, while the paler portion with a part of the original cell degenerates; D, numerous merozoites formed about the divided nucleus.

cytes, undergo a process of parthenogenesis (Fig. 113), whereby the vitality is again renewed and with this the ability to withstand the natural or acquired immunity of the host. Craig, on the other hand, describes the conjugation of two schizonts within the human blood cell, an observation which Ewing ('01) and Wright ('01) had also made, although in the last two cases in connection with the normal infection and not with recurrence, while the occurrence was stated as rare and exceptional. Craig ('05 and '07), however, claims that the union of schizonts is a normal process in every infection, and sees in this fact a means by which the organisms renew their vitality and thus bring about recurrence. Minchin doubts the interpretation of this fusion as given by Wright and by Ewing, and regards it as a process of plasmogamy without sexual significance. Craig's view is certainly enticing, but we must not forget that plasmogamy is a very common phenomenon throughout the group of protozoa and occurs frequently when

there is no subsequent reproduction. It happens in most of the common rhizopods, for example, and has been described for cases of arcella, difflugia, centropyxis, ameba, etc., and it has been shown that these unions have nothing to do with the actual process of fertilization. It is impossible to state that no stimulation whatsoever results from such a plastogamic union, especially if it is followed by nuclear union or karyogamy, according to the account given by Craig; but it is difficult to believe that two widely different processes of fertilization should exist in the same organism. My experiences with the free living paramecium in cases of depression where the organisms were stimulated to new activity and new reproduction by purely artificial means opens the possibility, at least, that some analogous stimulation in the human system may start up the flagging energies of the malarial parasites. It is not inconceivable that minute changes in the constitution of the blood, especially of the salt contents, act upon the parasites in the same manner that potassium phosphate acts upon the weakened paramecium.

Apart from the clinical effects of the different malaria parasites there is not much difference between them. The cause of quartan fever, *Plasmodium malariae*, for example, agrees in all of its phases with *Plasmodium vivax*, the most important difference being the period elapsing between successive sporulating phases, requiring seventy-two hours as against forty-eight. The forms assumed by the gametocytes agree in all essential features, and fertilization in the mosquito follows the same history as in *Plasmodium vivax*.

There is evidence that at least two kinds of parasites causing pernicious malaria exist, one giving rise to a daily and the other to a forty-eight-hour recurrence. The difference in form of the macrogametocyte was considered evidence of sufficient morphological value to justify a different generic name, and Grassi, therefore, gave it the name *Laverania malariae*. The grounds seem hardly sufficient for this, however, and the name *Plasmodium falciparum*, as given by Welch, is the one we adopt. (*Pl. immaculatum*, accepted by Schaudinn, was shown by Blanchard to be the name given by Grassi and Felletti to parasites occurring in birds.) In this parasite the macrogamete assumes the form of a crescent before maturity, but rounds out into a perfect sphere before fertilization.

The action of quinine on the malaria organisms is particularly interesting, since it is one of the best-known specifics against any of the protozoan diseases. Introduced into Europe, in 1640, by del Cinchon, it was immediately recognized as a specific and was used as a diagnostic therapeutic test for malaria. Just how it acts upon the malaria organism was, of course, unknown until more or less of the life history of the parasites was known. Marchiafava and Celli, Schaudinn, and, in short, all who have studied the matter carefully

have come to the same conclusion, that the drug acts directly upon the parasite, killing it with more or less distinct evidences of disintegration of the organism. Marchiafava and Celli conclude that the treatment is most effective during the period of sporulation and upon the young stages of the organism, and practically without effect during the period of pigment formation and full growth of the schizonts.

CHAPTER X.

THE PATHOGENIC RHIZOPODA.

THE biological conditions which underlie parasitism are but little known, but, as with free-living protozoa, the dominant factor is the problem of food-getting. The causes which lead an organism to invade a specific organ or tissue must, in the final analysis, be traced to this function, and reproduction leading to complete annihilation a cell or group of cells follows a parasite's life in a suitable food medium. There is a limit also to the kinds of parasites that can become cell-infesting forms, for the organism must have either the mechanical or cytolytical power of breaking down the barriers of a cell, and physical force enough and of a certain kind, to enable it to penetrate the cell membranes and cytoplasm. For such a function cilia are not useful, nor flagella, and we find that ciliates and ordinary flagellates rarely become intracellular parasites, and then only after losing their motile organs; unless, as in trichonympha, pyrsonympha, etc., they are provided with special anterior boring organs, by which they penetrate the cell membranes, or unless, as in spirocheta, they possess the power of undulatory motion independent of flagella action (Fig. 114). Spirochetes may thus become cell-dwelling as well as fluid-dwelling forms, and some, like *Sp. microgyrata* or *Treponema pallidum*, work their way through the tissues of an infected host and not infrequently bore into the cells themselves. The ciliated and flagellated protozoa, however, are typically fluid-dwelling forms, and when they attack the epithelial cells of an organ it is usually only for purposes of attachment, as in trichonympha and pyrsonympha. There is considerable evidence, however, to indicate that one of the ciliates, balantidium, is occasionally found inside the mucosa of the intestine, and even within the muscular coating of the colon, while collections often appear in the epithelial cells and, apparently, cause the ulcers that are found there. Two kinds of these ciliated parasites are common in man, *Balantidium coli*, frequent in the rectum, and *Bal. minutum*, and, according to Strong, Brooks, and Stengel, with others, the parasite becomes an important etiological factor in catarrhal inflammation of the intestine (Fig. 115). Other observers, including Malmsten, Opie, Dofflein, and others, hold that these forms are quite harmless, increasing in number with disorders of the digestive tract, and for this reason are not uncommon in the intestinal tract of victims of cholera, typhoid, dysentery, or diar-

FIG. 114

A, *Multicilia lacustris*, Lauterb. (After Lauterborn.) B, *Pyrsonympha vertens*, Leidy, with attaching organ. (After Porter.) x, vibrating band in the inner protoplasm.

FIG. 115

Flagellated and ciliated intestinal parasites. A, B, *Megastoma* (*Lamblia*) *entericum*, Grassi; C, *Balantidium entozoön*, Ehr.

rhea. Brooks has given strong evidence to show that *Bal. coli* was the cause of a fatal disease resembling dysentery, in some valuable apes belonging to the New York Zoölogical Society, and from his observations it is evident that these ciliates must be taken into account in searching for the causes of certain types of intestinal trouble, for, if not themselves the direct causative agent, they may be the bearers of some more pernicious organism.

While ciliates and flagellates are not adapted morphologically for an intracellular parasitic life, the rhizopods have no such disadvantage, and by virtue of their ameboid movements, and of the cytolytic ferment which they are apparently able to secrete, they make their way into tissues and cells and then live upon the fluid elements of the living protoplasm. Thus, *Plasmodiophora brassicæ*, while in the young amebula stage, works its way into the root cells of a cabbage or turnip plant, absorbs and grows upon the fluid protoplasm of the plant cells, forms a plasmodium, and reproduces within these cells (see p. 209). Certain human diseases, notably dysentery, hydrophobia, and smallpox, are characterized by the destruction of tissue cells, the agent being minute ameboid forms which we interpret as protozoa. In dysentery the organism causes the destruction of the epithelial cells of the digestive system; in hydrophobia, the nerve cells of the brain are destroyed, and in smallpox, the epithelial cells of the skin.

In none of these cases is it generally agreed that the structures found within the diseased cells are the causes of the several diseases, and, indeed, in the last two, hydrophobia and smallpox, pathologists do not agree that the structures found within the diseased cells are organisms at all, much less the causes of the troubles. Unfortunately, cultivation of such organisms upon artificial media, and in pure cultures, has never succeeded. Indeed, up to the present time no one has succeeded in cultivating a cell-infesting rhizopod, and Lühe goes so far as to state that it will never be done, although success with forms like the Leishman-Donovan bodies makes such sweeping generalizations unsafe. The only means of determining whether such things are organisms rests upon morphological evidence, and lacking cultural possibilities the only proof that they are the cause of disease is to find them in every case of the disease. The morphological evidence, to most pathologists, is insufficient, and to most of them these organisms are more probably artefacts or degeneration products of the human cells caused by the disease, than etiological factors. To a protozoölogist, however, the morphological evidence of organic structures of these protozoa is far more convincing, for he is familiar with the many variations in size and structure, in the different phases of the life history, of hundreds of different kinds of protozoa, and the structures seen in these questionable inclusions become to him convincing

evidence of their protozoan nature. Such is the situation at the present time in regard to the inclusions found in trachoma, molluscum contagiosum, hydrophobia, and smallpox, while those in dysentery (although still in dispute as to etiology) are universally recognized as ameboid organisms. In the present chapter, I purpose to give some of the evidence upon which the protozoölogist bases his conclusions that the more questionable inclusions referred to are actually organisms of the rhizopod type, and if, thereby, I am able to impart some of my personal convictions in regard to them, the matter of etiology will take care of itself.

In order to provide a basis for comparison of these disputed organisms it is necessary to consider first the variations in structure that occur during the life histories of widely different types of rhizopods, and then to show that, despite the minor differences, they all conform to a common type. The full life histories of many different kinds of rhizopods have been worked out on free living material, so that there is no ground for cavil as to whether such types are living organisms or artefacts.

As fully shown in Chapter III, the life histories of free living rhizopods, involving many form changes, are characterized, at certain periods of maturity, by diffusion of the nuclear material throughout the cell and by the formation of exceedingly minute gametes.

The curious diffuse idiochromidia are known to be no artefacts, nor abnormal features of the cell, but specific and highly important elements whose chief function is in sexual reproduction. It may be expected, therefore, and reasonably so, that similar structures should be characteristic of parasitic as well as of free living rhizopods, and the idiochromidia of chlamydophrys, of entameba, of neuroryctes, and cytoryctes, features of these organisms which many observers are reluctant to regard as evidences of organic structure, have the same importance as elsewhere. It is upon this feature of these organisms that we may reasonably depend for the assurance of the protozoa nature of the cell inclusions in trachoma, molluscum contagiosum, rabies, and smallpox.

There is no reason to believe that the life cycle of a parasitic rhizopod should be essentially different from that of a free living form, unless, indeed, there may be an acquisition of some special means of overcoming the unfavorable condition of parasitic life, such as exposure to antibodies, acids, alkalies, etc., in the body fluids of the host, or to difficulties in transmission from one host to another. These are, in the main, provided for by the phenomenon of encystment, the organism within its cyst being amply protected against unfavorable conditions. Such a function, however, is shared with the free living rhizopods, encystment playing an important part in the life history of both shelled and shell-less forms.

A transition from the free living to the cell infesting rhizopods is afforded by one species of shelled forms—*Chlamydomphrys stercorea*—and by different species of ameba—*Entameba coli* and *Entameba histolytica*—the life activities in all being singularly in conformity with the examples given above.

Chlamydomphrys stercorea, first described by Cienkowsky in 1876, is a rhizopod provided with a transparent glass shell of silica, found in animal feces. From its type of pseudopodia it would be classed with the reticulosa rather than with the lobosa or ameba type, and comes closer, therefore, to polystomella than to arcella or centropyxis. Schaudinn (loc. cit) found it in the feces of many different mammals, including cow, guinea-pig, turtles, and man, and was able to follow its life history by infecting his own digestive tract with encysted forms of the organism.

The protoplasm of the cell contains one nucleus, many fine particles, which are destined to form the shell of the daughter individual, contractile vacuoles (one or more), and idiochromidia in the form of a densely packed mass of granules about the cell nucleus. Like arcella, centropyxis, euglypha, and other shelled rhizopods, the organism reproduces asexually by budding division, the plasm flowing out of the shell opening until a daughter mass is formed equal in size to the parent; the nucleus then divides by mitosis, one-half passing into the bud organism. The idiochromidia do not flow into the daughter protoplasm with the protoplasmic streaming, as in euglypha and centropyxis, but adhere to the nuclear membrane, so that when the nucleus divides, the germ plasm is likewise divided into two parts, the daughter organism thus getting its proportion of the important idiochromidia. The sexual development is quite different from that of centropyxis. There is no dimorphism, and whereas in centropyxis the idiochromidia-bearing swarmers move out of the shell, leaving the disintegrating primary nucleus and residual protoplasm in control of the parental abode, here the residual parts are thrown out of the shell opening and the idiochromidia remain in the shell. The idiochromidia next give rise to a small number of secondary nuclei, usually eight, by segregation of the chromatin granules, and the protoplasm then divides into as many parts as there are nuclei. Each part assumes an oval form, develops two flagella at the pole, and swims out of the shell and away. Two swarmers (flagellispores) from different ancestors fuse, form a hard, protecting cyst which becomes brown in color and irregular in contour, and within these the fertilized cells with a high potential of vitality, live until conditions are again suitable for development. With characteristic patience and ingenuity Schaudinn kept these cysts in damp chambers for a period of many months without observing any change, and finally inoculated himself: "I swallowed on November 17, 1899, for the first time, the contents of

eight moist chambers, in which were innumerable permanent cysts of chlamydothryps, which had lain unchanged for two or three months, and found on the 20th two typical chlamydothryps in an infusion made from solid feces of the 18th, while by the 24th they were so numerous that every preparation contained from one to two individuals." (Schaudinn, loc. cit., p. 562). When he found that the organism would live in other digestive tracts, he gave up experimenting upon himself and used mice. One phase in the life history of this organism was earlier (1896) interpreted as a distinct species and named *Leydenia gemmipara*. (Schaudinn, 1903, p. 563).

Chlamydothryps, therefore, behaves like centropyxis and arcella in its vegetative activities, but resembles polystomella more closely in its formation of isogamous gametes. The chromidia are the same in all, being the substance of the nuclei of the conjugating cells.

A transition from the lumen dwelling to the intracellular rhizopods is afforded by the intestinal amebæ, which, since the time of Lösch, in 1875, have been closely associated with the problem of dysentery. These are minute amebæ which penetrate the tissues by forcing the cells apart, and although they apparently do not enter the cells, they cause destruction of the cells by cutting off the food supply, exposing them to the materials of the intestine, or disturbing the ordinary pressure relations by infiltration with round cells and edema. Different observers have described many kinds of ameba in the human intestine, both during health and disease, and while some of these observations warrant careful consideration, the majority of them are not zoologically satisfactory. There are few points of structure in the parasitic amebæ upon which to base species, and all attempts to create new species on account of size differences, nature of the pseudopodia, vacuoles, and the like, are insufficient; the only safe taxonomic basis is the life history, or the "individual" in the larger sense. At the present time very few of the many described amebæ have been followed in their life history, and, although there are probably more, we recognize only two species of intestinal amebæ, the one, *Entameba coli*, regarded by Casagrandi and Barbagallo, Schaudinn, Craig, and others as a harmless commensal in the human intestine, and *Entameba histolytica* (*dysenteriae*, Councilman and Laflour), regarded by pathologists generally as the cause of amebic dysentery. A third form, *Entameba buccalis*, is found in carious teeth (Prowazek). The life history in both of the intestinal species was worked out by Schaudinn, and the specific features were established by his demonstration of the characteristic differences in mode of reproduction.

Lösch, in 1875, was the first to describe the simple structures of these amebæ, which he also was the first to regard as an additional irritant, if not the cause, of dysentery. He named it *Ameba coli*. Later observers, finding the organism in so many cases of the normal

intestine, denied the pathogenic character of "*Ameba coli*," claiming that it is an organism of wide distribution and quite harmless. Casagrandi and Barbagallo were the first to prove, although not the first to suggest, that the ordinary form of the ameba is harmless, a proof which was confirmed by Schaudinn, who inoculated himself with *Entameba coli* and without any disturbance, a result which he also repeatedly obtained with young cats. From the medical side Councilman and Lafleur, in 1891, first demonstrated that dysentery is not all one type of disease, and that amebic dysentery is both clinically and etiologically different from other kinds. They suggested the name *Ameba dysenteriae* for the organism causing the intestinal ulcerations, and *Ameba coli*, Lösch, for the harmless form; but their suggestion was not followed by enough morphological data to warrant the creation of a new species, and zoölogists did not accept the new terms. Casagrandi and Barbagallo, working on *A. coli*, came to the conclusion that the generic name ameba should not be stretched to include forms like *Ameba proteus*, on the one hand, and these small intestinal parasites on the other, and so called the latter entameba, while the specific name *hominis* was substituted, without justification, for Lösch's term *coli*. Schaudinn, finally, overlooking Councilman and Lafleur's observations, adopted Casagrandi and Barbagallo's name entameba for the genus, and named the harmless form *Entameba coli*, and the pathogenic form *Entameba histolytica*, a better name, but not prior to Councilman's "*dysenteriae*."

Entameba coli is widely distributed in the human intestine, this distribution varying with the locality and with the people. Schaudinn found it in about 20 per cent. of the feces investigated by him in Berlin, while in the region about Rovigno, in Istria, he found it in 256 cases out of 385, and other observers have noted a like variation in the percentage of healthy persons infected. It is an organism to be obtained without much difficulty, and is more prevalent in persons suffering from intestinal disturbances. During the ordinary inactive phases there is little or no differentiation into cortical plasm (ectosarc, ectoplasm) and endoplasm, but when it moves, a hyaline sheet of protoplasm moves out from the body, and this is similar to the cortical plasm of fresh water amebæ. This ectoplasm is only momentary, however, for the endoplasm quickly flows into the advanced part. The nucleus is vesicular, with a distinct membrane and with one or more karyosomes of chromatin and plastin, while the numerous chromatin granules are distributed throughout the space of the nucleus, with a tendency—of frequent occurrence among the protozoa—to collect at the periphery. The abundance of chromatin makes the nucleus stand out prominently in stained preparations.

Multiplication of the parasite is accomplished asexually by simple division and by multiple division or spore formation into eight daughter

organisms. The centronucleus, with its single division centre divides, according to Schaudinn, by amitosis, but, as in the flagellates it is a primitive mitosis. Spore formation is accomplished after a peculiar fragmentation of the nuclear chromatin into minute granules which collect in a rim around the inside of the nuclear membrane, the cell body, in the meantime, throwing out all foreign matter and ceasing its movements. The peripheral chromatin next collects in eight centres, the nuclear membrane is ruptured, and the eight small nuclei pass into the cell body. The protoplasm divides into eight parts around the nuclei, and eight small amebæ finally creep out.

As with all protozoa that have been carefully investigated, the reproduction by asexual means, in this case leading to auto-infection of the host, cannot be maintained indefinitely, and there comes a period when the organisms encyst, the conditions under which encystment takes place being somewhat indefinite in Schaudinn's account. The cell throws out foreign matter and products of its own metabolism, and becomes more compact, smaller, and spherical, and then secretes a thick and slightly refractive gelatinous membrane. The nucleus then divides by primitive mitosis into two nuclei, which are separated from one another by the entire diameter of the spherical cell. The idiochromidia characteristic of the rhizopods is then formed by disintegration of the two nuclei, the protoplasm of the cell in the meantime dividing into two incompletely separated parts around the two nuclei. In some cases the entire nucleus disappears in a mass of chromidial granules, in other cases there appears to be a secretion of chromidial substance as in *arcella*, but in all cases a part of the nuclear material is thrown out of the nucleus to degenerate, and this portion represents the eliminated and unused nuclear parts of the free living rhizopods.

The fertilization process, following this preliminary division of the nucleus, is autogamous and similar to that in *Ameba proteus* and in the heliozoön *actinospherium*, as observed by Hertwig. The organism fertilizes itself in the following remarkable manner, the processes of maturation recalling those of the ciliate *paramecium*:

From the disintegrated chromatin or idiochromida of the divided cell within its cyst membrane a new and a smaller nucleus is formed in each of the halves. This divides by a primitive mitotic process into two nuclei, one of which immediately degenerates, the shrunken nucleus remaining as a highly refractive irregular mass in the cell body; the other daughter nucleus then divides again, so that three nuclei lie in each half of the double organism, or six altogether, two of these undergoing degeneration. Two of the remaining four nuclei then begin to shrink and to degenerate like the first one, until there are only two functional nuclei left. After this process, which Schaudinn interprets as equivalent to the reduction and polar body formation of metazoan cells, the final encystment takes place. The gelatinous

membrane disappears, and in its place is secreted a thin but much more refractive membrane, the definitive cyst membrane. The contents of the cyst become again closely united, and the two remaining nuclei are brought closely together. Then follows a third division by mitosis, characterized by long connecting strands which lie parallel with one another in the centre of the cell, so that the daughter nuclei of the two parent nuclei lie side by side in pairs. These nuclei then fuse, an eighth part of one of the original nuclei uniting with an eighth part of the other, while the outer membrane hardens and thickens. Each cyst thus contains two fertilized nuclei, the process recalling the phenomenon in paramecium where, from the same primary nucleus, a wandering and a stationary nucleus is formed. In the fertilized *Entameba coli* each of the two nuclei divides, forming four nuclei; then each of these divides again, making eight nuclei in the cyst, and in this condition the encysted parasite passes into the intestine of a new host, where the protoplasm of the cell divides into eight parts around the eight nuclei, the cyst membrane is dissolved off and eight small amebæ start a new infection with a new potential of vitality.

This complicated life history has been confirmed in part by other observers, Wenyon ('07) and Craig following out the sexual history in *E. muris* and *E. coli* respectively (see p. 142). The possibility of union of two amebæ before encystment is not excluded, nor is the possibility of pseudoconjugation, as seen in the gregarines, beyond question. Autoconjugation, while recognized in many different kinds of animals, is too unusual to be granted without the surest proof, and further research on the life history of these parasites is urgently needed.

The structure of *Entameba histolytica*, according to Schaudinn, is somewhat different from that of *E. coli*, and makes it better adapted for its cell destroying function. This is shown by its definite cortical plasm, a layer of firm protoplasm with distinctly higher refractive index than the internal protoplasm, which gives a more rigid character to the pseudopodia, by which the organism is able to force its way between the epithelial cells of the intestine and into the more deeply lying tissues. Schaudinn has watched the organism thus make its way into the epithelial tissue of a freshly extirpated, infected cat intestine, its active movements often lasting an hour, while its own body assumed the greatest variety of forms. The nucleus is difficult to see during life of the organism, a feature in marked contrast to the nucleus of *Entameba coli*, which Schaudinn recommends as a particularly favorable object for the study of the changes of the living nucleus. The nucleus of *E. histolytica* has very little chromatin matter as compared with the nucleus of the other species, but there is a single central karyosome and a slight collection of chromatin around the periphery. While the nucleus of *Entameba coli* is only slightly vari-

able, usually spherical, and without much change in position during the activities of the body, that of *E. histolytica* is highly variable, bending and turning with contact with objects in the cell, or flattening into a disk in the cortical plasm.

The ordinary vegetative increase of *Entameba histolytica* takes place by simple division or by budding on the periphery, the formation of eight spores never being seen. Division takes place while the organisms are lying between the cells of the gut tissues, and may be either equal or unequal, the unequal division passing by imperceptible grades into bud formation. The buds are apparently similar in their mode of formation to those of *acanthocystis* (see p. 31), the nuclei arising, according to Schaudinn, by amitosis (Fig. 32, p. 94.)

Permanent cysts are not formed during the height of the disease, but are first found during periods of healing, and after the organisms have reproduced again and again by division. The beginnings of the preparations for spore formation are first manifested in the nucleus. Here the peripheral zone of chromatin granules becomes thicker, the membrane of the nucleus disappears and the granules are ultimately disseminated throughout the protoplasm in a typical chromidium form similar to that of *centropyxis* (see p. 150), while the residual nuclear parts, with some protoplasm, degenerate. Spores are formed by the protrusion on the surface of the cell of small buds containing chromidia, and these buds are transformed into spores by secretion about themselves of a definite resisting membrane, while the central protoplasm, with the residual nucleus, degenerates. The further history of these buds was not ascertained by Schaudinn beyond the fact that they were capable of infecting normal cats with amebic dysentery, so that the processes of conjugation are still unknown. It will be an interesting study for some student of the group to see if conjugation follows the pattern of *Entameba coli* or that of *centropyxis*, where the idiochromidia bearing spores are gametes which unite after leaving the parent cells.

It is not the place here to discuss the question whether or not these parasites of the human intestine are the causes, or the sole causes, of acute enteritis in man.¹ Pathologists, in the main, are in accord that one type, at least, of dysentery is traceable to these rhizopods, but there is a difference in opinion as to whether the rhizopods create an enzyme or poisonous product which acts as a direct agent on the tissues, or whether they are passive in this respect, but cause mischief by the mechanical irritation of their movements between the cells. Shiga and Flexner have shown that one type of dysentery is to be traced to a bacillus, and Prowazek suggests that these parasitic amebæ may play an important part as carriers of bacteria into the deeply lying tissues

¹Prowazek has recently given evidence to support the view that flagellates of the genus *Lambliia megastoma* (Fig. 115) are capable of causing acute intestinal trouble of like nature.

of the intestine which they are incapable of reaching by their own movement. On the other hand, the nearly pure cultures of the ameba which Strong, Musgrave and Clegg, and others have succeeded in raising and in causing the disease in normal animals, and Schaudinn's experiments on kittens with dried spores of *E. histolytica*, speak for their specific pathogenic nature. Musgrave and Clegg ('04), indeed, are so positive of the pernicious effect that they maintain the pathogenic nature of all intestinal amebæ, and claim that ordinary pond or soil dwelling amebæ may become pathogenic on entering the intestine. Taking all into consideration, there is no doubt that the intestinal rhizopods are dangerous, and are either the causes of certain types of the disease, or pernicious accessories of the cause.

If skepticism exists as to the pathogenic nature of entameba and the causes of dysentery in general, what can be said as to neuroryctes and cytoryctes and the causes of hydrophobia and smallpox? With entameba, skepticism never reaches the level of denial of the organism, but with these other organisms not only does doubt exist as to their connection with disease, but their claims to relationship with living forms are widely denied. The problems are certainly very difficult, and with the immense numbers of degenerations, secretions, and the like which may be imagined in tissues under diseased conditions, it is easily possible to be mistaken when morphology is the sole criterion. But it is not inconceivable that these difficulties are overestimated, and that the questionable structures in diseased tissues are actual organisms.

Certainly no one doubts that rabies and smallpox are germ diseases, and it is equally certain that no other cause, apart from these cell inclusions, is known. There is a strong a priori reason, therefore, for believing that these intracellular structures in cells which are known to be the seat of the disease are the actual causes and not the product of the diseases. Thus, the Negri bodies (*Neuroryctes hydrophobiæ*) are constant inclusions in the brain cells of victims of rabies, and the Guarnieri bodies (*Cytoryctes variolæ*) are equally constant inclusions in the skin cells of man and apes infected with smallpox. So strong is the morphological evidence of the nature of these inclusions that there is no doubt whatsoever in my own mind as to their protozoan nature and to their affinities with entameba and other rhizopods.

The transition from the intercellular to these intracellular parasites of the rhizopod type is shown by such unquestionable ameboid forms as *Plasmodiophora brassicæ*, while recently a number of other forms of similar nature have been described. Among these the genus which Prandtl ('07) describes under the name of allogromia is very instructive. This is a parasite of free-living protozoa, such as *Ameba proteus*, arcella, nuclearia, or even paramecium, unicellular hosts which become infected with the sexual generation of the allo-

gromia. These grow to maturity and form gametes which escape and conjugate in the surrounding water, the resulting copula developing into a biflagellated organism which subsequently becomes ameboïd and grows into an adult allogromia (Fig. 116). While there is reason to doubt some of the developmental stages of this life history, the essential fact remains that here is a clearly defined rhizopod

FIG. 116

"Allogromia," sp. (After Prandtl.) A, an individual from *Ameba proteus* with nucleus undergoing fragmentation to form chromidia; B, aggregation of distributed chromatin into secondary nuclei; C, *A. Vampyrella*, sp., infected with *Allogromia*, sp.; D, allogromia from *Ameba proteus* shortly before ripening of the gametes.

FIG. 117

Single and multiple infection of ameba nuclei by *Nucleophaga amebæ*. (After Pénard.)

one stage of whose life history is passed as an intracellular parasite. The history of its nucleus is important as furnishing a possible interpretation of the distributed condition of the chromatin in neuroryctes and cytoryctes. The cell plasm of this so-called allogromia becomes filled with idiochromidia which are derived from the nucleus (Fig. 116, A, B).

It is probable, as Doflein points out, that this organism is not an

allogromia in the sense of Rhumbler's organism of that name, but that it is a species of a still more striking intracellular rhizopod first described by Dangeard in 1895, under the name of *Nucleophaga amebæ* and subsequently identified by Gruber, Pénard, and Doflein. It is a fairly common parasite of *Ameba proteus* and similar fresh-water forms, penetrating the nuclei and forming relatively large spherical reproductive bodies within the nuclear membrane (see Fig. 117). The nucleus becomes more and more hypertrophied with growth of the parasite, until finally the membrane gives way and the mass of spores is left in the enucleated body of the host. Under the name of *Karyoryctes cytoryctoides* the author described a similar

FIG. 118

Nucleophaga, sp., an intranuclear parasite in the macronucleus of *Paramecium aurelia*.
(After Calkins.)

intranuclear parasite of *Paramecium aurelia* in 1904 (Fig. 118). Being unfamiliar at the time with Dangeard's work, I was under the impression that the parasite in question was a new organism, and described it as such, pointing out its close resemblance to the intranuclear forms of the smallpox organism. There is no doubt, however, that the parasite is a species of nucleophaga, and the name karyoryctes must go. The striking similarity between the smallpox organisms and these intranuclear parasites leaves little room to doubt the close relations of the two, while the structures and life phases, also, of neuroryctes are almost identical with those of nucleophaga (Fig. 120). We are justified, therefore, at least until more convincing evidence to the contrary is forthcoming, in regarding the Guarnieri bodies of vaccinia and

smallpox, and the Negri bodies of rabies, as protozoan organisms of the nucleophaga type.

Neurorhynchus hydrophobiae, Williams, the "Negri body," offers the best evidence of the rhizopod affinities of these intracellular inclusions, the mammalian brain cells, better than the skin cells, lending themselves to rapid fixation and study.

When Pasteur and his immediate followers were working on the antirabic serum in connection with the cure of hydrophobia, they were obliged to wait from two to three weeks to tell whether the treatment they were giving a supposed victim was necessary or not. This was due to the fact that many days were required for the disease to develop in laboratory animals inoculated with the virus of the suspected animal, and, as may be imagined, it was a period of great suspense for all concerned. In 1898 the inoculation period was shortened to about nine days by Wilson's substitution of guinea-pigs for rabbits, these animals taking the disease more quickly than rabbits as used by Pasteur. Still, the time was far too long for diagnosis. Today it is possible to determine rabies in "mad" animals off the street in one-half hour. This wonderful practical advance in technical methods of the laboratory is due to the discovery by Negri, in 1903, of minute, characteristic inclusions in nerve cells of brain and spinal cord of animals with rabies, and by a special "smear" method of demonstrating them devised by A. W. Williams in 1904. The value of the Negri bodies in diagnosis was quickly recognized by pathologists throughout the world, and contributions confirming and extending Negri's discovery poured into the press. At the present time it is recognized that these characteristic structures occur in 100 per cent. of definite cases of street rabies, and that they are found nowhere else in diseased tissues. What claims have these specific structures to be regarded as organisms, and if organisms, where do they belong?

Negri regarded them as protozoa belonging to the class sporozoa, but was not particularly clear as to their classification. Previous observers, notably Di Veste, in 1894, and Grigoriew, in 1897, had mentioned structures in the nervous system of rabid animals and had described them as protozoa, but the things observed were apparently quite unlike the Negri bodies. Others, notably Volpino, in 1904, followed Foa, Schaudinn, and Prowazek in their interpretation of the Guarnieri bodies in smallpox, in believing that the real organism of hydrophobia is the granule, more often multiple, found in the substance of the "body," while the bulk of the "body" consists of material secreted by the cell (hence Prowazek's term "chlamydozoa") about the parasite. Williams' and Lowden's work, in 1906, and Negri's later papers leave no grounds for such an interpretation, the former believing that the granules represent distributed chromatin so characteristic of many forms of protozoa, and placing the Negri bodies as protozoa in the

suborder microsporidia, while Williams later gave the name *Neuroryctes hydrophobiae* to the Negri body.

The life history of *Neuroryctes hydrophobiae*, despite the admirable researches of Williams and Lowden, cannot yet be regarded as established, nor do I think the stages observed by Negri, Williams, and others justify us in assigning the organism to the sporozoa. The variable form, the uninucleate condition leading to the condition of distributed chromatin, and the budding phenomena are not characteristic of sporozoa, but are common to parasitic rhizopods, and the distributed chromatin is, in all probability, the idiochromidia, which, we have seen, is a characteristic phenomenon of all rhizopods.

FIG. 119

"Negri bodies," or *Neuroryctes hydrophobiae*, in different stages of chromatin distribution.
(After Negri.)

The organism is most abundant in the region of Ammon's horn, less abundant in the nerve cells of the cerebral cortex, cerebellum, medulla, and cord. In many cases, especially in street rabies, the organisms are large and ameboid in form, measuring up to $18\ \mu$ (Williams) (to $23\ \mu$, Negri), while minute forms, one-half a micron and less in diameter, are characteristic of the organism after the virus has been

repeatedly inoculated in animals of the same kind, and, owing to their very minute size, such organisms are easily overlooked in this "fixed" virus. It has been found by Remlinger, Schüder, Bertarelli, and others that the virus is still effective after filtration through a Berkefeld filter, a fact used as an argument against the specific pathogenicity of these structures; but the well-known variations in size of ameboid protozoa and the small size of some stages of the organism, combined with plasticity, which suggests ameboid movements, explains the ability to pass a filter. Other protozoa, notably spirocheta and trypanosoma, likewise pass through the Berkefeld. It is probable, therefore, that an organism as variable as neuroryctes in size would have some stages minute enough to escape filtration.

Negri was the first to make out the typical nucleus of the organism and to call attention to the distributed granules, although he did not

FIG. 120

Form and size changes of the organism of rabies, with evidence of budding in some cases. (After Williams and Lowden.)

interpret these correctly, Williams and Lowden, in 1906, being the first to interpret them as granules of distributed chromatin. Negri, in 1905, found that the nucleus has either a solid or reticular structure, according to the success in staining (Fig. 119), while the cell body contains a variable number of chromatin granules.

Reproduction of *Neuroryctes hydrophobiae*, according to Williams and Lowden, occurs by simple division and by budding. The division is either an equal binary fission, in which nucleus and chromatoid material are distributed to the two cells, although nothing like mitosis was observed. In budding, small buds are pinched off, these buds being single or multiple in number and containing granules of chromatin. The possibility of conjugation was suggested by Williams and Lowden, and illustrated by figures, but it is equally possible, and more probable, that the cases cited and illustrated were cells in division. Finally, what appears to be a spore-containing cyst (Fig. 120) was also described.

With the exception of the rhizopods, the entire range of protozoa offers no analogies to these stages of neuroryctes. The series of forms, following more or less closely the clinical history, agrees with the history of the parasitic amebæ so far as the general outline goes, while further details and careful study are necessary before the life history can be stated. With our present knowledge it appears that the organism, as seen in its smallest forms, is uninucleate; that as it develops into a larger ameboid form, the nucleus, either by fragmentation (as in *polystomella*) or by diffusion (as in *centropyxis* or *Entameba histolytica*), gives rise to the diffused chromatin or idiochromidia. In its mode of asexual reproduction it apparently follows *Entameba histolytica* in binary fission and in budding. Its sexual reproduction

FIG. 121

"Negri bodies in nerve cells." (After Wolbach.) A $\times 2000$; B $\times 1000$.

is as yet unknown, the union of two cells, as pictured by Williams and Lowden, being quite unlike any authentic account of conjugation in rhizopods or sporozoa. The nature of the "fixed" form, also, is enigmatical, but may be looked upon as a biological response on the part of a highly variable organism to long-continued conditions of the same nature.

Further work is needed on *Neuroryctes hydrophobiae* in respect to the mode of division and budding, and with especial reference to the nuclear phenomena; further, in respect to the nature of the permanent forms, encysted or otherwise, which might be expected to exist in animals shortly after recovery from rabies; and finally, work is needed in connection with the sexual phenomena whereby the potential of vitality of the parasite, and with it its capacity for further mischief, is restored.

The early illustrations published by Negri, Luzzani, and others of the organism of rabies showed an irregular body with numerous

vacuoles (Fig. 121), and sections of infected tissue not properly fixed and stained give no satisfactory pictures of the organism, the place of chromatoid granules and nuclei being taken by the vacuoles. Such a picture is duplicated by improperly fixed parasites of dysentery vacuoles appearing in the place of the formed parts of the cell. These, again, are duplicated by the ordinary appearance of the smallpox organism as it appears in sections of the skin (Fig. 123). This structure has been, and is still, next to the so-called protozoan inclusions in cancer, the most widely discredited cause of any malignant contagious disease. The reason for the skepticism on the part of pathologists generally is that the organism presents no appearance that can be identified with the ordinary cell, its lack of a vesicular nucleus, its highly vacuolated appearance, and its development in cells that are unquestionably pathological and degenerate, being, to them, evidence against its protozoan or parasitic nature.

While a great deal of the skepticism is due to traditional conservatism on the part of medical men and disinclination on their part to accept any but conclusively demonstrable evidence, it must be stated, with all respect, that there is among them a strong tendency to ignore such evidence as we do have in regard to the nature of these structures, and disinclination to accept such evidence as similar to structures in other protozoa. The difficulties attending the observations on the organism of smallpox are aggravated by the fact that it is apparently an exclusively human disease, and further, that the organism is an intracellular parasite which quickly disintegrates upon leaving its normal environment. One phase of the disease, however—vaccinia—is suitable for experimental study, but at best this is but a mild disorder when compared with variola inoculata of apes or with variola vera of man. Until some means of studying it on an experimental basis is established, we must make the best of the morphological evidence afforded by imperfectly fixed tissues from human beings, or from material in experimental animals with variola inoculata and vaccinia.

The cell inclusions in the Malpighian layer of the skin were early seen, interpreted as protozoa, and named *Monocystis epithelialis* by Pfeiffer, in 1887, but as he found so-called protozoa in all kinds of diseased tissue, his observation did not create much comment nor stimulate research. It was quite otherwise with Guarnieri, in 1892; this skilful investigator inoculated the corneal cells of guinea-pigs and rabbits with vaccine virus and with pustule contents, and found that the peculiar cell inclusions characteristic of smallpox and vaccinia reappeared in each new epithelium inoculated. He found that the structures appear with the greatest regularity in the vicinity of the nucleus, the largest forms appearing around the point of inoculation, while the most distant forms were the smallest. He regarded them as protozoa, naming the form as observed in vaccinia, *Cytoryctes vac-*

cinia, and in smallpox, *Cytoryctes variola*, but they were much more often referred to in subsequent investigations as the Guarnieri bodies. To Guarnieri, therefore, belongs the credit of placing smallpox and vaccinia among the experimental diseases, and the stimulus given by his work had an immediate effect. The majority of later investigators were opposed to his conclusions, although many, including Pfeiffer, Ruffer, and Plimmer, Clark, Monti, Wasielewsky, and others, believed that the parasitic nature of the inclusions had been demonstrated. The opponents based their criticisms upon the facts that no ameboid movement could be observed, nor division phases, nor cellular structures (Hückel, Foa, Mann, etc.), and they interpreted the Guarnieri bodies as special secretions or degenerations resulting from a peculiar transformation of a portion of the cell plasm under the stimulus of the vaccine virus. Wasielewsky, in 1901, brought new support to the view of Guarnieri by passing vaccine virus through forty-eight successive transplantations, the thirty-sixth giving a successful vaccination against smallpox. In each case the same inclusions were present in the epithelial cells and in approximately the same number, indicating that reproduction must have taken place. In 1903 Councilman, in coöperation with seven other investigators, published an exhaustive monograph on the pathology and etiology of smallpox, covering all phases of the pathology of the disease, Brinckerhoff and Tyzzer extending the experimental investigations of Guarnieri and Wasielewsky to apes, and Calkins working out a tentative life history of the parasite. Howard, in 1905, confirmed, independently, all of the findings of Councilman and co-workers, and identified every stage of life history of the organism.

So far as the organism is concerned, the most important discovery of these investigators was made by Councilman, Magrath, and Brinckerhoff, who found that in variola the inclusions are present both in the cell bodies and in the nuclei, while in vaccinia they are present only in the cell bodies. Councilman concluded that the intranuclear position indicates a phase in the life history of the parasite which is absent in the vaccinia cycle, and that this phase is responsible for the greater malignancy of smallpox.

Calkins interpreted the parasite as a sporozoan belonging to the group of microsporidia, and, as it now appears, gave an unnecessarily complicated account of the life history. Minchin ('06) regards it as more closely related to the haplosporidia, because of the absence of polar capsules and threads. The tentative life history worked out by Calkins was formulated before the observations on the chromidia of rhizopods were made and before the importance of this material of the cell was established. In the light of our present knowledge it is much more probable that the Guarnieri bodies are rhizopods, and that the complicated changes which were earlier interpreted as pansporoblast

formations are phases in the development of the idiochromidia. Without going into the controversy again as to whether or not these bodies are organisms, a matter, I may add, which is not yet settled to the satisfaction of either pathologists or biologists, I will here give only an interpretation of the questionable structures on the basis of their probable relationship to neuroryctes and the other parasitic rhizopods like nucleophaga, a relationship of which I am fully convinced.

The youngest forms of the parasite are small, spherical, and apparently homogeneous granules measuring about half a micron. In slightly larger forms a central granule can be detected more easily in the cornea cells of inoculated rabbits than in the human skin. Differentiation of the organism follows with growth, two substances of the cell indicating differentiation. One of these is distinctly chroma-

FIG. 122

Section of the lower part of the epidermis, showing the cytoplasmic stage of cytoryctes in the epithelial cells. $\times 1000$.

toid, and becomes diffused throughout the body of the parasite at first in irregular clumps (Fig. 122), later in a fine network (Fig. 123). Such a structure is to be compared with the chromidiennetz of the rhizopods. As with the chromidia material of the free forms, small, spherical, deeply staining nuclei are formed out of this chromidial substance, the organism then assuming an appearance strikingly like the figure of arcella as given by Hertwig, in 1899 (compare Figs. 46 [p. 118] and 123). These granules are not artefacts, but developmental stages of the organism. The proof of this is given by the fact that they may be distinguished after any of the ordinary differential nuclear stains, but more surely by the fact that their presence is indicated by photographs made with the ultraviolet rays from unfixed and unstained living tissue of the inoculated cornea. These granules were interpreted as gemmules in 1904, and as vegetative spores or merozoites I would

similarly interpret them today. The body ruptures and the spores are liberated, to be carried by the blood into new regions of the skin, where the cytoplasmic cycle is repeated.

In vaccinia it is apparent that this vegetative cycle is the only phase of the life history, and this, in the same host at least, is limited in extent. In variola, however, the vegetative cycle is repeated many times, but finally the nucleus becomes infected and the parasites,

FIG. 123

Cytoryctes variolæ in different stages of multiplication, outside first three figures and outside last two figures of the nucleus. (Alter Calkins.)

FIG. 124

Two of the larger cytoplasmic forms of cytotryctes in the epithelium. The two dark bodies in the middle showing reticular structure are the parasites. $\times 1000$.

like nucleophaga, develop in a more definite manner. Chromidial fragments are formed, varying in size and character, while a residual portion of the chromatin, analogous to the residual nucleus of free living rhizopods, remains unformed and apparently useless (Fig. 123). The many rings, vacuolated structures, etc., which earlier were interpreted as developmental phases of sporoblast and spores, I now believe to be degeneration forms assumed by the parasite, possibly due

EXPLANATION OF FIGURES IN PLATE IV. (After Mallory.)

The drawings were made with the Abbe camera lucida; projection on to table. Zeiss apochromatic homogeneous immersion 2.0 mm., apert. 130, compensation ocular 6.

FIGS. 1 and 2 show numerous large and small scarlet fever bodies (stained light blue) in and between the epithelial cells of the rete mucosum. In Fig. 1 is a large body in a lymph space of the corium just underneath the epidermis. Several of the bodies suggest fixation while in amœboid motion.

FIGS. 3, 5, and 6 are coarsely reticulated forms which may be degenerated forms of the scarlet fever bodies, or stages in sporogony.

FIGS. 4, 8, and 9 probably represent stages preceding the radiate bodies. In Fig. 9 the bodies lie in a lymph space. It shows also four small forms which have just got free from a rosette.

FIGS. 7, 10, 11, 12, 13, 14, and 15 show different stages in the development of the radiate bodies.

Fig. 10 is the earliest stage: there is a distinct central body and a definite, regular arrangement of granules at the periphery. FIGS. 7, 11, and 12 show a little later stage of development; 11 and 12 are optical sections, while 7 is a surface view. Moreover, in Fig. 7 the body lies free in a lymph space in the corium. The segments begin to show a certain amount of lateral separation from each other. Fig. 13 is a still later stage: the segments are increasing in size and are more or less free from each other, although most of them are still attached to the central body. In Fig. 14 the segments are all free and enlarging, although still grouped around the central body. In Fig. 15 the bodies are still grouped around the central body, which is free and stains deeply with eosin.

PLATE IV

to the toxins, infective material, etc., of the developing pustule, or possibly to ill preservation of the tissues. They are characteristic of the later pustules, and their vacuolated appearance may be ascribed to the same causes as that which produces poorly fixed and stained amebæ, or poorly stained Negri bodies. In the latter the better technique of recent methods has shown that what appear as vacuoles in the photographs are actually chromatin fragments (see Fig. 125), and by analogy I would prophesy that when better methods of fixing and staining the intranuclear form of cytoryctes are devised, a similar chromatin distribution will be discovered. The tissues, such as we worked upon four years ago, show many cellular structures like those of the well-fixed and stained Negri body (compare Figs. 121, 122, 124 and 125), and although these were regarded formerly as aberrant forms of the sporoblast structures, many of them were figured and described.

FIG. 125

A large cytoplasmic form of *Cytoryctes variolæ*.

As with neuroryctes, further study with better methods must be undertaken to complete the life history of cytoryctes; the important sexual stages must be found, a hint to this end being given by the changed nuclear phenomena of the intranuclear form (see difference in nuclear processes of vegetative and sexual phases of entameba).

In this same category, finally, must be placed the interesting organisms discovered by Mallory ('04) in the skin cells of scarlet fever victims, and named by him *Cyclasterion scarlatinalis* (Plate IV). Also the curious structures described by Prowazek in trachoma, forms

similar to cytoryctes and neuroryctes, and all of which, together with the cell inclusions of molluscum contagiosum, Prowazek includes under the name of chlamydozoa or "mantle-covered" organisms. This name represents a point of view held by many protozoölogists that the real organisms are the chromatin granules, while the material about them is only coagulated nuclear material. The entire absence of fortuitous strands of such nuclear material in the cell, apart from the enclosed granules, together with the definite history which corresponds exactly with the idiochromidia formation in other rhizopods, renders this interpretation improbable.

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ABBREVIATIONS.

- A. A. Anatomischer Anzeiger.
- A. B. Archives de Biologie.
- A. A. P. Archiv für Anatomie und Physiologie.
- A. M. N. H. Annals and Magazine of Natural History.
- A. m. A. Archiv für mikroskopische Anatomie.
- A. N. Archiv für Naturgeschichte.
- A. Entw. Archiv für Entwicklungsgeschichte.
- A. Z. E. Archiv de zoologie expérimentale et générale.
- B. C. Biologisches Centralblatt.
- B. M. J. British Medical Journal.
- C. R. Comptes Rendus de l'Académie de Sciences.
- C. R. S. B. Comptes Rendus de la Société de Biologie.
- J. M. Journal of Morphology.
- J. Z. Jenaische Zeitschrift.
- M. A. Müller's Archiv.
- M. J. Morphologisches Jahrbuch.
- Q. J. Quarterly Journal of Microscopical Science.
- Z. A. Zoölogischer Anzeiger.
- Z. w. Z. Zeitschrift für wissenschaftliche Zoölogie.

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