PROTISTS AND DISEASE

A Border in which a Didymium Lived.

[Frontispiece.

PROTISTS AND DISEASE

VEGETABLE PROTISTS; ALGAE AND FUNGI, INCLUDING CHYTRIDIINEAE; VARIOUS PLASSOMYXINEAE, THE CAUSES OF MOLLUSCUM CONTAGIOSUM, SMALLPOX, SYPHILIS, CANCER, AND HYDROPHOBIA; TOGETHER WITH THE MYCETOZOA AND ALLIED GROUPS

Being the Sixth Book published
for the Author on the same Subject

BY

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MENS · SANA · IN · CORPORE · SANO ·

LONDON
BAILLIÈRE, TINDALL AND COX
8, HENRIETTA STREET COVENT GARDEN

1922

29368

PRINTED IN GREAT BRITAIN

PREFACE

A CHANGE of title from "Protozoa and Disease" of four preceding books to "Protists and Disease" has become necessary by reason of my having found that the pathogenic organisms dealt with are not protozoa, but protists allied to Synchytriaceae. In other words, in making comparative study of alien bodies that abound in the lesions of molluscum contagiosum, cancer, and other diseases, we have examined protozoa with great care when we should have given the closest attention to every detail of the life-history of certain vegetable protists, especially Synchytriaceae and some Olpidiaceae.

But in spite of our having searched in the wrong department of biology the nature of cancer, smallpox, and other human diseases would before now have become plain to all, had there not been some weakness in the foundations of biology. The nature of this weakness is not obscure: it consists in a serious defect in the cell-theory, a defect the bearing of which is shown in chapters of this book, and which may also be briefly outlined here.

If we bear in mind that the word "nucleus" denotes a morphological conception, the cell-theory in its present accepted form may be defined as consisting of Virchow's "*Omnis cellula a cellula*" (1855) plus Flemming's "*Omnis nucleus a nucleo*" (1882).

The original theory comprised two propositions; firstly, that the ~~nucleus~~ is the unit of structure and function in both plants and animals; and, secondly, that new nuclei and new cells arise in an amorphous basis. The first part was at once recognized by Virchow as a great advance in science, but he did not attach importance to the second part, which indeed was later found to be untrue for the forms of life to which it was applied. In its present state as defined above the theory has proved to apply to nearly all known living things, and it has been the instrument by which so much imperishable biology has been built up that the fact that it is contradictory of dominant phases of well-known groups of organisms has not yet received due attention.

It applies as far as is known to all Metazoa, and to all plants above the Thallophyta, and to most of these, and to many Protozoa; but it is patently at fault when it is applied to dominant phases of certain protists, some thallophytic and others protozoan.

That no qualifying clause has been added to the cell-theory to meet this position is strange seeing that gametogenetic chromidia are now commonplaces in biology, and that these vital processes translate terms such as "free nucleus-formation" into prophecies fulfilled.

In Nature variation is so great that misstatements concerning reproductive processes of some forms of life have proved to be accurately descriptive of what occurs in other forms.

Denial of the existence of non-nucleated phases of some protists renders complete comprehension of many diseases impossible. In the following pages I show once again how the plasson state plays a leading part in the lives of the parasites which cause molluscum contagiosum, cancer, smallpox, &c.

Had English pathology been conversant with Synchytrium from the year 1892 to the year 1895, the immediate causes of cancer, smallpox, and syphilis might have been agreed upon during that period, and, incidentally a new province of biology would have been then acquired.

The first practical aim of this book is once more to beg those who in this country direct investigations into the immediate causation of disease to arrange for systematic examination of molluscum contagiosum. This minor malady, which shows in the skin like dimpled pearls, deserves more consideration than all the pearls in the world for the light it sheds on other far more serious diseases. If unprejudiced and willing examination of molluscum bodies is made in the simple way described in this book, it will be recognised in the course of a few days that these bodies are parasitic organisms. In molluscum we have an easy key to smallpox, the filtrable organisms, and to cancer.

Another aim has been to establish the biological relationships of the pathogenic organisms dealt with; and I trust that readers who are trained in the biology of cryptogams will condone the verbal and ideal stumblings of a beginner; and that others, to whom the matter of this part of the book may be as new as it was to the author two years ago, will follow with sympathy and some profit the progressive, if limited, illumination that has been attained.

In order to make this attempt possible, it has been necessary for me to consult literature outside that contained in our professional libraries, and I am under particular obligation to the officers of the library of the Linnaean Society of London, and of the Cryptogamic Botany Department of the British Museum. Both for direct information and for guidance in the choice of literature Mr. J. Ramsbottom's articles in the "Transactions of the British Mycological Society" have been indispensable to me.

My indebtedness to colleagues and others who have assisted me with gifts of material and specimens is acknowledged in the text, but I must make additional reference to the skilled help generously given me by Dr. J. Herbert Perkins, pathologist to the Hampstead and North-West London Hospital.

J. JACKSON CLARKE.

LONDON, *September*, 1922.

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PROTISTS AND DISEASE

CHAPTER I

FOUNDATIONS

Civilisation still expects accredited instruction upon the real nature of cancer and other tumours; smallpox and other fevers.

If these capital diseases were but fugitive discomforts, it would still be of interest, even of importance, to inquire into their causes for the purpose of adding to existing biological knowledge.

These facts demand that we review the rudiments of our biological information, and that we convert as much of it as circumstances require or allow into knowledge by personal practical work.

Protists.—We need to know some very primitive forms of life, some of those that are conveniently grouped in our minds under the designation of protists. This name is not now used in its original sense as invented by Haeckel, synonymous with "Monera," to apply to a proposed new kingdom consisting of organisms devoid of nuclei; but as one designation for the two overlapping sub-kingdoms or phyla, the Protozoa and the Thallophyta, the latter term applying to the algæ and fungi, including the diatoms, the desmids, and the bacteria. With Haeckel the Third Kingdom was to be that of the Monera, and any non-nucleated cell was to be termed a cytode.

The question of the Monera was touched upon in 1877 by Huxley, but at that date the chromidial state of living organisms was unknown and the data for even a partial solution of the problem were wanting.

In 1896 the position was summed up judiciously by Delage and Hérouard: "For a long time great importance was attached to the cytodes and the Monera of Haeckel . . . but it has been found that this supposed absence of a nucleus is explained in many cases by defective methods. Since nuclei have been discovered in the most of the Monera, of the cytodes, and even in bacteria, some people, by rather hasty induction it seems to us, have denied the existence of non-nucleated organisms." Remembering that filtrable organisms, chromidia, and akaryote phases of protists such as *Sorosphaera* (see Chapter VII) were unknown when the passage just quoted was written, we cannot but admire the moderation of thought and expression shown by the French zoologists.

The Cell-Theory.—A study of protists must include an examination of the cell-theory of life as it exists at present in biology. The nucleus was recognised as a normal feature in the epidermal cells of orchids by Robert Brown in 1833.

In 1839 Schwann's view was stated thus: "The Cytoblastema, or amorphous substance in which new cells are to be formed, is found either contained within cells already existing, or else between them in the form of intercellular substance. . . . In cartilage it is very consistent and ranks among the most solid parts of the body; in areolar tissue it is gelatinous; in blood quite fluid. . . . In plants, according to Schleiden, the nucleolus is first formed, and the nucleus around it: The same appears to be the case in animals."

In 1841 Remak described direct division of nuclei, and in 1873 Anton Schneider described indirect division (karyokinesis or mitosis). Coming to a more recent date I will refer, and I do so with affection and gratitude, to Edmund B. Wilson's "The Cell in Development and Inheritance," 2nd edit., 1904. It is to be remembered that when Wilson wrote generative chromidia had not been discovered.

This premised, one passage may be quoted, p. 294: "It may now be taken as a well-established fact that the nucleus is never formed *de novo*, but always arises by the division of a pre-existing nucleus."

Now, many chromidia, such, for instance, as some of those of the protozoon *Mastigella vitrea*, arise for the very purpose of making nuclei *de novo*, which is the same thing as free nucleus-formation.

Disagreement between facts such as this and the generalisation just quoted are considered in Part III, 1912. Some points call for restatement. Let us glance again at pictured examples of two modes of origin of new nuclei, and we see a great contrast. In Fig. 1 are seen three stages of regular mitosis in a protist: chromosomes, centrosomes, spindle, etc. In Fig. 2 new nuclei are being formed from a chromidium, a segregated mass of akaryote germ-plasm that has been extruded from the nucleus of its parent protist

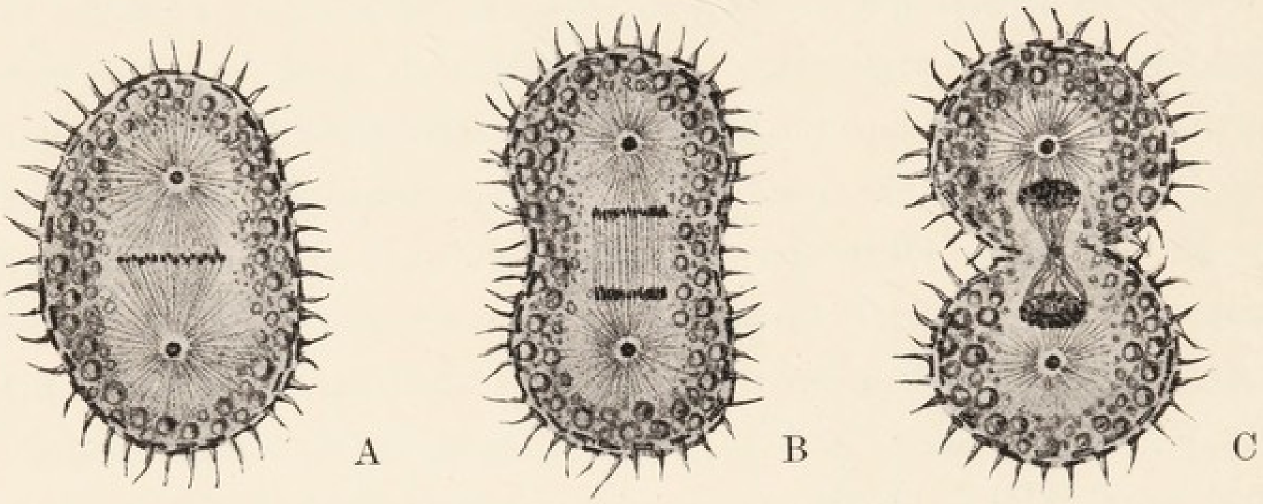

Fig. 1.—Stages of Mitosis in Acanthocystis aculeata, a Heliozoon: *a*, metaphase; *b* and *c*, anaphases. (From Doflein, after Schaudinn.) From Part III.

and lies in the cytoplasm, equivalent to a metazoan ovotestis. In the vegetative part of its life *Mastigella* undergoes mitosis quite like that of *Acanthocystis*, Fig. 1.

Chromidia.—An akaryote structure destined to produce gametes illustrates one kind of chromidium, the generative; the other kind is the vegetative. Generative chromidia have been recognised (H. Wager) as of two kinds; firstly, those that replace nuclei, and secondly, those that are capable of being re-formed into nuclei. Of the latter the gametogenetic chromidium of *Mastigella* is an example; in this case the origins of the new cells, Fig. 2, *2*, Chr., are not differentiated into nucleus and cytoplasm.

Vegetative chromidia are again of two kinds; firstly, portions of a nucleus extruded diffusely into the cytoplasm, and secondly, chromatin accumulated in nucleus-like structures.

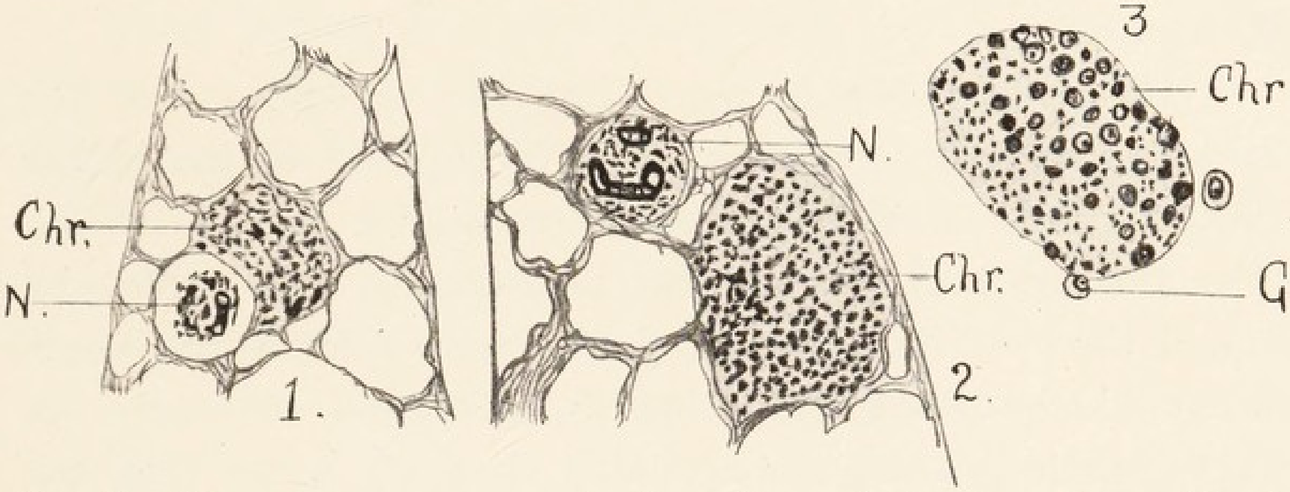

Fig. 2.—Mastigella vitrea: 1. Formation of the chromidium (Chr.). 2. The chromidium increased in size. 3. The chromidium alone more highly magnified, to show free nucleus-formation. N, nucleus. G, gamete. (After Goldschmidt, from Doflein.) From Part III.

Once it is fully developed the chromidium, Fig. 2, Chr., is an independent organism much as is the prothallus of a fern. If we could suitably subdivide it, each segment would doubtless live and be able to produce gametes.

The foundation of all biology and pathology, the cell-theory, as it exists to-day, is unsound in that it is contradictory of akaryote states which are dominant in the lives of certain protists.

Plasson.—If we look at Goldschmidt's drawing, Fig. 2, *2*, of the origins, or primordia, of the gametes of *Mastigella*, when first they appear in the matrix of the chromidium, we can see in them no differentiation of structure; each gametoblast is a particle of apparently structureless material. For such substance Haeckel used the term "plasson." This totipotential form of living matter has been defined in an article on the Proteomyxa by S. J. Hickson:

"It seems probable then that the protoplasm of the Proteomyxa really represents the protoplasm of the higher Protozoa and Metazoa plus the substance of the nuclei. It is the substance which van Beneden, in 1871, proposed to call the 'plasson,' that is, the formative substance which is capable of becoming, either in ontogenetic course or in phylogenetic course, monocellular elements, after that the chemical elements of the plasson have been separated to constitute a nucleus and a protoplasmic body."

Haeckel was right in that non-nucleated organisms exist, but those we know have also phases in which they have nuclei; therefore he was mistaken in assuming that his cytodes were of necessity devoid of nuclei at all stages of their life-history; indeed, many of them have been found to have nuclei at all stages.

In estimating Haeckel's work we should remember that he was the first to note the inception of foreign particles by leucocytes; the bearing of this function on immunity was first indicated by Carl Roser in 1881, and later named phagocytosis by Metchnikoff.

Filtrable Viruses.—The living virus of foot-and-mouth disease was found by Loeffler and Frosch (1898) to pass through filters which arrest any known bacteria. Since that date a number of other living poisons have been found to be filtrable, including those of measles, smallpox, and molluscum contagiosum.

Many pathologists now write with facility of "invisible micro-organisms," so it is of value to examine this very important field. M. Arthus, in a most interesting book published last year, writes thus: "We admit that the causal microbe exists, but suppose that it is so small that it eludes microscopic search, and this hypothesis—for it is nothing more than a hypothesis—is supported by the facts elicited in the study of pleuro-pneumonia of cattle." The author goes on to say that in the serous exudation of this disease fine granules can be seen with the highest powers of the microscope; they can be stained, though with difficulty, and they can also be cultivated on very special media. Arthus asks whether we may not see in the virus in question a link between the visible and cultivable organisms on the one hand, and the invisible organisms on the other hand.

We find no word here of these most minute of known living units having any phases other than the filtrable. And this is the usual view, though it is proved to be an error by what is seen in simple water cultures of molluscum bodies: see Chapter VI.

It is as if a new "Third Kingdom" were being erected to replace that of the Monera. Just as there are cytodic (plasson, chromidial) stages of some protists which have obvious nuclei at other stages, so there are filtrable stages of parasites such as molluscum corpuscles, which are relatively large bodies; and, just as some living organisms may conceivably be persistently akaryote, so others may possibly exist only in the filtrable phase.

Protoplasmic Motion.—"The colloidal nature of protoplasm is manifested in many of its properties. Its power of adsorption which lies at the basis of many cell reactions and certain staining processes, is similar to that of other colloids. . . . The permeability of a vacuolate cell is in general the resultant of the permeabilities of the ectoplast, cytoplasm, and tonoplast" (L. W. Sharp, 1921). The chief cause of stability in colloids is Brownian movement (Bayliss, 1920, *q.v.*). This movement is seen in many living cells, *e.g.* in the protoplasmic strands of *Spirogyra*. Streaming is probably present in the protoplasm of all active cells. It was first recorded in *Chara* by Corti in 1774. In some cases, as in the cells of the staminal hairs of *Tradescantia*, the stream does not carry the nucleus with it; in other cases, as in the leaves of *Vallisneria*, the nucleus moves with the rest of the protoplast.

Most impressive are the to-and-fro currents of the plasmodium of Mycetozoa: in these the outward current lasts over a minute, the return a little under a minute. In

Fig. 38 is copied de Bary's instance of a rotatory movement replacing the alternate currents in a mycetozoan plasmodium.

In a subsequent chapter an account is given of a streaming or circulation of protoplasm in the bodies peculiar to the disease molluscum contagiosum.

This streaming may last for several days and may be accompanied by the presence of bud-like protrusions and of separate minute spheres with a similar streaming. Apart from other evidences in molluscum bodies this streaming is a strong witness to life; and it is a phenomenon any one who may have the material can reproduce.

Besides such movements of protoplasm of a cell there are currents in channels or linear vacuoles: watching a large fungus hypha growing in a slide-and-cover culture I have seen in minute parallel channels steady currents pass some in the direction of growth, others the opposite way; one such hypha was undivided when first seen, later septa were formed, but these did not appear to affect the circulation.

By fixing root-tips in a mixture of neutral formalin (10 c.c.), K_2CrO_4 (2·5 g.), $HgCl_2$ (5 g.), H_2O (90 c.c.), Bensley demonstrated the channels he named canaliculi, the presence of which is an indication that intracellular circulation of liquids exists. In mitosis the centrosomes and the two spindle poles have been found to be alike electrically, and, if so, they cannot represent an ordinary electromagnetic field. The filament that connects a nucleus and the blepharoplast at the base of many flagella suggests that the movement of these is effected by the same apparatus as mitosis. As is shown in Fig. 22, *b*, zoospores of synchytrians penetrate their host by a nuclear structure which resembles a blepharoplast with its connecting filament; other chytridians and, as Waterhouse found, the sporidia of *Puccinia graminis*, appear to effect penetration in a similar way.

Simulation of Life.—In his book, "The Protozoa," 1901, Calkins gave a good account of the life-simulating states of dead matter: amœboid movements simulated by a drop of olive oil in water; Buetschli's mock-protoplasm, olive oil mixed with salt or sugar in water; Rhumbler's shellac-coated spicule of glass incepted by a drop of chloroform in water and ejected when the shellac is absorbed, with many other instances.

I have made Buetschli's experiment both with salt and oil, and sugar and oil. A few crystals that project from the oil into the water are seen to dissolve, causing active currents of very brief duration; and slow local passive currents are seen in both oil and water; there was nothing that should be mistaken for active living matter.

Culture of Metazoan Cells and Tissues.—Every cell of a living metazoon is an animalcule, and with minute care to exclude bacteria (see Lee's "Microtomist's Vade Mecum," 1921) such cells may be kept alive and cultivated artificially. Nerve-cells from the embryo frog were first grown by Ross

Harrison in 1907, the medium being lymph from adult frogs. Next Burrows grew tissues from embryo chicks on blood plasma in plate cultures. Using the same method Carrel and Burrows cultivated tissues (kidney, spleen, bone-marrow, etc.) of adult cats and dogs.

Connective-tissue from the embryo chick has been kept in culture for as long as eight years by Ebeling. An incubation of three days was observed by Busse before the cells of fragments of rabbit's heart or aorta developed new cells. The latter are branched and anastomose, but when bacteria are placed near them they become detached from one another and round; they are attracted to the bacteria (chemiotaxis) (see the *Lancet*, April 29, 1922, p. 856). All these features are in keeping with what is familiar in the study of granulation-tissue by other methods. When the infecting organisms of molluscum contagiosum and kindred affections are more generally appreciated, such cell-cultures in practised hands may be useful in the study of some details of the stages of infection.

Cellulose and Chitin.—Chemically isomeric with starch, cellulose is one of the most abundant of the products of life. Like starch it grows by intussusception, and from it are made a number of degradation products such as the jelly so freely produced by many algae, etc. In the form of cotton-wool it responds readily to the usual tests, turning blue with iodine solution followed by sulphuric acid diluted with water; equal parts (Bower) or one of iodine solution to two of acid (Chamberlain), also with chloro-iodide of zinc and iodine; and dissolving in saturated solution of copper hydrate.

Where fungi are present one might expect these reagents would reveal them by colouring their cellulose, but the reactions are of value only when positive. It is rare to find fungi that respond. Fungal cellulose often requires long maceration in 15 per cent. potash solution before it may react. In Mycetozoa de Bary obtained the reaction but once in the innermost layer of the sporangium wall of *Trichia varia*, and had many negative results. The capillitia of *Didymium* and *Stemonitis* changed to violet with the acid alone.

Woody fibre is composed chiefly of lignin, a modification of cellulose, but does not react in the same way. In protozoa, chitin, a nitrogenous substance akin to keratin, takes the place of cellulose, though one Rhizopod, *Chlamydomyxa*, is partially enclosed in a capsule of cellulose. Cellulose also forms the tunic of Ascidians, animals near the vertebrates in organisation.

Chromatin and Nuclei.—The living nucleus can be studied easily in many protists and cells. It contains refracting material which stains readily; hence called chromatin (Flemming, 1879). When this is not diffused through the whole nucleus we see in fixed preparations that it lies in a net which is continuous as a rule with the cytoplasm through the nuclear membrane. This supporting net is called linin, doublet of linen (Schwarz, 1887).

In mitosis spindles are formed by re-arrangement of linin fibres. Spindles are usually formed inside, but sometimes outside the nuclear membrane.

The substance that separates two nuclei after mitosis comes from the linin spindle.

In the following pages where my own observations are referred to and it is not otherwise stated, cells, plasmodia, or tissues were stained with acid hæmatoxylin after fixation by Foa's solution: see note at the end of this book. With this stain pure chromatin is of a deep blue colour.

Ehrlich divided dyes into basic and acid according as the colouring matter plays the part of an acid or a base the compound employed (Wilson). Ehrlich's statement only applies to cover-glass preparations dried and fixed by heat without the use of reagents (Lee's "Microtomist's Vade Mecum").

Heidenhain termed the chromatin of Flemming "basichromatin" to distinguish it from nucleoli, cytoplasm, and other parts of cells which take acid dyes, such as acid fuchsin and eosin. He concluded that basichromatin is rich in nucleic acid, which Bayliss defines as a compound of phosphoric acid with a 5-carbon sugar and a purine derivative.

The staining reactions of nuclei of the same organism may vary: thus Barrett found in the chytridian *Olpidiopsis Saprolegniae* that after the gonoplasm has passed into the oogonium the nuclei in the resulting oospore stained more deeply with safranine than they did before fertilisation.

As Wilson points out, there is no specific stain for chromatin. Similar staining reactions do not necessarily mean chemical identity; both the nuclei and matrix of cartilage stain alike with methyl green.

Staining reactions have their value and we should be able to make use of every technical resource; at the same time we must not forget that a process which reveals one feature brilliantly will conceal others of equal importance; nor that technique is a means, not an end.

Bodies suspected of being protists should invariably be first carefully examined unstained in conditions as nearly as possible like those in which they are discovered, and then in water. In this way alone can we let them show by their own activities if they are living organisms. They should also be tested by attempting cultures in water as described below in Chapter VI.

Intravital Staining.—Methylene blue, neutral red, and other dyes are used to stain living tissues and organisms. Their applicability is limited; it is generally agreed that when a nucleus takes a stain it is dead. Neutral red stains granules, and it has the advantage of being a test of acid or alkaline reaction, becoming brighter red with the former, yellow with the latter. It is a basic dye, its name referring to its tint. Janus green (Hoechst) has been found to stain the mitochondria of living human lymphocytes; mitochondria, being soluble in alcohol, chloroform, and ether, are destroyed by the ordinary processes.

Medicine a Branch of Biology.—Members of the medical profession have direct access to a wide field of biological experience and material from which the academic biologist is debarred. Our daily study of our patients is a biological occupation. Each advance a doctor makes in skill is a biological achievement. If such advance is a discovery new to his science, and is published, it is a contribution to the humane applied biology we call Medicine. "Pathology is by no means the smallest branch of that beautiful science biology," Virchow wrote in 1855.

To have in the foundations of our consciousness the well-established principles of general biology is necessary to every medical practitioner. But principles cease to guide if they are not revived from time to time by personal experience; to spend an occasional hour or two watching with the aid of a microscope living algae, fungi, Mycetozoa, etc., brings real refreshment. In order to be able to share any new knowledge we may gain in matters that pertain to general biology we must use terms that cannot be misunderstood; for this purpose we must learn the rules of technical biological conversation, and by so doing we shall obtain for ourselves the key to the treasures stored in systematic biological literature. The responsibility of instructing the rest of the community on the subject of disease must be ours. Ours also is the duty of investigation; lay biologists have voluntarily come to our aid from time to time; and too often from being untrained to know or to counter the dangers that they ran, their zeal has cost both them and us their lives.

Classification or Taxonomy.—Any collection of objects can be classified in various ways; *e.g.* by size, shape, or colour. Classification is abstract, artificial: as Delage and Hérouard have written, "Only individuals exist." All the same we know that without systems of grouping there could be no natural science. On this subject Huxley may be quoted: "Each such assemblage is in fact a 'natural order' in the sense in which that word is used by botanists, and although the number of these natural orders may be increased by the discovery of new forms, or diminished by the ascertainment of closer bonds of union than are at present known to exist between the orders already discriminated, yet the morphological types which they represent will always remain, and therefore the knowledge of their characters, once acquired, will be a permanent possession."

As far as knowledge allows organisms are grouped according to the stock they come from; their *stirpes*, as John Ray (1628–1705) called them. Ray was the first to distinguish in flowering plants the two groups, mono- and dicotyledons.

After the publication of Darwin's "Origin of Species" in 1859, classification became more consciously guided by phylogeny.

The immense array of living organisms is first broadly separated in our minds into the two kingdoms of plants and animals respectively.

The animal kingdom is divided into two sub-kingdoms or phyla, the protozoa and the metazoa. Botany has no group that corresponds exactly to protozoa, indicating a division in which the greatest structural differentiation is reached in unicellular bionts. The term protophyta was used by Sachs to denote a joint group of algae and fungi; blue-green algae (Cyanophyceae) like *Chroococcus*, *Nostoc*, &c., and green algae (Chlorophyceae) such as *Pleurococcus;* together with the bacteria and yeast-fungi.

The Thallophyta in botany are a section of the cryptogams, and include all plants lower in organisation than the mosses.

The body of such plants is termed the thallus (Gr.=green bough). The vegetable protists include Sachs's Protophyta and filamentous algae such as the Ulotricheae, the Conjugateae (Zygnemieae, desmids, and diatoms), and others such as *Vaucheria*, which produce oospores, together with the corresponding fungi, Phycomycetes, and a number of other groups of algae and fungi. The commoner grouping of organisms in descending scale is into classes, orders, genera, and species; secondary groups being intercalated as affinities require. The terms genus and species as defined by Linnaeus are unchangeable; as regards the remainder custom varies with different authors, but the terms class and order are fairly constant in application.

In botany names of orders are Latin adjectives ending

2

in -ae with "Plantae" understood; *e.g.* Rosaceae, Compositae. For cryptogams the ending -ales is also used.

The word family was first used as equivalent to order; so by J. H. Balfour (1855), and so in the British Museum 'Handbook of British Lichens,' 1921; but in Bennett and Dyer's *Sachs* (1875) we read "Smaller groups which are called families," and some writers still follow them.

The class is always a wide group. All the Protozoa are made into only 4 classes: Rhizopoda, Flagellata, Sporozoa, and Ciliata. All the plants fall into 4 great groups: Phanerogamae (flowering plants), Pteridophyta (ferns &c.), Bryophyta (mosses &c.), and Thallophyta (algae, fungi, lichens &c.). The secondary groups of Thallophyta: algae, fungi, lichens, Mycetozoa, &c., constitute each a class. As to the proper term to apply to the next subdivisions sub-class is obvious; between it and the order groups are variously named; series (desinence -eae) and sub-series (desinence -ineae) are convenient terms.

In grouping the protists the Mycetozoa must be given a central position as being about equally animal and vegetable in constitution, and we may arrange on one side of them a series of vegetable, and on the other side a series of animal protists.

Terminology.—The names used for different phases and organs of protists are complicated by some groups being included both in botany and zoology. The well-known *Volvox* is an alga in botany, a phytoflagellate in zoology.

In botany its motor organs are termed cilia, in zoology flagella. There is no essential difference between the organ indicated by cilium (eye-lash) and flagellum (whip-lash), but it is useful to use words to distinguish the single or few motor organs of the Flagellata from the relatively smaller and more numerous organs of the Ciliata. In this book the word flagellum will be used for motor organs of the zoo-spores of algae, and Phycomycetes, &c., where "cilium" is usually written.

Difference in the use of terms is nowhere more marked than in the use of the word "spore." In zoology it is now but little used: asexual reproductive elements are merozoits to distinguish them from sexually formed sporozoits. What used to be called a spore in gregarines is now a sporocyst, and its contents sporozoits. In Myxosporidia and Haplosporidia the first subdivisions are pansporoblasts, which produce the sporoblasts or spores. Pansporoblasts with many minute spores recall the sporangia, which are grouped in the sorus of synchytrians.

In botany there is need of names for a wide range of reproductive elements. De Bary gives an account of his interpretation of the word spore. Though known in a general way respectively as zoospores and spores the asexual reproductive cells of algae are termed gonidia (Gr. *gone*, offspring), and of fungi, conidia (Gr. *konis*, dust). The term swarm-spore is sometimes used as equivalent to zoo-spore. The fertilised ovum, as in *Vaucheria*, is called an oospore; the fused gametangea of *Mucor*, &c., form a zygospore.

When the spore of a fungus germinates by the formation of a promycelium from which arises reproductive elements different in shape from the ordinary conidia they are called sporidia.

Nomenclature.—It is imperative that there should be a uniform system of names in biology. The binominal or Linnaean system is universally adopted. Linnæus (Carl A Linné, 1707–1778) defined his system thus:—

"Diagnosis sic plantæ consistit in affinitate *Generis* et in discrimine *Speciei*. Nomen plantæ itaque, ut utramque diagnosin indigitet, duplex erit: *Genericum*, cognomen gentilitium. *Specificum*, Prænomen triviale."

At the International Botanical Congress, 1905, it was recommended that specific names begin with a small letter, except those which are taken from names of persons (substantives or adjectives) or those which are taken from generic names. This was to apply to the vascular plants in particular.

The Medical Research Council ('Notes upon the Preparation of Monographs, &c.,' 1921) directs that all specific names be written with a small initial letter, even when they are formed from the names of persons or places. This is the practice in zoology. Thus in the British Museum 'Guide to the Mycetozoa' (1919) we read *Diderma Trevelyani*, and *Reticularia Lycoperdon*; and, in zoology, *Trypanosoma brucei*, and *Spirochaeta anodontae*. The latter method has the attraction of uniformity, but the other is that usually adopted by the botanists to whom we owe our knowledge of the Mycetozoa and the vegetable protists. In this book specific names are written with small initials except in Chapter VIII, where, in order to give an example of the customary botanical method of writing names, these are written as in A. Lister's monograph on the Mycetozoa.

There is scope for a joint conference of cryptogamic botanists and zoologists to decide on a common plan of taxonomy and of nomenclature for protists.

CHAPTER II

SOME ALGAL AND FUNGAL PROTISTS

PROTISTOLOGY has been defined by Pavillard as the comparative cytology of primitive forms of life. It is this and more: it includes the natural history of the organisms, and, when they are parasites their relation to their hosts.

Algae

With the known exception of one parasitic species noticed below, all algae contain chlorophyll and so have that most important of all physiological properties, the power of decomposing CO_2 in sunlight, the oxygen being liberated and the carbon either used in metabolism directly or built up into starch, $C_6H_{10}O_5$, as reserve nutriment. In a word they are holophytic, except one or two parasitic species.

The starch in many cases is deposited in chromatophores in refractive bodies, called pyrenoids, which stain purple, blue, or even black with iodine solution. A few typical algae and fungi may now be reviewed briefly in order to lead up to a closer study of some of their congeners, which are pathogenic to man.

Ulothrix zonata is to be found in spring and summer attached to stones and other objects near the surface of slow streams or ponds; if brought in the morning from a cool to a warm place, it forms its reproductive elements. A filament mounted in water and covered may show both macro- and microzoospores, Fig. 3, A; *ma, mi*; from two to eight of the former, and from four to sixteen of the latter being produced from a single cell. The macrozoospores, which have a contractile vacuole, sink, and lose their flagella, in place of which an organ of attachment or rhizoid is developed, the element germinating directly as a new plant, Fig. 4, *A*. The microzoospores conjugate by their anterior ends, then lose their flagella, coalesce completely, and the resulting zygospore secretes a cell-wall and matures slowly for germination next spring. In other terms the microzoospores are gametes, their fusion is isogamy, and the zygospore is a zygote. Both kinds of zoospore in *Ulothrix* are at first enveloped in a thin capsule which is ruptured to allow the active cell to escape. The same feature is observed in the zoospores of the fungus *Achlya*, Fig. 4, *C*.

The macrozoospores of *Ulothrix* correspond to the parthenogonidia of *Volvox*, the sexual elements in the latter being differentiated as oogonia and spermatozoids.

Another alga, *Vaucheria sessilis*, is a species belonging to a group called the Siphoneae. It grows on damp earth and can often be found in greenhouse flowerpots, and a plant is easily isolated by unaided vision. The thallus is a tube, simple or branched, at first without septa. It is usually sparsely branched and some of the branches may be modified into rhizoids, Fig. 3, *B*, *r*. The protoplasm lines the cell-wall and contains nuclei in its inner layer, and

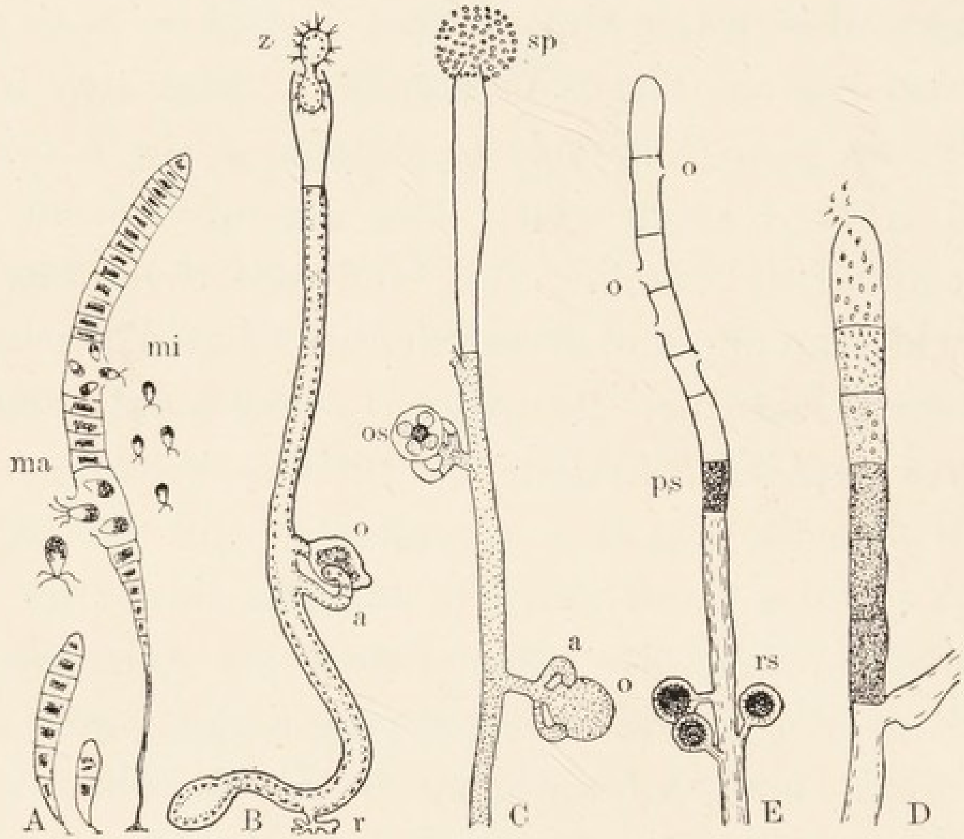

Fig. 3.—Some Algae and Fungi. *A*, diagram of a filamentous alga; *ma*, macro-, *mi*, microzoospores; *B*, *Vaucheria;* *r*, rhizoid; *z*, zoogonidium; *a*, antheridium; *o*, oogonium; *C*, end of a hypha of *Achlya;* *sp*, spores; *a*, antheridium; *o*, oogonium; *os*, oospores; *D*, end of a *Saprolegnia* filament infected by *Rozella septigena;* *E*, later stage of an infected hypha; *ps*, parasitic segment; *o*, *o*, openings whence zoospores have escaped; *rs*, resting spores of *Rozella;* *c–d*, after de Bary and M. Cornu.

chlorophyll corpuscles and oil-drops externally. The middle of the filament is occupied by a sap-vacuole.

This arrangement, a multinucleate non-septate condition seen in Siphoneae, is also found in Phycomycetes such as *Pythium.* Such organisms are termed coenocytes (Gr. *koinos*=shared in common).

Those who can recall their early botanical studies may remember what happens when a *Vaucheria* is incised under water; part of the protoplasm oozes out in lobed masses, which show amoeboid movements for from half an hour to an hour. A sharply, often doubly contoured skin-layer or ectoplasm, like that seen in the mycetozoan plasmodium is the surface of this naked protoplasm, portions of which may become detached as rounded bodies, in which vacuoles appear. The layer of protoplasm limiting a vacuole is called the vacuolar membrane or tonoplast. Many of the amoeba-like fragments disintegrate, but some which contain nuclei renew their growth and secrete a new cell-wall. When *Vaucheria sessilis* is grown in water in sunlight vegetative reproduction by zoogonidia occurs. These are multinucleate bodies with a pair of flagella opposite each nucleus. A terminal part of a filament is shut off by a septum before the zoogonidium is formed, and the motile body escapes by an aperture in the cell-wall. This happens when the plant is exposed to light after being in the dark for some hours.

After swimming awhile the zoogonidium sinks, loses its cilia, secretes a cell-wall, and germinates by one or more sprouts. The zoogonidium remains as a permanent part of the thallus thus developed.

Sexual reproduction is by gametangia: an antheridium and one or two oogonia are formed as lateral branches shut off by septa, Fig. 3, *B*, *a*, *o*, and Fig. 4, *B*. The gametangia appear in the evening and are complete next morning.

The modes of reproduction of Volvocineae and of *Hydrodictyon* (the water-net) should be recalled. In the latter the contents of a single cell divide simultaneously into from 7,000 to 20,000 zoospores, which join together to form a new net (Bower). A cell of the same organism in its sexual

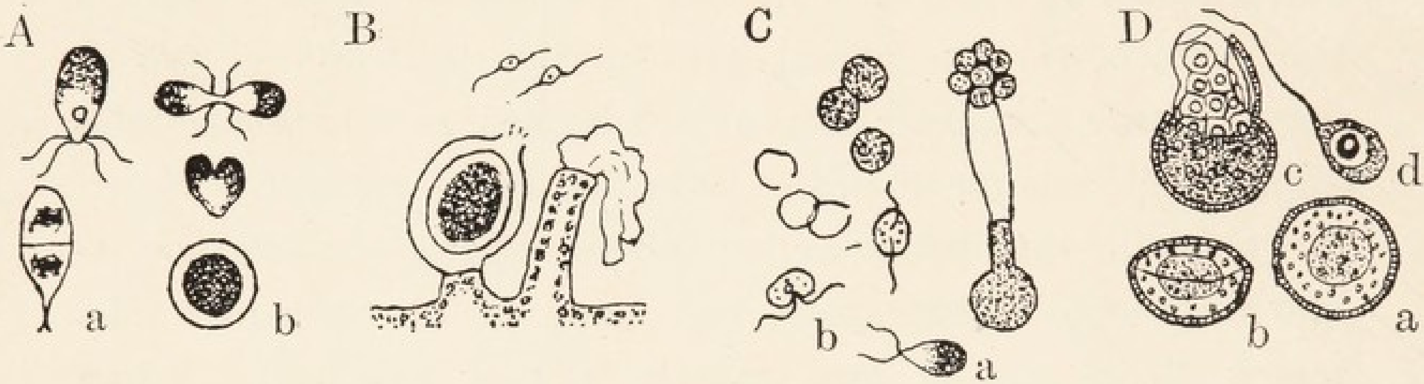

FIG. 4.—STAGES OF SOME ALGAE AND FUNGI. *A*, diagram, *a*, of algal macrozoospore and young plant, and, *b*, of conjugation of micro-zoospores and resulting zygote ; *B*, *Vaucheria*, empty antheridium, oospore, and antherozoids ; *C*, conidia of *Achlya* changing to zoo-spores, *b* ; germination of oospore ; and, *a*, the first form of a zoospore of *Saprolegnia*, the second being like *b* ; *D*, stages in the germination of the resting spore of a cladochytridian, *a*, end view, *b*, side view, *c*, sporangium forming, *d*, zoospore ; *C* and *D* after de Bary.

phase produces upwards of 30,000 zoospores which conjugate in pairs or greater numbers (van Tieghem).

The cells of simple green algae, *e.g. Pleurococcus, vulgaris*, are arranged in twos, fours, &c., or separately from one another. *Pleurococcus* shows as green powder on tree-trunks, &c. Its cells have nuclei and chromatophores. In some young algal cells chlorophyll seems to impregnate the whole cytoplasm, there being no chromatophores. Some algae grow in pure cultures like bacteria. Charpentier found that *Cystococcus humicola* grown in a liquid medium containing 1 per cent. of glucose besides the usual salts (phosphates, nitrates, and sulphates) would live in the absence of light, taking its carbon from the glucose. Grown in the light the plant multiplied more rapidly and produced no starch, which was abundant when the plant was grown in the dark. He regards *Cystococcus* as a " plante de passage " prepared to adapt itself to a mode of life like that of the fungus, *Mucor*.

Able to take carbon from the air green algae seldom adopt a parasitic life, but one of them, *Chlorochytrium lemnae* is parasitic on duckweed. The genus was founded in 1872 by Cohn, who, as B. M. Bristol informs us, described it as follows: " Endophytic green unicell, in which multiplication takes place by means of numerous zoogonidia produced by free cell-division, first into large segments, later into innumerable pear-shaped bodies which are extruded through the tubular process on the cell-wall." The nucleus in Miss Bristol's illustration is central and has a large nucleolus.

Another parasitic alga, *Rhodochytrium spilanthidis*, deserves close attention. It resembles the fungi in being devoid of chlorophyll, and is strictly parasitic. Common in N. Carolina and Equador it has been reported in only one other district. *Rhodochytrium* occurs in different host-plants.

To the naked eye it appears as a small bright red spot, as told us by R. F. Griggs, some of whose illustrations are copied in Fig. 5. It developes starch abundantly, the grains being built up in the protoplasm as Charpentier found to be the case with *Cystococcus*.

The colouring matter is a lipochrome. The plug at the surface end, Fig. 5, *C*, *p*, melts away when the ripe sporangium is placed in water. *Rhodochytrium* is not an intracellular parasite like *Woronina* and *Rozella*, but it makes room for itself in the intercellular spaces of the host plant. At its broad deeper end it pushes out rhizoids, which are shut off by septa when the alga becomes either a resting-spore or a zoosporangium. The smallest resting-spore is 70μ long. At maturity the nucleus of the resting-spore shrinks, Fig. 5, *B*, or collapses, to expand again when the spore germinates in water. The zoosporangium has other peculiar nuclear characters: on entering the host-tissues the nucleus increases rapidly in size, from 4 or 5μ in the zoospore to 50 or 60μ in the zoosporangium; such a growth of a nucleus is only equalled in *Synchytrium*. It is not in size alone that the nuclei of *Rhodochytrium* recall those of *Synchytrium*: the peculiarly large nucleoli, Fig. 5, *D*, and the disordered-looking primary mitosis, *E*, have a likeness to features in *Synchytrium*. Now, though *Rhodochytrium*, an intercellular parasite, is different in habit from the intracellular *Synchytrium*, these nuclear processes show that there is a probable near kinship.

If we compare the parasite, *A*, with a zoospore, *G*, a great difference in size is apparent, but the protoplast[1] in *A*

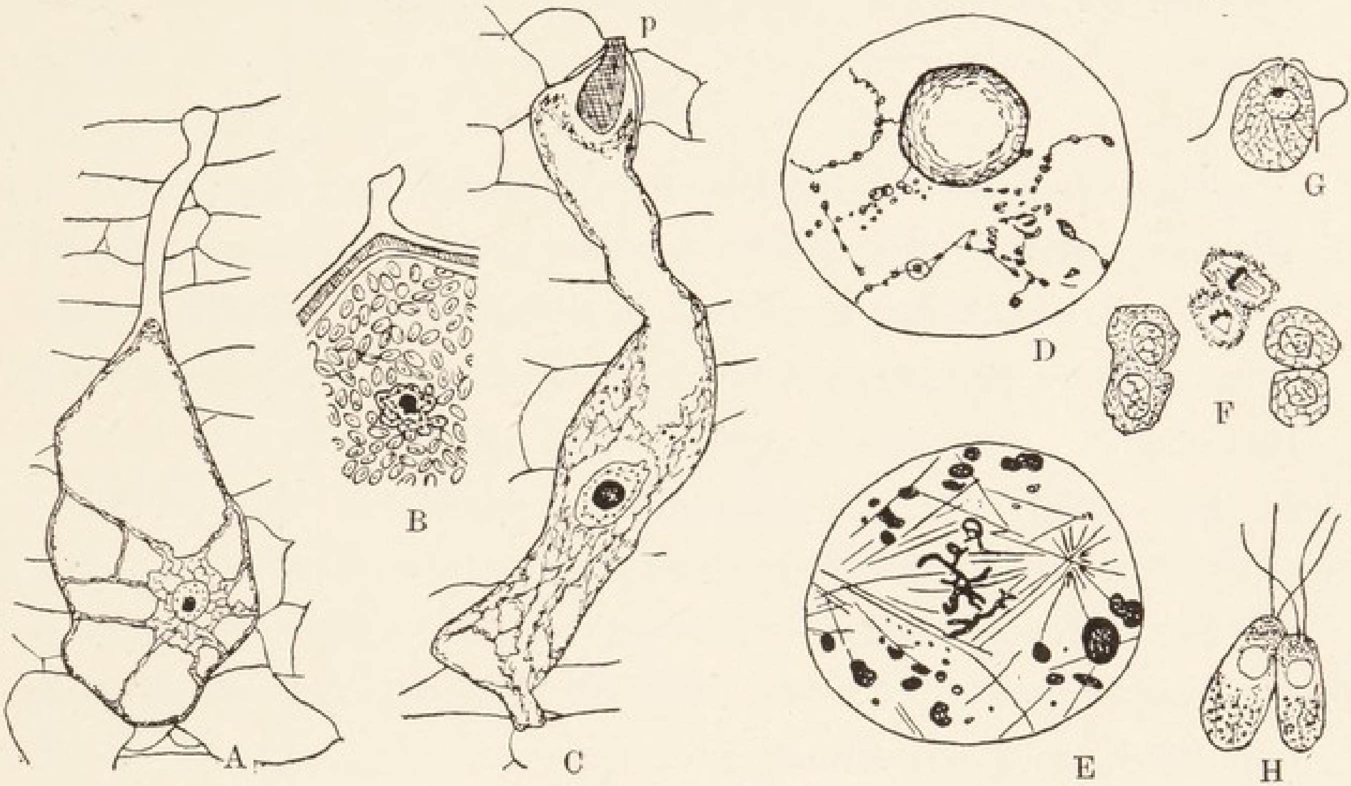

Fig. 5.—Rhodochytrium Spilanthidis. *A*, form of the parasite destined to become a resting spore, rhizoids not in the section; *B*, segment of a ripe resting spore, with shrunken nucleus, starch grains, and triple capsule; *C*, form of parasite destined to become a zoosporangium, a large vacuole occupies the outer half, and a thickening in the form of a plug closes the surface end; *D*, a resting nucleus, a large nucleolus and beads of chromatin on linin threads, from a half-grown zoosporangium; *E*, the primary mitosis, at one end of the spindle the aster is wanting, and there are coarse linin threads and chromatin masses outside the spindle; *F*, the last mitosis and the two last states of subdivision; *G*, zoospore; *H*, conjugation of two zoospores. Reduced to $\frac{2}{3}$, after R. F. Griggs.

is only slightly larger than that of the zoospore; the increase in size is due to large vacuoles filled with cell-sap.

[1] The term protoplast in botany signifies the active protoplasmic cell-contents, *i.e.* the nucleus and cytoplasm of nucleated cells (Hanstein, 1880).

That this non-chlorophyllous plant can produce starch is explained by its being a parasite and using the juices of its host for nutriment.

Fungi

Fungi never possess chlorophyll and hence, like animals, they are entirely dependent for their nutrition upon organic matter, living or dead. They do not produce starch, though some possess glycogen, which, like starch, is easily converted into sugar.

Fat, either as granules or oil drops, is the principal substance they store as reserve nutriment. The dry thallus of *Penicillium* after completion of the vegetative period contains 50 per cent. of fat. The oil of many fungi is coloured, constituting lipochrome.

The Phycomycetes include such genera as *Saprolegnia*, *Peronospora* and *Mucor*. The name was given them by de Bary from their affinity to the algae. The Chytridiineae have since been included in this group.

Saprolegnia.—All species of this genus are aquatic, the thallus being attached by a rhizoid to decaying matter. One species is found as an epiphyte on ulcers of diseased salmon. The hyphae may be $\frac{3}{4}$ inch long. Dying gold-fish are often covered with a woolly growth of *Achlya prolifera*. These fungi are very like *Vaucheria* in organisation. To obtain specimens place some dead insects or boiled white of egg in a quart of pond water. After 24 hours rinse in clean water and transfer to tap water. Sporangia appear in 24 hours (Chamberlain). The thallus is normally an undivided sac, simple in *Saprolegnia*, branched in *Achlya*. Septa are formed to shut off reproductive organs or to repair injury.

In agamic reproduction the end of a filament swells as a long sporangium, a septum isolating its contents, which subdivide into many uninucleate segments. In *Saprolegnia* these escape directly as bi-flagellate zoospores, in *Achlya* in a mass held together by a jelly, Figs. 3 and 4.

The large sporanges of *Saprolegnia* with their central vacuole, cylindrical or irregularly linear, according to the thickness of the protoplasm, lend themselves to the study of the mode of origin of zoospores; this has been described by M. Hartog and others; seeing that corresponding stages are seen in *Pythium* and Chytridiineae, the leading features may be enumerated, following E. J. Butler's account: 1, Preliminary separation of spore-origins by concentration of protoplasm around each nucleus, linear vacuoles occupying the intervals; 2, homogeneous stage caused by swelling of spore-origins and disappearance of the sporangial vacuoles; this stage has been explained as being due to rupture of the skin-layer admitting water to the spore-origins; 3, vacuolation; in the origins are formed a number of vacuoles, which grow, fuse, and disappear; discharging water into the exterior where the escaped cell-sap attracts bacteria; 4, final separation of zoospores by surface grooves meeting newly-formed linear vacuoles, which re-appear in the protoplasm.

In *Saprolegnia* the zoospores leave the sporangium as motile egg-shaped bodies like those of algae, Fig. 4, *C*, *a*. They have two flagella at their narrow end which is foremost in progression, and three vacuoles in the granular plasm. After a few minutes the zoospore comes to rest, its form changes to a sphere and a cell-wall of cellulose is secreted. After some hours or even days the contents escape again as a zoospore, this time kidney-shaped like those of *Achlya*. The second mobile stage may be omitted, the spore germinating directly as a hypha.

This transformation in the zoospores of *Saprolegnia* is termed "diplantish" by de Bary, suggesting that at different stages the zoospores gave the impression that they belonged to two different plants. The somewhat consonant term "diplanetism" is now applied to the same phenomenon, signifying a double period of motility.

In *Dictyucus* the zoospores instead of escaping from the sporangium in one mass are retained in the sporangium where they germinate and escape by penetrating the sporangium wall, or, failing in this, they perish, the fate of the majority.

Resting conidia.—In old growths, especially of *Saprolegnia*, the thick hyphae break up transversely into segments, which are cylindrical, round, or barrel-shaped. These segments are resting conidia ; they are rich in protoplasm and often have thick walls. In *Achlya prolifera* de Bary saw them form acrogenously. They germinate either in clean oxygenated water or in suitable nutrient fluids, producing either new hyphae or sporangia.

In the sexual process antheridia and oogonia are formed and shut off by septa. The contents of the oogonium divide into several oospores, which require a variable time to ripen. They germinate by forming a sporangium, which produces a bunch of conidia, which become zoospores, Fig. 4, *C*.

Biologic forms of Fungi.—Before passing to the consideration of some parasitic fungi the subject of what is known as biologic variation should be mentioned. The facts have been well stated by Massee as follows: "In the case of many parasitic fungi certain members of a given species have become so modified and specialised in their parasitism, that they can only infect a given species of host-plant, or, at most a few closely allied species.

"Such are termed biologic forms, on account of their speciality in this direction being of a purely physiological nature. . . . No morphological differences are presented by biologic forms belonging to the same species. . . . The morphological species called *Erysiphe graminis* is parasitic on barley, oats, wheat, and many wild grasses. Culture experiments have proved, however, that the particular form parasitic upon any one of the plants enumerated above cannot infect any of the other plants."

Parasites of Saprolegnia.—Chytridian parasites such as *Woronina* and *Rozella* often occur in cultures of different species of Saprolegnia. It is *Rozella* that causes the curious septate condition shown in Fig. 3, *D* and *E*. The number of compartments found in any one filament varies; it depends on the number of parasites that penetrate: the more the parasites the more the compartments, though not in strict numerical proportion because fusion of parasites occurs. Each compartment is a sporangium of the parasite, its wall adherent to the cell-wall of its host. The zoospores of the Chytridian are evacuated in from 60 to 90 hours after infection through a hole at the side, Fig. 3; *E*, *o*, *o*. Resting spores are also formed: lateral shoots are protruded and typical chytridian resting spores are formed, causing a curious resemblance to the hosts' ovum in the ovary, as in Fig. 3; *E*, *rs*; but Cornu noticed that the protrusion containing the parasite is not shut off by a septum as the ovary is.

Peronosporeae.—The next group of the Phycomycetes is called the Peronosporeae, and includes the genera *Pythium*, *Peronospora*, *Phytophthora*, and *Cystopus*.

Pythium.—The genus *Pythium* was founded in 1858 by Pringsheim as subordinate to *Saprolegnia*; the aquatic species having been studied before the terrestrial. The genus is a small one (18 species) though of world-wide distribution. Butler found in pond-water in India the same species as he had found in England. *Pythium* affords a basis for comparison with algae and with kindred genera of primitive fungi. Facts mentioned below combine to make this genus one of the most important for practical study.

Specimens of water-species can be obtained by the same

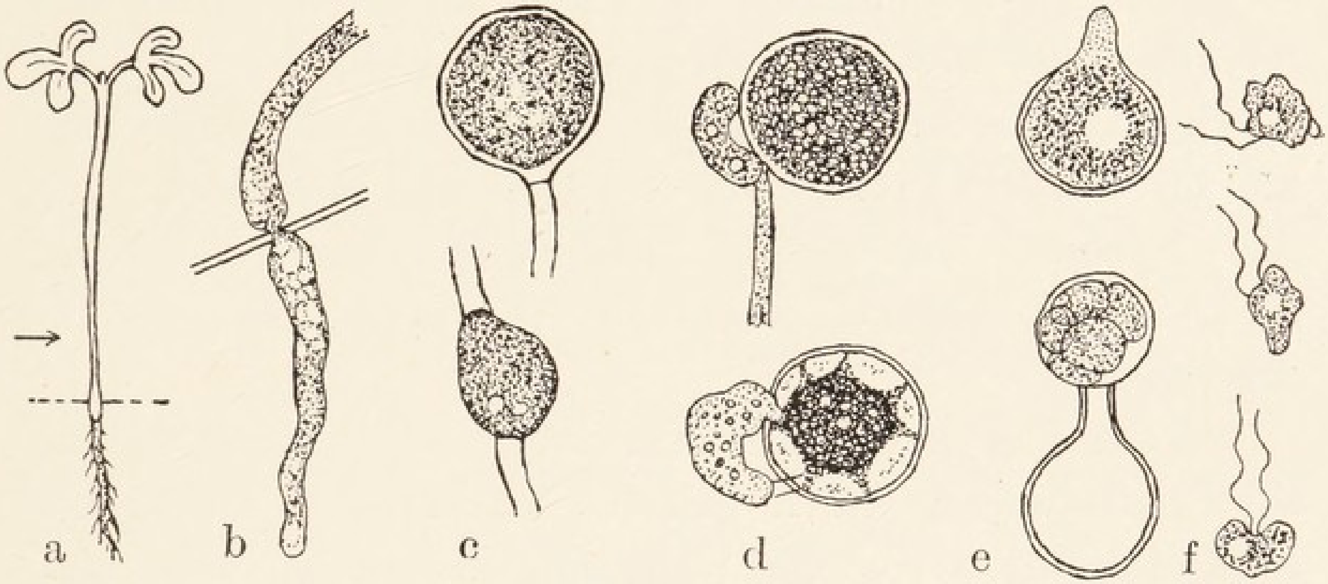

Fig. 6.—Pythium. *a*, seedling of cress, the arrow points to the part invaded by the parasite; *b*, end of a hypha which has pierced the wall between two host-cells; *c*, conidia of *Pythium debaryanum*, above, ripe terminal, below, early interstitial; *d*, oogonia and antheridia, above early stage, below, oosphere contracting, fertilisation canal formed; *e*, two stages of the germinating zoosporangium of *P. proliferum*; *f*, zoospores. Portions of Marshall Ward's illustrations.

means as *Saprolegnia*, a slice of raw potato makes a good substratum. One sub-genus, *Aphragmium*, by its mode of reproduction is very like a filamentous alga: without septa even being formed, an unaltered portion of its mycelium is transformed into zoospores, which escape at a hole in the wall. The slender pythium-filaments are multinucleate.

Writing of the genus *Pythium* in general Dr. E. J. Butler states: "All the species which have been investigated are capable of living saprophytically. Many are capable in addition to attack and destroy living tissues; but they are hemisaprophytes, for even the most destructive, *P. debaryanum* (Hesse), attains its maximum development and reproductivity when cultivated saprophytically."

One species has been found to attack and destroy the vinegar eelworm; the body of which was filled with very fine hyphae. In this *P. anguillulae aceti* sporangia, conidia, and oogonia formed at the same time, not in succession.

Parasites of Pythium.—The kindred of *Pythium* include the Chytridiineae, and it happens that fungi of this latter class often infect common pythiums, so that likenesses and differences between host and parasite can be studied; but care not to mistake normal for parasitic features is required: thus of a species of *Pythium* that kills palm-trees Butler wrote: "The hyphae are at first always unseptate and crowded with a dense protoplasm. Fat is abundant, glycogen also occurs in highly-refractive droplets, whose resemblance to Chytridiaceous fungi, parasites of the genus, is remarkable."

Pythium debaryanum.—In any sowing of cress on garden soil a few seedlings may be found bent down. The stalk is narrowed at the bend: such a plant straightened is shown in Fig. 6; *a*, where the narrow invaded part is indicated by an arrow, the interrupted line showing the level of the soil. If seed is sown thickly on soil half filling a flower-pot and kept well watered and covered by glass nearly all the plants may be affected. Gardeners call this "damping off." Bower describes the last stage thus: "If the cress culture be kept damp for some days longer, a thick felt of hyphae will be formed, which will bind the seedlings together: and finally the disorganisation will spread throughout the seedlings, causing complete rotting." In a sowing made on sterilised sand I found that no infection occurred.

Marshall Ward found that a healthy seedling placed in water beside a diseased one was infected in from 12 to 24 hours. The hyphae of *Pythium* are not, like those of *Saprolegnia*, content to form an attachment by a rhizoid, they penetrate and traverse the host-cells, Fig. 6, *b*; and ramify in and between them and on the surface of the plant. "The cells are killed very shortly after the fungus reaches them, and there is no attempt to react in any way to the invading organism; no hypertrophy appears nor any attempt at cell-division" (Butler).

An infected cress-plant placed on a slide shows numerous delicate non-septate hyphate, which after a day or two will be found to have round swellings at the ends of some hyphae, and round or oval swellings in the course of (interstitial or intercalar) other or the same hyphae. These swellings at first look very much alike, but soon in some a large vacuole and a beak-like process of the inner wall develope, Fig. 6, *c*, showing that it is a sporangium; the other unspecialised swellings are called conidia; in shape these are not at first different from the oogonia, but the latter appear a little later than conidia and are soon distinguished by the presence of an antheridium, Fig. 6, *d*, near each of them, and the subsequent formation of encapsuled oospores within them, Fig. 7, *f*. Left alone the conidia remain dormant, and they are able to resist frost, but not complete desiccation. If fresh oxygenated water be added they germinate by extrusion of the endospore into a hypha. If they are detached as soon as ripe and placed in fresh water they produce each a brood of zoospores. We have seen that a single zoospore of *Saprolegnia* could be replaced by a hypha, and here it is seen that a single Pythium-hypha may be replaced by a group of zoospores. Some conidia of *P. debaryanum* have the power of remaining dormant for long periods, and such are at times distinguished by a slightly thicker wall, and are termed "resting conidia."

Sporangia germinate as a small brood of zoospores, if they are supplied with plenty of well-oxygenated water in a strong light. The stages are like those of Saprolegnia with an additional feature, the distension of the apex of the beak into a bubble or vesicle by cell-sap, and the passage of the sporangial protoplasm into the vesicle there to break up into zoospores, Fig. 6, *e*. Rotation occurs before the protoplasm passes into the vesicle during the stage of early segmentation; the homogeneous stage occurs about 5 minutes before the vesicle is formed. In the vesicle a rolling of the contents is observed before subdivision.

Zoospores are at first amoeboid then kidney-shaped with 2 flagella attached at the hilum. They escape by rupture of the vesicle, and tend to collect at the surface of the water. They become encapsuled before germination; diplanetism has been observed by Butler in one species.

In most species germination is by a branched hypha, but in *P. tenue* and others parasitic in *Vaucheria*, *Spirogyra*, &c. the encysted zoospore having settled on a host filament forms a penetration-tube like that of some olpidians.

The gametangia, Fig. 6, *d*, and Fig. 7, consist of a round oogonium and an antheridium which arises close to the oogonium from the same hypha or from an adjoining hypha. The tip of the antheridium touches the oogonium into which it sends a process, the fertilisation canal, through which in hanging-drop cultures the gonoplasm can be seen to pass.

The brooch-like arrangement of the gametangia is the origin of the name of the group. Sometimes two or more antheridia are attached to the same oogonium.

Amoeba-like movements of the oosphere in the course of preparation for fertilisation are described by Marshall Ward, who observed that the passage of the antheridial contents requires about an hour. The periplasm from which the outer wall of the oospore is formed is scanty.

The ripe oospore of *Pythium* after a long resting period germinates in water by developing a hypha. In some kindred genera, *e.g.* *Cystopus*, Fig. 8, *g*, the oospore pushes out a process which becomes a zoosporangium.

Nuclear Processes in Pythium.—The nuclear changes in the sexual process of *Pythium*, Fig. 7, have been studied by A. H. Trow, and by Kiichi Miyake (1901). Young oogonia measure from 20μ to 25μ, their nuclei are larger

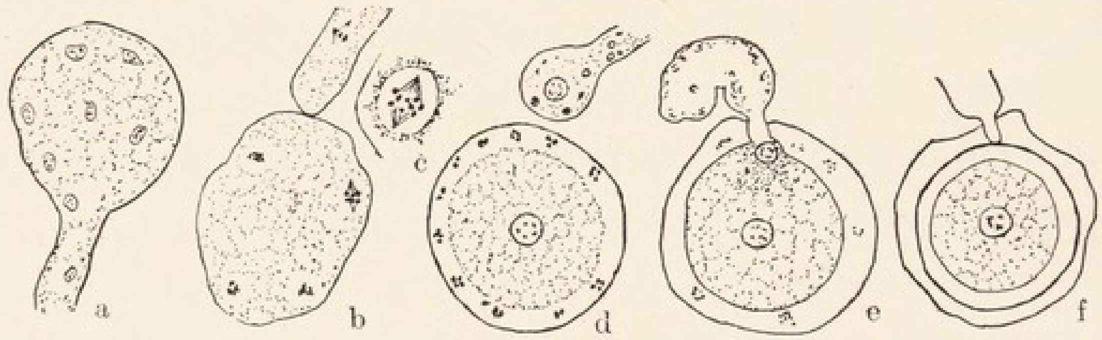

Fig. 7.—Nuclear Processes in Pythium debaryanum. *a*, young oogonium ; *b*, nuclear divisions in both oogonium and antheridium ; *c*, early anaphase, from oogonium ; *d*, one nucleus in each gametangium is intact, the rest disintegrating ; *e*, passage of male nucleus into oosphere ; *f*, ripe oospore with membrane and end of empty antheridium. After Miyake, reduced to $\frac{2}{3}$.

than those of the hyphae. When the ovum is nearly mature its nuclei divide by karyokinesis, and all save one move to the membrane and disintegrate, *d*. The spindle is intranuclear. Eight chromosomes were counted ; the author could not detect a reduction. The remaining nucleus lies in the middle of the ovum. Events in the antheridium are similar to those in the oogonium.

Near the single remaining nucleus of the oosphere A. H. Trow observed the formation of a coenocentre in *P. ultimum*.

"The wall of *Pythium* is usually assumed to consist of cellulose. However, as Trow has observed in the case of *P. ultimum*, the cellulose reaction is often difficult to obtain. The blueing with chloriodide of zinc is often faint or only got after long treatment" (Butler).

Phytophthora infestans is the cause of "blight," the most serious disease of the potato. The leaflet issued by the Ministry of Agriculture and Fisheries tells us that it was first noticed in Europe and America in 1840 and that it caused famine in Ireland in 1845. It is now always present in the British Isles. The mode of entry into the host-plant is shown in Fig. 12, *a*. The first sign of the disease is the presence of brown or black patches on the leaves, which look as if charred in places.

At the margin of these places on the under side of the leaf, and, in wet weather, on the upper side also, a white powder is seen. This whiteness is the conidia, which are borne on branched stalks pushed through the stomata from within. Conidia germinate in the same way as those of *Cystopus*, Fig. 8, *b*, *c*.

Details of the nuclear processes in a kindred species, *Phytophthora erythroseptica*, which also destroys potatoes, are given by P. A. Murphy (1918). The oogonium is peculiar in that it pierces the antheridium to develope on its farther side. Nuclear reductions occur leaving one nucleus in each gametangium, these nuclei coalesce to form the single nucleus of the oospore as in *Pythium*.

Cystopus candidus.—This fungus, which is parasitic in Crucifers, is a common parasite in the weed, shepherd's purse; it attacks also crucifers of economic value: cabbage, radish, horse-radish, cress, &c. The conidia form beneath the epidermis causing the appearance known as "white rust." They are really sporangia, which, on germination under water, produce a number of bi-flagellate zoospores. These in turn lose their flagella and germinate as shown in Fig. 8, *d*.

Details of structure in Oospores.—In *Pythium* the oospore or fertilised ovum, Fig. 7, *f*, secretes a stout cell-wall inside that of the original oogonium; there is but little periplasm, and no thick exospore is produced. In *Cystopus candidus* a thick exospore is formed from the periplasm. After a long resting period the oospore germinates in water as is shown in Fig. 8, *g*, *h*: the innermost wall of the spore bulges with its contents, becoming a sporangium, from which zoospores escape.

Nuclear processes in Cystopus.—In *C. Candidus* the gametic nuclei behave as in *Pythium*, and there is an additional feature in the presence in the ovum of a deeply-staining body, the coenocentre, Fig. 8, *i*, which, as Wager has suggested, may be the equivalent of a vegetative chromidium.

In *C. bliti* the nuclei of the gametes subdivide mitotically and conjugate in pairs.

A second simultaneous division of nuclei occurs in the oosphere before fertilisation.

The greater the number of nuclei that remain in the ovum of any species, the smaller is the coenocentre.

Equivalence of a germ-hypha to a brood of Zoospores in Phycomycetes.—An instance of the equality of germ-hypha and zoospore has been mentioned above: the first

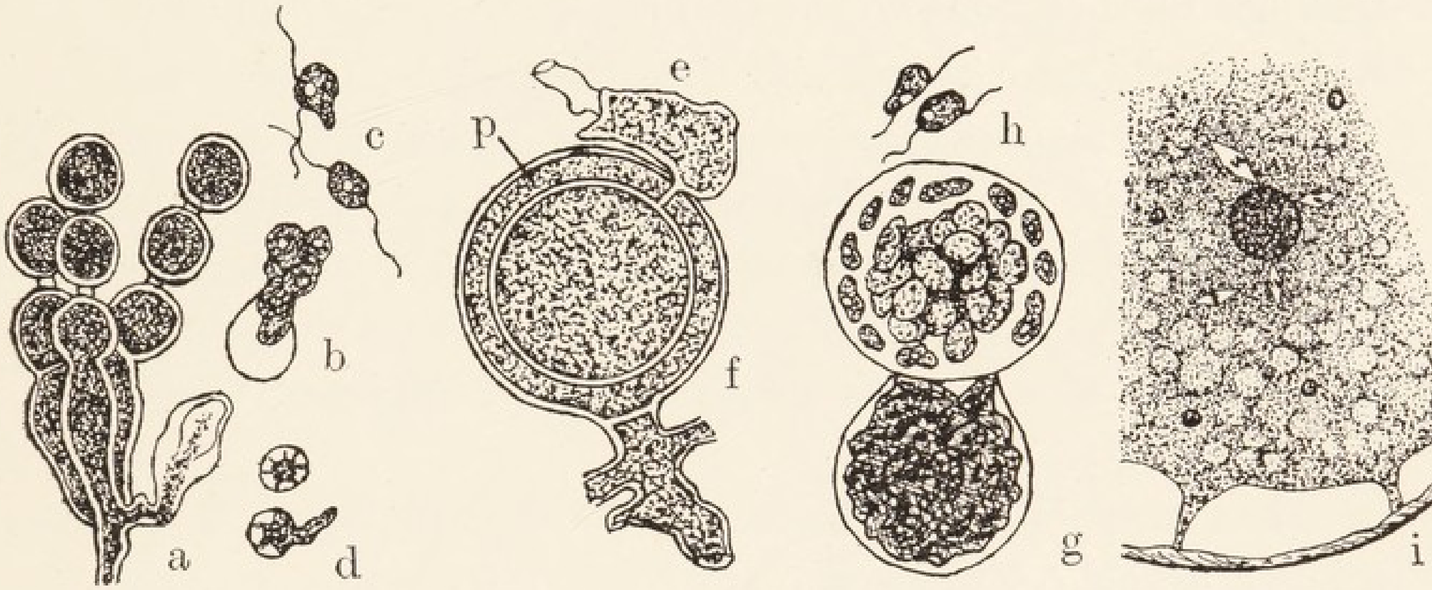

FIG. 8.—CYSTOPUS CANDIDUS. *a*, end of a hypha with 4 branches (conidiophores); *b*, germination of a conidium; *c*, zoospores; *d*, germination of zoospores; *e*, end of antheridium attached to *f*, the oogonium, the fertilisation-canal is formed, *p*, periplasm; *g* germination of oospore; *h*, zoospores; *i*, part of an early oogonium, showing in the middle the darkly-stained coenocentre and near it 4 mitoses, farther out, small nuclei. *a-h*, after de Bary; *i*, modified from Stevens.

alga-like form of zoospore of *Saprolegnia* secretes a capsule from which either a kidney-shaped zoospore or a germ-hypha may be produced; and in *Pythium* a germ-hypha may be alternative to a zoosporange. A similar alternative is found in *Phytophthora*, the conidia of which in water produce a group of zoospores, but in nutrient media a simple germ-hypha.

Zygomycetes. — This group includes Mucorineae and Entomophthorineae. The former agree with Oomycetes in general structural plan, but they differ in the absence of zoospores and in forming zygospores in place of oospores.

Material for study is easily obtained: a piece of bread that has been exposed to the air for 2 or 3 days is dipped in water, placed on a saucer and then covered by an inverted tumbler; in 4 or 5 days a growth of mould will appear. *Mucor mucedo* can be recognised by its tall unbranched conidiophores and dark round sporanges. The former are generally about half an inch long, but I have seen the growth like a piece of a sheep's fleece with conidiophores nearly 3 inches long.

The mycelium or thallus is non-septate save when injured or old. Zygospores are formed by fusion of projections from neighbouring hyphae, Fig. 9. They form more readily in some species than in others, thus they abound in *Sporodinia grandis*, which is parasitic on fleshy Hymenomycetes, such as Bolets and Russulas; while in *M. mucedo* their presence is exceptional. The reason of this was shown in 1894 by Blakeslee, who discovered that in this and some other species of *Mucor* conjugating filaments belong to different strains of mycelium; a more vigorous, or +, mycelium, and a less vigorous, or −, mycelium.

If growths of a + and a − strain are started on opposite sides of a culture-plate, they will meet near the middle and form a dark line of zygospores, seen as black spots projecting a little above the substratum. Mucors that produce zygospores from the same thallus are termed homothallic, those that do so only from different strains, heterothallic.

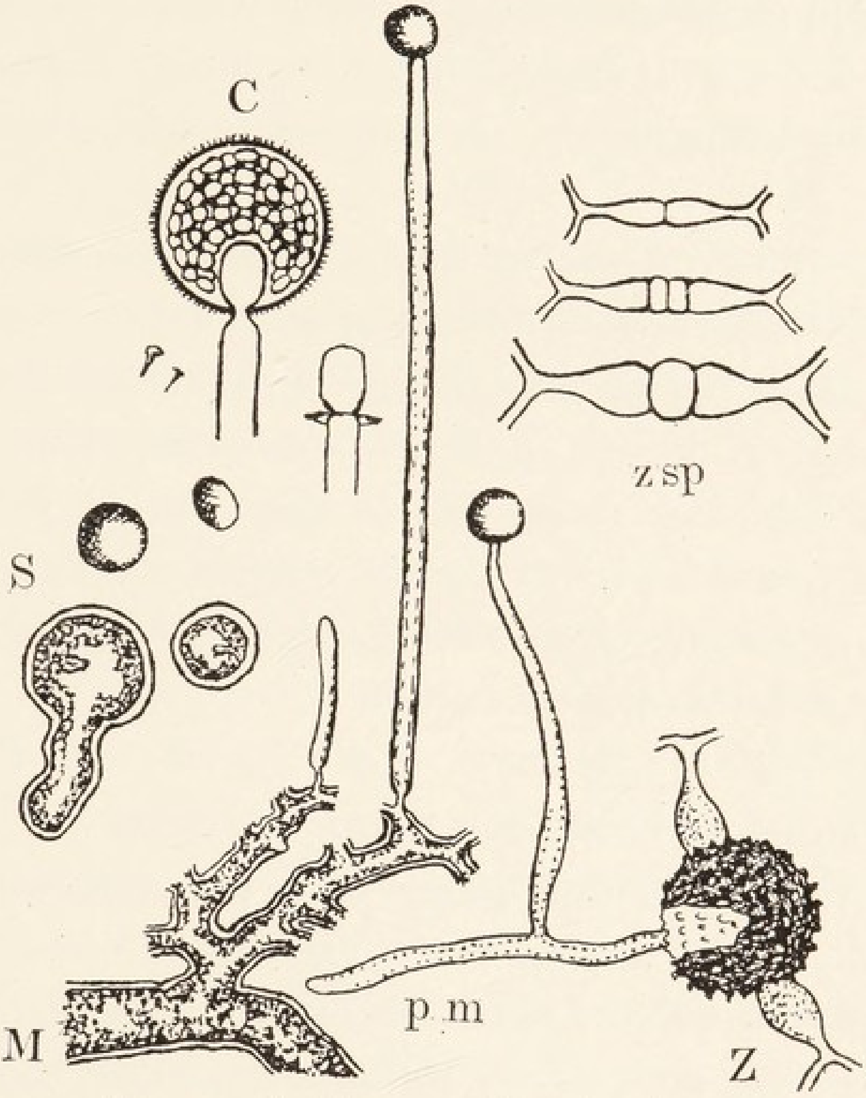

FIG. 9.—MUCOR MUCEDO.] *M*, mycelium bearing two conidiophores; *C*, sporangium with spores, two crystals more magnified, and end of conidiophore with part of membrane and columella; *S*, 4 spores, the two lower germinating; *z sp*, stages in the formation of a zygospore; *Z*, zygospore, which has germinated by the promycelium; *pm*, which bears a conidiophore. Mainly after de Bary.

In *M. mucedo*, Fig. 9, the conidia are formed simultaneously in a sporangium, the wall of which may be beset with crystals of oxalate of lime. The septum at the base bulges into the cavity constituting a columella.

The zygospore requires some months to mature. It germinates by sending out a single hypha called a promycelium, which produces a sporange full of conidia, but when the zygospore germinates in a nutrient medium it forms a mycelium.

Spores.—Two classes of spores are to be distinguished in fungi by their mode of germination: in one the whole of the protoplasm passes into the germ-hypha and the empty capsule is left to decay; in the second group, that to which *Mucor* belongs, the spore retains part of the protoplasm, grows, and remains part of the developing plant.

Spores of the first group will germinate in water, those of the second require nutrient media.

Four spores are shown at *S* in Fig. 9; the two upper from a sporangium needled in a drop of water, the two lower in a weak infusion of plum-jam. Only the latter are changed being swollen and having a double contour and a central vacuole. From one a hypha has grown, some neighbouring spores had two, and others three such. Two days later a hypha which had eight lateral branches was sketched. In its central part granules were seen to flow a considerable distance towards the growing point, and at the same time other granules were flowing in the opposite direction. Not more than one per cent. of the spores in this culture germinated.

Gemmae.—Spores of *Mucor Mucedo* sown on glucose solution with free access of air produce the usual non-septate mycelium, and oxygen is absorbed; but if the growth is submerged or air is replaced by hydrogen, the hyphae become septate and break up into segments, which multiply by budding, like yeast, to form a scum of large cells or gemmae. This condition is easily produced in *M. racemosus.* Alcoholic fermentation is caused by mucors in this state. Spores placed directly in glucose solution free from oxygen produce gemmae instead of hyphae. If air is admitted, gemmae produce ordinary mycelium.

Sporidia grown from promycelia of some species of *Ustilago* multiply in a similar yeast-like way in nutritive media such as manure heaps (Massee). Gemmae were seen by Butler in *Pythium rostratum.* A different sort of element is also termed a gemma: it is found in old badly-nourished mucors, and it is produced by a segment of mycelium being isolated by septa. Such elements are really a sort of conidia.

Parasitic Mucors.—One of the parasitic Mucors is illustrated in Fig. 10. *Piptocephalis* has for its host a *Mucor* of another species. Its haustoria are seen as blunt projections provided with very fine branches which lose themselves in the plasm of the host.

Nuclei of Mucor.—The tip of a hypha of *Sporodinia Grandis* is shown in Fig. 11, *a.* Its point consists of protoplasm without either nuclei or cell-wall. In the rest of the hypha nuclei are numerous especially near the point, where they are very small. They consist of nucleolus, nuclear plasm, and membrane, resembling those of *Saprolegnia, Ustilago* (smut), and *Uredo* (rust). The nuclei may become

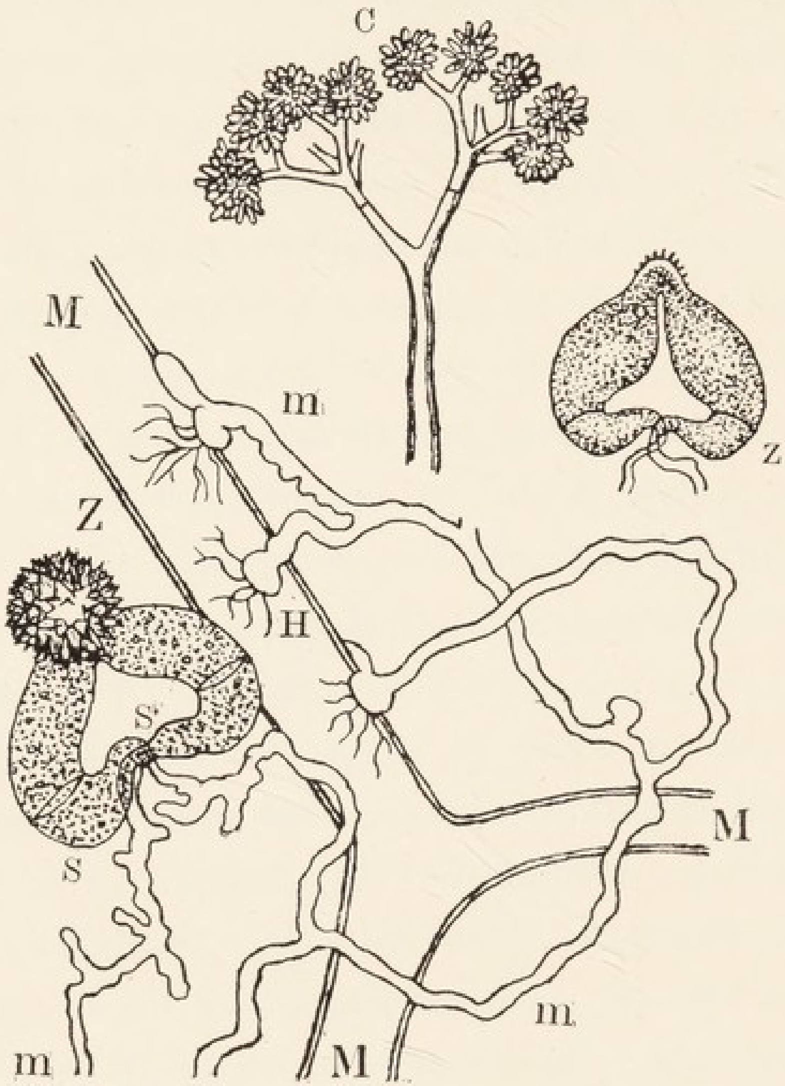

Fig. 10.—Piptocephalis Freseniana. *M*, hypha of *M. mucedo* ; *m*, hyphae of *Piptocephalis* ; *H*, haustoria ; *Z*, zygospore ; *s*, suspensors ; *z*, stage in formation of zygospore ; *c*, conidia. Modified from de Bary, after Brefeld.

elongated in the direction of the hypha. Similar nuclei are found in the sporangium, the plasm of which surrounds a vacuole, and subdivides into spores, each of which contains several nuclei. In the conjugants similar nuclei crowd the plasm on each side of the septum, and when the latter has been absorbed some of the nuclei would appear to fuse in

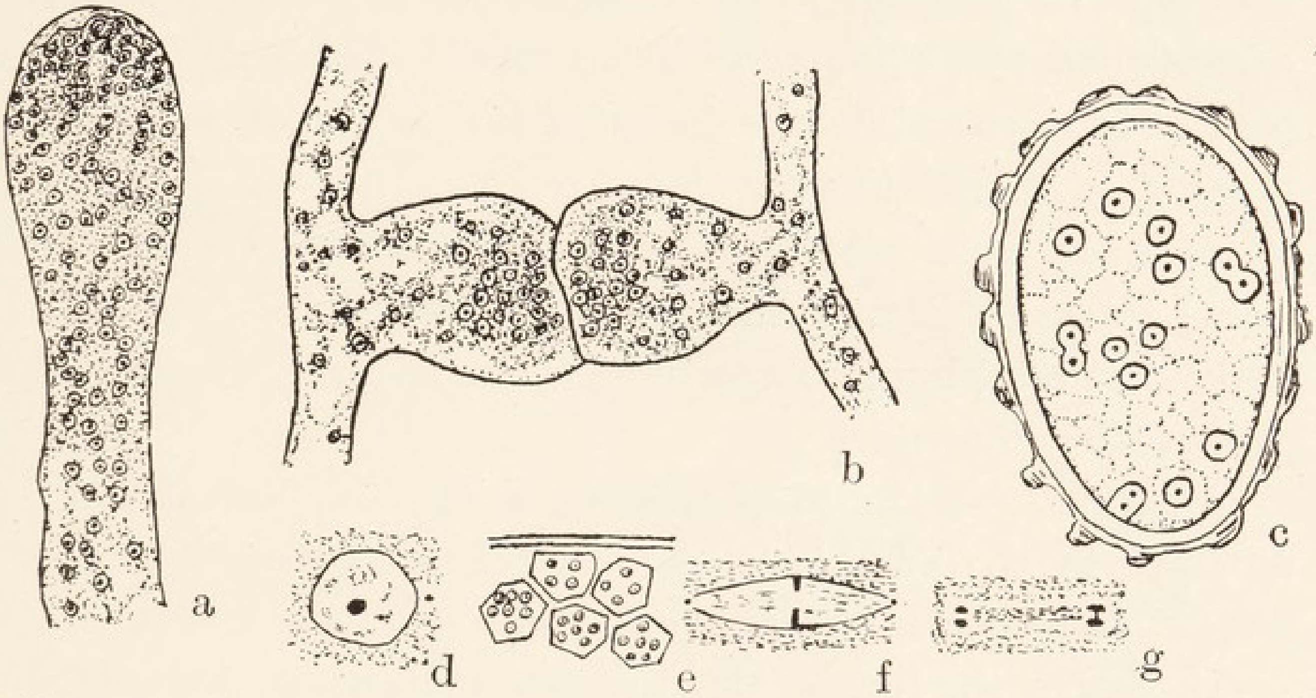

FIG. 11.—NUCLEI OF MUCOR. *a*, end of hypha; *b*, conjugation; *c*, (?) fusion of nuclei in zygospore of *M. fragilis*; *d*, resting nucleus with nucleolus and centrosome; *e*, part of sporangial membrane with spores; *f*, mitosis, metaphase, in *M. sylvaticus*; *g*, anaphase in *Phycomyces nitens*. *a–c* after Dangeard, the rest after Moreau.

pairs; the remainder disintegrate. All the details have not yet been made clear.

Some Features of Parasitic Fungi.—Spores of some parasitic fungi may go through the first stage of germination if placed in water, but they soon die unless they are brought into contact with their proper host. Such organisms are necessary parasites. The special organs of the simpler parasitic fungi may be considered before passing to examples of more intimate adaptation.

4

Haustoria, Fig. 12, *b*, *c*, are processes growing from a parasite into or among the cells of a host. They may be simple and knob-like, or branched. De Bary mentions that the type of haustoria just referred to in *Piptocephalis* is exceptional. The haustoria by means of which the chytridian *Polyphagus* sucks the juices of its prey must approach those of *Piptocephalis* very closely.

Another parasitic *Mucor*, *Chaetocladium*, is said (de Bary) to make direct communications between its own and its host's protoplasm at apertures where hyphae of host and parasite are in contact.

Symbiosis.—Akin to parasitism is the relation of fungi to algae in the symbiosis seen in lichens, of which about 2000 British species are known.

In lichens the fungus alone retains the property of sexual reproduction, the alga being restricted to vegetative growth by the control of the other symbiont, but when gonidia of lichens have been isolated and cultivated they have produced sexual generations.

The hypha of the germinating fungus-spore grows around its proper alga or algae and the joint vegetation is a lichen-thallus, Fig. 12, *d*, *e*.

Nowhere is the power of protistic life more prominently shown than by lichens. Towards the arctic *Cladonia rangiferina* (reindeer moss) grows luxuriantly making life possible for beast and man; and in sub-tropic deserts *Lecanora esculenta* grows loosely attached to limestone rocks and, scattered by winds, serves as "manna" in case of need. This *Lecanora* when dry is said (A. Lorrain Smith) to contain over 60 per cent. of crystals of oxalate of lime. The real nature of lichens was first explained by Schwenender in 1867, but it was many years before his view was accepted in England. The word symbiosis was created by de Bary

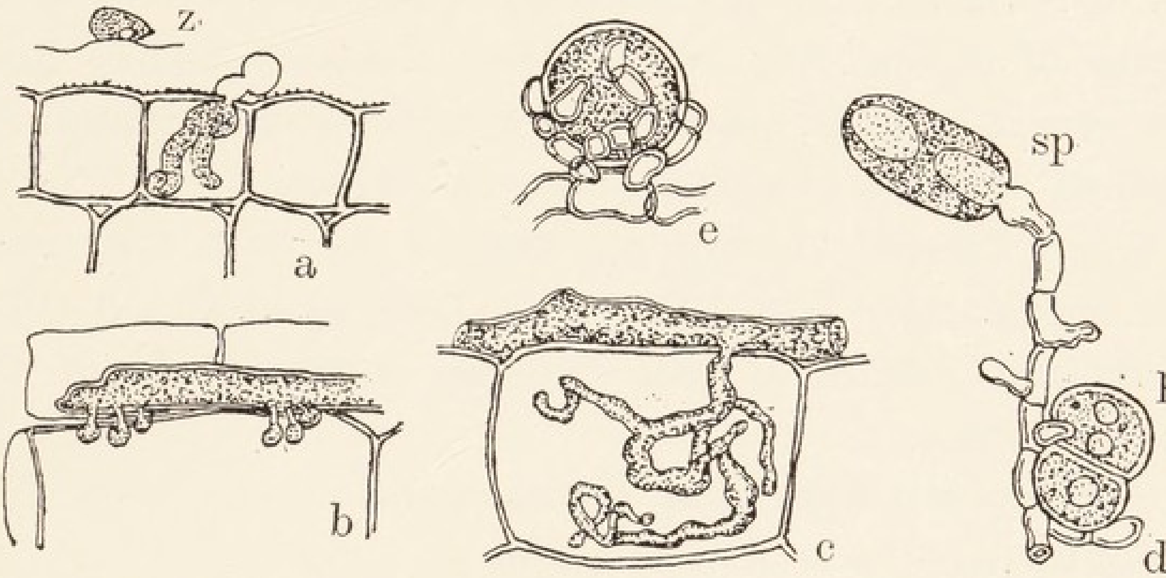

FIG. 12.—SOME FEATURES OF PARASITIC FUNGI. *a*, *Phytophthora infestans*, the zoospore, *z*, in germinating secretes a capsule which it leaves and penetrates into an epidermal cell of a potato-plant; *b*, end of a hypha of *Cystopus candidus* with knob-shaped haustoria penetrating into a cell of its host; *c*, *Peronospora calotheca* with a branching haustorium; *d*, the beginning of the lichen *Physcia parietina*, *sp*, spore of the fungus which has germinated, its hypha sending branches round two cells of the alga, *Protococcus viridis*, *p*; *e*, fungal hyphae and *Protococcus* from the lichen *Cladonia furcata*. After de Bary.

to express the relationship of the two participating organisms or symbionts of lichens.

Other examples of symbiosis are shown by the zoochlorels, unicellular algae found in symbiosis with many fresh-water protozoa and invertebrates; and by the zooxanthels, cryptomonads, found symbiotic with marine protozoa, &c. In these cases the symbiosis is not so perfect as that of lichens: the animal digests its companions when starvation threatens.

The green or yellow organisms have the structure of algae: nucleus, chromatophore with pyrenoid, and cell-wall of cellulose. Their occurrence is not constant in every species of animal with which they associate. They have been seen to divide inside their symbionts, and Famintzin obtained divisions in a slide-and-cover culture.

When infested animals are kept in the dark they eject their zoochlorels. The latter have been isolated and cultivated, and identified with *Chlorella vulgaris* one of the Protococcaceae.

Caullery mentions that these organisms also were long denied their real status in England.

Mitochondria.—In the higher animals and plants bodies which stain like chromatin with iron-haematoxylin are found in the cytoplasm. These bodies are called mitochondria or chondriosomes. They undergo changes in the vital processes of the cells. Bayliss states that they are composed of albumen and lecithin. Some of them resemble bacteria very closely and one of the many theories of cancer, that of Portier, is based on the assumption that they are symbiotes, which cause cancer by assuming parasitic characters. Facts that are recorded below render any theory of cancer unnecessary.

The present position of questions relating to mitochondria are given by Sharp (1921), who concludes that some structures included in that term should be regarded as cell-organs, though, like centrosomes, they may be not permanent.

CHAPTER III

CHYTRIDIINEAE

THE Chytridiineae, also called the Archimycetes, are a group of microscopic, parasitic fungi. They are named from a feature common to them all: propagation by zoospores formed in receptacles,[1] which have walls of cellulose. Their systematic definition by Engler and Prandtl, 1897, reads: "Mycelium wanting or in the form of fine protoplasmic strands. Sporangia always produce zoospores."

The protoplasm which fills the sporangium is rich in fat. Considered by themselves they present biological and cytological features worth careful study; their affinities to algae, Peronosporineae, and mucors on the one side, and to the Mycetozoa and Protozoa on the other give them added interest.

The genus *Chytridium* was founded by A. Braun in 1850. Before that time the sporanges of these parasites had been mistaken for reproductive organs of their hosts; a mistake

[1] The Greek word *chytridion* means a little pot, and *angeion* and *soros* mean vessel or urn. In botanical writings the adjective chytridiacean is used; by a simple elision I have shortened this, and in this book the words chytridian, olpidian, and synchytrian are used both as adjectives and nouns.

that was repeated in regard to Protozoa as hosts, and was corrected by Dangeard in 1894, as is explained below.

One group, the Synchytriaceae, differs so widely from average fungi and approaches the Sporozoa so closely in constitution and life-history that errors are likely to have been made. Most Synchytrians do not appear to inflict on their hosts more than local damage, but *Synchytrium endiobioticum*, by attacking certain kinds of potato threatened to destroy the crop over large areas of England. The parasite causes tumours which resemble cancer more nearly than does any other tumour of plants.

In other Chapters of this book I give reasons for my belief that the parasites which cause cancer, sarcoma, syphilis, smallpox, and other diseases in man and animals are allied to the genus *Synchytrium*.

Wide differences are found between forms at the extremes of the Chytridiineae, and, though linking species exist, it has been doubted whether the grouping represents a natural affinity, that is a common descent, or merely a likeness of form caused in organisms of varied descent by their living under like conditions. De Bary recognised four main groups, now made into as many orders: Rhizidiaceae, Cladochytridiaceae, Olpidiaceae, and Synchytriaceae.

The two former have a mycelium, and hence are grouped as Mycochytridiinae; the two latter without mycelium, hence Myxochytridiinae.

The mycelial forms may be akin to the genus *Ancylistes*, allied to *Pythium* in organisation, which attacks single cells of algae, sending out rhizoids that pierce adjoining cells.

Olpidiaceae agree with Synchytriaceae in other ways besides the absence of mycelium; and a descent from an alga such as *Pleurococcus* has been suggested; a view that has some confirmation for Synchytriaceae in features of the parasitic alga *Rhodochytrium*, see above p. 27.

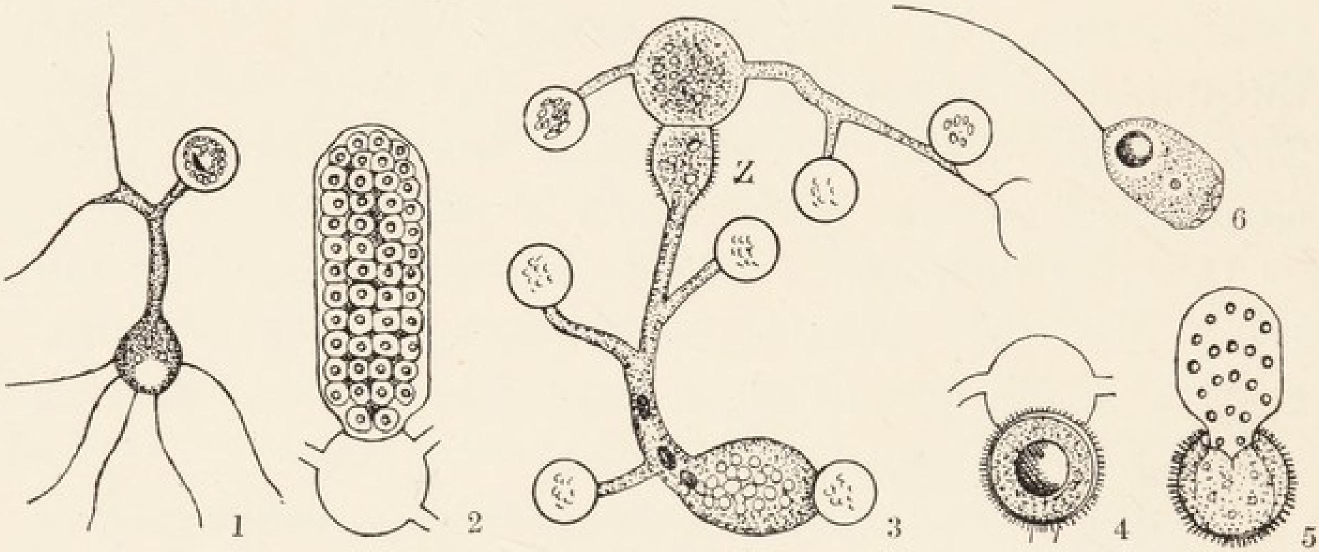

Fig. 13.—Diagram of Polyphagus Euglenae as Seen Alive. The small circles represent encysted *Euglenae* on which the fungus feeds. *1*, mycelial stage; *2*, sporangium with empty section of mycelium; *3*, conjugation, the lower plant is the male, the upper the female; commencing zygospore at *Z*; *4*, the ripe zygospore with parts of empty tubes; *5*, germination of zygospore, sporangium forming; *6*, zoospore. Modified from de Bary after Nowakowski.

Rhizidiaceae.—The first order is illustrated by *Polyphagus euglenae*. In this organism, Fig. 13, the zoospore, after a dancing motion of about a quarter of an hour, becomes amœboid, and sends out "rhizoids," resembling filamentous pseudopodia. The organism creeps about until one or more of the rhizoids find an encysted *Euglena viridis*—this they pierce and suck its substance by means of haustoria. The rhizoids are branched, and each branch may find a victim. The body of the parasite grows, and part of it bulges into a more or less cylindrical sac or sporangium, into which all the protoplasm passes to divide into a fresh brood of zoospores, which are liberated to repeat the cycle through several generations.

The passage of all the protoplasm into the sporangium or into the zygote accounts for the empty tubes seen in Fig. 13, *2* and *4*. Watched under the microscope in the living state, numerous bright globules, which change in size and arrangement, are seen in the sporangia: they are oil drops: Fig. 4, *D*; and Fig. 13, *2* and *5*. The nuclei are not so prominent and for the most part are seen only in stained preparations.

When food becomes scarce conjugation supervenes. In this process a rhizoid of one amœboid individual joins the body of another, and the protoplasm of the two flow to an enlargement in the joining rhizoid at the point of union to form a zygote or resting spore, which has a thick wall usually studded with short spines. After a resting period it germinates forming a sporangium the contents of which subdivide into zoospores. The oil-drop is absent from the zoospores of some chytridians.

The zoospores of *Polyphagus* are phototaxic as are the *Euglenae* it feeds on (de Bary).

Wager found that cultures of *Euglena* obtained from a sewage-farm were heavily infected; their normal grey-green colour changing first to yellow and finally to a dark brown. The same author has given a full account of the cytology of *Polyphagus*. The zoospore germinates as soon

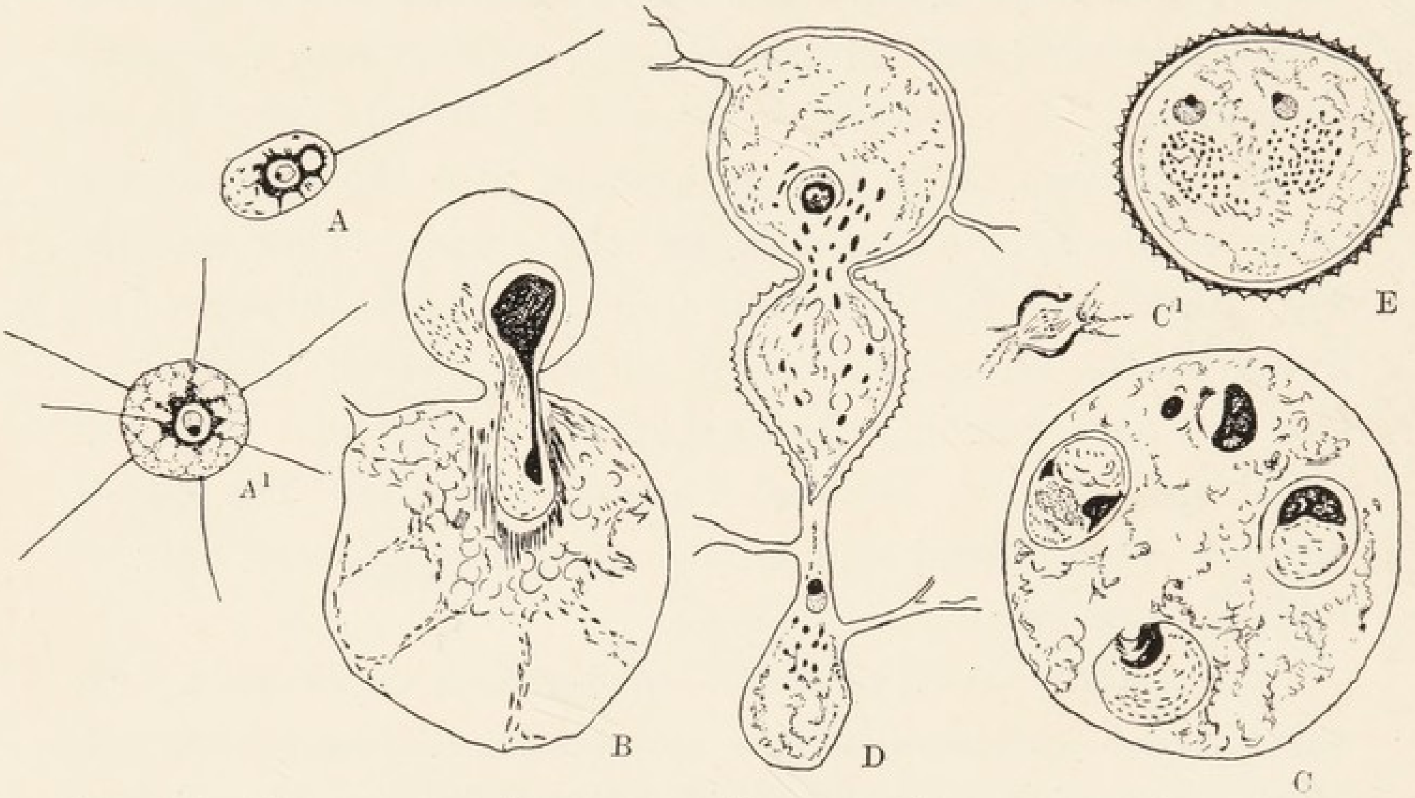

FIG. 14.—POLYPHAGUS EUGLENAE, NUCLEAR PROCESSES. *A*, zoospore with its nucleus and fat-drop surrounded by a chromidium; A^1, young plant soon after germination, nucleus and chromidium; *B*, beginning of vegetative spore-formation, half the nucleus is in the sporangial diverticulum; *C*, vegetative sporangium with 4 resting nuclei; C^1, a sporangial nucleus at metaphase; *D*, conjugation, some of the chromidial granules of the female (upper) gamete have entered the swelling which becomes the resting spore or zygote; *E*, zygote, with 2 small nuclei and their chromidia. Reduced to $\frac{2}{3}$, after H. Wager.

as it comes to rest; in external form it resembles the common protozoon *Actinophrys*, a Heliozoon, and having a well-marked chromidium it is very like a Rhizopod, Fig. 14, A^1.

The nature of chromidia or collections of extranuclear chromatin is discussed in Chapters I and IV.

Encysted *Euglenae* alone are attacked by *Polyphagus*, the rhizoids of which may attach as many as fifty. From the end of the rhizoid a branched haustorium pervades and soon exhausts the prey. The protoplasm is dense and contains many oil drops. The nucleus and chromidium pass with the protoplasm into the sporangial diverticulum.

The chromatin of the resting nuclei is massed at one side of the nucleus, Fig. 14, *C*. In mitosis the greater part of this chromatin lies around the spindle, in optical section showing as a dark crescent on each side at some distance from the spindle, Fig. 14, C^1.

The zygote is formed from two ordinary vegetative cells; the male puts out a long slender rhizoid on which a swelling arises at the point where it comes in contact with the female gamete, Fig. 14, *D*. The male nucleus enters the swelling first, and, after a series of changes in which the nuclei are reduced in size, the two chromidia fuse into one mass at the sides of which the two nuclei are embedded. Fusion of the nuclei appears to occur only after germination has begun. Nuclear divisions take place only in sporangia. The chromosomes number 10 or 12 and are very small. The spindle is internal. The chromatin which surrounds the spindle is used in part as nutritive material in the growth of the sporangium. The chromidium surrounds both nucleus and oil-drop in the zoospore of *Polyphagus*, Fig. 14, *A*.

2. **Cladochytriaceae** (Gr. *klados* = branched).—This group is described by de Bary as having a delicate, rhizoid-like, richly-branched, wide-creeping mycelium. Nowakowski first found them in decaying marsh-plants. Sporangia are either terminal or interstitial.

Of *Cladochytrium iridis* only resting spores were known, see Fig. 4, *4*.

Cladochytrium graminis "attacks the root and leave

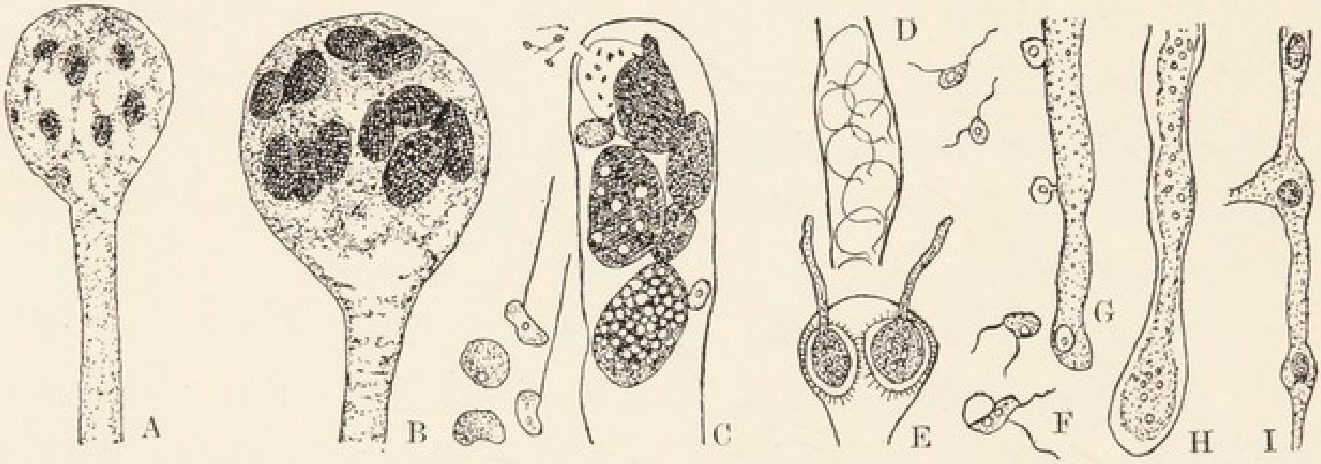

Fig. 15.—Olpidiopsis Saprolegniae. *A* and *B*, stages of parasites in the same host-filament; *C*, in a host-filament, the protoplasm of which is exhausted, are six parasites at different stages, the uppermost discharging zoospores; below, zoospores more magnified; *D*, part of a filament with empty sporangia; *E*, at the end of a filament are two parasites with prickle-walls, and spore-ducts; *F*, four zoospores; *G–I*, stages of the same three parasites in the same host-filament. *A–D*, after M. Cornu; *E–I*, after A. Fischer. Reduced to $\frac{2}{3}$.

of different kinds of lawn and pasture grasses: the disease spreads from a centre, killing off the herbage and leaving naked patches. . . . If the dead basal leaves are examined, the tissues more especially along the edges of the leaf, are seen to be crowded with the resting spores of the fungus" (Massee).

Urophlyctis.—This genus also belongs to the Cladochytriaceae. *U. alfalfae* causes the crown-gall of lucerne; *U. leproides*, beetroot tumour.

3. **Olpidiaceae.**—This order and the next have been made for chytridians without mycelium. Olpidians were first discovered by M. Cornu in *Saprolegnia*-filaments. The damage they can cause is often very great, *e.g.* in the disease of seedling cabbages mentioned below. They are not entirely restricted to plants as hosts: the kindred *Sphaerita endogena* attacks protozoa, and *O. gregarium* develops in the eggs of certain rotifers; Butler depicts one egg being attacked by four zoospores, and others filled with sporanges and resting spores.

The zoospores of some members of the order are uniciliate, of others biciliate. After swimming for a time zoospores come to rest as a particle of naked protoplasm, which in some cases exhibits amoeboid movement for a time before it becomes of a rounded shape.

If situated on a suitable host-cell a penetration-tube is formed and the parasite's plasm passes into that of the host, Fig. 15, *G.* If they do not find a host quickly they break up.

Some of the names used for olpidians call for explanation: species that bear the order-name are in the minority. The generic diagnosis states that resting sporangia are smooth and without appendage cell in *Olpidium*; this is so in Butler's drawing of *O. gregarium.* In *Olpidiopsis saprolegniae* the resting spores are spinous and have an appendage cell, denoting a zygospore. In *Pseudolpidium*, e.g. *Ps. Pythii*, there is no appendage cell. In all these three groups all stages of development from zoospore to sporangium or sorus, *i.e.* group of sporangia, or to a resting-spore can be traced, because the parasite-plasm remains separate from the host-plasm, and the sporangia are separated by an interval from the cell-wall of the host.

Very different is the case of *Rozella*, Fig. 3, *D*, *E*, where the individuality of the parasite is lost and its plasm finally replaces that of the host, of which the cell-wall is inseparable from that of the parasitic sporangium. The genus *Pleolpidium* was made by Fischer for several species of *Rozella*.

Woronina, named after the Russian botanist, appears to differ from *Rozella* in that its sori lie free in the host-cells. After the entrance of a single zoospore into a *Saprolegnia* from 2 to 14 sporanges of *Rozella* have been seen to arise.

Plasmodium-formation.—A true union of the bodies of several parasites appears to occur in both *Woronina* and *Rozella*, but even in these genera this fusion is apparently not a constant occurrence.

When the plasmodium is formed it behaves like a single organism, becoming either a single sporangium or a sorus or a resting spore. In the case of *Rozella* an additional point of interest is seen; the parasite fuses so completely with the host-plasm that, according to Fischer, another kind of plasmodium is produced.

A careful study of the nuclear processes of these alleged plasmodium-forming genera is greatly to be desired.

Sorus-formation.—It is shown in Fig. 16 how a single zoospore of *Olpidiopsis* without loss of individuality developes into a single sporangium ; in an allied genus, *Pseudolpidium*, either a single sporange or, as an alternative, a group of sporanges or sorus may be produced. Sorus-formation is the rule in *Woronina*.

Course of the Disease.—The effects of infection by the olpidians on their hosts constitute a disease, and the remarks of E. J. Butler are of importance : "Cases were observed in which the presence of the parasite did not interfere with the normal life of the host, even to the extent that normal sporangia and zoospores were formed, and it was only later on when secondary sporangia were forming, that the parasite gained the upper hand. . . . The zoospores of the parasite often infected *Saprolegnia* zoospores during their first encystment without checking the process of diplanetary formation of secondary zoospores, though whether the latter developed into a mycelium was not ascertained."

With the aid of a pocket-lens Cornu was able to separate Saprolegnia-filaments in which were older parasites, but the microscope was required to detect their earlier stages. When the parasites first become visible under the microscope they look like condensed portions of the host's plasm, Fig. 15, *A* ; a little later they become more definite, *B* ; later still, as at *C*, they are large, well-defined, and some contain bright globules. In this stage in Cornu's drawing they look exactly like bodies from the cysts in a case of cystic disease of the human urinary tract examined by myself (1892) in the fresh unstained condition. These bodies are termed " psorosperms " by some English pathologists, see Chapter X.

Olpidiopsis saprolegniae [1] produces two kinds of sporangia, smooth-walled and prickle-walled, Fig. 15, *D* and *E*. Fischer's description of the zoospores differs from Cornu's, as shown at *B* and *E* respectively. Zoospores from prickle-sporangia are said to be double the size of those from the smooth. Fischer described an alternation of generations, the zoospores from the smooth sporanges growing into prickle-sporangia and *vice versâ*. In Fig. 15, *E*, is shown the mode of formation of sporoducts by the prickle-generation. The contained plasm of the duct-process like the rest of the parasite subdivides into zoospores.

Cornu observed that the zoospores have a jerky movement for a short time and that they disintegrate rapidly if they do not succeed in finding a host. He also comments on the resemblance of the plasm of *Olpidium* to that of the Mycetozoa.

Fischer described the protoplasmic motion that occurs in the parasites as they break up into spores ; it would appear to be similar to that I described in molluscum bodies in 1895. Fischer states that countless spores are evacuated

[1] Differential features of *Olpidium* and *Olpidiopsis* are given on p. 61.

in the course of an hour and that many remain in the sporangia and perish. Thus in disease caused by parasites akin to Chytridians we may find extensive areas of necrosis composed of dead parasites.

Olpidium brassicae. This parasite attacks seedling cabbages causing damage which resembles that due to *Pythium* and which is included by gardeners under the name of "damping off." The sporanges have sporoducts resembling those of *O. saprolegniae.* Zoospores are uniciliate. Multiple infection occurs.

Olpidium viciae. Kusano described conjugation of zoospores in 1912. The zoospores develope into ordinary sporangia, the zygotes into resting spores; later findings (1920) in a *Synchytrium* accord with this observation (Fig. 22).

Nuclear Processes in Olpidians.—The development and sexuality of some species of *Olpidiopsis* have been examined by J. T. Barrett. The zoospores are bi-flagellate, Fig. 16, *a*, *b*. The sexual process is by zygospore, usually in the form of the prickle-sporangium of Cornu, but occasionally sporangia are smooth.

When first liberated the zoospores swim actively for from 2 to 5 minutes. They then become attached to the slide and a rocking motion begins, the flagella becoming shorter. After 7 to 10 minutes they cease to move for a space of 5 to 20 minutes and one or two contractile vacuoles appear in them and a second period of movement begins. This sequence of events has been compared to the diplanetism seen in *Saprolegnia*. The point of attachment of the flagellum is hard to see.

Only a small percentage of zoospores effect an entry

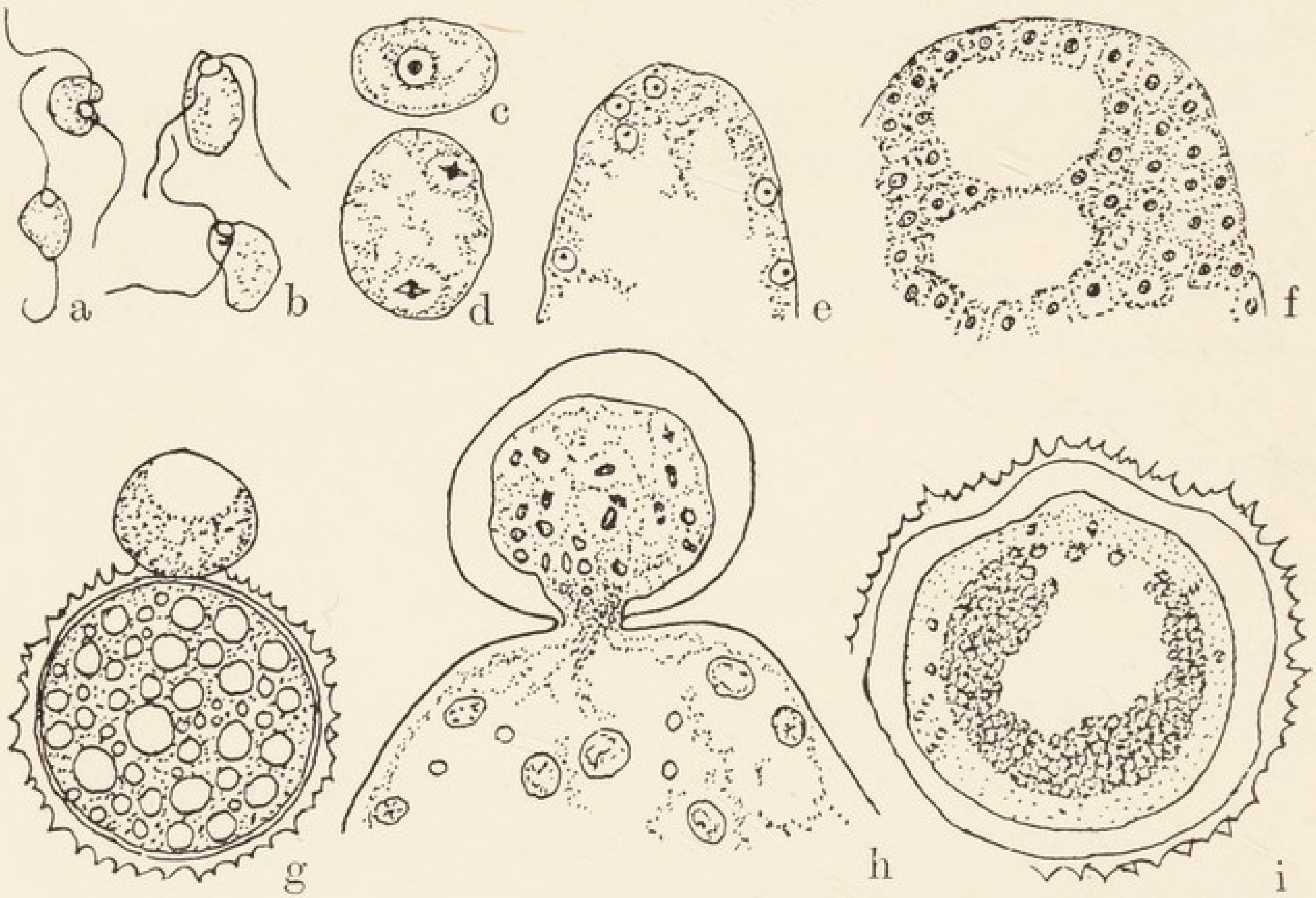

FIG. 16.—NUCLEAR PROCESSES IN OLPIDIOPSIS. *a*, zoospores of *O. Saprolegniae*; *b*, the same of *O. vexans*, the lower one stained; *c*, early stage, non-sexual; *d*, nuclear division; *e*, early sporangium, resting nuclei and internal vacuole; *f*, sporangium, separate zoospores formed; *g*, zygospore, contents of antheridium passing into oogonium; *h*, the same stained; *i*, ripe oospore. After J. T. Barrett.

into a host. Entry is as shown in Fig. 15, *G* to *I*. The parasite at first contains but few granules, these grow and coalesce into oil-like drops, which are replaced by vacuoles at maturity when exit tubes are formed.

The nuclei divide simultaneously by karyokinesis, Fig. 16, *d*. Sexual stages were obtained by supplying fresh food (ant larvae) to old cultures of *Saprolegnia*. The sex-cells especially in *O. saprolegniae* and *O. luxurians* are easily distinguished from the vegetative cells by their vacuolated coarsely granular plasm. All the contents of the antheridium pass into the oogonium, Fig. 16, *g* and *h*, from one to several hours being required. The nuclei of the antheridium are small and in an active state, those of the oogonium are large and resting. The ripe oogonium, Fig. 16, *i*, has a thick endospore, inside this a finely granular layer containing the nuclei, inside this again a coarsely granular layer, and in the middle a large oil-drop, as in the zygospore of *Polyphagus*.

The staining reactions of the nuclei of the zygote vary at different stages.

Barrett did not see amoeboid changes in, nor fusions of, the larger non-sexual parasites; nor could he confirm Fischer's view about an alternation of generations.

Sphaerita endogena.—This parasite was discovered in a culture of *Euglena viridis* by Dangeard, who also described another chytridian, *Nucleophaga amoebae*, considered below in Chapter X. For biology the value of these two genera consists in the light they shed on processes previously supposed to be modes of reproduction in protozoa and which are now known to be instances of parasitism.

Sphaerita grows in the cytoplasm of its host, the nucleus of the host-cell remaining unchanged. The nucleus of the parasite consists at first of nucleolus, nuclear cavity, and membrane.

Later the chromatin is arranged in groups of granules, six granules in two rows appear to represent a primitive mitosis.

A stage with many definite nuclei is followed by subdivision into spores, liberated as bi-flagellate zoospores by rupture of the host-cell. Dangeard also found a series of stages that he was in doubt whether to attribute to *Sphaerita* or to some other chytridian. No prickle cells (resting sporanges) are described. Being devoid of mycelium *Sphaerita* belongs to the Myxochytridinae and it differs from *Olpidium* sufficiently to constitute a separate genus. Chatton and Brodsky found a *Sphaerita* with the nucleus situated eccentrically, and Dobell found a *Sphaerita* in *Endolimax nana*, an amoeba parasitic in the human intestine.

4. **Synchytriaceae.**—This order of the Chytridiineae is distinguished by the prominence and regularity of sorus-formation in the life-cycle. In some species, *e.g. S. taraxaci*, this segmentation occurs within the original capsule of the parasite; in others, *e.g. S. succisae*, the parasite leaves its capsule, Fig. 19, *C*, before subdividing into sporanges.

Synchytrians are frequent parasites of a great number of flowering plants. Some are limited to one species of host, whilst others have a wider choice: thus *S. aureum* has been stated to have been found on 180 different host-plants. Rytz concluded that this species has but few chief hosts, but in favouring weather it infects other plants. In marshy land near Berne the creeping loosestrife (*Lysimachia nummularia*) was the chief host; in the Bernese Oberland it was *Saxifraga aizoides.* Some species of *Synchytrium* are of economic importance from their infecting food-plants; the potato, the cabbage and the strawberry.

Schroeter states that their occurrence is often very regional, infected plants being abundant in one district, whilst the same kind of plant in other similar regions is free. Dampness of the soil favours them: in a low-lying part of a meadow he found many infected scabious plants, but in higher parts of the same meadow the scabious was free from infection. Rytz states that *Synchytrium alpinum,* parasitic in *Viola biflora,* is very common in Switzerland up to 2400 metres, far above the tree limit.

Many Synchytrians are coloured; yellow, orange, and red being common. They owe their colours to lipochromes. In the spirit in which infected leaves are kept drops of coloured oil may collect at the bottom of the containing vessel.

Their effects on the host-plants vary. Often they are not very damaging, causing only a nodular thickening of the edges of leaves, which are rolled inwards. With the naked eye sometimes, always with the aid of a pocket-lens, the coloured or pearly points can be seen on leaf or stem or both. They are sometimes obscured by hypertrophy or proliferation of the cells around those that harbour parasites: small galls, Fig. 18, *G*, are thus produced. In the common potentil this hypertrophy takes the form of hair-cells. In the potato *S. endobioticum* causes warty cancer-like tumours. Some kinds of potato are immune, but it is not clear in what this immunity consists.

The Life-cycle of Synchytrians.—The life-cycle of Synchytrians varies in different species. In some, such as *S. anemones* and *S. mercurialis*, only resting-cells are formed.

These are dormant all winter in the decaying remains of the host-plant and germinate in spring, their zoospores entering epidermis cells of young plants. From their condensed life-cycle de Bary named these Pycnochytria. They always pass through a soral stage in germinating. The remainder, or Eusynchytria, have a double cycle, like many Sporozoa. In suitable weather a series of generations follow one another before the resting cells are formed. The Eusynchytria again fall into two groups: in one group, which may, perhaps, be named Pleochytria, and which includes *S. stellariae* and *S. oenotherae* (evening primrose) a sorus is formed in both parts of the cycle: in the other group the sorus is omitted from the germination of the resting cell. To this latter group belong *S. taraxaci*, *S. succisae* (scabious), and *S. endobioticum*, &c.

S. aureum belongs to the pycno-group, having one brood of swarm-spores in the spring of each year. One pycnochytrian, *S. Wurthii*, is peculiar in having several broods annually.

There is a wide range of nuclear form and mode of division in different Synchytrians. Stevens reminds us that larger nuclei are found in these fungi than in any other plants: the synchytrian nucleus often measures 35μ, the nucleolus 11μ in diameter; an average vegetative nucleus

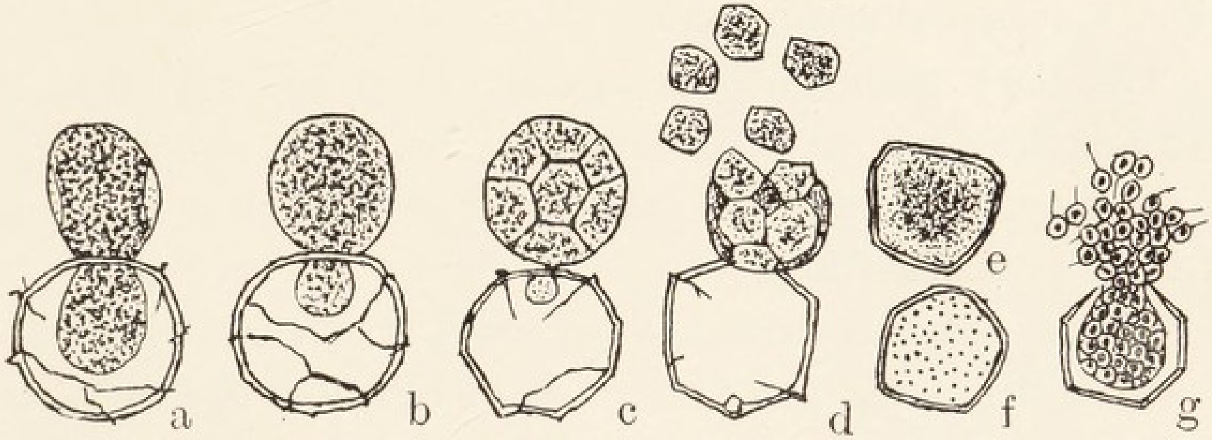

Fig. 17.—Synchytrium stellariae, from Stellaria Media. Germination of resting cell in water after it had been kept dry all winter. *a*, more than half the protoplasm has streamed out of its capsule; *b*, 4 hours after *a*; *c*, 7 days later, the sorus had been subdivided into sporangia for 5 days; *d*, sorus completed, sporangia separating; *e–g*, formation of zoospores; 2 hours between *e* and *f*; 45 minutes between *f* and *g*. *a–d* $\times$ 145, *e–g* $\times$ 375. After de Bary.

in plants being from 5μ to 9μ. The host-cell increases from 15μ to 100μ.

We may now consider a few details of the life-cycle in this genus. First, as is desirable in all study of protists, we may consider changes as seen in the living organism.

An account of the germination of the resting cell of *S. Stellariae* as seen in the living state has been left us by de Bary.

The ripe resting cell is enveloped in the remains of the host-cell externally, and internally by its proper capsule consisting of two layers, exospore and endospore. In germination the endospore bulges at one small area very much as happens in the germinating oospore of *Cystopus*, Fig. 8. The leisured rate of the process as depicted by de Bary is noteworthy. The first three stages shown in Fig. 17 occupied seven days.

The dark granules seen in Fig. 17, *f*, are oil-drops, as also are the dark spots in the zoospores. The redistribution of fat plays an important part in the reproductive processes of these parasitic fungi.

Of the eusynchytrian, *S. succisae*, an account has been given by Schroeter, some of whose illustrations are suggested in Fig. 18.

Schroeter found synchytrians so easy to study that he almost apologises for the lightness of his task. He found it easy to infect young leaves, Fig. 18, *A*, by placing on them water containing zoospores. The plant-tumour or gall, *G*, deserves careful scrutiny: the shrunken capsule of a summer or soral sporange lies in the middle; projecting above it are hypertrophied host-cells; beneath it and at its sides are infected host-cells containing resting-spores in course of development. In view of what follows we may regard the resting stage as being developed from zygotes, *i.e.* paired zoospores.

Recognition of synchytrian infection in wild plants may be of great importance; thus, for example, infection of the potato by *S. endobioticum* has been found to be associated with infection of hedge-row plants, the woody and the black nightshades, *Solanum dulcamara* and *S. nigrum* respectively.

At this point we can profitably select information on

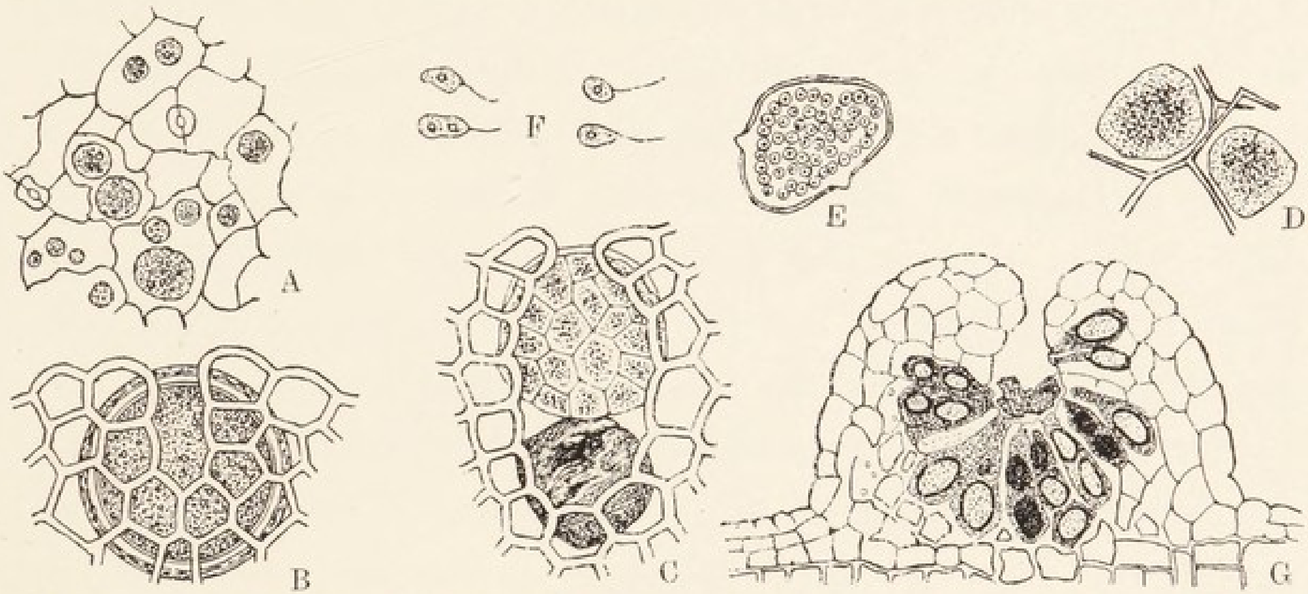

FIG. 18.—DETAILS OF SYNCHYTRIANS. *A*, young parasites in the epidermis of a scabious plant; *B*, a ripe parasite in its natural site; *C*, the typical germination of a synchytrian: the sorus formed; *D*, two segments of the sorus of *S. globosum* (of the dog-violet), showing interstitial substance; *E*, a sporangium of *S. succisae*; *F*, four zoospores; *G*, a gall, showing the capsule of the original parasite and, in the cells, stages of resting-spores. After Schroeter, reduced to $\frac{2}{3}$.

that important parasite *Synchytrium endobioticum*, the cause of wart disease or black scab of the potato. Taking first information given in Leaflet No. 105 issued by the Board of Agriculture and Fisheries: "The young warts may easily be seen in the eyes of the tubers. . . . They increase in size and subsequently become irregular excrescences, which often run together forming large masses. In certain varieties (*e.g.* Arran Chief) all resemblance to a normal tuber may be lost, the entire tuber being transformed to a coralloid mass. The warts are at first white, but as they become old they begin to turn black and finally form a putrid mass from which dark brown liquid exudes."

The parasite was first described in 1896 by Schilbertzky in Hungary as a chytridian. John Percival, Reading, recognised it as a synchytrian and named it as above.

A monograph by Dr. K. M. Curtis was published in 1921. It is a record of a very thorough biological study: from it a scheme of the life of the organism can be constructed as is done for Sporozoa and other protists, Fig. 19.

Curtis found that zoospores can live nearly a week in an intact sporangium of *S. endobioticum*, if the sorus is not ruptured. If water is added the sporangia burst and the zoospores rush out. They are very active moving to and fro for from 10 to 20 minutes. In 30 to 40 minutes movement ceases, and unless they are in contact with a suitable host-cell they disintegrate.

Unripe sporangia need more time to discharge and the zoospores are sluggish at first, and some die either in the sporangium or near it. Living zoospores, Fig. 20, *a*, are $1{\cdot}5\mu$ long and have a bright spot, the nucleus, at the anterior end.

It seems that zoospores from the same sporangium do not pair. Before conjugation can occur a preparatory

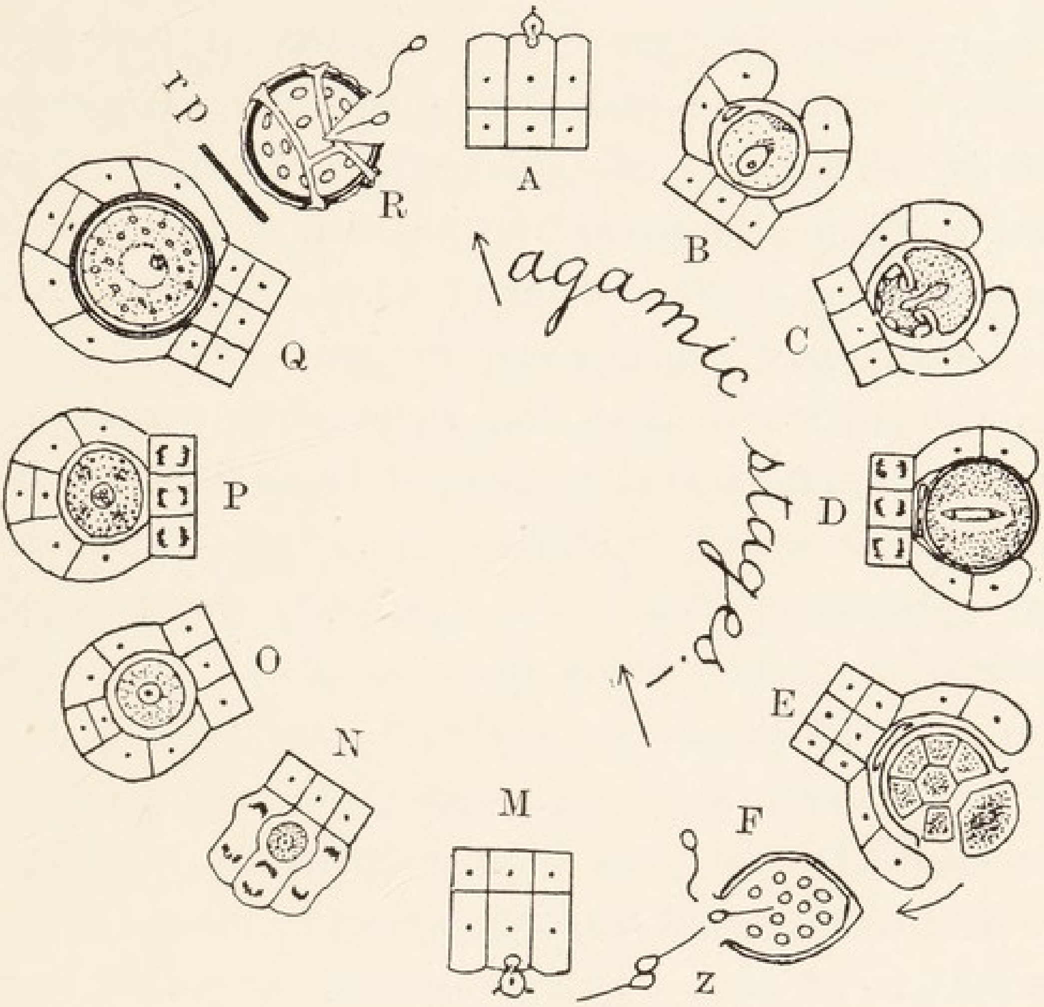

Fig. 19.—Diagram of the Life-cycle of a Fungus, Synchytrium Endobioticum. *A*, parasite with remains of flagellum still present entering an epidermal cell; *B*, parasite much larger with large nucleus and granular plasm at the surface of and above the nucleus, the lateral host-cells hypertrophied and projecting as the "rosette"; *C*, sorus-formation, more than half the parasite has passed out from its original cell-wall; *D*, first mitosis of sorus; *E*, sorus completely subdivided into sporangia; *F*, single ripe sporangium, zoospores escaping, two forming a zygote at *z*; *M*, zygote entering epiderm cell, its two flagella showing as remnants; *N*, parasite grown larger lies on the deep side of the host-cell nucleus, which, with those of adjacent cells, is in mitosis; *O*, parasite still larger with granules in its plasm; *P*, definite chromidia in plasm of parasite, cells of host-plant deep to it in mitosis; *Q*, parasite full-grown with large nuclear cavity and nucleolus at one side of it; *rp* indicates a resting period of $2\frac{1}{2}$ months; *R*, ripe resting-sporangium germinating, zoospores escaping. Based on K. M. Curtis's descriptions.

pause is required between the formation of zoospores in two separate sporangia and their discharge. The conjugation is selective isogamy, the chief details of which are shown in Fig. 22, which also shows the relation of the parasite to the host-cell, and its development to a chromidial resting-cell or sporangium in which zoospores are formed.

Miss Curtis found that the maturation-period of the resting-sporangium from the time of the formation of the outer membrane was $2\frac{1}{2}$ months. Rain-water produced the quickest germination. A very significant point regarding the maturation of the resting sporangium of *S. endobioticum* is mentioned. E. S. Salmon (1908) as the result of a series of infection experiments concluded that resting sporangia, after exposure for $1\frac{3}{4}$ hours to a temperature ranging from $-5°$ C. to $-6°$ C., could dispense with the winter dormancy and germinate at once. In Nature the sporangia ripen in the host-tissue; when this decays they are liberated.

Shortly before maturation the sporangium enlarges rapidly, sometimes doubling its diameter. At this stage the two outer membranes are soft and the innermost is hyaline. This distention gives the zoospores more room and their flagella attain their full length. Rupture of the sporangium is by a slit. The zoospores are in active movement before the rupture, and at suitable temperatures, 12° C. to 14° C., continue to move for 2 hours. Zoospores from resting sporangia measure 2μ, one-third larger than those from the sorus.

Curtis explains short flagella such as those in Fig. 18, *F*, as being due to immaturity.

Entry into the host-cell is similar to that of the zygote, Fig. 22, *b*. Having entered, the parasite first approaches, Fig. 20, *b*, then sinks below the host-nucleus as at *c*. Growing rapidly the nucleolus discharges chromatic material at intervals into the nuclear cavity and some of it enters the cytoplasm. The chief features of what has been recorded of the synchytrian nucleus is given in the next Chapter.

Referring again to Fig. 19, *D*, and *N*, we note that the parasite seems to be able to excite just such cell-divisions in the host-tissue as serve its changing needs. Only actively dividing regions of the host-plant are attacked. At *B* in Fig. 19 the lateral cells project to form a cup; this serves to catch rain for the hatching of zoospores shown at *F*. At *D* host-cells deep to the parasite are in mitosis causing a tension towards the surface where the summer sporanges are to be formed: at *N* the surface cells are dividing, placing the winter sporange deeper in the tissue of the host to mature thus protected.

CHAPTER IV

THE SYNCHYTRIAN NUCLEUS

SOME protists have two modes of reproduction, firstly by mitotic cell-division, and secondly by the way of a generative chromidium, see Figs. 1 and 2. Both these modes are found in the genus *Synchytrium*.

A great variety of nuclear processes has already been made known by different observers, and the bearing these have on general cytology and pathology makes their close study necessary.

F. L. and A. C. Stevens commented on the work of Dangeard and of Rosen on *S. Taraxaci* in a note to the effect that current theories of the nucleus would suffer violence if the conditions reported by these authors really existed. Dangeard had described as an occasional occurrence a direct division of the nucleus by inflexion of the nuclear membrane. Rosen found that in the primary nucleus the chromatin forms a spireme, and the nucleolus divides, the halves migrating to form daughter nuclei, thus completing a nuclear division in the spireme condition without the aid of the usual achromatic structures. The subsequent successive divisions Rosen found to assume more and more the character of an ordinary mitosis. The Stevenses gave an account of the first mitosis in the soral sporangium of *Synchytrium decipiens* of *Falcata comosa*, the hog pea-nut, an American plant.

Nuclear Divisions in the Sorus.—The division of the primary nucleus of *S. decipiens* is mitotic. The nuclear

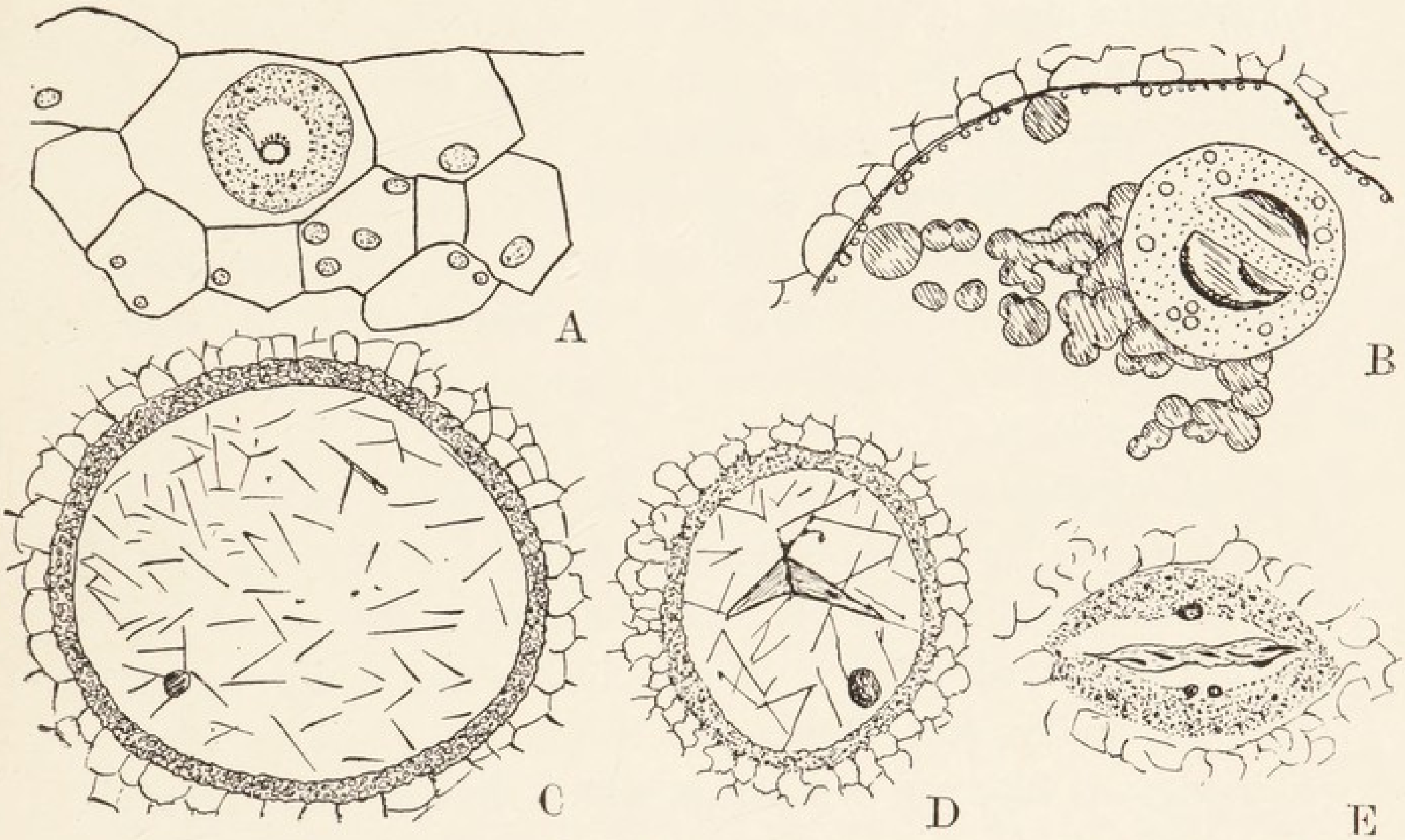

Fig. 20.—The Primary Nucleus of Synchytrium Decipiens. *A*, Parasite in an epidermal cell; *B*, nucleolus and part of nuclear membrane, masses of chromatin are emerging from one side of the nucleolus; *C*, spireme stage, nucleus surrounded by a chromidial zone; *D*, spindle forming in middle; *E*, mitosis nearing metaphase. After F. L. and A. C. Stevens.

membrane being gradually dissolved (from the outside the authors thought), becomes gelatinous and stains more deeply. The nucleolus from one side discharges its chromatin in large lumps, Fig. 20, *B*, and these appear to break up into globules and accumulate in the gelatinous nuclear envelope. Finally the nucleolus, save for a few globules, entirely disappears leaving the nucleus as shown in *C*, which the authors regard as the typical spireme of the *S. decipiens*. The nucleus shrinks as the spireme threads coalesce to meet in the middle, *D*. Finally an intranuclear spindle appears, the chromosomes, about three in number, at first forming a cap at each end. The spindle becomes constricted at its middle, *E*, and the nucleus divides.

If we compare the nuclear processes just described with those Wager met with in *Polyphagus*, Fig. 14, it is obvious that the accumulation of granules about the nucleus is a chromidium.

Other phenomena observed in *S. fulgens*, *papillatum*, and *decipiens* by F. L. Stevens are detailed in Chapter V.

Sorus-formation in S. endobioticum.—The nucleolus having increased greatly in size lies eccentrically in the nuclear cavity and discharges chromatic granules along linin strands into the cytoplasm, which thus becomes highly chromatic, and a fine cell-membrane is secreted. Granules collect at the upper part of the membrane and over the upper end of the nucleus, Fig. 21, *c*. The membrane is then dissolved at a pore and first part of the cytoplasm, next more plasm and part of the nucleus, and finally the rest pass out of the membrane into the upper half of the host-cell, *d*. The nucleus now shows linin-threads, *e*. Soon an intranuclear spindle with 5 chromosomes is formed and the first division occurs, *f*. Successive simultaneous mitotic divisions follow till the sorus subdivides into sporangia, *g*, containing a great number of small nuclei. The young zoospores are at first close-packed but, water

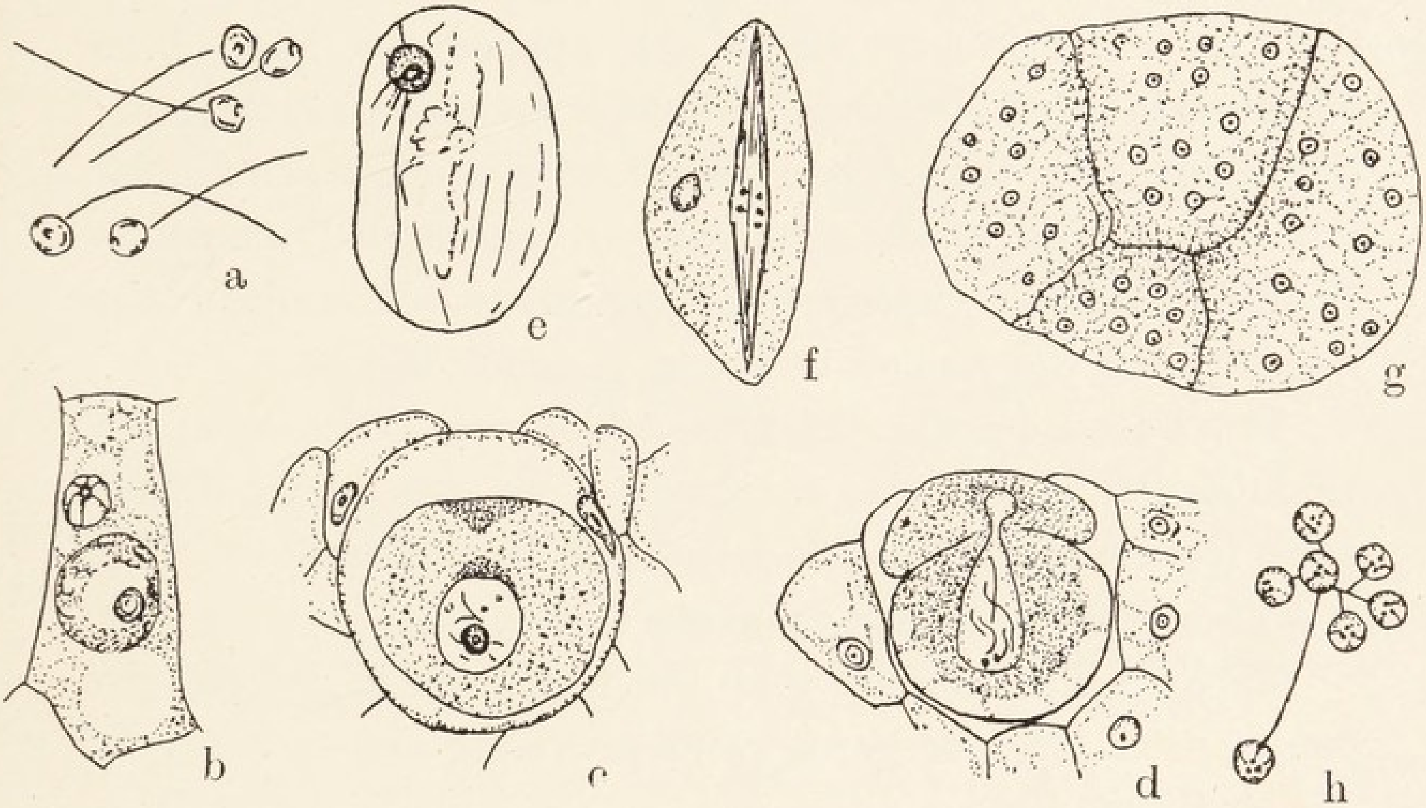

FIG. 21.—SYNCHYTRIUM ENDOBIOTICUM, AGAMIC STAGES. *a*, zoospores, living state ; *b*, young parasite nearing host's nucleus ; *c*, full-grown parasite ready to develope into a sorus, pyramidal heaping of granules ; *d*, a stage in formation of the sorus, the nucleus beginning to pass through the opening in the capsule ; *e*, primary soral nucleus, late prophase ; *f*, the same metaphase, spindle with 5 chromosomes ; *g*, early stage of segmentation of sorus ; *h*, zoospores nearly full-grown, the stands joining them become flagella later. After K. M. Curtis.

being absorbed, they become more loosely arranged and are joined by fine strands which become the flagella entering each zoospore at the point where the blepharoplast is seen.

In the agamic stages *S. endobioticum* contains a good deal of chromidial material derived from the nucleus, but no definite chromidial zone enveloping the nucleus such as Stevens found in *S. decipiens* is produced. The passage of chromatin into the nucleus by a series of discharges rather than in a continuous stream as appears to have been observed in other species is perhaps a variable phenomenon: the difference is that in one case there is a series of unipolar mitoses, in the other a stream of chromidial elements. Possibly equable experimental conditions determined the regularity of the various stages in *S. endobioticum.*

The Gametic Stages of S. endobioticum.—The complete examination of this one species that we owe to Dr. Curtis calls for comparison with what has been found in other species and for reflection upon the facts.

There is little doubt that the formation of zygotes by conjugation of zoospores will prove to be general in *Synchytrium.*

In Pycnochytria (in de Bary's sense) only the gametic part of the life-cycle will probably be found, and it will be interesting in this group, and in that division of the Eusynchytria which produce a sorus from the resting-cell (*S. stellariae* &c.) to see what exactly are the nuclear and chromidial processes in the gametic sorus. Cells infected by zygotes divide leaving the parasite in the deeper daughter cell, Fig. 22, *e.* This division is repeated till the infected cell lies under two or more layers of uninfected cells.

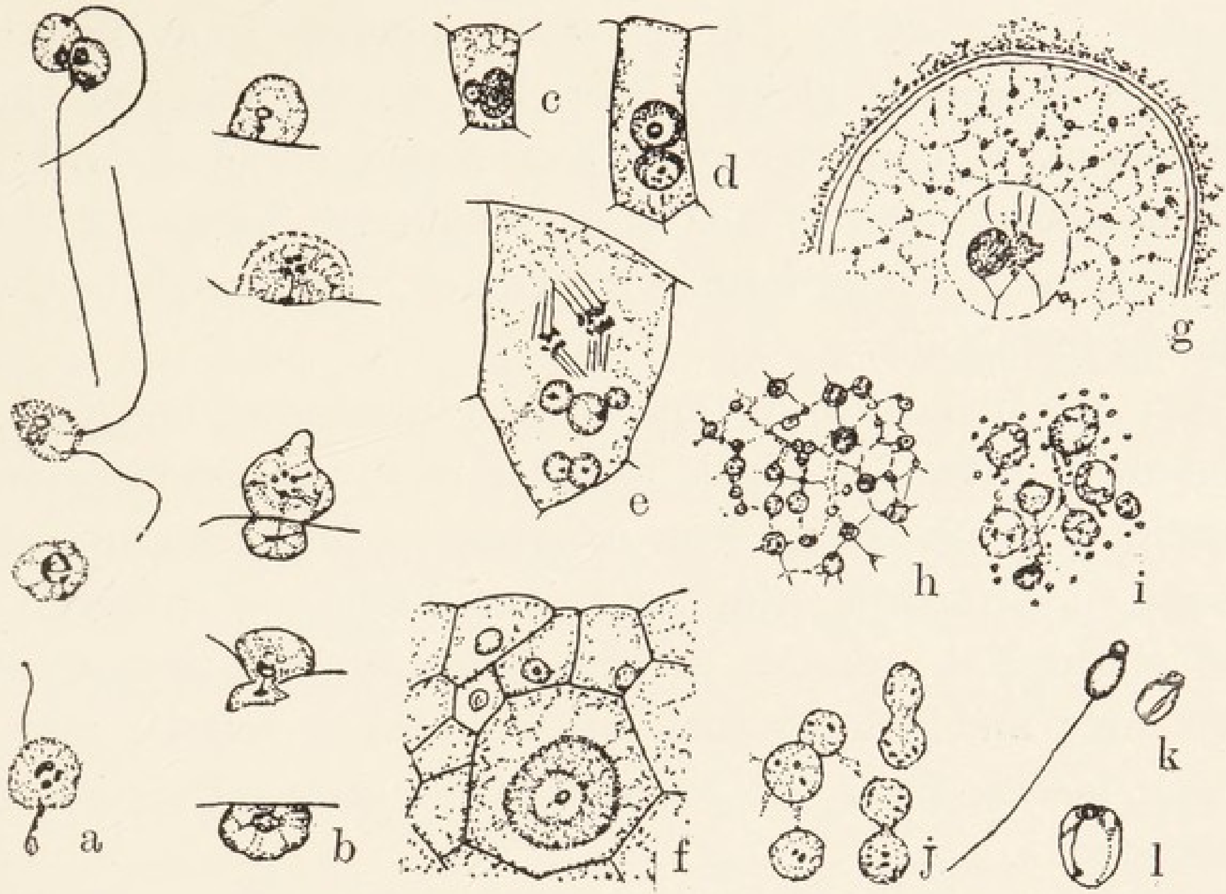

Fig. 22.—S. Endobioticum, Sexual Generation. *a*, 4 stages of syngamy, the lowest shows recession of the 2 flagella; *b*, 5 stages in penetration, the transverse line is the surface of a host-cell; *c*, cell with young zygote smaller than cell-nucleus (× 400); *d*, the parasite is larger and lies below the cell-nucleus; *e*, division of host-cell containing 5 parasites, the latter remain in the lower half of the cell; *f*, cell with large parasite now separated from the surface by previous divisions of host-cell; *g*, part of a parasite, it has its own capsule and, outside that, a second derived from the host-cell, its nucleolus has discharged chromatin into the cytoplasm, where chromidial granules are seen, × 800; *h*, zoospores beginning to be formed from chromidia, × 1650; *i*, *j*, further stages in zoospore-formation; *k*, zoospores × 780, living (left) and fixed and stained (right); *l*, zoospore × 1650, nucleus, blepharoplast, and joining strand seen. After K. M. Curtis.

The Gametic Nucleus.—Fusion of the two gametic nuclei is shown in four stages at *a*, and the part played by the nucleus of the zygote during penetration of the host-cell in five stages at *b*. The parasite's nucleus is at first a "single chromatic globule lying free in the mass of the cytoplasm." A nuclear space soon forms, *c* and *d*. Later the nucleolus becomes the most prominent feature of the nucleus, as at *f*, and from it strands of linin stretch to the nuclear membrane.

In the parasite's cytoplasm are numerous chromatic granules; a feature which becomes more prominent later. Continuous with the nucleus, is a mass of linin fringed with points of chromatin (the "amœboid body" of Percival); the chromidial granules in the cytoplasm are now more numerous and the parasite has secreted a capsule upon which matter from the remains of the host-cell is being deposited, *g*.

The Generative Chromidium.—The chromidial granules arise, Dr. Curtis states, by some six or seven intermittent discharges from the nucleolus. They first accumulate in estricted equidistant areas, Fig. 19, *P*, but afterwards completely replace the cytoplasm, the remains of the nucleolus then seems to be of no active service. The chromidial sporangium is thus established. The granules of the chromidium at first stain uniformly with iron-haematoxylin and hence, as is explained below, the chromidium is at first in the plasson state. The granules are the nodal points of a fine reticulum and they soon show central chromatin granules with a clear space around them, and outside a lightly staining zone, Fig. 22, *h*.

At this stage some of the granules, now zoospore-origins, or "primordia," show signs of subdivision. Later still the plasm of the primordia becomes vacuolated and granules of chromatin are expelled, *i*; by this process the primordia become equal and spherical and their chromatin is reduced to a few granules, from which the single nucleus of the zoospore is formed. The young zoospore's plasm first becomes dense, then vacuolated, *j*. The nucleus in the zoospore has a clear central area with chromatin granules around it, *k*, and a chromatic strand stretches from it to the blepharoplast at the opposite end of the body of the zoospore.

A species of Synchytrium in a Thistle.—For an opportunity of studying some aspects of the cytology of *Synchytrium* and of comparing it with such records as I have read and with the structure of *Plasmodiophora* and Mycetozoa I am indebted to the kindness of Professor V. H. Blackman and Assistant Professor R. J. Tabor of the Imperial College of Science and Technology.

The host-tissue was a thistle-leaf [1] which had been fixed in 95 % alcohol. With a pocket-lens the parasites were seen as brick-red points. Sections were cut for me by Dr. Perkins and stained with H. and E. Some of the results are shown in Fig. 23.

The walls of the host-cells are thick and stain deeply as

[1] Not having been able to identify the species of either host or parasite in this instance I refer to the parasite in what follows as the thistle-*Synchytrium*.

also do the special capsules of the soral sporangia. Of the latter some are somewhat shrunken by dehydration as shown in Fig. 23, *a*.

In some sections clusters of three sori are found. It will be noted that the cells around the sorus do not project to form a rosette. The rigid nature of the host tissue caused the parasitic elements to fall out of a number of the sections so that a continuous series of sections of any one element was not obtainable, still it is easy to recognise two separate processes; one the formation of sori, the other the formation of single or direct sporangia in some of which the zoospores are completely formed. In this thistle the soral sporangia and the non-soral (here termed "direct") might belong to different species, though this would not rob their minute structure of interest and meaning. The earliest stages are not present: the youngest direct sporangium met with is shown in Fig. 23, *c*, *d*, where three nuclei are seen, all having relatively large nucleoli. The nuclear membranes are merely surfaces of plasm bounding sap-vacuoles in each of which a nucleolus lies eccentrically. The nucleoli have the same general characters as those of *S. endobioticum*. What looks like a vacuole in Fig. 23, *e*, is really a blunt process like others which are seen in profile in the same element, studded at their surface with granules, the latter on the left side being continuous with the adjoining part of the nuclear membrane. In *S. endobioticum* the nucleolus (according to Curtis) discharges chromatic matter intermittently to form chromidia in the reticulum, whereas in this thistle-*Synchytrium* the nucleolar substance has the appearance of being converted continuously into a chromatic reticulum in which separate chromatin granules are not recognisable.

The next stage of the direct sporangium is that shown in Fig. 23, *f*. The nucleoli are not yet wholly absorbed, but the reticulum has changed, having become finely granular and less deeply stained, and in every part definite equal grains of chromatin are evenly distributed in the reticulum of the developing sporangium. Stages between that just described and the formation of separate zoospores, Fig. 23, *h*, *i*, are not to be traced clearly in the sections of the thistle.

The zoospores are typical; but in this case the wall of the direct sporangium is much thinner than that of the soral sporangia. Beside the intracellular parasites in the thistle are larger intercellular bodies, stained purple and of a texture resembling akaryote plasmodia. These structures call for further examination.

The Nuclear Cavity.—With regard to the nuclear cavities Fig. 23, *d*, and *f*, they are obviously nothing more than sap-vacuoles; and in this relation it is of interest to recall the view expressed in 1903 by A. A. Lawson, and supported by careful cytological observations. Lawson concluded that the nuclear cavity of the cells of the higher plants is only a water-cavity similar to a cell-vacuole; in other words the nuclear membrane is a cytoplasmic structure.

The three nucleoli seen in Fig. 23, *d*, have probably arisen from the division of a single nucleolus and have become separated by the formation from their own substance of

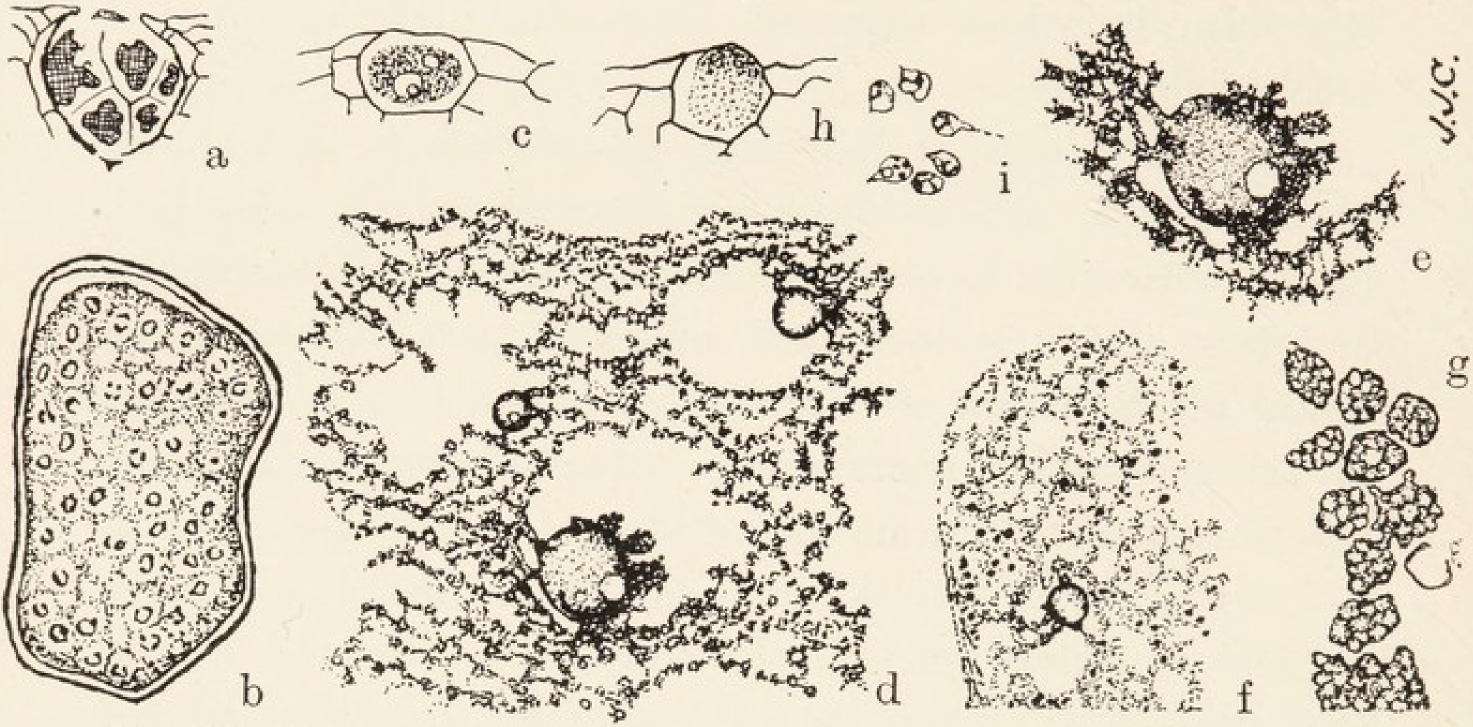

FIG. 23.—DETAILS OF A SPECIES OF SYNCHYTRIUM IN A THISTLE-LEAF. *a*, sorus subdivided into sporangia ; *b*, a soral sporangium, zoospores nearly formed ; *c*, early stage of direct sporangium ; *d*, part of *c* more magnified showing three nucleoli ; *e*, a single nucleolus still more magnified ; *f*, a stage later than *d*, a single nucleolus and grains of chromatin in a finely granular plasm ; *g*, a stage later than *f*, protoplasm segmented prior to zoospore-formation ; *h*, direct sporangium ; *i*, part of *h* more magnified, zoospores. Magnification : *a*, *c*, and *h*, × 80 ; *b*, *d*, *f*, *g*, and *i*, × 1000 ; *e*, × 1500.

new reticular plasmodium. One of the nucleoli, the uppermost in *d*, has a projection not quite separated from the rest.

Assuming that both the soral and the direct sporangia of the thistle-*Synchytrium* belong to the same parasite, an assumption that makes no difference in regard to the cytological points to be considered, we may compare some features of this infected thistle-leaf with those described by Curtis in the potato.

Apart from the want of a "rosette" and there being no evidence of cell-proliferation on part of the host-tissue, the formation of the sorus and the character of the nuclei in the nearly ripe soral sporangia is the same in the two parasites.

Very different is the rest of the picture; and yet the character of the nucleoli, and the processes of chromidium-formation show that the direct sporangium in this thistle-*Synchytrium* corresponds with that sporangium of *S. endobioticum* that developes from a zygote.

Stages of the two may be compared as far as a very partial examination of the thistle-*Synchytrium* allows. Fig. 23 is reproduced from the "Lancet," vol. ii. p. 495, 1921.

In the thistle-*Synchytrium* at a certain stage no separate chromatin points are to be seen, so the term "plasson" is the one I would suggest to describe the state of the sporangium as shown in Fig. 23, *d* and *e*. It becomes chromidium at the later stage, *f*. The terms plasson and chromidium are used here as defined above, Chapter I, pp. 4–6.

The Synchytrian Nucleolus.—The nucleolus is the dominant cell-organ in *Synchytrium*; from it are developed the chromidia; vegetative in the agamic, generative in the gamic stages; and in the former also the chromosomes and spindle. The nucleolus is the centre of growth as well as of reproduction in *Synchytrium*.

The synchytrian nucleolus is composed of living matter with the least recognisable differentiation of structure and of the greatest potential ; all cell-organs being formable by it ; it is totipotential protoplasm ; plasson.

An instance is given in the next Chapter of a whole synchytrian nucleolus expanding on all sides simultaneously into a spreading mass, which infiltrates the cytoplasm. In that and other similar cases the nucleolar expansion appears to have replaced a primary and several subsequent mitoses, smaller nuclei being formed from the expansion as bird's-eye bodies.

In the direct sporangium of *S. endobioticum* expansion occurred by a series of what may be regarded as unipolar mitoses ; in that of the thistle-*Synchytrium* by a gradual and continuous expansion from the surface of the nucleolus.

To the original unexpanded form of plasson as seen in the nucleolus the adjective pycno (Gr. = dense) may be applied, the expanded state as seen in Fig. 23, *d*, being designated chasmatoplasson (Gr. *chasma* = an expanse).

If we imagine a degree of parasitism even more intimate than is to be found in *Synchytrium*, we should expect to find the parasites reduced in some stages of their existence to the condition of the synchytrian nucleolus. In Chapters VI and X it is shown that the causal parasites of molluscum contagiosum, smallpox, syphilis, and cancer are reduced to this state in their earlier stages.

CHAPTER V

"CANCER-BODIES" IN SYNCHYTRIUM AND IN SYPHILIS

Bird's-eye Nuclei in Synchytrium.—In three species of *Synchytrium* F. L. Stevens found nuclear structures the nature of which, he stated in 1907, was far from clear:—

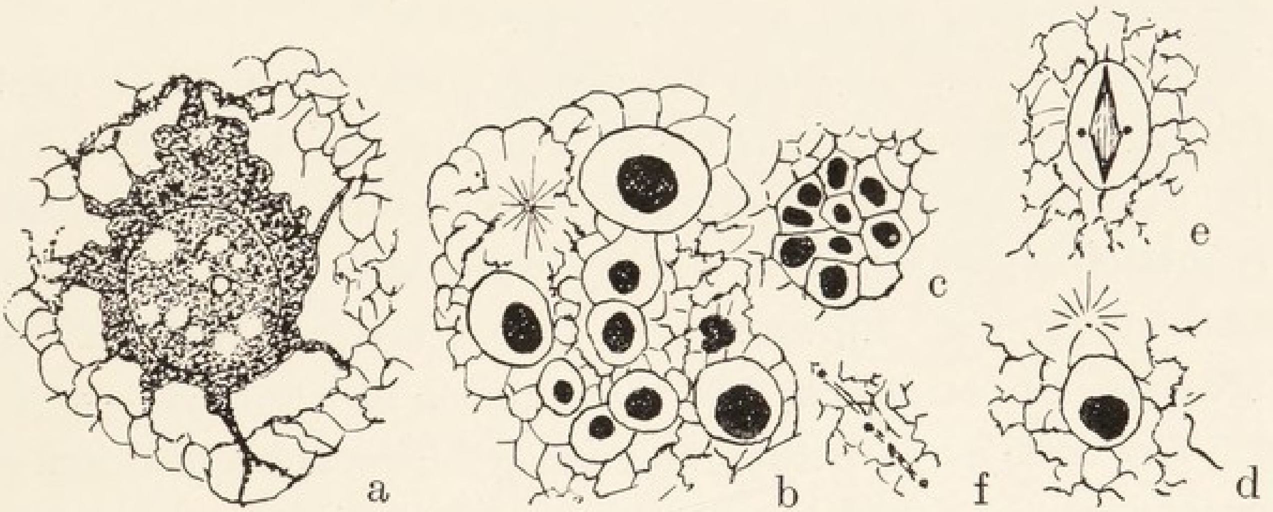

FIG. 24.—SOME NUCLEAR PROCESSES IN SYNCHYTRIUM DECIPIENS. *a*, Irregular nucleolus extending into the cytoplasm by radial extensions; *b*, group of bird's-eye nuclear bodies with an aster; *c*, cluster of bird's-eye bodies such as are often seen in cancer; *d*, bird's-eye body with aster attached; *e*, mitosis, metaphase; *f*, mitosis, anaphase. After F. L. Stevens. From the "Lancet."

"The structures were atypical, strikingly peculiar, and quite without parallel in any plant or animal as yet described." Some of these bodies are shown in Fig. 24, *b*, *c*, *d*.

The large nucleolus, Fig. 24, *a*, is continuous with the cytoplasm by radial extensions across the remains of the nuclear cavity. The latter is seen as in the direct sporangium of the thistle-*Synchytrium* described at the end of Chapter IV to be nothing more than a sap-vacuole. Stevens terms the irregular body, Fig. 24, *a*, a nucleus, but there is no doubt that it corresponds to the nucleolus of Fig. 20, *B*.

The nucleolar substance instead of growing into the cytoplasm by intermittent mitotic discharges as in *S. endobioticum*, or by continuous expansion as chasmatoplasson in the thistle-*Synchytrium*, has become amoeboid and is streaming on all sides into the cytoplasm.

Together with nuclei such as that just described Stevens found homogeneous nuclear bodies, Fig. 24, *b*, surrounded by a clear space and bounded by a delimiting membrane. Sometimes, as shown at *c*, they were met with in closely packed groups. Stevens also found astral bodies either near, as in *b*, or attached to these nuclei, *d*.

The Bird's-eye Bodies of Cancer.—The abovementioned curious nuclei found by Stevens in *Synchytrium* are nothing more nor less than the homologues of the "cancer-bodies" or bird's-eye bodies of human pathology. In 1892 and for several years after that date they were claimed by some pathologists to be the only true "protozoa" to be found in cancer.

They are not peculiar to cancer, nor are they to be found in their typical form in all cancers, though they are remarkably abundant in many.

Their appearance in many cancers, *e.g.* in the cancer of the breast shown in Part III, Plate VII, is even more like Stevens's nuclei in *Synchytrium* than are those shown here in Fig. 25.

As they are seen in cancers these bodies have been very frequently described in pathological writings from 1892 onwards, but I have never seen so good a description of them as that Stevens wrote :—

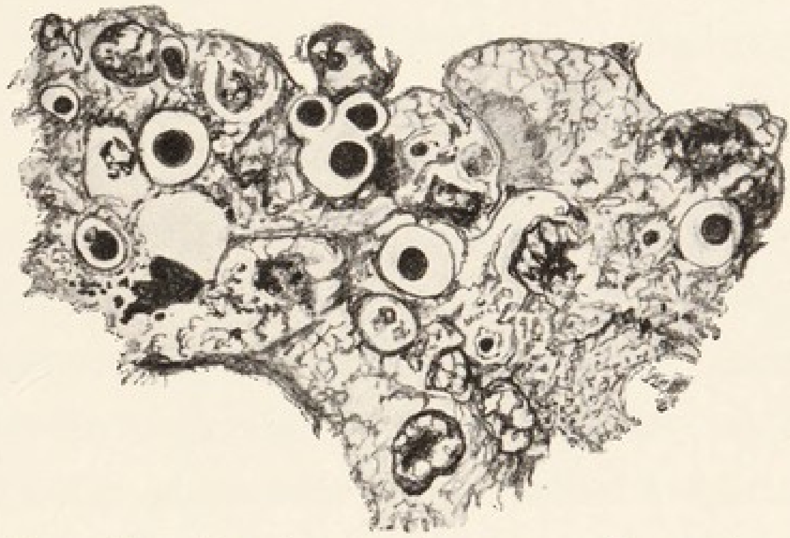

Fig. 25.—Bird's-eye Bodies. Part of a section of a cancer of the breast. After F. B. Mallory, from Part IV.

" Large homogeneous nuclear bodies consisting of nucleoli or chromatin, or both, are scattered in the protoplasm of the parasite. They are surrounded by a clear sap-space which is ordered by a definite nuclear wall. In some cases no wall was discernible."

Stevens found asters associated with many of these bodies as shown at *b* and *d*, which are copied from portions of Stevens's drawings of larger areas containing many such elements, concerning which he writes : " They undoubtedly do arise sometimes by successive divisions of the nuclei arising from the primary division as described in 1903. Stages showing 1, 2, and 4 spindles per cell prove this, but if such were the prevalent mode stages between elements like Fig. 24, *d* and the binucleate stage would be common, and they are not; I am led to consider the possibility of a second mode of division of the primary nucleus consisting of a simultaneous breaking of the one nucleus into many."

I think the puzzling bodies are nuclei usually originating in a chromidium, as I saw it in the living state in syphilis. The following account is copied from Part II, but was first published in 1907. Before passing to this description I would again refer to the nucleus of *S. decipiens*, Fig. 24, *a*, remembering that the nucleus there depicted corresponds with the soral nucleus in *S. endobioticum*, Fig. 21. We see that, instead of dividing, the nucleolus of *S. decipiens* as seen by Stevens became plasmodial and was streaming into the cytoplasm, where new nuclei were formed as they are from generative chromidia. I have seen similar nuclei or bird's-eye bodies form in molluscum corpuscles after they have been in water for some days.

Living Bird's-eye Bodies in Syphilis.—The production of these bodies in living intracellular parasites in syphilis is so important that I will insert the account almost as it stands in Part II; the observation was made long before the introduction of rapidly acting arsenical compounds in the treatment of syphilis.

The groups of oscillating granules which I saw associated with the formation of bird's-eye bodies would probably be equivalent to the asters Stevens described in *Synchytrium*.

I had for a long time desired to watch these bodies under conditions similar to those that obtain during life, but so great are the obstacles to such work in professional life, and so reluctant is one to withhold treatment from a patient, and so rapidly in syphilis are lesions usually removed by treatment, that it was not until August 31, 1901, that I was able to do this. Among my out-patients at the N.-W. London Hospital was a man who had some obstinate tertiary syphilitic lesions on the face and in the mouth that resisted full doses of the biniodide of mercury and potassium, which I had given to him by the mouth for several weeks. These lesions were subsequently completely cured by intra-muscular injections of sal-alembroth. I arranged for this patient to attend at about 7 p.m. on the date mentioned, and after wiping the surface of a warty lesion of the hard palate with a dry sterilised swab I took a scraping with a clean knife, not going sufficiently deeply to cause any bleeding. The material thus obtained I mounted in its own liquid exudation, covered it with a thin cover-glass, and examined it on a Stricker's warm stage. With the objects before me I made the graphic and written notes which are reproduced in Fig. 26 and the accompanying description.

What are the bodies? They are certainly of kindred nature to those I described in cancers in 1892, but it is to be noted that on the warm stage these bodies from the tertiary lesion exhibited what could only be vital processes.

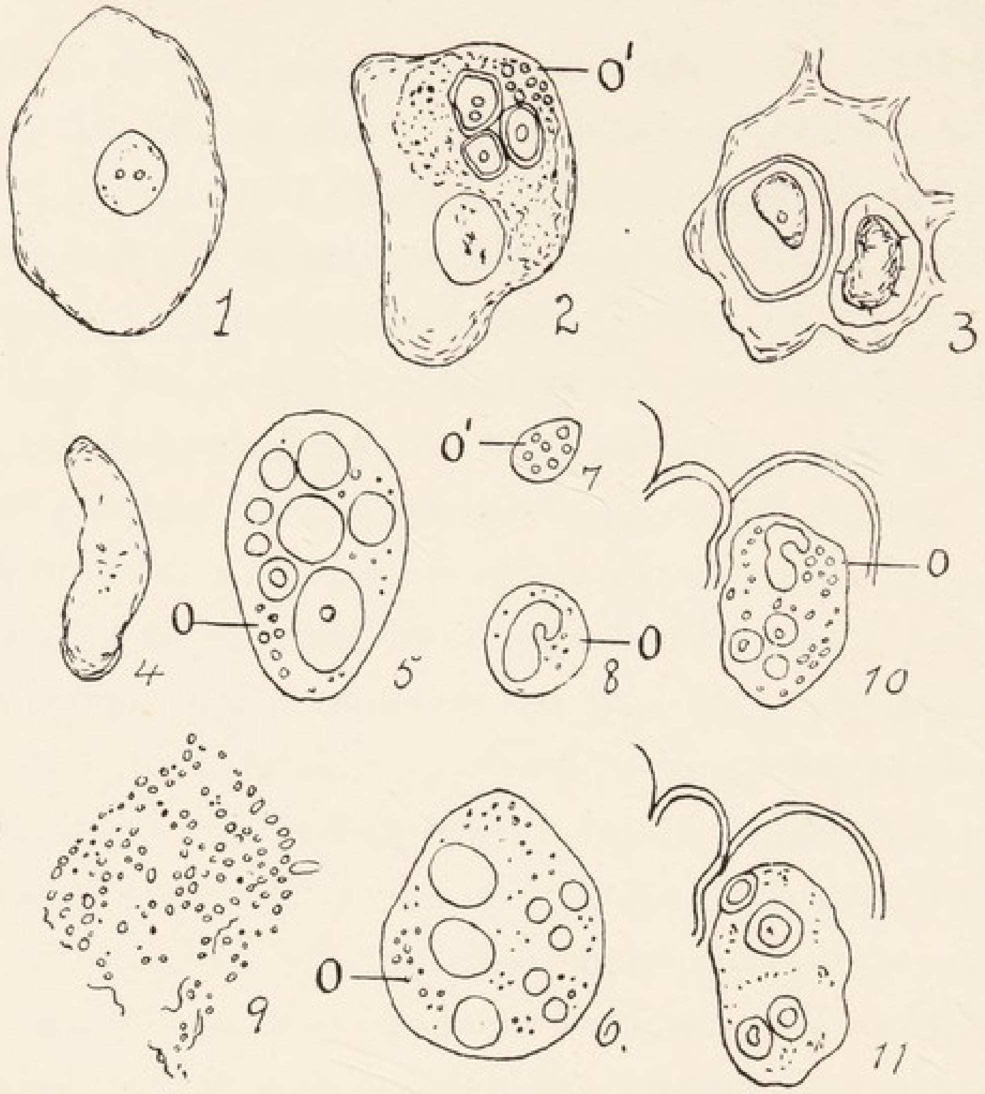

FIG. 26.—BODIES OBSERVED IN A SCRAPING OF A TERTIARY SYPHILITIC LESION. *1*, An ordinary squamous epithelial cell devoid of movement; *2*, a similar cell, but containing in optical section a granular mass in which are three typical bird's-eye bodies, and at *o′* a group of granules in lively oscillation; *3*, another epithelial cell, the nucleus of which is not seen, and which contains two highly refracting inclusions; *4*, a free body of similar optical characters to the inclusions in *3*; *5* and *6*, structures resembling an epithelial cell in size, but containing large globules of a bright greenish appearance, and at *o* and *o′* groups of smaller globules in oscillation; *7* and *8*, small bodies resembling parts of *5* and *6*, lively oscillation in one, and oscillation and a greenish nuclear body in the other; *9*, a large body, the pale central mass of which is not shown, but globules and granules and a few wavy lines are seen on the surface; *10* and *11* are the same body, *10* as seen at 7.20 p.m., and *11* as seen at 10.45 p.m., when the preparation has cooled down. When first seen, there was a group of oscillating granules, *o*, and a single greenish curved structure to the left of them; later, the preparation having cooled down, the oscillating granules had disappeared, and the body seemed to have divided into two. Seen by $\frac{1}{12}$ in. oil-imm. objective. From Part II.

Each group of oscillating granules could only represent a focus where a process equivalent to mitosis was going on. The mass invading the epithelial cell shown in Fig. 26, *2*, contained three typical " bird's-eye bodies," and the cluster of granules in violent oscillation at *o′* marked the spot where another bird's-eye body was in process of formation. I had previously concluded from histological study that these bodies are thus formed, and this observation of the living cells confirms my view. Bodies such as *5* and *6* are met with in sarcoma and cancer as well as in syphilis. The clear circles within them represent greenish globules, which are formed in the same way as the bird's-eye bodies and the oscillation at *o*, in these two bodies marked I do not doubt the formation of new globules. The two smaller bodies, *7* and *8*, as shown in the sketch, are not unlike leucocytes, but in the living state their absolute identity in physical characters with the larger bodies and their green colour [1] and oscillating granules distinguished them from leucocytes. As to the bodies *10* and *11* the partial capsule at their upper end is probably the remains of host-cells, and the evidence detailed in the description strongly supports the view that the curved greenish body near the group of oscillating granules at *o* divided into two parts.

"**Cells of endogenous origin.**"—These nuclear structures described by Stevens in *Synchytrium*, by many pathologists

[1] A similar green colour is to be seen in the early intracellular stage of the parasites of smallpox and in some of those of cancer, &c. It is also seen in the nuclei of human epithelial cells when teased out in water, especially at the metaphase stage of mitosis.

7

including myself in cancer, and by myself in syphilis are nothing more than Virchow's "cells of endogenous origin." In 1851 Virchow summed up his observations thus: "A portion of a large cell with granular contents, perhaps an altered nucleus, the dimensions of which it has, becomes homogeneous and clear like water. This portion has at first a sharp and stout wall, which very soon by the addition of fresh layers is thickened and becomes doubly-contoured, in every way like a cartilage cell." The "granular contents" of the foregoing description agrees with the appearance shown in Fig. 26, *2*. The "cells of endogenous origin" are nuclei formed in parasites in the chromidial state, and they are only temporary nuclei comparable, perhaps, to the tropho-nuclei of Plasmodiophoraceae (Chapter VII). They are occasionally seen to form in molluscum-bodies in water-cultures, see Fig. 32. They do not represent a necessary or invariable phase in molluscum, or in cancer, or in *Synchytrium*; but what may be termed an optional or alternative phase.

CHAPTER VI

PLASSOMYXINEAE AND MOLLUSCUM CONTAGIOSUM: MICROHENADS

THE term Plassomyxineae first used last year to designate a group of pathogenic organisms, of which the casual parasite of Molluscum contagiosum is the most easily studied, I have defined as follows :—diagnosis : *Plassomyxineae, nomen novum, applicandum quaedam ad protista parasitica et pathogenica, generi Synchytrio affinia, quae interdum in statu plasson dicto sunt.*

The name first given to any group of organisms, if it is not based on misconception and hence misleading, must stand as the name of the group in question. Now in using the name Plassomyxineae, I am well aware that the term Chlamydozoa has been previously applied to the molluscum body and kindred structures. Let us trace this idea to its source. It had been found that molluscum bodies resist the action of strong acids and alkalies, &c., and it was inferred that they could not therefore be anything but colloid. The matter was conscientiously examined by L. Pfeiffer, who compared the behaviour of Coccidia with that of the molluscum body when treated by the same chemical reagents. He found that coccidia were altered in appearance, but not the molluscum. His conclusion was that the molluscum body could not be a *Coccidium*, but that within the opaque colloid covering of the molluscum body there was concealed a parasite. This same view was adopted later by von Prowazek with the addition that he identified the parasite with some alleged minute granules in the middle of the body, and he made the name Chlamydozoa for the group to which the molluscum body belongs.

Now, seeing that except for a thin cortex the middle of the body is like the rest, and that the parasites appear from what follows to be most closely related to the genus *Synchytrium*, and so not protozoa at all. Prowazek's name is based on misconception ; it is misleading, and should be replaced by some more appropriate term.

Plassomyxa contagiosa.—This name I have made to apply to the causal parasite of molluscum contagiosum, and seeing that some of its phases are easily studied in the living state, the behaviour of the organism in water-cultures must be given in some detail although they have already been considered to some extent in Part IV.

Fortunately, the bodies characteristic of this disease in the human subject when full-grown are relatively large, Fig. 27, and firm of texture, and hence it is easy to watch what happens to them when they are placed in surroundings that make it possible for them to show that they are living things; that is when we place them suitably in water, thus using the experimental method.

Those observers who have studied lesions in their earliest stages have found that the first site of the parasite is inside the host-nucleus, and its escape into the cytoplasm has been seen. Some subsequent stages are shown below in Fig. 30.

Cultures. — When we are planning experiments to decide whether any object is alive we must first consider the conditions in which it is found in nature.

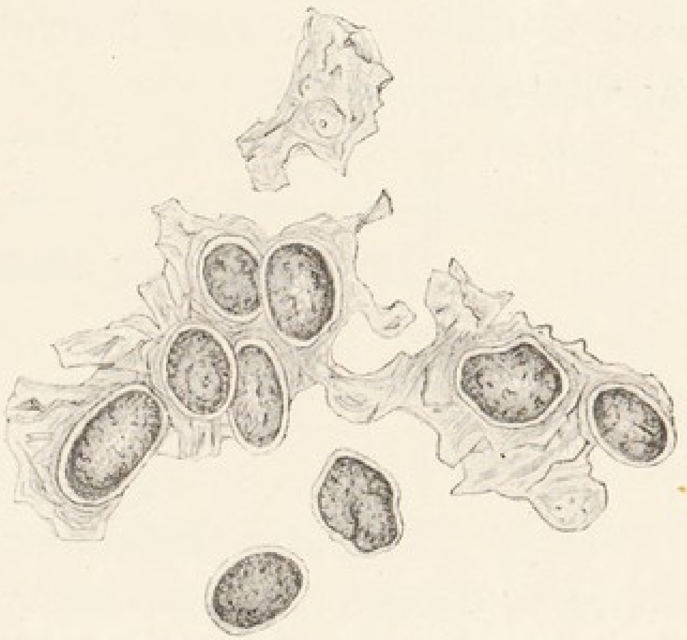

FIG. 27.—MOLLUSCUM BODIES AND SQUAMOUS EPITHELIAL CELLS. From a teasing of a freshly excised nodule smeared on a slide and fixed with perchloride of mercury solution and mounted in glycerine and water. Drawing eye-piece. × 400 diams. From Part IV.

The earliest stages of *Plassomyxa contagiosa* as seen in sections of the tumour are found inside other cells, hence in anaerobic conditions: the ripe parasites on the contrary are extruded from the body and their subsequent development is most likely to be both aerobic and aquatic, the latter probability being supported by the observation that the disease is sometimes contracted in Turkish baths. Aerobic water-cultures are thus indicated. Seeing that we are dealing with an obligatory parasite only a certain limited number of stages are to be expected in such cultures.

Considered as isolated phenomena the subjoined facts are evidence of vitality in molluscum bodies: taken together they either prove this vitality or they prove the microscope to be useless for the study of protistic life. Personal practical work of the simplest kind is required for any one to witness the vital phenomena.

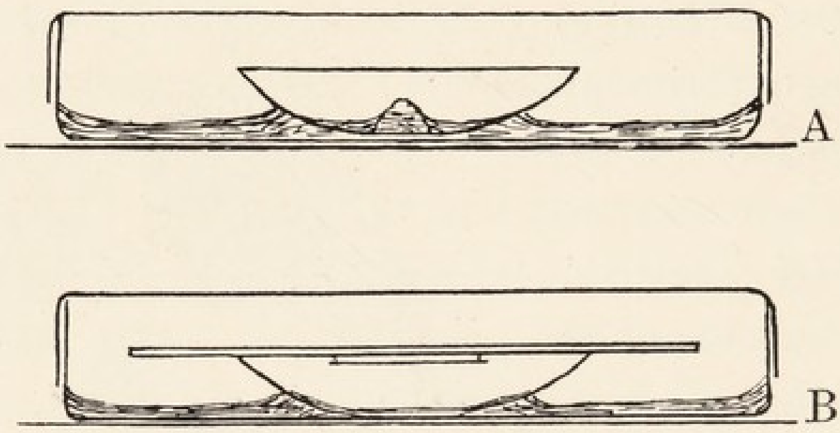

Fig. 28.—A Method of Water Culture. *A*, the material to be cultivated is heaped in the middle of a watch-glass and water, but not enough to cover the material, has been added drop by drop; *B*, after teasing in a drop of water on a slide, a particle or drop of the material in *A* is covered and examined microscopically, then the preparation is inverted over a watch-glass containing water and put between Petri dishes for re-examination later.

The culture must be kept at room temperature, because incubation favours the growth of bacteria, which, when abundant, kill the specific parasites. Occasionally a culture apparently free from bacteria is obtained, but usually bacteria are present; in moderate number they do not prevent vital changes from occurring.

It is important to make clear drawings of objects at the time they appear under the microscope, and it assists the eye, if the main features of each stationary part are traced by means of a drawing eye-piece or other similar optical instrument.

Enumeration and Details of Vital Changes.—Some vital changes which I have already observed are shown in Fig. 29; they are:—1, streaming of protoplasm with, 2, subdivision by budding; 3, formation of a supporting framework or of protective capsules; 4, formation of bird's-eye bodies; 5, vacuolation with oscillation of granules; 6, formation of active flagellate or spirillar bodies.

1. **Streaming.**—The reality of the phenomenon being proved by facts recorded below a few details may be added. Fig. 29, *a* shows the aspect of a corpuscle just before streaming begins; its texture becomes more granular: this is succeeded by a disappearance of granules from either part of, as at *b*, or throughout a corpuscle, as at *c*, *e*, and *f*. The fresh corpuscle often presents indications of being segmented, and a regular segmentation is sometimes seen in water cultures; in one such I found the rotary motion affected one only of the segments, Fig. 29, *d*.

There are no separate granules to be seen in the moving substance, which must be composed of extremely minute elements, but large granules are sometimes seen on the surface of the flowing part of a corpuscle, such granules I have observed to be carried round one segment of bodies such as that shown in Fig. 29, *c*.

2. Budding.—From some of the bodies with streaming protoplasm part of this can be seen to protrude as in Fig. 29, *f*; and in the neighbourhood are smaller spheres

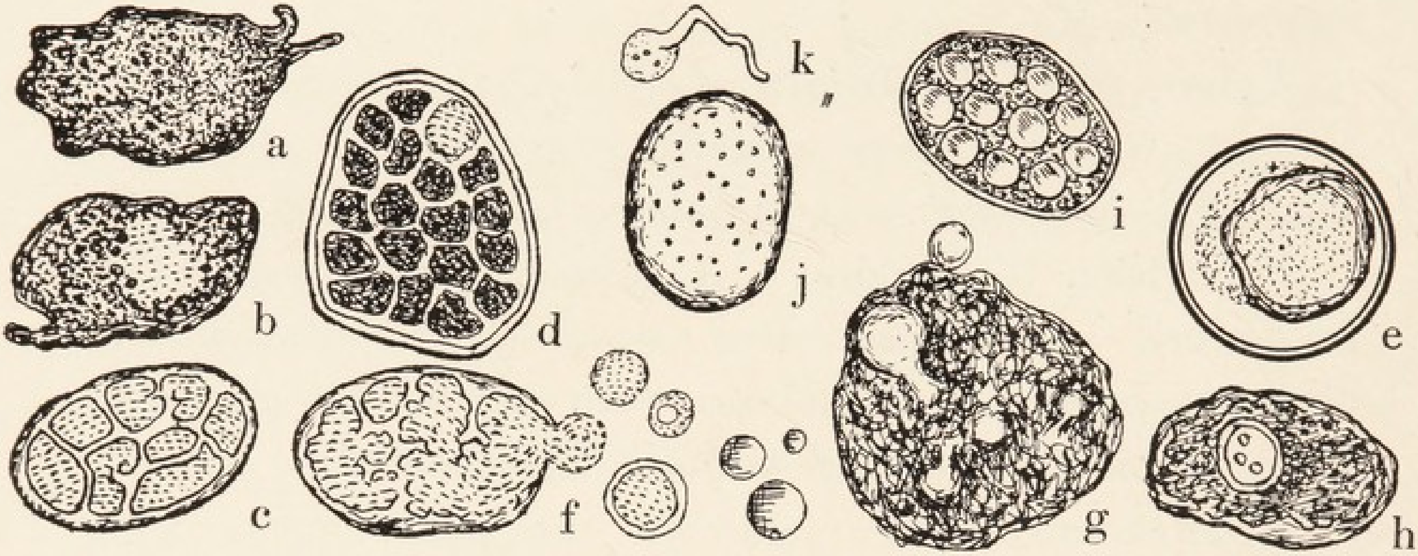

FIG. 29.—VARIOUS ACTIVE PHASES OF PLASSOMYXA CONTAGIOSA. *a*, molluscum body unchanged save that the cortex is more definite and the granules are larger than in the fresh state; *b*, a clear area has formed and in it the protoplasm is circulating; *c*, an internal framework continuous with the cortex has formed and the whole of the protoplasm is circulating; *d*, a capsule has formed and within it the protoplasm is subdivided into segments, in one of which only the protoplasm is circulating; *e*, a spherical capsule has formed, the protoplasm is circulating and is in part protruded beyond the cortex; *f*, the whole protoplasm is circulating and a small protrusion is present, and there are three separate subdivisions with circulating protoplasm, one vacuolated, another with a clear outer zone, and, to the right, three subdivisions as they appear when they come to rest clear and refracting; *g*, a body in a surface view of which five buds are seen in process of formation; *h*, a body in which a nucleus-like structure has formed; *i*, a body with vacuolated protoplasm, the intervening granules in Brownian movement; *j*, body with violent agitation of protoplasm; *k*, flagellated body with agitated granules at its expanded end. *d*, *f*, and *g*, × 800; the rest × 500.

with streaming; in some of the latter there is a bright outer zone with no streaming; some of the small streaming spheres have a vacuole.

All three kinds when they are observed to come to rest assume a uniform refracting appearance and have a green tint. Several times I have watched protrusions of streaming protoplasm such as are shown in Fig. 29, *f* and *g*, having in contact with them a small separate sphere of streaming protoplasm; and this sphere has, as if by the rotation of its substance, moved slowly from its first position. Though I have not watched the first formation and the actual separation of one of the small spheres I have no doubt these arise as buds from the molluscum bodies.

3. **Doubly Contoured Capsules.**—These are rare in fresh material, but many develop in water cultures, Fig. 29, *e*. They seem to be formed as a defence against bacteria: I have seen many with bacteria on the outside of the capsule but never inside.

4. **Internal Framework.**—Irregular processes extending inwards may be formed apparently in the spaces between the segments of a corpuscle. They are sometimes regular and anastomose to form a net, Fig. 29, *c*; or the framework may be scanty as at *f*. When a capsule is secreted the framework is not formed, and *vice versâ*.

5. **Nucleus-like Bodies.**—These are not present in the fresh corpuscle. Granules appear in an area where a nucleus or bird's-eye body is to be formed. Oscillating motion may be seen among the granules as the nucleus appears in a clear space bounded by a membrane. This stage represents in my opinion a low state of vitality of the molluscum body.

6. **Vacuoles.**—These I have seen once as sketched in Fig. 29, *i*. The granules between the vacuoles were in a state of oscillation or Brownian movement. I have seen a similar appearance in a sclerotium of *Badhamia utricularis* after it had been 12 hours in water.

7. **Flagellate Bodies.**—This striking feature I have only once observed in a culture on the fourth day. The material was from abundant lesions, some of which I heaped up in the hollow of one of the cupped slides for hanging-drop preparations, and placed in a moist chamber as shown in Fig. 28. The result I described in the *Centralblatt für Bakteriologie*, 1895, vol. i. p. 245.

The description, slightly condensed, runs—

"The most remarkable appearance consists in the presence of a great number of actively-moving flagellate bodies. They have a roundish head of the size of a red blood corpuscle, and a single powerful flagellum, and under a one-twelfth immersion lens were easily seen and unmistakable; many passed across the microscopic field and then escaped from sight. Many of the molluscum bodies were unchanged; of others, but a thin shell remained; still others had apparently undergone a liquefaction in their central part, and in this area were numerous highly-refracting oscillating granules."

I may add that in what I called the "heads" of the motile bodies Fig. 29, *k*, there were moving granules quite like those in the molluscum bodies. The "heads" may be only residual, the flagellum becoming free as a spirochaete or spirillum.

This state of reproduction I regard as the acme of vitality in the parasite, the streaming state with budding coming next.

Reaction to Iodine-Sulphuric Acid Mixture.—In a water-culture of some molluscum lesions with which Dr. Ernest Dore kindly supplied me last year I tested for cellulose some of the bodies in which a framework had formed, by adding a few drops of a mixture of one part of iodine solution to two parts of sulphuric acid. Parts of the previously colourless framework changed to a greenish blue colour: as much reaction as I have ever found in moulds, mildews, &c. With the same reagent the previously unchanged molluscum bodies became a deep purple, which lasted a few days; the epithelial cells became pale yellow and swelled to oval form. Some *Synchytrium taraxaci* was treated in the same way, and I found its capsules were not stained at all; the sporangial contents became dark green. For the *S. taraxaci* I am indebted to Mr. J. Ramsbottom.

Not a Coccidium.—Many have tried to identify the molluscum body with the familiar genus *Coccidium*. That attempt has failed and worse than failed; it has delayed the recognition of the real nature of the parasite.

Only by purely objective study can we know the characteristics of an organism, and having ascertained them with minute care we must, if necessary, make a new group in classification. The group to which the causal parasite of molluscum belongs has not as yet, perhaps, been adequately introduced to cryptogamic botanists.

The Molluscum Body is not a Symbiotic Cell.—Arguing from the fact that a 1 in 10,000 suspension of a ground-up molluscum tumour gives a virulent filtrate, and that when stained by Giemsa's method the molluscum body shows a certain minute structure M. Borrel concludes that this body is the result of symbiosis between an invisible microbe and an epidermal cell. The observations recorded above of the behaviour of molluscum bodies in water-cultures negative this idea.

Microhenads.—The word microhenad I suggest as the designation for the phase in which the filtrable micro-organisms are at the time they can be pressed or aspirated through a filter that arrests bacteria. This suggestion is made on the supposition that the "filter-passers" have not yet been named in biology. The word is made from the Greek ἑνάς, a unit.

It is not needful to give here the already long and growing list of organisms that have been found to have a microhenad phase or phases; the details of the technique of filtration as used for molluscum virus is given below, p. 117.

The Kinds of Filter used for Virus-filtration.—Besson lays down that too fine a filter must not be used; a Berkefeld 'V,' or a Chamberland 'F.' The former is made from infusorial soil and hence is composed largely of silicious skeletons of diatoms, the Chamberland is of porcelain, and the 'F' is its ordinary form. The filter must be new and be sterilised before use. Filtration must be done soon after the liquid to be filtered is prepared: Dr. Perkins tells me that with time typhoid bacilli will grow through any filter. As a control the water used for dilution may contain motile bacteria.

Historical Notes of Virus Filtration.—The virus of foot and mouth disease was the first to be filtered by Loeffler and Frosch. Borrel found no cell-inclusions in this disease, but areas of leucocytic invasion between the layers of epidermal cells.

Pleuropneumonia of cattle was filtered by Nocard and Roux in 1898. This virus in rabbit's serum requires to be diluted with 20 to 30 vols. of water before it can pass into a collodion sac.

Saponin does not affect bacteria, but it has been found to kill microhenads except those of trachoma and cyanolophia gallinarum. Saponin is a nitrogen-free glucoside obtained from the horse-chestnut and many other plants.

Remlinger, who first showed that rabies virus can be filtered, began his account:—"It is classic to say that the virus of rabies is stopped by filters." In the same way we can say that it is "classic" to say that the virus of syphilis is not filtrable. Now, from the presence of large plasson bodies in this disease, I am sure that, if taken at the right

On p. 109 mention should have been made that in 1892 Ivanovsky demonstrated the filtrability of mosaic disease in the tobacco plant.

stage, this virus also will be filtered, indeed one positive result has already been recorded by Jahnke.

Remlinger used immediate rapid filtration through a Berkefeld 'V.' In prolonged filtration this filter becomes imperfect. Fixed virus obtained from the brains of rabbits the spinal cords of which had been used in antirabic treatment was the material he employed. We may best appreciate the facts connected with filtration of living organisms by studying some of the records. A method used for molluscum is given below in this Chapter.

Sheep-pox virus Borrel got by scraping the under surface of skin removed from a large pustule 5 to 6 cm. across. This mixed with 100 c.c. of tap-water proved to be virulent after filtration through a Berkefeld filter, the filtrate giving no growth on the usual media. Rous found a transplantable spindle-celled sarcoma in chickens; it had a filtrable virus, which reproduced similar tumours in fowls; and when injected into eggs, on the membranes, and sometimes on the body of the chick.

The Size of Microhenads.—Speculations on this subject have been numerous; R. T. Hewlett has given us data by which we can form a mental picture on the supposition that microhenads are rigid solid bodies:—"Visually, when an object comes into the region of a little under one fifth of a micron you cannot see it, even though you use the very best lenses. Although an object of such dimensions is very small, compared with molecules it is of course very large;

you may have a very considerable number of ultimate particles of matter in a body of such dimensions. So that there is no inherent difficulty in conceiving that micro-organisms may possess such small dimensions as those I have just given. (A micron = $\frac{1}{25000}$ inch.)"

Anyone who has watched the streaming subdivisions of *Plassomyxa contagiosa* is forced to conclude that such plastic living matter may pass through a filter in masses easily visible, equivalent to many microhenads joined together.

The filtration of molluscum virus has as yet been effected only after comminution, and thus another possibility arises, namely, that the virus may be in fractions of units, which recombine in the filtrate.

Notes on the Histology of the Molluscum Tumour.—This aspect of molluscum has been dealt with in Part IV, where it is shown that the accepted notion that the molluscum body is a degenerated cell is based on superficial and misleading histology: only a few details need mention here.

Part of a section of a lesion of pin's-head size is shown in Fig. 30. Parasites at *b* and *c* have escaped from the host-nucleus and expanded in stellate form, staining less deeply than still younger forms. The larger parasite near the middle of *a* shows chromatic points throughout, these suggest that a readjustment of chromatin occurs at this stage. The granular chromatic matter in the oldest bodies

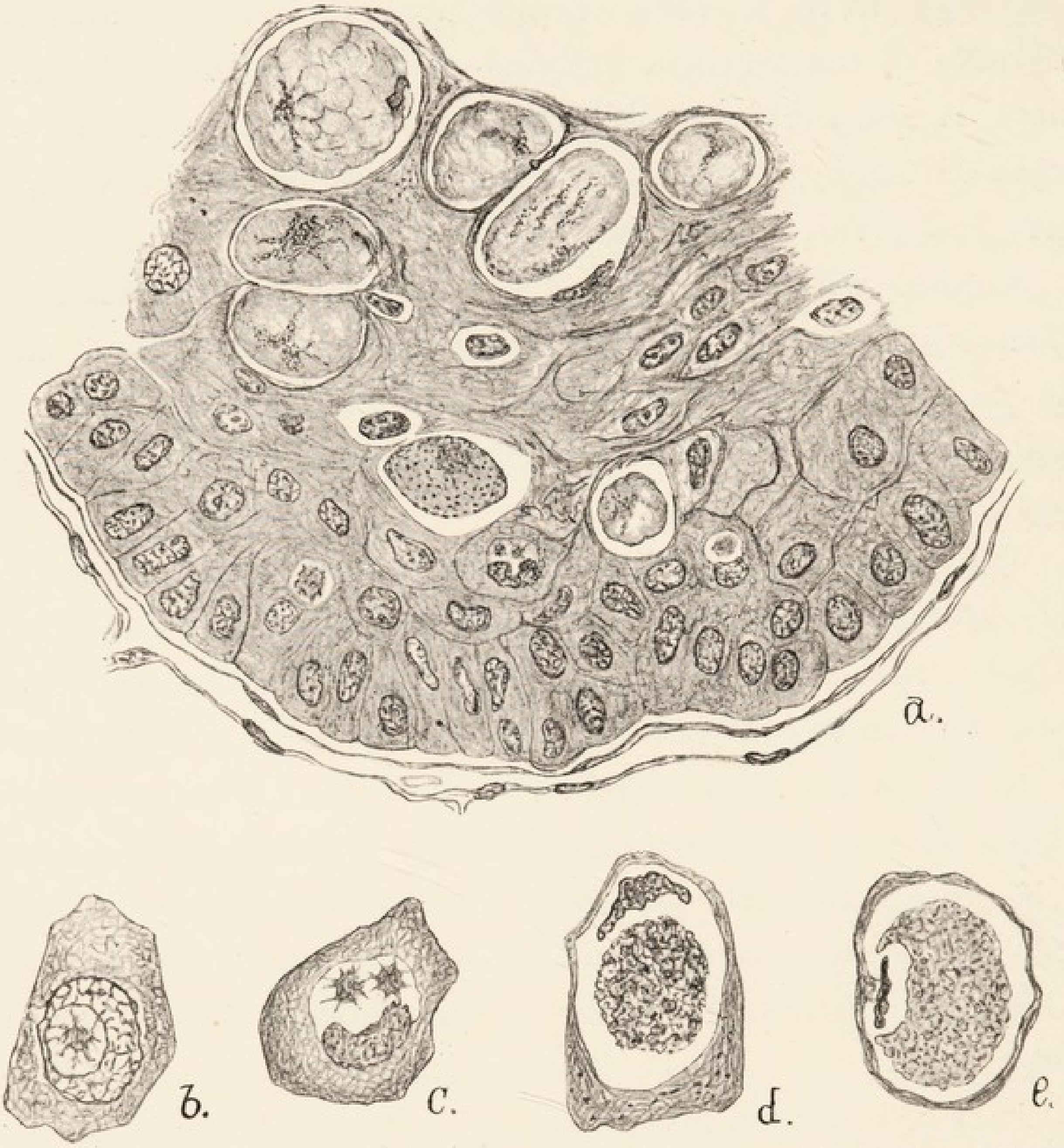

FIG. 30.—*a*, PART OF A SECTION OF A MOLLUSCUM TUMOUR. Various stages of the molluscum bodies are shown in the epithelial cells, in most of them chromatin in the form of chromidial granules is present; *b*, a very early stage, a non-nucleated stellate amoebula apparently within the nucleus of the epithelial cell; *c*, two amoebulae between the nucleus and the cytoplasm of an epithelial cell; *d*, the molluscum body is larger and consists of a plasmodium of extremely small stellate amoebulae; *e*, a still larger parasite; the individual amoebulae have fused together and the body is finely granular. *a*, × 600 diams., the rest × 800 diams. Drawing eye-piece. From Part IV.

is, I think, extruded to construct the capsules, &c. that are formed in water-cultures.

In a valuable piece of work noticed below on the method of filtration of the virus of molluscum the authors begin with the premise: "It has been conclusively demonstrated that they (the molluscum corpuscles) represent peculiar forms of epithelial degeneration;" and as the basis of this belief, they refer to "the excellent pathologic studies of"—here follow five names, none of which I have found in any list of those who have added to our knowledge of Phycomycetes and Mycetozoa. The authors who took great care in making one set of experiments evidently omitted the simple precaution of examining for themselves by the best methods the minute structure of the molluscum tumour. It is in this way that errors are perpetuated.

Further Cultural Results.—For the material on which some of my latest cultural observations were made, I have the pleasure of thanking Dr. G. Pernet, physician in charge of the department for diseases of the skin at the West London Hospital, and the house-surgeon, Dr. Barker. I have also to thank Dr. Beverland and Mr. H. K. Shaw, resident medical officers at the Hampstead General Hospital, for helping me with the preparations. The lesions were placed in covered Pétri dishes, one without any addition on a cupped slide, the others on tea-leaves, as described in Part IV, with the addition of a sterilised solution of glucose and peptone, each 0·2 per cent., which had been prepared beforehand. All the preparations gave positive results.

I was able to demonstrate the movement on the third, fourth, fourteenth, and fifteenth days to seven different trained observers, four of whom are expert in pathology. In these demonstrations the movement was shown under a magnification of 500 diameters with a dry objective. The oil-immersion lens when used for preparations simply mounted in water has the drawback of dragging on the cover-glass and causing movements in the objects beneath it.

This set of preparations I last examined on the thirty-fifth day after making the cultures. On this occasion commotion was present in a few only of the corpuscles, and the rest were obviously degenerating and becoming stained by the colouring matter of tea-leaves on which they were placed. So long as they remain in full vitality they do not absorb the colouring matter.

In addition to facts mentioned above, Fig. 31 shows the appearance and distribution of contaminating bacteria in molluscum cultures.

To anticipate what will become clear after smallpox, syphilis and cancer have been considered in Chapter X, it may be observed that *Plassomyxa contagiosa* passes through certain phases in water outside the host-body, whereas the parasites of smallpox &c. pass through the corresponding phases inside the host-body.

The Incubation-Period of the Filtered Virus.—One of the latest communications on Molluscum Contagiosum that have come to my notice is a paper by Udo. J. Wile and Lile B. Kingery. The authors' task was to test the question of filtrability of the virus. They quote Marx and Sticker on molluscum contagiosum of birds: "sterile" filtrate

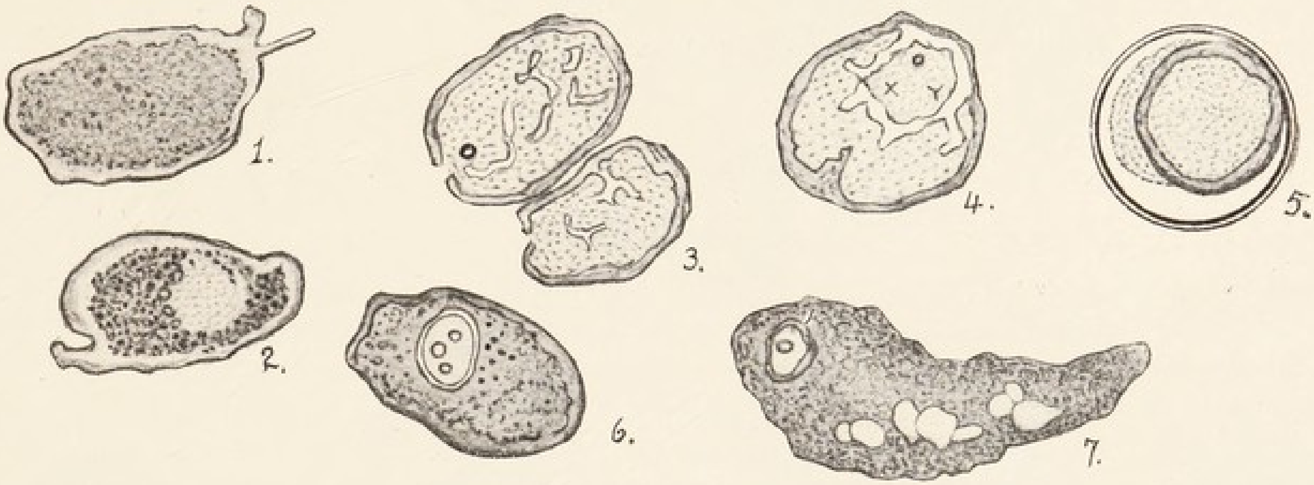

FIG. 31.—VARIOUS CHANGES IN MOLLUSCUM BODIES AFTER BEING KEPT IN WATER AT ROOM TEMPERATURE FROM SIX TO FORTY-SIX DAYS. *1*, Unchanged save cortical layer clearer; *2*, motion in clear central part; *3*, two with motion throughout, except in some skeletal parts and in the cortex, which shows an opening in both. The granule in the upper body was of a deep crimson colour; *4*, motion throughout except in the skeletal part—the granule above X, Y, was first seen at "X," it moved to "Y," and then to where shown; *5*, encapsuled body, motion in middle part and in the part which bulges into the intracystic space; *6*, body in which a trophonucleus had appeared after slight motion in adjoining granules; *7*, body with trophonucleus and several clear spaces but without motion. × 500 diams.

rubbed into scarified areas on the comb of fowls having produced lesions in eight to ten days. Marx and Sticker found that the virus was extremely resistant to heat, light, and cold, and that its infectivity was maintained when the material had been kept for a considerable time in glycerine.

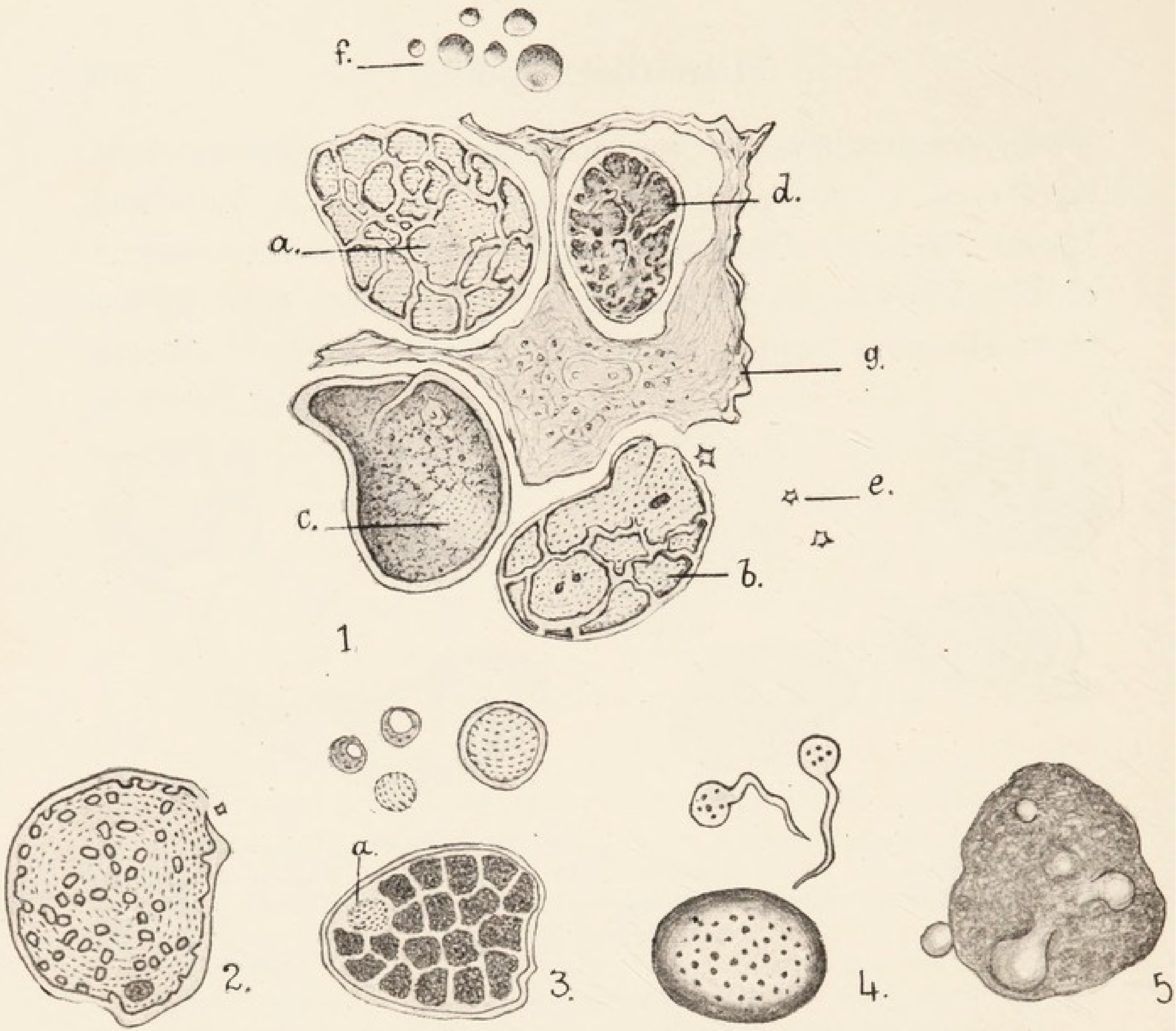

FIG. 32.—PLASSOMYXA CONTAGIOSA IN CULTURES. *1*, Bodies seen in a teasing of a bacteria-free culture on the fourth day ; *a*, a body with framework developed and which showed protoplasmic movement throughout ; *b*, a body similar to *a* ; *c* showed protoplasmic movement in part only of its extent ; its framework is imperfectly formed ; *d*, a body which showed a framework but no motion ; *e*, one of three minute motile bodies ; *f*, highly refracting globules ; *g*, epithelial cell ; *2*, body with imperfectly formed framework which had all its protoplasm in motion, and a minute body escaping at a gap in the contour ; *3*, molluscum body segmented, the protoplasm of one segment only in motion ; smaller spherical bodies, two with vacuoles and two with protoplasmic motion ; *4*, two zoospores with oscillating granules and molluscum body with similar moving granules ; *5*, molluscum body extruding globules similar to those in *1*, *f*. All the foregoing drawn by the aid of the drawing eye-piece ; × 800 diams.

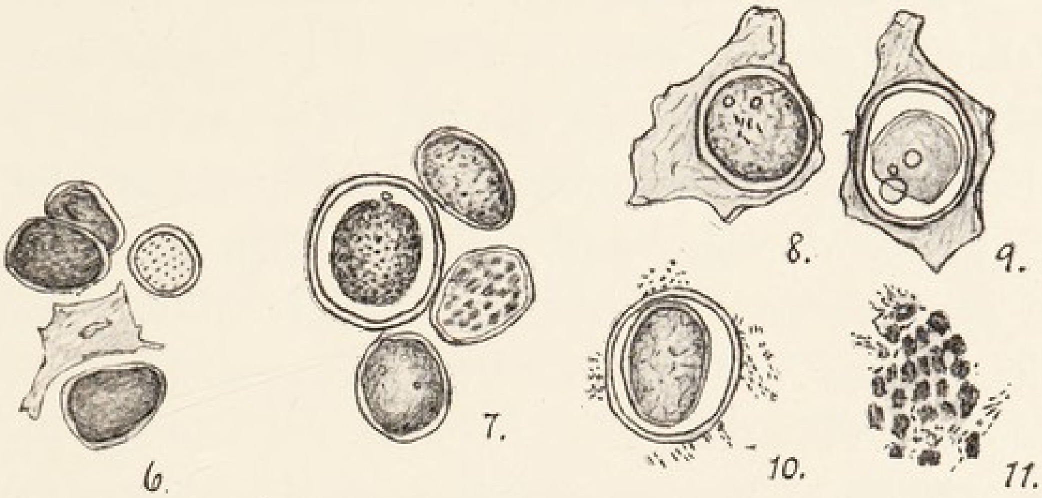

FIG. 32—*continued.*

6, four molluscum bodies and an epithelial cell as seen with a dry lens; one molluscum body showed protoplasmic motion; *7*, four molluscum bodies, one encapsuled, another segmented; *8*, molluscum body encapsuled within an epithelial cell; *9*, similar to *8*, but showing a globule being extruded; *10*, encapsuled molluscum body, bacteria outside but not inside the capsule; *11*, unencapsuled molluscum body segmented and invaded by bacteria:—this is the end of a culture that is contaminated. Figs. *6* to *11* × 400 diams. From Part IV.

Tumours were ground up with 1 to 2 c.c. of saline solution by means of a pestle and mortar, and filtered through the smallest available Berkefeld filter, the porous surface being reduced by paraffin. The emulsion was filtered by suction and divided into two portions, which were injected by a fine needle intradermally into the backs of two volunteers, after having been found to give no growth on agar jelly. In the first case, typical lesions were obtained in fourteen days, in the other case, faint erythematous patches were observed after twenty-five days, and for three weeks these remained stationary, and then became typical lesions. Histological examination confirmed the clinical diagnosis. The article is fully illustrated, and shows, in the authors' opinion, that the lesions obtained were developed in connection with hair-follicles and sebaceous glands. The authors believe that the lesions are recognisable clinically before the molluscum bodies develop, and conclude therefrom that the bodies are degeneration-products. Their results confirm previous findings to the effect that the virus of Molluscum Contagiosum is filtrable.

The Identity of Plassomyxa Contagiosa with the Filtered Virus.—When one examines in sections a fully-developed tumour, *e.g.* Plate III, Part IV, it is seen that the proportion of parasites to host-tissue is very considerable. Roughly, one-third of the weight of the tumour must consist of the parasites, and a casual relationship of the latter to the disease is thus seen to be more than probable.

Again, it is certain that parasites which cause the disease are contained in the filtrate of experiments, such as that of Wile and Kingery.

There is no inconsistency in the two groups of facts. After the use of the pestle and mortar biological units, capable of being drawn by suction through the pores of the filter, are free in the liquid medium. Such units, I do not doubt, are a plasson phase of *Plassomyxa contagiosa.*

Synchytrium and Plassomyxa contagiosa compared.—Comparable features in synchytrians and the causal parasite of molluscum are found in the nucleolus of the former and the whole body of the latter. Invisible in its earliest stage the synchytrian nucleolus appears and grows rapidly by general increase: *Plassomyxa* appears first in the host-nucleus, is next seen in the cytoplasm where it grows rapidly in the nucleolar state until it is full-grown; reproductive action by the zygotic nucleolus of *Synchytrium* produces the generative chromidium. The later changes in *Synchytrium* end in the production of zoospores when the ripe resting-cell has been in water some days: the formation of flagellate zoospores occurs also in water-culture of *Plassomyxa,* but the latter has alternative phases, *e.g.* gemmation of the parasite with streaming protoplasm. The formation of the capsule of the synchytrian zoosporangium is repeated in *Plassomyxa,* definite doubly-contoured capsules being frequently formed; the formation of a supporting framework is an alternative to this.

The Geographical Distribution of Molluscum.—The late S. von Prowazek [1] found that the occurrence of this disease was variable in the tropics. It had been met with in Samoa, Brazil, Java, Sumatra, New Guinea, China, and abundantly in East Africa.

Avian Molluscum or Bird-pox

This disease affects domestic poultry of all kinds, cage-birds, and wild wood-pigeons. It so closely resembles human molluscum that the question of identity arises.

[1] S. von Prowazek, one of the editors of the "Archiv für Protistenkunde," died of typhus whilst investigating that disease in 1915.

A case in which a lady appears to have caught the disease from a canary has been recorded, so Dr. Pernet informs me.

Marx and Sticker found the virus to be filtrable by a Berkefeld filter. They found it more resistent to cold, sunlight, drying, and heat than any other filtrable virus. The filtrate dried in vacuo resisted heating to 100° C. Passage of the virus from pigeons through hens diminished virulence for pigeons. An extensive attack conferred immunity.

Burnet found that inoculation of the cornea produced bodies like the Guarnieri bodies in the vaccinated cornea.

L. Pfeiffer found that in hens and turkeys the disease ran a chronic course and the lesions were confined to the comb, wattles, &c., not invading the mouth. Young pigeons are especially disposed: tumours as large as hazel-nuts forming about the head and anus. Lesions form at the angles of and inside the mouth, sometimes extending along the trachea and intestine and causing rapid death. Pfeiffer found a close relationship between molluscum and what he named flagellate-diphtheria in pigeons, an affection in which diphtheria-like membranes cover the mucosa of the mouth, trachea, and intestines. Millions of flagellates crowd the membranes. He identified them with *Trichomonas.* His drawings show large multinucleate bodies with one or two flagella, some appear to have been attached by a pedicle. In view of the flagellate bodies which I saw develope in water-cultures of human molluscum Pfeiffer's disease of pigeons calls for re-investigation. Burnet quotes Carnwath as having found the virus of bird-pox to be the same as that of flagellate-diphtheria.

Pfeiffer described the healing of lesions in pigeons to be like that of human vaccine vesicles.

The molluscum body in birds has been described as " melting away " when placed in water. I have seen nothing like that in human molluscum ; but it is quite possible that in birds the bodies grow more rapidly, are softer, more labile, and in water break up quickly into microhenads.

Inoculation of a bird, M. Borrel observes, is easy : all that is required is to rub a little virus on a feathered part and multiple tumours follow just as vaccine is followed by vesicles if rubbed on the shaven skin.

Bird-molluscum must be one of the easiest of all diseases to investigate. All the necessary material can be obtained in the course of treating an ailing pigeon medically, with as much consideration as though it were a human being. No pain need be caused. No license to experiment on living animals is required for most of the work.

Further Details of Culture-Method.—In giving these simple instructions I have a hope that others who have access to material may be led to repeat and extend, or to correct my observations, which have been too often interrupted by other affairs.

The necessary apparatus need not occupy more than a square foot of space in a consulting-room and cannot but be pleasing to the eye: two pairs of Petri dishes and two watch-glasses being all that is required besides the microscope, slides and covers, mounted needles, wire loop, &c. which already find their places.

The patient's skin should be cleansed with ether and absolute alcohol, no strong antiseptic being used. Water should be taken after the tap has been allowed to run a few seconds, in order to remove traces of copper, and boiled, as also should all watch-glasses, Petri dishes, slides, &c.

A watch-glass is placed in the lower of each pair of dishes and some water (not enough to float or cover the watch-glass) is poured into the lower dish, Fig. 28. One dish is left covered by its upper half; into the watch-glass of the other is placed a little heap of excised lesions cut open from the surface downwards; or of the white wax-like material squeezed out of ripe lesions. For small portions a cupped slide may replace the watch-glass. A few drops of water, but not enough to cover the heap, are now added and the upper half of the dish is placed over the lower. Rain-water might suit better than tap-water. Streaming occurs with normal saline, and the addition of a trace of bicarbonate of soda to the latter favours subdivision of the bodies into segments.

The cultures must be kept at room-temperature.

CHAPTER VII

PLASMODIOPHORACEAE

This name was given by Zopf in 1885 to a group of organisms related to Mycetozoa. The prototype genus of the group is *Plasmodiophora*. At a later date the same group was called Phytomyxineae, a supposed genus "Phytomyxa" being based upon a misinterpretation of objects seen in sections of root-tubercles of leguminous and other plants: "plasmic masses" and rod-shaped and angular "spores" being described. These were the bacteria that cause the tubercles, the plasmic masses being their mucous secretion.

The bacteria of root-tubercles benefit their hosts and the land on which these grow. The Plasmodiophoraceae, on the contrary, are destructive parasites. The group is divided into genera according to the form and arrangement of the spores, thus:—1, *Plasmodiophora*, with free regularly shaped spores; 2, *Tetramyxa*, spores in groups of four enclosed in a membrane; 3, *Ligneria*, variously shaped spore-groups; 4, *Sorosphaera*, spores grouped in a hollow sphere; and 5, *Spongospora*, spores forming a sponge-like ball.

Its relationship to the Mycetozoa proper gives this group a theoretical interest; its power to cause disease in food-plants a practical economic interest; and in addition, the likeness the trophic nuclei have to the "endogenous cells" of cancer &c. gives these parasites a definite interest for students of human pathology.

As examples a member of genera 1, 4, and 5, will be briefly described here.

Plasmodiophora Brassicae (Woronin, 1877).—This parasite causes irregular lumpy growths, known as "finger and toe," or "anbury," on the roots of several cruciferous plants; cabbage, turnip, etc. In certain localities it has done serious damage, numbers of the infected plants being killed, and others rendered useless for the table. The appearance of the rootlets of a diseased cabbage is shown in Fig. 33: *1*, *a* and *b*. The spores are said to remain dormant in the earth during winter, and infect the next spring crop. The cortical layers of the root are irregularly thickened and of an opaque grey colour. The spore hatches out a zoospore, *3*, which penetrates a cell near the growing point of a rootlet. The host-cell has a nucleus surrounded by a sheath of cytoplasm, strands of which join that lining the cell-wall; sap-vacuoles occupy the intervening spaces. In this environment the parasite becomes amoeboid, and in the earliest stage recognisable by the microscope, it possesses two or more nuclei, each with a nucleolus, round which is a clear space separated by a sharp boundary from the cytoplasm, *1*. These nuclei are trophic, and have been compared to the "bird's-eye" bodies of cancer, and with which, I believe, they may be homologous. In this intracellular stage the parasites appear to subdivide without any nuclear division. They occupy

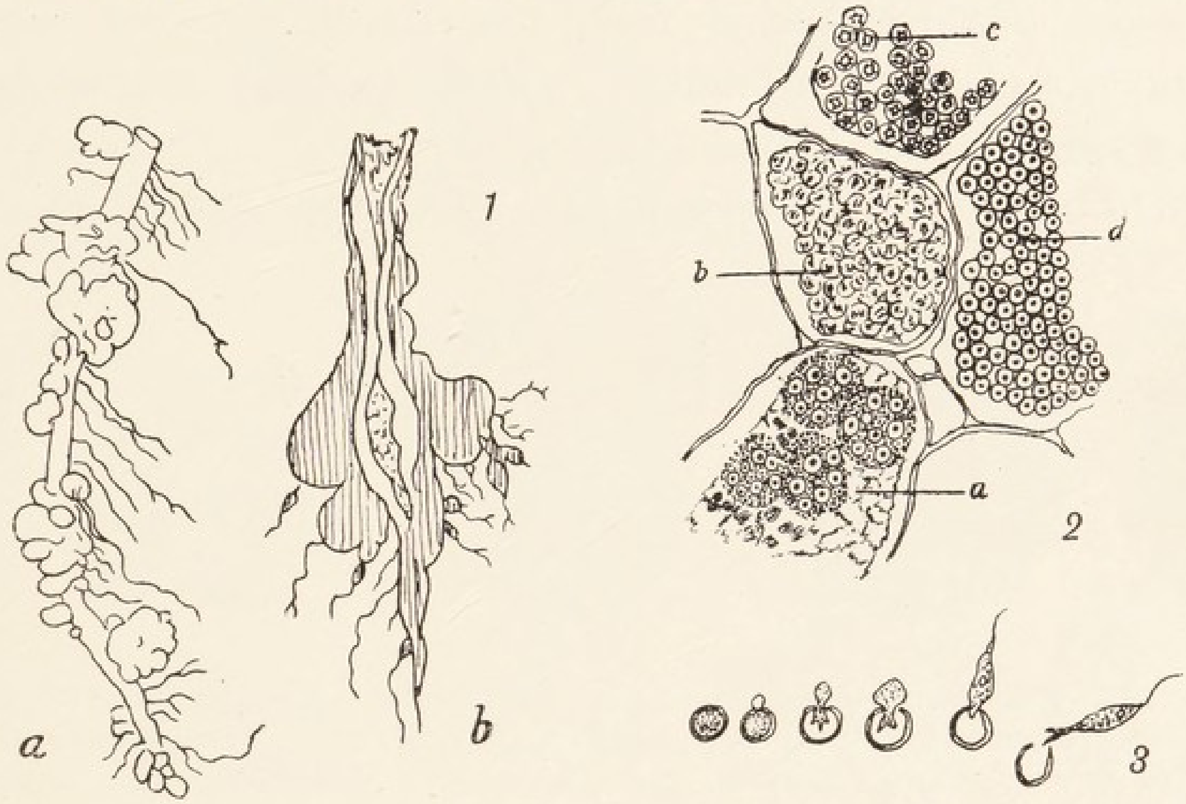

Fig. 33.—Plasmodiophora Brassicae. *1*, Rootlets of infected cabbage; *a*, surface view, after Woronin; *b*, section, after L. Pfeiffer; *2*, section; *a*, 4 multi-nucleate parasites in a cell; *b*, plasmodium, the nuclei subdividing; *c*, and *d*, spore-formation; *3*, stages in the hatching of a zoospore. × 250 diams. From Part I., modified from Doflein after Woronin.

an increasing amount of the space in the cell, and are said to fuse together into a plasmodium, *2*, *a* and *b*.

The nuclear processes in *Plasmodiophora* are shown in Fig. 34.

The trophonuclei lose their chromatin, which passes into the cytoplasm, *1* and *2*. The reproductive nuclei are then formed. They differ from the trophonuclei by having no nucleoli. Two successive karyokineses then take place, and the plasmodium subdivides into spores, *4*.

The absence of a Capillitium from the life of *Plasmodiophora* distinguishes it from the Mycetozoa, but such distinctions are not radical: such features might be suppressed in parasitic adaptation.

The absence of a sporangium allows the spores, which

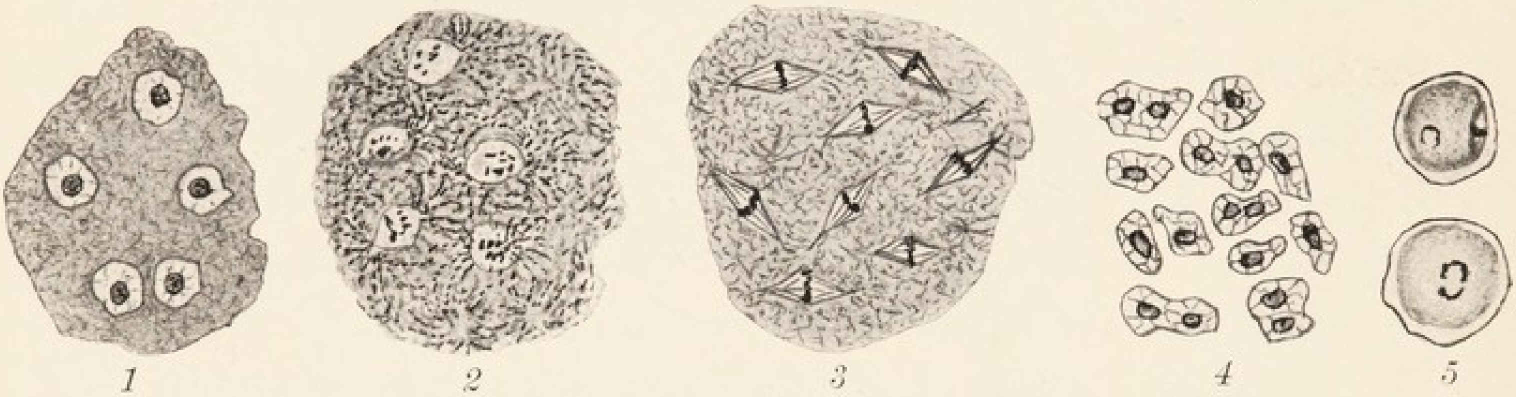

Fig. 34.—Plasmodiophora, Nuclear Processes. *1*, Amoeboid stage with trophonuclei; *2*, chromidial or akaryote stage; *3*, karyokinesis in new nuclei; *4* and *5*, spore-formation. From Part III. After v. Prowazek.

are in great numbers, distending the otherwise empty cell-walls of the host, to collect into roundish masses moulded to the space in which they are contained.

The individual spores have a thin doubly-contoured capsule. In August, 1918, a friend gave me some badly diseased cabbage-roots from his garden at Chesham Bois, Bucks.—a rather heavy, chalky, and flinty soil. I made many observations on scrapings from a slice of root kept partly in and partly out of water, but I did not succeed in seeing the hatching of a zoospore, though many small monads were present.

Sorosphaera Veronicae.—This parasite has been de-

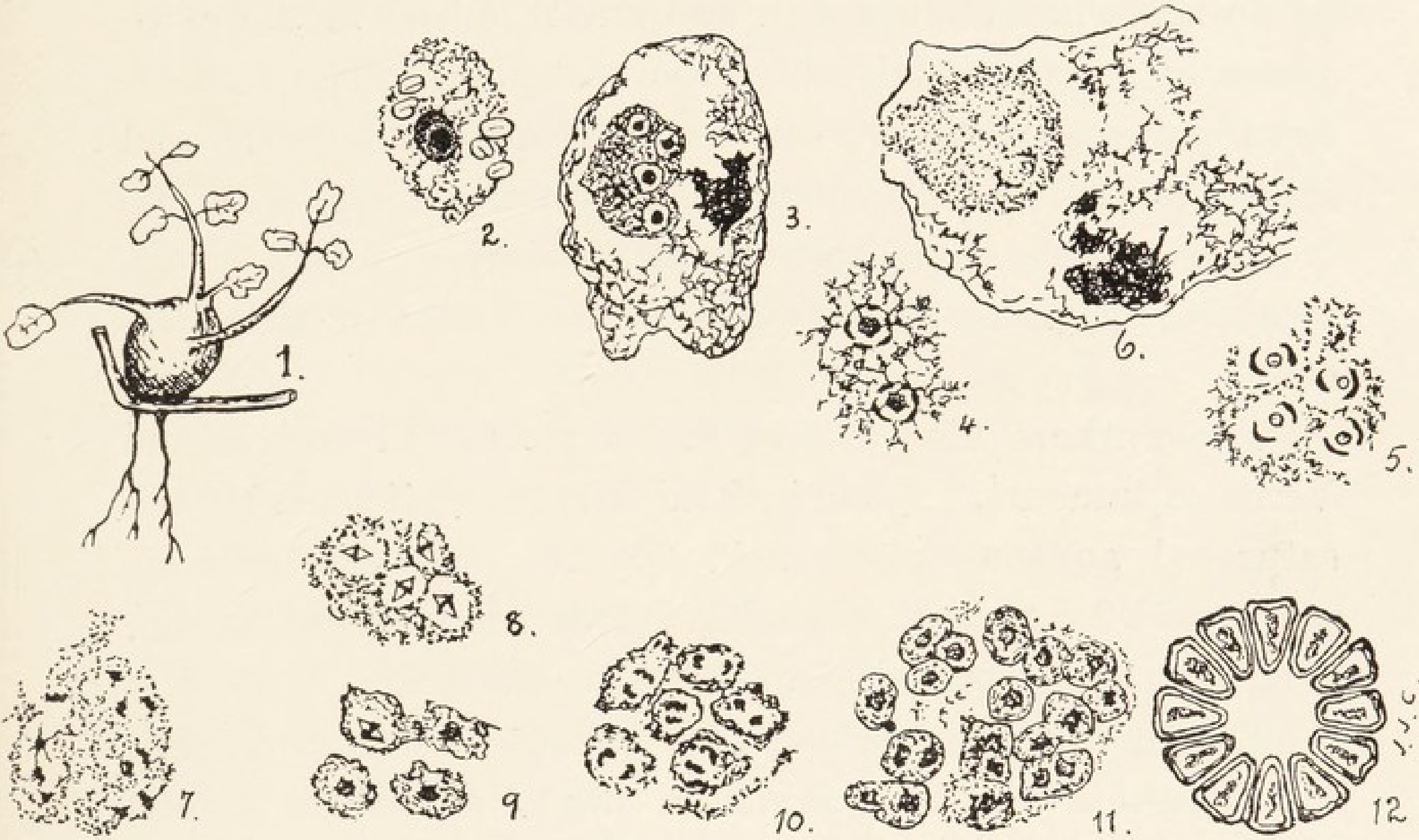

Fig. 35.—Sorosphaera Veronicae. *1*, A tumour on a Veronica plant; *2*, plant-cell with nucleus and starch grains in protoplasm; *3*, multinucleate parasite in host-cell, in which are no starch grains; *4*, two trophonuclei with surrounding plasmodioplasm; *5*, prophase of trophonuclei; *6*, akaryote stage; *7*, new nuclei forming from akaryote state; *8*, metaphase of reproductive nuclei; *9*, plasmodium separating into nucleated subdivisions, one nucleus at metaphase; *10*, the second simultaneous division of reproductive nuclei; *11*, spores formed; *12*, spores arranged in spherical sorus. *1*, nat. size, after Blomfield and Schwartz; the rest × 1000 and drawn from a preparation by Blomfield and Schwartz.

scribed by Blomfield and Schwartz. The host-plant shows tumours in various parts, Fig. 35, *1*, on stems, petioles, and leaves. Seedlings were infected by sprinkling them with water in which tumours which had been dried and pounded in a mortar were suspended. The vegetative period begins as an amoeba having one or two nuclei lodged in the cytoplasm of a procambial cell of the host-plant. The amoebiform parasite grows, its nuclei increasing in number, and it is termed a plasmodium. Several such may be contained in the same host-cell which is then greatly hypertrophied. Mitoses are frequent in the nuclei of the host-cells.

The authors kindly gave me a section of one of the Veronica tumours. Fixed in Bouin's solution (formol 10 c.c., saturated aqueous picric acid 30 c.c., and crystallisable acetic acid 2 c.c.), and stained in Benda's iron haematoxylin, it shows the features described by the authors very clearly.

The dividing trophonuclei at metaphase are cruciform, the elongated nucleolus having a ring of chromatin around it. The nuclei shown in Fig. 35, *5*, are in a prophase: Maire and Tison give a similar drawing and state that it shows "separation of idiochromatin from trophochromatin in prophase of nuclear division."

The spherical sori with spores arranged peripherally round a central space are a striking feature in the sections. A chromidial stage followed by two ordinary reproduction-mitoses ends in spore-formation, which differs from that of Plasmodiophora only in the formation of a sorus.

Spongospora scabies is the cause of corky scab of the potato. Massee informs us that Berkeley, who first described this parasite, and others classed it with the smuts because the spores are formed in groups.

The lesions on the potato vary from small scabs to cavities lined with a dense layer of snuff-coloured spores.

Massee states :—" The plasmodium appears to be active only during the period when the tuber is growing, and passes into a resting condition when the tuber is dormant during the winter. In the spring, when the potato commences to sprout, the plasmodium again becomes active and migrates from the old tuber or set into the new tubers formed during the process of growth."

The foregoing calls for re-examination : it is probable that infection of the young tubers is produced by hatching of zoospores from the infected " set."

Whether some species of this order attack animals is a question worth considering. Léger and Hesse described " A New Protist Parasitic in *Otiorhyncus*." The parasite, *Mycetosporidium talpa*, infests the epithelium of the whole of the intestine of the beetle, and the authors leave it doubtful whether it is a Haplosporidian or a " Mycetozoon."

Several points connected with this group still require to be made clear. Do the zoospores multiply by fusion and conjugate ? What are the earliest intracellular states of the parasite ?

Schwartz doubts the conjugation that v. Prowazek described in the course of formation of spores.

At the close of the chromidial stage Prowazek and subsequent writers found that the new nuclei appeared in the old nuclear cavities. In this section all the chromidial parasites are finely granular throughout, Fig. 35, *6*, and no remains of nuclear cavities can be traced ; also the nucleated state is reconstituted by the fusion of granules at equidistant foci, Fig. 35, *7* : a good example of free nucleus-formation.

Opinion as to the relative position of this group is in favour of its being placed between the Chytridians and the Mycetozoa proper.

CHAPTER VIII

THE MYCETOZOA

THE group of organisms which de Bary named Mycetozoa (fungus-animals) in 1859 are also known as Myxomycetes (slime-fungi). Without practical knowledge of this group no one can command a right perspective of the Protista.

The commoner kinds are to be found without much trouble in pleasant places. A. Lister told us that *Lamproderma scintillans* is a most abundant species in England, and how it appears in countless numbers in heaps of dead leaves: in a dark fir plantation the stones and herbage by the side of a rivulet appeared hoary over an area of many square yards with the young rising sporangia, and a little search showed the mature forms in equal abundance. *Mucilago spongiosa* may be unpleasantly abundant: G. Massee wrote:—"Its plasmodium often creeps up the stems of living grasses, and forms spore-masses up to 2–3 inches in length and an inch in diameter. . . . The dense masses of spores are said to injure vegetation by a process of suffocation. . . . Horses and other animals, have suffered, even died, from the effects of having eaten the masses of spores adhering to grass &c."

Mycetozoa are counted among the lowest forms of life and yet they exhibit a wonderful range of form, and a series of physiological processes of the highest interest.

They make a small group, about 180 British species being known, hence they offer a compact example for classification.

To have written a fitting account of them would have made this the longest chapter of the book. But, seeing that the " British Museum Guide to the Mycetozoa " can be bought for one shilling, and that it is a guide to both the biology and the systematic arrangement, great pruning is possible. No guide, however, can take the place of practical personal study.

In their active states mycetozoa are aquatic and semi-aquatic in habit. A long spell of wet weather favours them.

Stages in the life-cycle.—To-day a mycetozoon may be a plasmodium varying in size from a mere speck of soft protoplasm in some cases to an area of a square foot or more in *Fuligo septica*, the 'flowers of tan'; to-morrow, perhaps, it is one or many brittle sporanges full of spores; and, a day or two later, with rain, the spores become shoals of flagellate zoospores ready to recommence the cycle.

In size and colouring, and in the sculpturing of their outer coat the spores resemble those of many fungi. If kept dry they remain alive for over three years. Many species pass their period of growth, *i.e.* their plasmodial stage, among decaying leaves, others grow hidden within rotten wood, emerging only to form sporangia. Shady spots where leaves are left in heaps suit such species as live among dead leaves: the stages of one of these are shown in Fig. 36, and in the Frontispiece a place in which it throve. The zoospores swim and creep alternately; owing to frequent binary division they are often seen in pairs joined base to base, struggling to be free from each other. With patience even in simple slide-and-cover cultures the fusion of two zoospores that have withdrawn their flagella may be seen; thus is formed the zygote or plasmodium at its inception.

On cultivating spores in water we notice how variable is the hatching process both in the same and in different species; the spores of *Reticularia Lycoperdon* usually have the spore-capsule thinned at one side, and they often hatch in an hour.

Most species require 12 hours, some much longer. When hatching has occurred we note that the characters of zoospores of different species vary: in the winter species, *Trichia varia*, for example, the zoospores move in a leisured way, and the nucleus is hard to detect; those of *Didymium* and other genera move briskly and their nucleus and nucleolus are plain to see.

Stages of sporange-formation.—As seen in a species of Stemonitis stages of sporange-formation are shown in

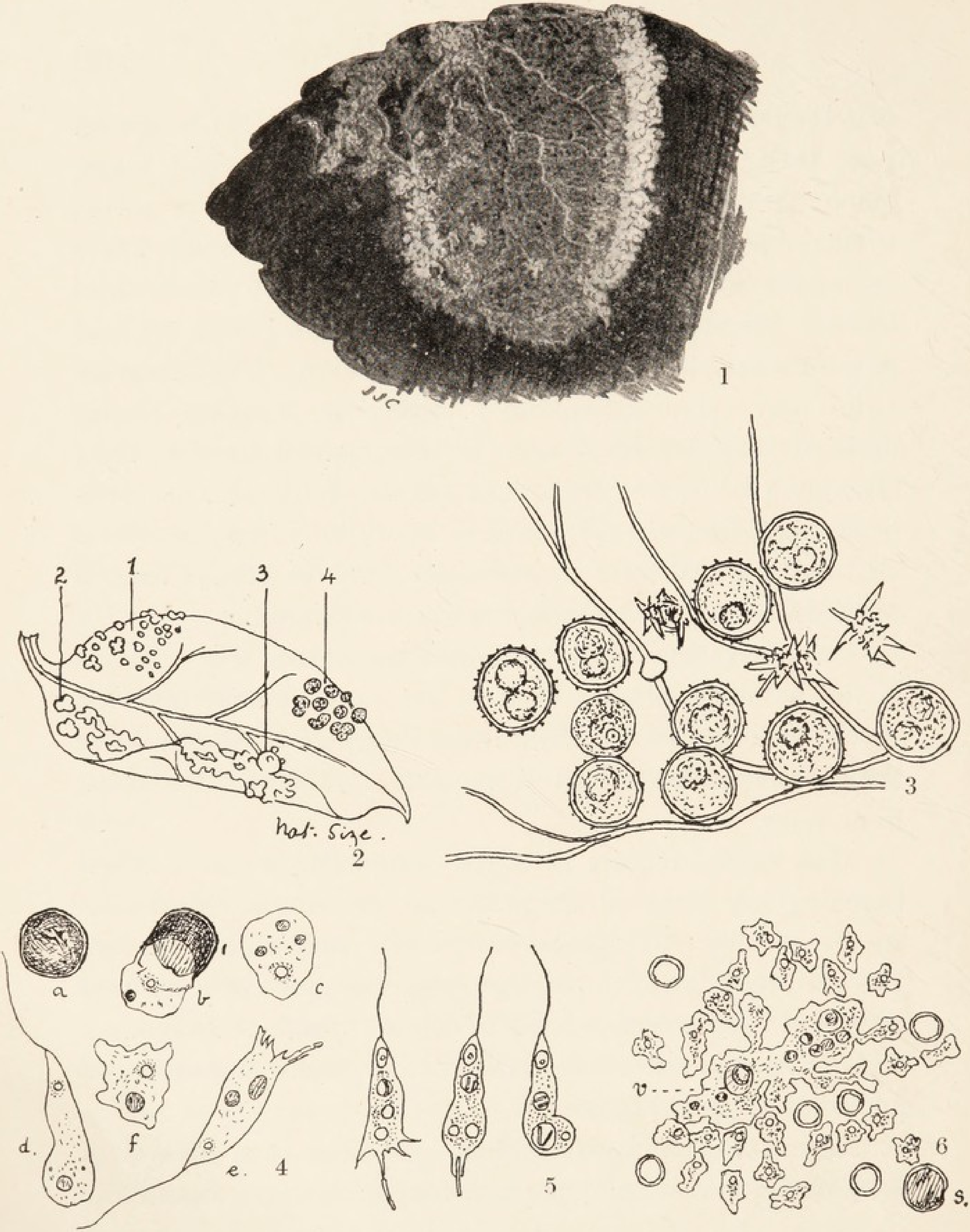

FIG. 36.—Description on opposite page.

FIG. 36.—STAGES IN THE LIFE OF DIDYMIUM DIFFORME, DUBY.

1, PLASMODIUM OR CREEPING FILM ON A DEAD LEAF ;

2, DIFFERENT VARIATIONS AND STAGES SHOWN AS IF ON THE SAME LEAF : *1*, the smallest discrete elements ; *2*, larger discrete elements separated from a continuous patch, which at one point *3*, is heaped up in a rounded nodule with small secondary projections ; *4*, sporangia, dark from the purple spores ;

3, SPORES, CAPILLITIUM FILAMENTS, AND CRYSTALS : one of the spores has not yet formed its capsule, the beginning of which process I watched under the microscope. In its early stage the capsule is of a rose-colour, later the pigment is purple-brown and is condensed in the points which project from the surface of the capsule. Drawing eyepiece. × 800 diams. ;

4, THE HATCHING PROCESS : *a*, spore , *b*, swarm-cell escaping from spore-case ; *c*, newly hatched swarm-cell containing a nucleus and three vacuoles ; *d*, flagellated swarm-cell ; *e*, swarm-cell with two vacuoles containing bacteria, and produced at the posterior end into pseudo-podia, to one of which a bacterium is attached : *f*, amoeboid swarm-cell. × 720. After A. Lister ;

5, STAGES IN THE INCEPTION OF A BACILLUS BY A ZOOSPORE. After A. Lister ;

6, YOUNG PLASMODIUM WITH ATTENDANT AMOEBOID SWARM-CELLS, SOME OF WHICH HAVE TURNED INTO MICROCYSTS : *v*, vacuole containing a microcyst ; *s*, empty spore-cell. × 450 diams. After A. Lister.

Fig. 37, based on the drawings and description of Mr. A. E. Hilton, who kindly gave consent. The specimen was found in the Highgate woods; six weeks of wet weather had preceded its appearance. The plasmodium had grown in a tree-stump and on emerging it had crept upon the surface of a flat fungus and had assumed the cushion-shape

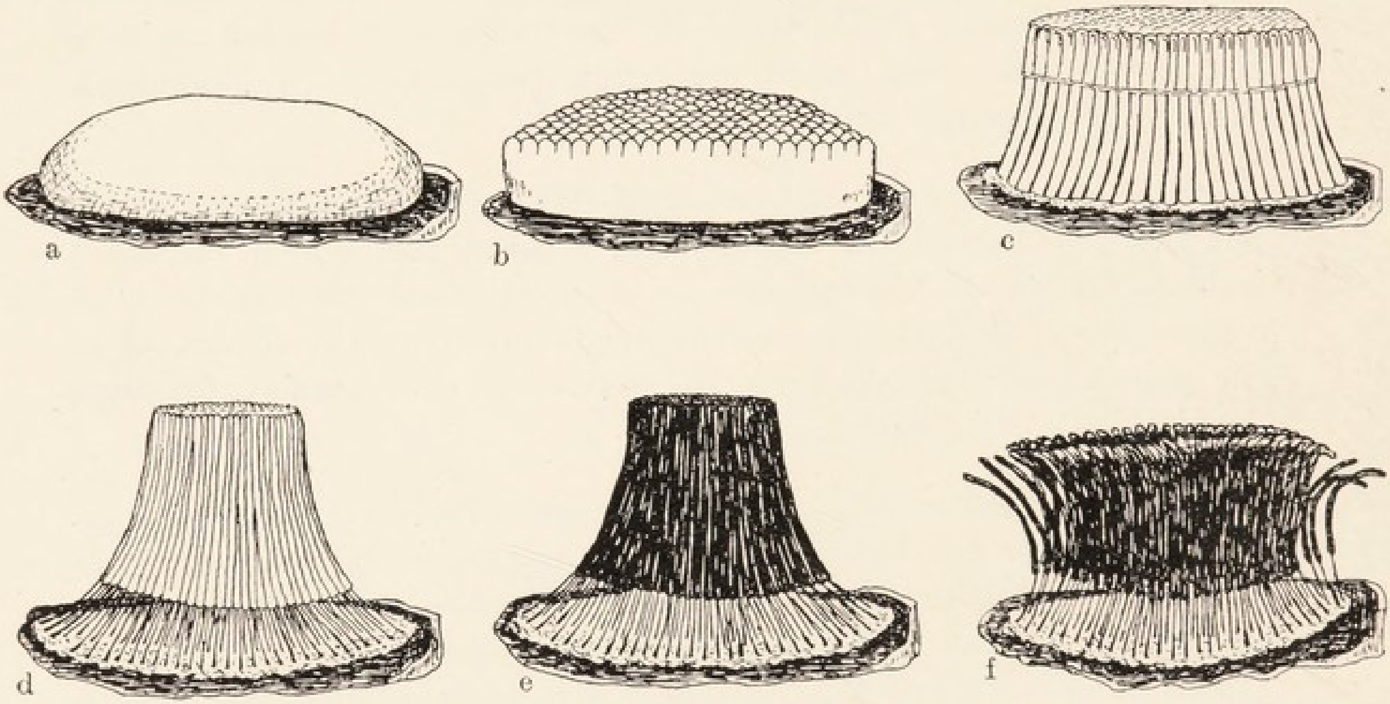

Fig. 37.—Stages in Sporange-formation in a species of Stemonitis. The time between *a* and *d* was 20 hours. *a* to *d*, after A. E. Hilton; *e* and *f*, from the same author's description.

shown at *a*. Smooth at first, its surface soon became subdivided into dome-shaped segments; the mass then increased in height and shrinking at its base, leaving traces, called the hypothallus, on the substratum. As the changes in form proceeded the colour changed from white through deepening shades of brown to black, then to brown again as the sporanges dried, becoming separable, brittle, and easy to detach from their stalks.

The manner in which the stalks are formed and prolonged into the columella as seen under low magnification is shown in Fig. 38, *7* to *10*.

A small plasmodium may become a single sporange. Sporange-formation as seen in *Trichia*, *Arcyria*, and other genera with clustered fruits begins in the same way as in *Stemonitis*. In some genera such as *Reticularia*, and *Fuligo* the sporangia fuse into a mass called an aethalium.

The most familiar example of an aethalium is perhaps that of *Lycogala epidendrum*, whose plasmodium emerges from rotten wood as coral beads and whose fused sporanges make rounded bodies like small puff-balls, Fig. 43, *15*, and change from rose-colour, through grey to brownish. The collection of grey-pink spores within the tough membrane of the ripe aethalia farther reminds one of puff-balls and explains how the whole class of Mycetozoa were at one time called Myxogastres, and were thought to belong to the same group of fungi as the Gasteromycetes.

In *Ceratiomyxa fruticulosa*, Fig. 45, the single species which constitutes the exosporous subdivision of the Mycetozoa, the sporangia are not closed; the spores being formed at the end of short stalks on finger-like sporophores.

The *life-history* of Mycetozoa is still imperfectly known: I have found spores unchanged in the gut of earthworms;

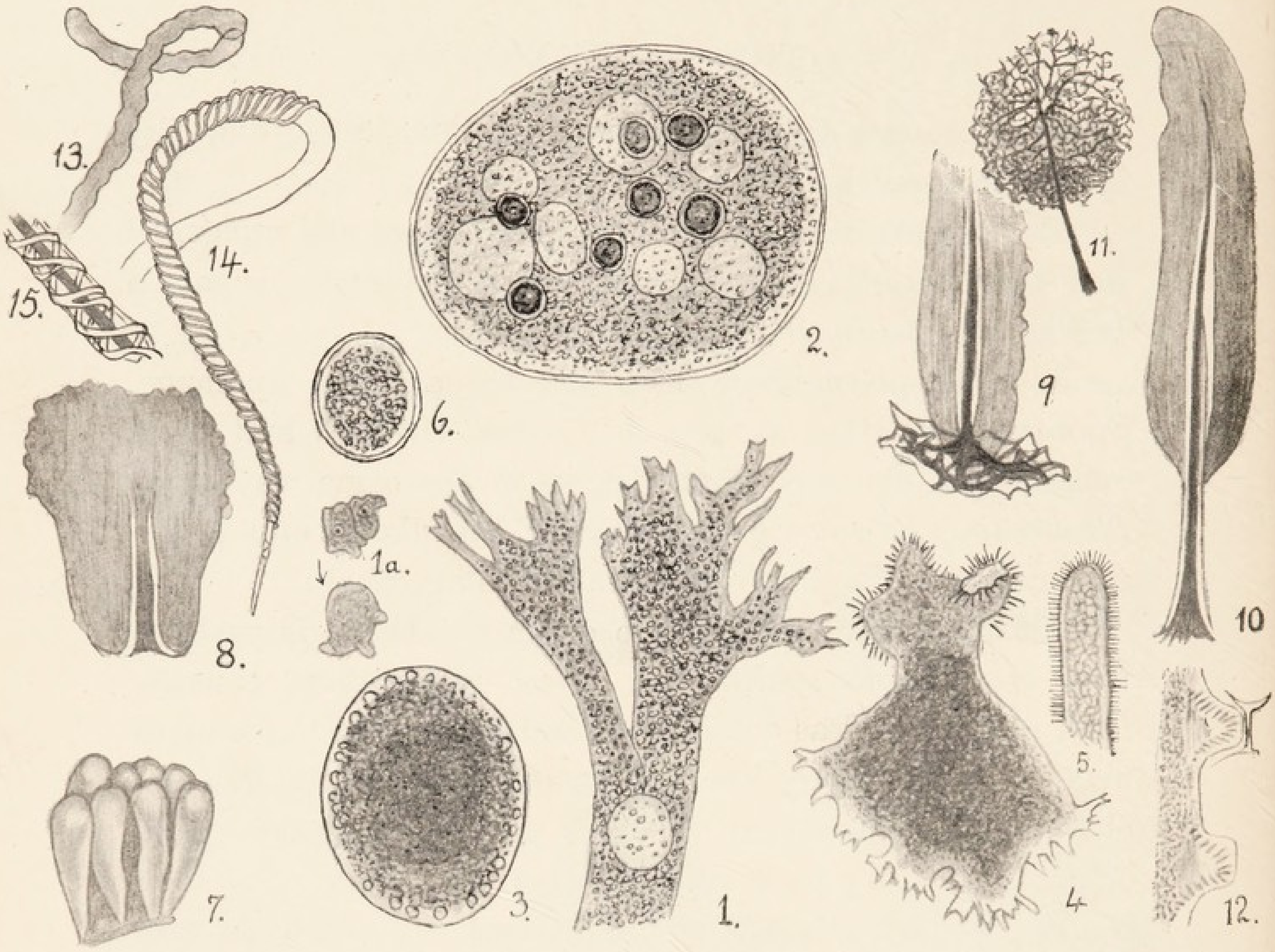

FIG. 38.—SOME DETAILS OF MYCETOZOA. *1*, Branched end of a plasmodium growing on a microscope slide; *1a*, two amoebulae, which united to form the plasmodium (below); *2*, encysted plasmodium which has incepted seven spores (these were carried round in continuous rotation); *3*, a young plasmodium in a resting state; *4*, the same plasmodium active, its hinder (upper in this Fig.) end is studded with hair-like processes; *5*, the end of a receding strand with similar processes; *6*, element from a sclerotium; *7*, cluster of young sporangia of *Stemonitis ferruginea*; *8*, optical section of one element of the same, showing a central axis separated to form the beginning of the columella; *9*, sporangium of the same species further advanced, shows the continuity of the columella with the general framework below; *10*, the same still older, showing the protoplasmic body has drawn upwards leaving the lower part of the central support free as the stalk; *11*, stalk, columella, and capillitium of a fully developed sporangium of *Comatricha nigra*: note absence of the sporangium wall and of spores; *12*, two of the tubercles, by which one *Stemonitis* sporangium abuts on another; *13*, first stage of the middle of an elater of *Trichia varia*; *14*, half a fully developed elater; *15*, part of the same after treatment by potash solution. After de Bary; *1a*, After Cienkowski, from de Bary.

these animals may play a part in the conservation of some species.

The plasmodium.—The first picture of the beginning of a plasmodium was that of Cienkowsky reproduced in Fig. 38, *1a*; the latest is that of Jahn, Fig. 40, *E*. Jahn's picture shows that the spores of Mycetozoa are encapsuled gametes, and that the plasmodium is at first a single zygote.

De Bary describes the grown plasmodium as consisting of a ground-substance with granules scattered through it in greater or less proportion, according to the species. At the surface the ground-substance may be quite clear, forming, except over the young pseudopodia, a doubly-contoured line of ectoplasm.

Alcohol, glycerine, and zinc chloride solution cause the inner protoplasm to shrink and remain attached at a few points only to the cortical layer or hyaloplasm.

The Granules.—The larger of these consist of carbonate of lime in the Calcarineae, the other granules being very small. Where pigment is present it is in fluid form, and it invests the lime granules.

Colours.—The plasmodia in many species are white, but others are yellow, pink, purple, or green, and owe their colour to a fluid pigment scattered in small drops through the protoplasm. The whole plasmodium of *Lycogala epidendrum* is rose-red. The spore-capsules are coloured in most species, and in *Didymium difforme* colour is recognisable in the hyaloplasm, out of which the spore-capsule is secreted.

De Bary mentions that some of the colouring matter is soluble in alcohol.

Protoplasmic motion or Streaming.—This is seen in most plasmodia. The stream is in the course of the strands of the network, and it follows their subdivisions, but it may be arrested in a main strand and continue in side branches. At the edge of a plasmodium streams may run in opposite directions in neighbouring pseudopodia.

Streaming may be modified into rotatory motion. In Fig. 38, *2*, is an instance. In an encysted plasmodium the endoplasm and incepted bodies were in "perpetual rotation." Another resting plasmodium is shown in *3*; *4* is the same plasmodium become active, and it has fine processes resembling cilia covering its hinder third. A similar condition is seen in *5*, which represents the end of a receding strand.

A. Lister filtered through wet cotton-wool a plasmodium of *Badhamia utric.* containing spores of *Stereum.* The spores were left behind, the plasmodium passed through emerging in separate parts which fused together again.

Plasmodia of different species vary greatly in consistence. From those of *Badhamia utricularis* and *Didymium difforme* I have been able under water to detach portions by a needle, float them on to a slide; and, when covered, they have resumed all their activities. In another species I found abundant on straws of a recent part of a heap of horse-stable manure the plasmodium crept actively from the straws over the wet surface of a Petri dish and examined there under a magnification of 60 diams. showed typical streaming, but so fragile was the plasmodium that on covering with a thin cover-glass after adding a drop of water it broke up into chalky subdivisions.

The sclerotium.—This resting state is one of the most important phases of Mycetozoa. It develops only from mature plasmodia, and was discovered and named by de Bary. The beginning resembles the first step towards spore-formation: the plasmodium concentrates at points and breaks up into segments, Fig. 39, *a*; all food remains are rejected. Most, but not all, mycetozoan sclerotia may be regarded as a protective encystment, and compared to the formation of microcysts or to hypnocysts in protozoa.

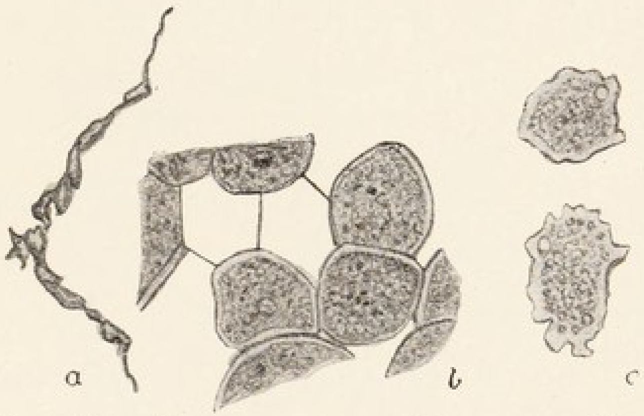

FIG. 39.—SCLEROTIUM OF DIDYMIUM COMPLANATUM. *a*, The whole sclerotium about natural size (it had formed on a moss-stalk); *b*, part of a section, from which the contents of two cysts have fallen out (× 250 diams.); *c*, return of two elements to the amoeboid state. × 250 diams. From Doflein, after de Bary.

On being completely dried the sclerotium assumes a horny brittle consistence. The minute subdivisions are either round or oval, or, from mutual pressure, polyhedral in form. In the latter case a section may have a curious resemblance to the parenchyma of a plant, but on being soaked in water the cysts separate, *b*, *c*. I have examined many sclerotia of *Didymium difforme* but in none have I found this regular segmentation. In *Badhamia* the segments recall the multinucleate conidia of *Mucor*. Reviving sclerotia should be watched in slide-and-cover preparations mounted in water. The protoplasm absorbs water to form vacuoles, round which groups of oscillating granules are seen; after this the segments coalesce and streaming begins.

Nuclear processes in Mycetozoa.—The zoospores have a contractile vacuole and a nucleus, which is placed close to the insertion of the flagellum at the narrow anterior end of the organism. A. Lister described the nucleus of the zoospores of *Reticularia Lycoperdon* (Journal of the Linnaean Society, 1893) at rest and in division and Jahn's description and illustrations, Fig. 40, *a* to *d*, confirm Lister's, and his account of conjugation of swarm-cells with karyogamy at the beginning of plasmodium formation has been accepted in biology.

Both de Bary and Cienkowski believed that the nuclei of the swarm-cells disappeared when they coalesced to form the plasmodium, Fig. 38: *1a*. In 1893 the fact that some plasmodia have nuclei had been established by Schmitz and Strasburger, as quoted by A. Lister, who wrote: "It may be presumed that they are the persistent nuclei of the swarm-cells and the results of their divisions."

There is a simultaneous karyokinesis of nuclei in the

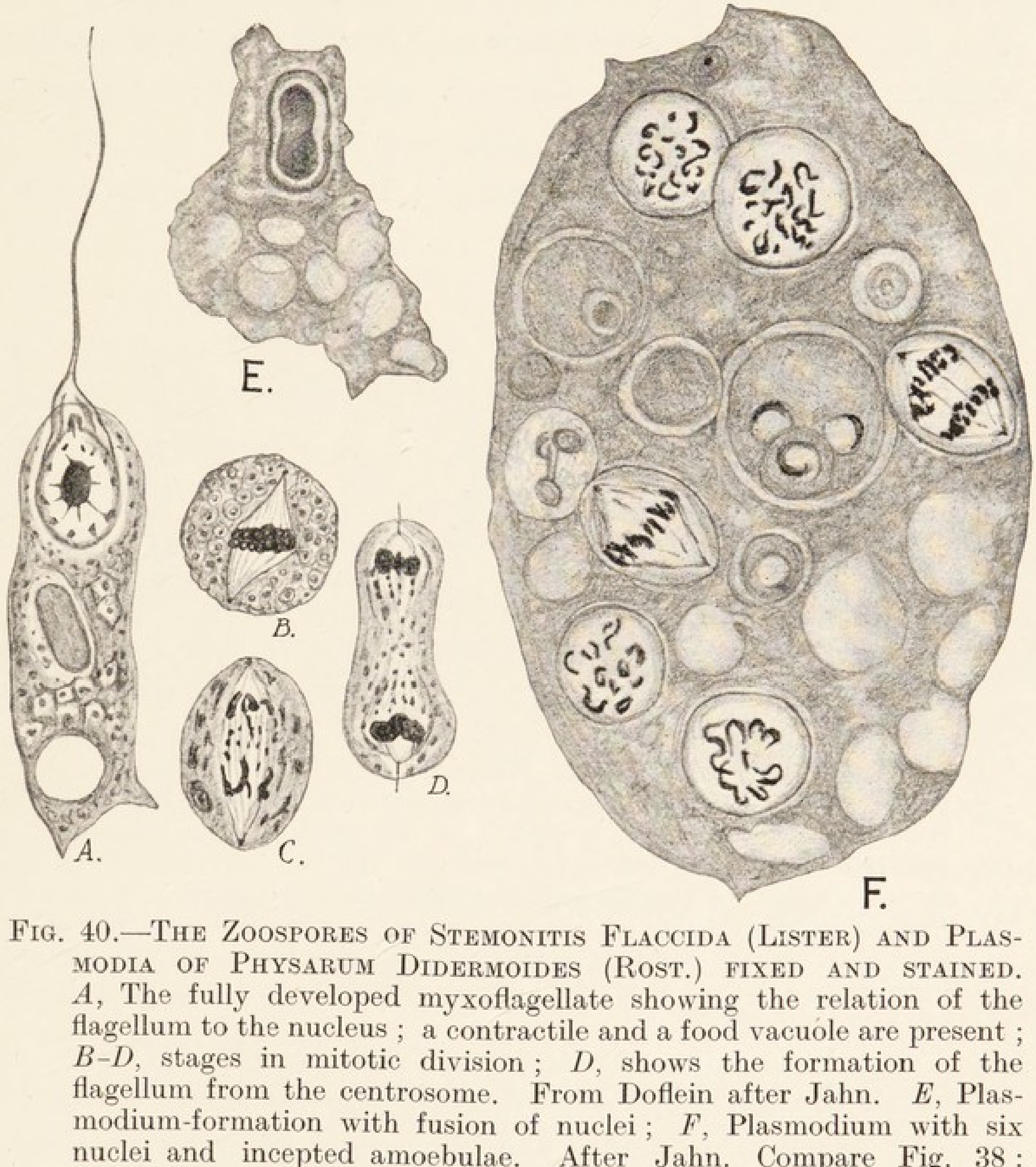

FIG. 40.—THE ZOOSPORES OF STEMONITIS FLACCIDA (LISTER) AND PLASMODIA OF PHYSARUM DIDERMOIDES (ROST.) FIXED AND STAINED. *A*, The fully developed myxoflagellate showing the relation of the flagellum to the nucleus ; a contractile and a food vacuole are present ; *B–D*, stages in mitotic division ; *D*, shows the formation of the flagellum from the centrosome. From Doflein after Jahn. *E*, Plasmodium-formation with fusion of nuclei ; *F*, Plasmodium with six nuclei and incepted amoebulae. After Jahn. Compare Fig. 38 : *1a* and *2*.

sporangium about an hour before spores are formed, see Fig. 41.

In a section of a sporangium of *Lycogala* Vonwiller found that the nucleus of every spore had beside it a small darkly staining body which he terms a sphaeroplast; A. Lister described the same body in 1893.

The nucleated substance left in the stalk and to some

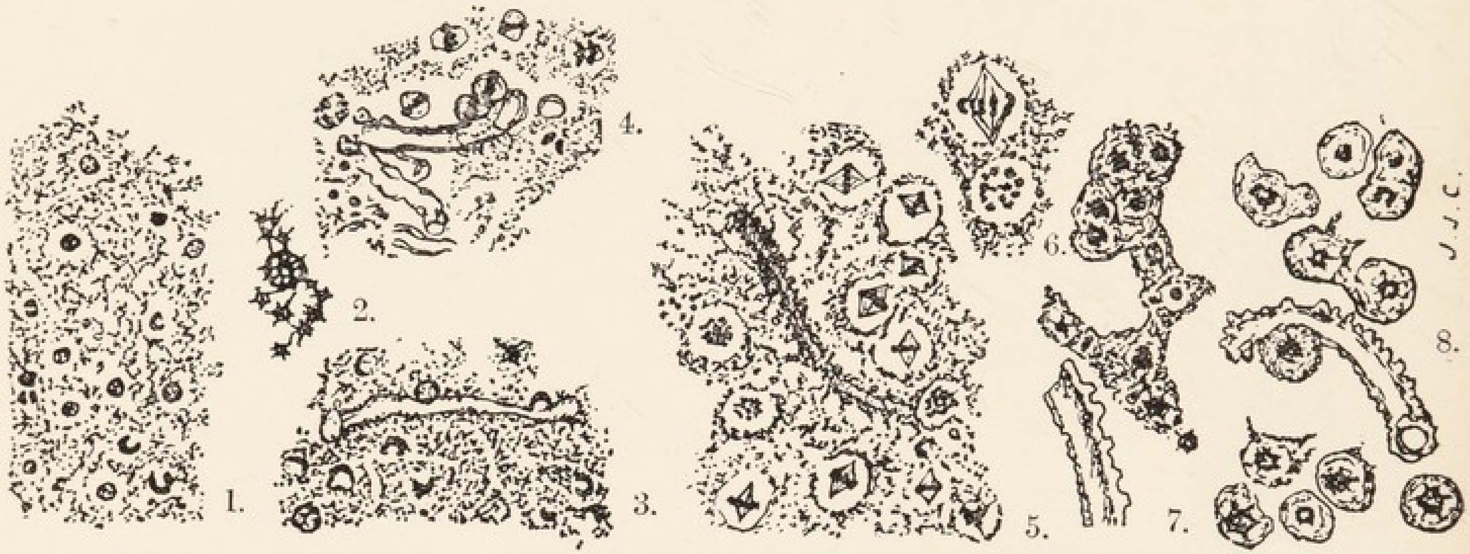

FIG. 41.—NUCLEI IN SPORANGIA OF ARCYRIA INCARNATA. *1*, Stage with many small nuclei and finely granular plasm; *2*, a nucleus from *1*; *3*, stage with fissured plasm and larger crescentic nuclei, an early stage of capillitium-tube present; *4*, the first mitosis, blunt-ended spindles, capillitium-tubes formed; *5*, the second mitosis, sharp-ended spindles, a capillitium fibre apparently being formed by confluence of granules; *6*, two nuclei more magnified; *7*, nuclei of individual spores formed, spores not yet separated, a fully-formed capillitium-tube; *8*, individual spores separated. *2* and *6*, × 1000; the rest, × 500.

extent in the hypothallus of *Arcyria* appears to be a simple residue, the nuclei having the characters of resting nuclei. They may perhaps be compared to the nuclei of *Mucor* that remain in the conidiophore.

Jahn has found many small degenerating nuclei in sporangia, the element shown in Fig. 41, *2*, may be the converse of this: the formation of a nucleus by the confluence of chromidial granules.

In Fig. 41, *5*, the formation of a capillitium tube by the confluence of chromatic granules is evident.

The details in Fig. 41 were drawn from one section of a group of sporangia of *Arcyria incarnata*, the different sporangia present being at different stages of evolution: they are numbered as I think they come in order of age beginning with the youngest.

Fertilisation.—In Fig. 40, *E*, is shown the union of two amoebulae with fusion of the nuclei; the converse of cell-division. The amoebulae are gametes and the fusion of the two nuclei marks the completion of fertilisation in the zygote. The chromosomes in the nuclei before conjugation Jahn found to number 8, and after conjugation, 16; the number lost in the reducing divisions being restored on conjugation. Nuclei with the reduced number are termed haploid, in distinction from the diploid or unreduced nuclei.

In many algae and fungi reduction takes place after fertilisation.

Chromidial elements.—Anyone who may have the good fortune to find *Didymium difforme* growing profusely from year to year a few yards from his study door, and will examine every phase in the living state and in sections, will, I do not doubt, find the chromidial stages described in Part IV. Nodules such as that shown in Fig. 36, *2*, *3*, appear to become a kind of sclerotium; it was from one such that the large double cells Fig. 18, Part IV, developed.

In Fig. 26, Part IV, is shown a section of an early sporangium; in a plasmodial lobe are two large chromidial bodies, and in a space between the lobes is what I called a spore, but the different nature of which has been explained by a later experience. In 1920 on teasing in water one of a group of ordinary-looking sporangia of *Didymium difforme* I found instead of the usual mass of dark brown spores there were only one or two such in the field and no capillitium fibres; but many concentrically striated bodies. The latter in a slide-and-cover preparation first swelled, and on the 4th day, expanded into streaks with a red core; the streaks changed into ordinary capillitium fibres. The red colour was no doubt a prismatic effect; the coloured parts looked black when out of focus.

The chromidial processes appear to be used largely for the production of capillitium elements; I would designate such chromidial matter skeletoplasm. I was unable to find another sporange in which the concentric bodies were present, but I found later that, if slides washed in water and then wiped dry after they have been in 5 % solution of lysol are used, sporangia of various Mycetozoa give when teased in water a simulation of the red streaks with some imitation of capillitium-formation. Lysol is made by boiling either linseed oil or resin with tar oil, alcohol, and potash.

Mitochondria were found to be abundant in Mycetozoa by Cowdry (quoted by Sharp).

Conditions of life or bionomics, &c.—From what has been given above many of the habits and life-relationships of Mycetozoa will have been inferred. In the growth-period those that can be watched from their developing among decaying leaves or on the surface of rotting wood, etc., are attracted by moderate degrees of light and heat, and repelled by extremes. Grown on glass with a minimum of food a plasmodium of *Fuligo* sent out pseudopodia vigorously into a solution of glucose or an infusion of tan brought into contact with a part of it.

The plasmodium is rheotropic: when a gentle stream of water is directed over a slide or through a strip of linen or filter-paper and this is brought into contact with a plasmodium, the latter moves against the stream on to and over the substratum.

As to *nutrition*, the zoospores and plasmodium are holozoic in many species, solid ingesta being digested in food-vacuoles, the contents of which are acid, though the reaction of the plasmodium itself is alkaline. Mycetozoa have a facility for digesting cellulose, whether extraneous or of their own making. The hyphae of *Stereum hirsutum* can be seen to dissolve as the hyaline border of a wave of plasmodium of *Badhamia utricularis* advances over them (A. Lister).

W. T. Elliott found that the plasmodium of *B. utricularis* placed on fungi other than *Stereum* varies considerably in its relation: it crept all over a *Coprinus*, but was destroyed, if it had not crept off when the fungus decayed; it consumed every part of *Boletus flavus;* but did not consume any part of *Lycoperdon gemmatum.*

The nutrition of species that grow in woody tissue has been inferred to be derived from solutions. De Bary gives the appearance of plasmodia of *Lycogala* in a section of fir-wood—lobulated protoplasmic masses, with no definite relation to the cell-walls of the wood.

The chemical composition of the plasmodium of *Fuligo* as described in 1881 is still quoted as being characteristic of protoplasm in general.

A parasitic mycetozoon.—*Hymenobolus parasiticus* was found in Austria by Zukal in 1893; the species has been found also in Scotland. The plasmodium of *Hymenobolus* has no rhythmic circulation, and instead of spreading in a network it forms a compact mass which eats its way into the lichen or alga on which it feeds. In drought it contracts to form a rose-coloured macrocyst. Its sporangia, which usually have a lid, are drab or, from refuse matter, black. *Hymenobolus* has no capillitium.

Culture of a Mycetozoon.—Some Mycetozoa in cultures pass rapidly into the sporangial state, but *B. utricularis* can be kept in the plasmodial state almost indefinitely by supplying it regularly with fresh *Stereum.*

Fragments of this fungus placed with water in a vessel such as a Petri dish and a small flake of sclerotium placed on the fungus will in a day or two produce a plasmodium, which in the course of a week in warm weather will cover the greater part of the *Stereum.*

A square of glass should be placed over the culture in such a way that a very small air-way is left at each side.

At a point near the middle of the glass the *Stereum* may be brought into contact with it; when hungry the mycelium will spread widely over the glass. Now, if fresh *Stereum* is added to that in the dish, the plasmodium will leave the glass to form a dense layer over the fresh food.

When the plasmodium is spread on the glass this may be raised a little above the edge of the dish by placing a rod under the glass at each side; this measure will allow the plasmodium to dry slowly. As it dries it shrinks to form a sclerotium. After a day's gradual drying the glass may be removed and the sclerotium dried completely. It can then be scraped from the glass in flakes like shellac and kept dry for future use.

Sporangia.—There is a wide range of form in the sporangia of some species: thus in *Physarum nutans* and *Didymium squamulosum* stalked and sessile sporangia and vein-like plasmodiocarps are often formed from the same plasmodium.

The elements of which Fig. 42 is made up are parts chosen from seventy-seven double plates in the first edition of A. Lister's book. The plates are collotype reproductions of water-colour camera drawings. The small portions here

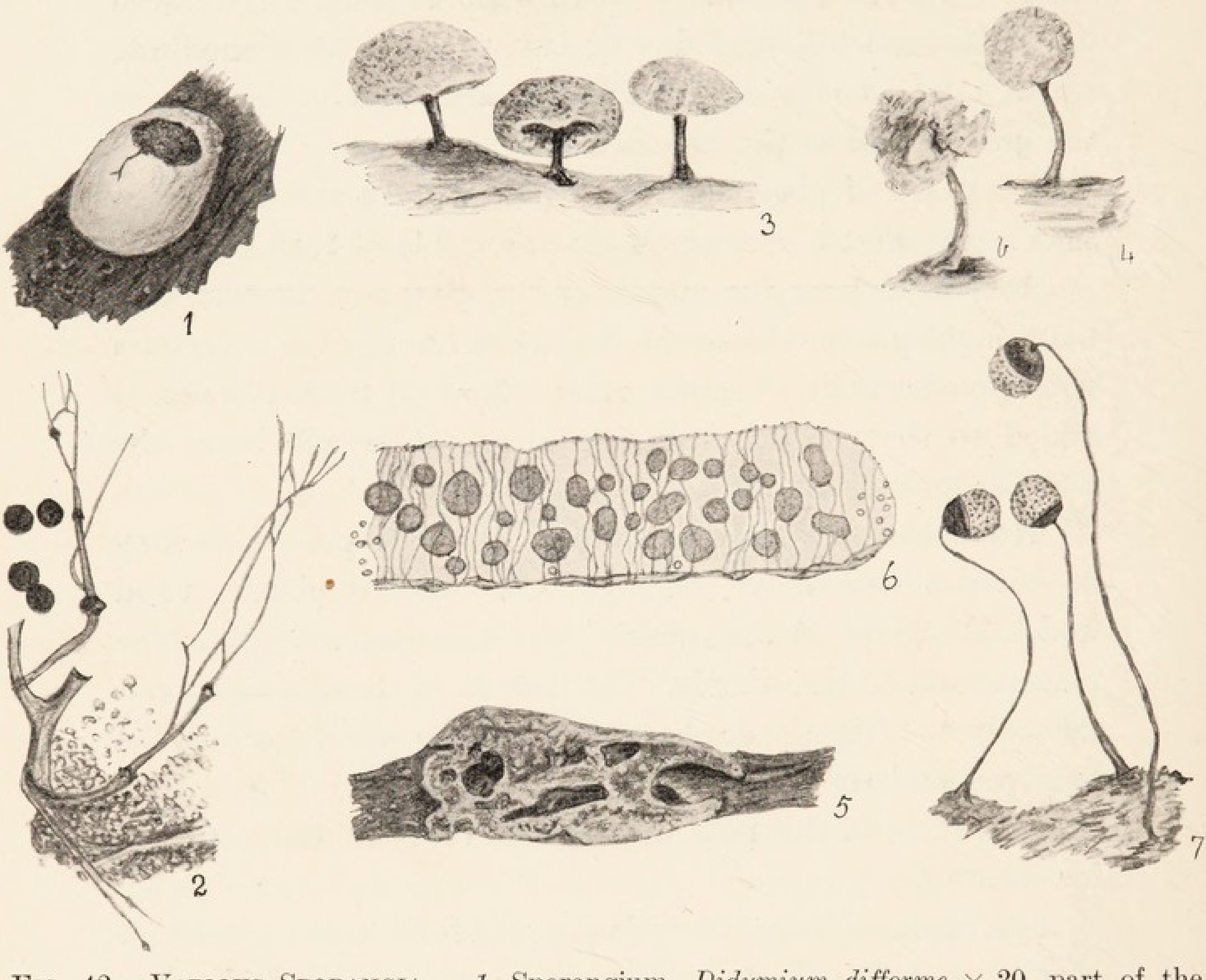

FIG. 42.—VARIOUS SPORANGIA. *1*, Sporangium, *Didymium difforme* × 20, part of the outer membrane removed; *2*, Capillitium of the same × 280, the fibres have nodes and are dichotomous; the thickened margin of the base passes into the wall of the sporangium; *3*, *Didymium Clavus*, sporangia × 20; *4*, *Didymium nigripes* (*xanthopus*), sporangia, *a*, intact, *b*, broken open, showing the white columella; *5*, *Didymium complanatum*, plasmodiocarp, × 2; *6*, section of the same × 80, showing capillitium and large vesicles; these are filled with yellow obscurely granular matter, and are peculiar to this particular species; *7*, *Cribraria languescens* (United States) sporangia after dispersion of spores × 20. After A. Lister.

shown I copied by making tracings, and transferring these to cardboard. In the process much of the delicacy of the originals has been lost. Miss G. Lister very kindly gave me permission to use this illustration.

Those who desire to appreciate fully the morphology of Mycetozoa should study A. Lister's monograph, 2nd edition, revised by Miss G. Lister, 1911.

Further morphological details.—The elements in Fig. 43, represent types, for the most part as diagrams, based on the works of A. Lister, A. de Bary, T. H. MacBride, and others; they are not intended to be accurate copies, and to some I have added features to illustrate certain points. Readers will, I hope, be led to consult the original documents. The degree of magnification varies: small designs placed at the side of larger ones, *9* and *11*, are only a trifle above the natural size. Spores and capillitium are shown magnified about 300 times. In *Badhamia* (named after Badham, an English mycologist) lime granules pervade the whole capillitium, whilst in other Physaraceæ the lime granules are limited to expanded parts of the capillitium, as in *2*, *3*, *4*, and *5*, and to parts or the whole of the Sporangium wall. In *Diderma, 6*, one genus of the Physaraceæ, the capillitium is devoid of lime-knots, but the outer of the two layers of the sporangium wall contains granules of lime. In *Diachaea*, *7*, the stalk and columella are charged with lime, which is absent from the purple capillitium. Of *Lycogala*, three unequal aethalia (copied from A. Lister's book) and part of

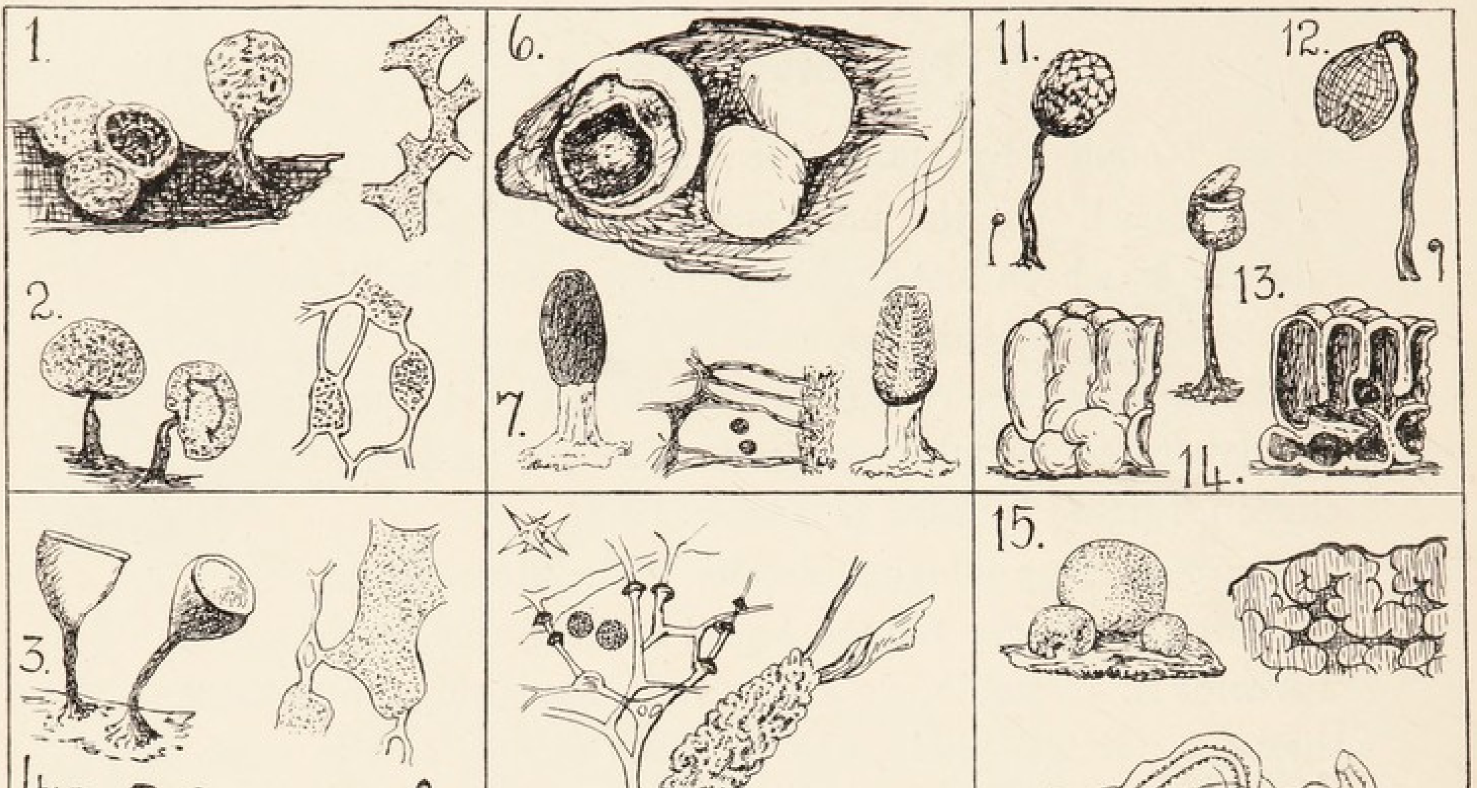

FIG. 43.—MORPHOLOGICAL DETAILS OF MYCETOZOA. *1, Badhamia macrocarpa*; *2, Physarum nutans*; *3, Craterium minutum*; *4, Leocarpus fragilis*; *5, Fuligo septica*; *6, Diderma testaceum*; *7, Diachaea leucopoda*; *8, Mucilago spongiosa*; *9, Comatricha typhoides*; *10, Comatricha nigra*; *11, Cribraria vulgaris*; *12, Dictydium cancellatum*; *13, Orcadella operculata*; *14, Tubifera ferruginosa*; *15, Lycogala epidendrum*; *16, Trichia varia*; *17, Arcyria incarnata.* Made diagrammatic from various sources mentioned in the text.

the convoluted early sporangium and the capillitium tubes perforating the inner wall of the fully formed sporangium (from de Bary) are shown in *15*. A cursory view of the illustration as a whole may leave the impression of a number of apparently unrelated structures, but if what has been stated in this chapter is kept in mind, the diversity is not inexplicable. Details given in Fig. 41 show that before spores are formed the protoplasm of Mycetozoa separates into two distinct substances. The first, to use in a particular sense a term employed by Lionel Beale, we may call Bioplasm; the other part destined to form the supporting structures may be called Skeletoplasm. The bioplasm is used for the formation of the spore contents, and, with one queried point, it is nucleated throughout. The skeletoplasm from the beginning of sporangium-formation is, I think, in the chromidial condition; from it the capillitium as well as other parts of the skeleton are formed.

It is easy to understand structural diversity if a Mycetozoon such as *Comatricha nigra*, *10*, is studied. The series of sporangia were drawn from nature. At first a sticky sessile white bead, the sporangium soon becomes elevated on a brown stalk, which can be seen to be prolonged as the columella, owing to the transparency of the substance of the young sporangium. The latter next becomes opaque and of a glistening black; a state which lasts but a short time: the sporangium wall is evanescent and leaves a dull dark brown surface when it disappears. A portion of this surface

(×370) is shown, *10*, to consist of capillitium fibres, in which four spores are supported, but no trace of a membrane is present.

If the rising sporangia of *Comatricha* instead of growing perpendicularly were to grow interwoven in all directions and fused together an aethalium such as that of *Fuligo*, *5*, or other similar form, would be the result. When the skeletoplasm is used entirely in the formation of coherent sporangium walls a condition like *Tubifera*, *14*, ensues. The quaint genus, *Orcadella*, *13*, is still more easily derivable from the type of a *Comatricha* ; and, without its stalk and with its lid replaced by a less definite form of dehiscence, it would become like a *Licea*, which has the simplest sporangium of all the Mycetozoa.

CHAPTER IX

ACRASIEAE: AFFINITIES AND PHYLOGENY OF MYCETOZOA, &c.

The Acrasieae are a very small group made for organisms which were formerly included in the Mycetozoa of de Bary. In zoology they are termed Pseudo-plasmodida (Delage) to distinguish them from the Euplasmodida or Mycetozoa proper. They have also been called Sorophora.

The only state in which they are visible to the naked eye is when they have formed spore-clusters, which resemble small mycetozoan sporanges: round, and about the size of pins' heads, or, sometimes streak-like; they are white, red, or yellow in colour.

With the exception of *Acrasis*, which grows on the yeast of beer, the known species live on decaying vegetable matter and the dung of the horse and the cow.

They begin their career by hatching from a spore-case as amoebulae; they do not develop a flagellum, but creep like amoebae.

They are depicted by Brefeld with short pointed pseudopodia. They have also a nucleus and a contractile vacuole. Once hatched they multiply rapidly by simple division.

When nutritive material is used up, so states van Tiegham, the myxamoebae converge on certain centres and become apposed without fusion ; each such aggregation erects itself perpendicularly, the elements climbing over one another to take characteristic shape in a spore-apparatus. The long plasmodial stage accompanied by growth seen in the Mycetozoa is wanting in the Acrasieae.

Spores are constituted by encapsulation of separate

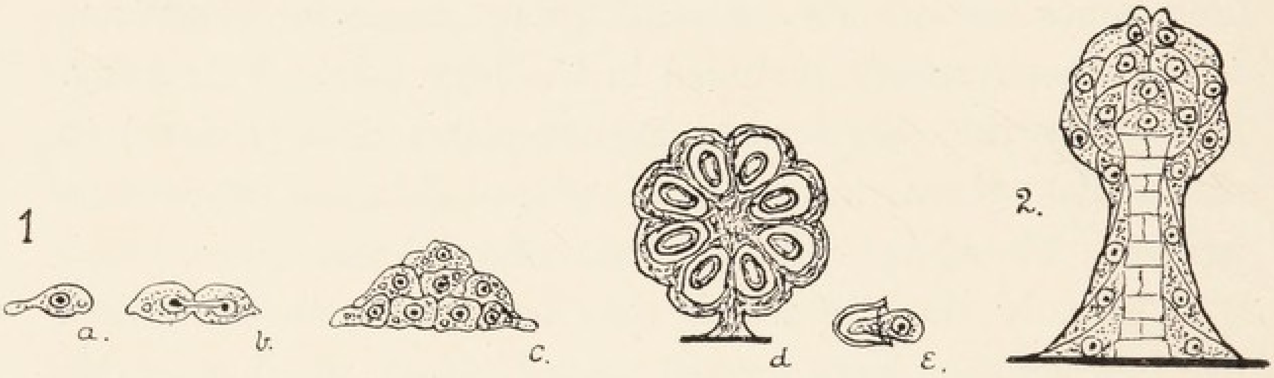

FIG. 44.—DIAGRAM OF STAGES OF ACRASIEAE. *1, a*, Amoebula ; *b*, binary division of same ; *c*, false plasmodium (cohesion without coalescence) ; *d*, formation of sorus and spores. *2*, Stage in sporangium-formation in the *Dictyostelidae*—massed amoebulae of which the innermost are transformed into a skeleton up which the rest climb.

amoebulae in the aggregations. They are held together by glutinous material. There is no sporangial membrane like that of the endosporous Mycetozoa ; van Tiegham compares them in this respect to the exosporous Mycetozoon, *Ceratiomyxa*, Fig. 45, *6* to *11*.

In unfavourable surroundings the myxamoebae form protection-cysts, which may be two or even three-fold in *Guttulina*.

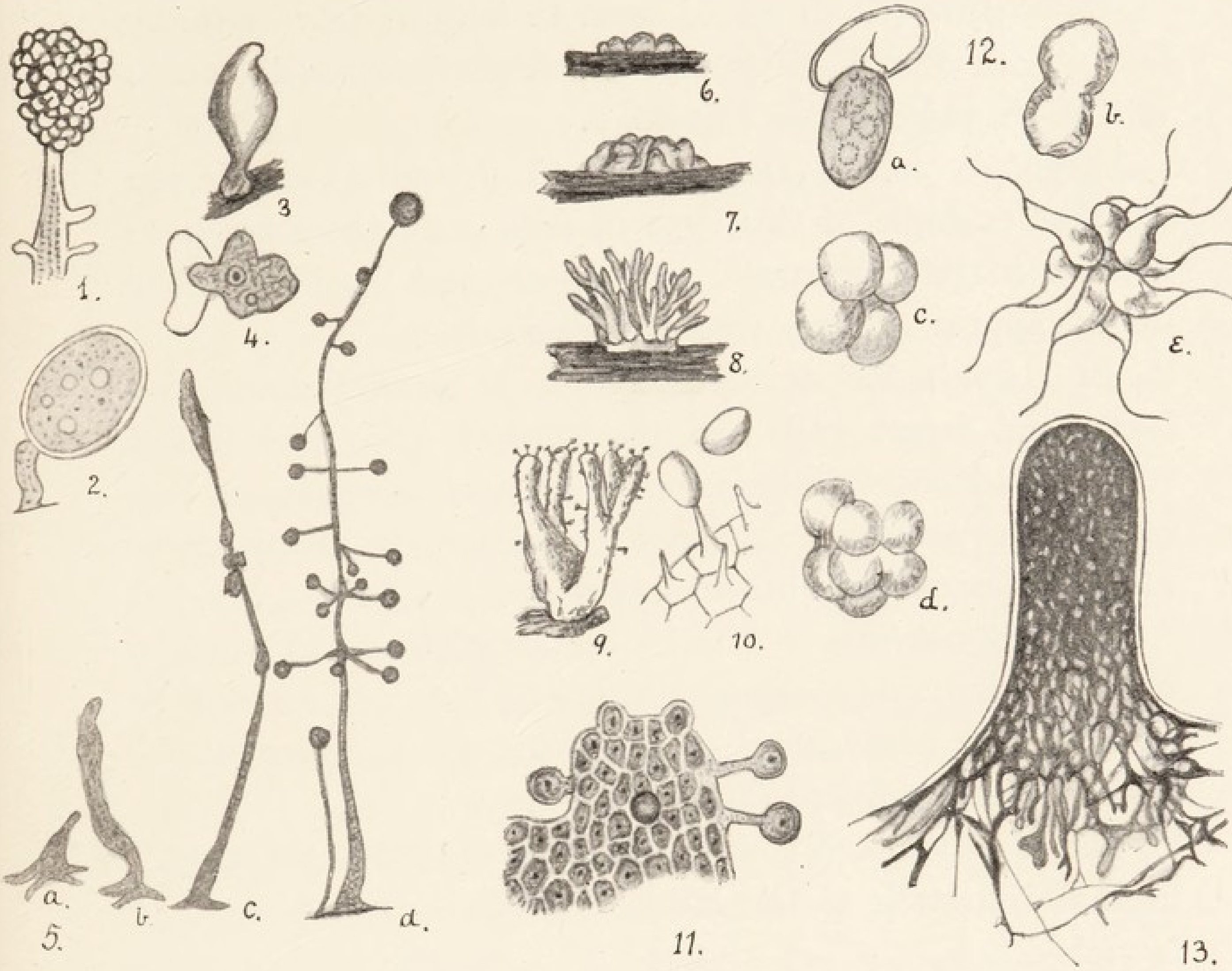

FIG. 45.—*1* to *5*, ACRASIEAE ; *6* to *13*, THE EXOSPOROUS MYCETOZOON, CERATIOMYXA FRUTICULOSA. *1*, *Sappinia pedata* (Dang) cysts ; *2*, the same, single element encapsuled ; *3*, *Copromyxa protea* (Fayod) sorus ; *4*, the same, amoebula escaping from spore-case ; *5*, *Polysphondylium Brefeldi*, *a*, *b*, *c*, *d*, stages in the formation of sorus, which is branched, from massed amoebulae—from Doflein after Olive and Brefeld ; *6*, *7*, and *8*, stages in the formation of sporophores in *Ceratiomyxa* ; *9*. the same × 40 ; *10*, the same × 480 ; *11*, the same, cells on surface, some being modified into spores, × 120 ; *12*, *a*, *b*, *c*, *d*, *e*, stages in hatching, and subdivision of spore and formation of zoospores, × 1200 ; *13*, young sporophore showing plasmodial net in process of forming the cellular surface of the sporophore—the hyaloplasm is drawn thicker than in the original, × 68. *6* to *8*, After de Bary ; *9*, *10*, and *12*, after A. Lister ; *11* and *13*, from de Bary after Famintzin and Woronin.

Copromyxa (Zopf) would seem to be a variety of *Guttulina*: both are of milky aspect and produce sessile round, or club-shaped colonies, Fig. 45, *3*.

Sappinia pedata (Dang), Fig. 45, *1* and *2*, affords the simplest example of this type of organism: the amoebulae become encysted separately, on straws and the like. They do not appear to form a pseudo-plasmodium.

In *Acrasis* the amoebulae form a vertical row. The lower and larger cells are transformed into thick-walled strongly adherent cellulose cubes full of clear liquid, the rest of the amoebulae arrange themselves in chaplet-form at the upper end and become spores.

In *Dictyostelium* a similar differentiation takes place but the supporting cellulose column, Fig. 44, *2*, may be composed of many rows of cells. De Bary compares the stalk of *Dictyostelium* to that of *Stemonitis*.

Dictyostelium and *Polysphondylium* (Brefeld) differ only in that the stalk of the latter, Fig. 45: *5*, *a*, *b*, *c*, *d*, is branched.

I have not been able to find living examples of this group. The impression left by reading accounts of Acrasieae is that much more remains to be learnt about them.

Notes on some affinities of Mycetozoa

Watching living zoospores of any Mycetozoon under the microscope we see them incept and digest bacteria, and we see them divide, so that it would be easy to think that we had before us a simple flagellate or monad.

Again we see a mycetozoan plasmodium incept whole colonies of bacteria or other solids by its pseudopodia, and digest them in vacuoles, so that we could well believe it to be a rhizopod protozoon.

Examining the stages of spore-formation we cannot fail to be reminded of the same process in a puff-ball, a synchytrian, or a sporozoon.

Mycetozoa have affinities with the vegetable protists especially Chytridiineae on the one hand, and with the animal monads, rhizopods, and sporozoa on the other hand.

The likeness that is seen on comparing the texture of the protoplasm of chytridians with that of Mycetozoa has often been remarked upon. The protoplasm of the thistle-*Synchytrium* is very like that of many mycetozoan structures. We may compare the sexual stages of *Synchytrium* to the mycetozoan plasmodium, both result from isogamy; the subsequent numerical increase in the synchytrian is by generative chromidia; in the mycetozoan by mitotic nuclear divisions, such chromidial processes as I have observed being chiefly for the production of capillitium elements.

The nucleus of *Polyphagus euglenae* is more like the mycetozoan nucleus than is that of *Synchytrium* when in mitosis, the former having 10 or 12 chromosomes, the latter in the asexual sorus of *S. endobioticum*, only 5.

The conjugation of similar zoospores to form the mycetozoan plasmodium is an algal trait, which is found also in some monads. We may compare the growth of the synchytrian zygote with that of the plasmodium of a parasitic form more justly perhaps than with free-living mycetozoa. The synchytrian grows by chromidial or plasson extensions from a nucleolus to form what is really a plasmodium limited in extent by a cell-wall. Plasmodiophorans grow by nuclear divisions, the chromidial phase being assumed as a brief stage after growth is complete. Zygote formation has not as yet been seen in them.

The Animalia monadida fall into two groups, Acraspedina and Craspedina, according as the flagellum has not, or has, a collar (Gr. *kraspedon*) round its base. The Monadida Acraspedina include free-living forms such as the Rhizomastigina, of which a phase of one species, *Mastigella vitrea*, has been mentioned in Chapter I; together with many parasites, such as, of blood-parasites, the genus *Trypanosoma* (see Part I), causes of sleeping-sickness, &c.; tissue-parasites, such as *Leishmannia donovani*, the cause of black-fever and splenomegaly (see Part II); and innumerable intestinal parasites such as *Copromonas subtilis* of the frog.

Two monads described by Cienkowsky, *M. parasitica* and *M. amyli,* have a course of life that approaches that of Mycetozoa very closely.

The convincing resemblance that the mycetozoan plasmodium bears to rhizopods needs no emphasis. The absence of mention of chromidia in most accounts of Mycetozoa might be regarded as pointing to an essential difference, but chromidial features which I found in *Didymium difforme* (Part IV) remove some of this seeming distinction.

Of all Sporozoa, the Haplosporidia ; that heterogeneous group founded by Caullery and Mesnil in 1899, are the most likely to furnish examples of kinship to mycetozoa. One haplosporidian, *Schewiokovella schmeili,* a parasite of copepods, has an unsporozoan character in the presence of a contractile vacuole, and a mycetozoan character in that the young animals sometimes fuse into a plasmodium, which becomes encysted and subdivides into spores. Another Haplosporidian, *Rhinosporidium kinealyi,* is the cause of infective tumours in the nasal fossa and other parts of the human body. In this species also, as is shown in Part IV, there is evidence of a contractile vacuole at one stage, while at another stage the parasite assumes a chromidial or even a plasson state.

From some simple alga or alga-like fungus such as *Aphragmium* (p. 35) may have evolved both Acrasieae and Mycetozoa.

A Note on the Basis of Classification of the Phycomycetes and the Mycetozoa

The name Phycomycetes or alga-like fungi was made by de Bary to include forms like *Saprolegnia*, *Pythium*, and *Peronospora*. It has since been extended to embrace the Chytridiineae.

The systematic arrangement of the Mycetozoa has to be viewed from two different aspects, the botanical and the zoological respectively.

A complete modern classification of the Phycomycetes was given by J. Ramsbottom in the Transactions of the British Mycological Society, Vol. VI, Part II, 1916.

The subjoined zoological classification of the Mycetozoa is that given by Delage and Hérouard, Zoologie Concrète, Tome I, 1896.

PHYCOMYCETES=*OOMYCETES*+*ZYGOMYCETES*.

OOMYCETES=CHYTRIDIINEAE+ANCYLISTINEAE+MONOBLEPHARIDINEAE + SAPROLEGNIINEAE +PERONOSPORINEAE.

ZYGOMYCETES=MUCORINEAE+ENTOMOPHTHORINEAE.

CHYTRIDIINEAE=MYXOCHYTRIDIINAE+MYCOCHYTRIDIINAE.

MYXOCHYTRIDIINAE = Olpidiaceae + Synchytriaceae.

MYCOCHYTRIDIINAE = Rhizidiaceae + Cladochytriaceae.

Olpidiaceae = *Olpidium* + *Olpidiopsis* + *Pseudolpidium* + *Pleolpidium*, &c.

Synchytriaceae = *Synchytrium*.

Synchytrium = (Pycnochytria) = *S. anemones* + *S. aureum*, &c. + (Eusynchytria) = *S. taraxaci* + *S. succisae* + *S. endobioticum* + *S. stellariae*, &c.

Botanical.

MYCETOZOA = *PLASMODIOPHORACEAE* + *ACRASIEAE* + *MYXOMYCETES* (*MYCETOZOA* of de Bary).

Zoological.

RHIZOPODA = *PROTEOMYXA* + *MYCETOZOARIA* + *AMOEBINA* + *FORAMINIFERA* + *HELIOZOA* + *RADIOLARIA*.

MYCETOZOARIA = PSEUDOPLASMODIDA (ACRASIEAE) + FILOPLASMODIDA (LABYRINTHULEA) + EUPLASMODIDA (MYXOMYCETES or MYCETOZOA of de Bary).

The position of the Plassomyxineae must be next to the Synchytriaceae, and near the Plasmodiophoraceae.

CHAPTER X

NOTES ON SMALLPOX, SYPHILIS, CANCER, ETC.

Cystic disease of the urinary tract.—Sometime before 1892 representative London pathologists had sought the opinion of T. Spencer Cobbold upon some microscopic bodies found in two instances of disease; one of these was a condition similar to that of which some details are shown in Fig. 46. Cobbold pronounced the bodies to be "psorosperms," meaning that they were sporozoa of some sort. This opinion was accepted by English pathologists. The fullest account of my case is in Part II, where the difficulty I had in adopting the accepted view is explained. When I realised that it is parasites one sees in those cysts, Fig. 46, *1*, I did not think I was merely in presence of a rare disease, which by reason of its rarity would, in itself, have the less importance for mankind; but I felt that what I was studying was probably a rare manifestation of several common diseases, such as some forms of kidney-disease, papillomas of the bladder, and adenomas of the prostate.

Last year for the first time I saw Cornu's illustrations of *Olpidiopsis;* some of them are suggested in Fig. 15, p. 60.

Here for the first time in biological literature I saw something relating to organisms of the same category as Cobbold's psorosperms.

Can one identify the cyst-contents of this disease with the Olpidiaceae ? There are two difficulties ; firstly, the cytology of such olpidians as *Rozella septigena* &c. that appear to form

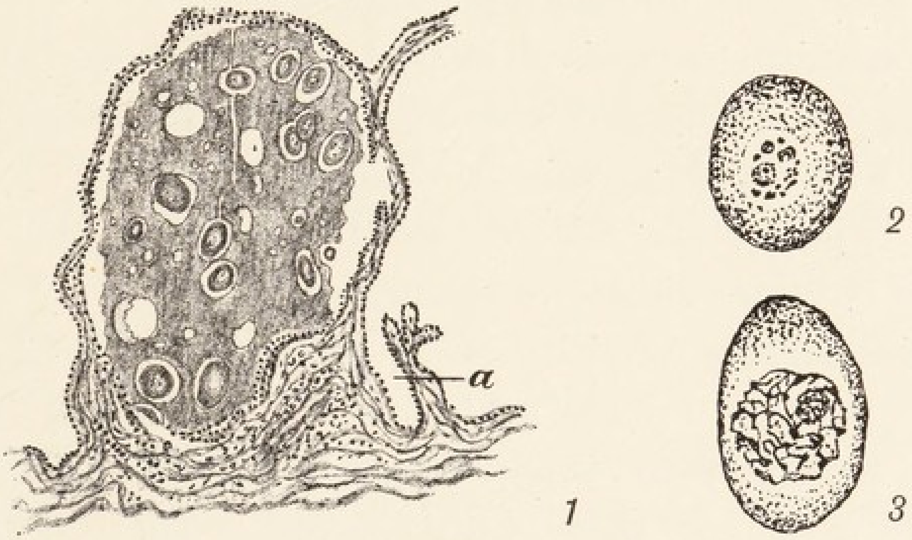

FIG. 46.—CYSTIC DISEASE OF THE URINARY TRACT. Section of a cyst in the pelvis of the kidney under a low power, showing the general character of the cyst-contents. At *a* is an invagination of the mucous membrane, as if preparatory to formation of a new cyst (from Part II) ; *2*, parasited with a nucleus of fragmented chromatin ; *3*, ibid. with a well-formed nucleus ; *2* and *3*, × 1000 diams., from the Trans. Path. Soc. 1892.

plasmodia with one another or with host-cells has not been fully established ; secondly, the cystic disease has only been examined 24 hours or more *post mortem.* At present we can hardly assign a definite place in taxonomy to the parasite.

All known Olpidiaceae, whether they form plasmodia or not, produce zoosporangia. Now, though there is evidence of the formation of capsules round some of the oval bodies, reproduction in the cysts is chiefly by plasmodia subdividing directly into amoebulae, a fact that suggests the Plassomyxineae as their proper group; but early intracellular stages not being traceable, the classification must be uncertain until more information is available.

An organism recognised in pathology for over 30 years should have a name and without prejudice to future identification with some parasite previously described, I would suggest *Olpidiiforma cobboldi* as the name of the causal parasites of cystic disease of the urinary tract.

A false analogy.—With respect to the parasites of cancer &c. the position I have maintained is that they are of kindred nature with those of cystic ureteritis. Several observers identified them with coccidia. This Coccidium-idea was successfully opposed by Fabre-Domergue and others. Their task was a very easy one, that of destroying a false analogy, a very different matter from disproving the fact that the bodies in question are parasites.

Virchow compared the bodies of molluscum contagiosum with *C. oviforme* and finding no clear correspondence decided illogically that the bodies must be altered epidermal cells, a decision that is accepted in pathology to-day.

That earnest pioneer, L. Pfeiffer, making the same comparison on other grounds, decided that the virus must be concealed in the interior of the molluscum body and thus was laid the foundation of the delusive Chlamydozoa-theory of von Prowazek.

Again, in an article published in the British Medical Journal, 10th Dec. 1892, Metchnikoff left the impression that he regarded as coccidial the bodies described by Soudakewich in cancers. In the next issue appeared a letter of mine stating that they are not coccidia "in the biological sense of the word."

At the present time one investigator writing with seeming indifference to the history of the subject, and apparent neglect to examine epithelial lesions, claims *Cytoryctes luis* as a *Coccidium*.

This protozoon has had such a disastrous effect on pathology that I am almost ashamed to have given the first description of its early microgametogenesis.

The sporozoa idea not only diverted me from the right path but also led me into error; thus in 1893, in the course of an examination of an adenoma of a cat's lip among the sections of the tumour which show cysts that contain bodies like those of cystic ureteritis, another of a seminal vesicle of an earthworm found its way, probably from the razor of the microtome. Too keen on the mistaken trail of Sporozoa it was this particular section that, not suspecting what it was, I chose to show at a meeting of the Pathological Society, and a drawing of it disfigures p. 93 of my "Morbid Growths and Sporozoa."

So long as I accepted *Olpidiiforma* and kindred bodies as sporozoa I was studying fungi and calling them protozoa all the time, *i.e.* for nearly 30 years ; and this has been the case generally, with the result that we have importuned protozoologists for enlightenment, which could only have been given us by cryptogamic botanists.

Smallpox

Guarnieri's experiment.—Twelve hours after inoculation of a scratch in the corneal epithelium of any rodent with smallpox or vaccine lymph minute foreign bodies can be found close to the nuclei of the epithelial cells. With the point of a sharp knife or needle a linear scratch is made vertically across the cornea through the epithelium without incising the fibrous layer. At the desired time the animal is killed, the eyeball excised and placed at once in the fixing fluid, or scrapings can be made for immediate examination. This is Guarnieri's experiment.

After 24 hours the corpuscles are larger and the experiment can be used for diagnosis, scrapings showing the bright greenish bodies clearly. At this stage sections show that the bodies in cells close to the incision are larger than those farther away, those in cells next the apparently uninfected ones being very small, many of them subdividing by fission.

At the end of 48 hours the bodies are very obvious and in well-stained sections show very clearly as in Fig. 47. They stain well with methylene blue and with haematoxylin and eosin, taking the basic and acid stains about equally.

Mitoses in epithelial cells.—In good sections at this stage in a narrow zone separated by a definite interval from the area in which the parasites are visible the epithelial cells are swollen and many of them are in mitosis. The cells occupying the interval between the advanced line of visible parasites and the active epithelial cells probably contain

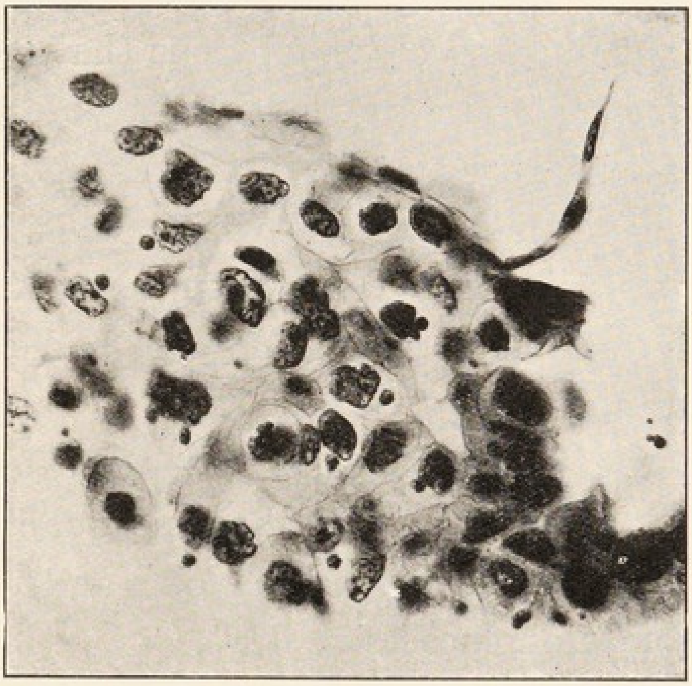

Fig. 47.—A Portion of the Epithelium of a Rabbit's Cornea Forty-eight Hours after Vaccination. Guarnieri's bodies are seen as roundish dark objects near the nuclei of the epithelial cells. From a photograph of one of the Author's preparations made in 1894. H. and E. × 400.

parasites in the microhenad stage and the activity of the cells is defensive.

Variations in the parasites.—Though they look quite different the two sets of elements shown in the two parts of Fig. 48 represent the same fact, the passage of the parasites from the pycno- to the chasmatoplasson state with the appearance of chromatin, repeating stages of the thistle-*Synchytrium* previous to its subdivision into zoospores. Such forms as those in Fig. 48, *1*, are hard to find because parasites as they mature fall off with their host-cells. In some of the elements of Borrel's drawings, *e.g.* Fig. 48, *2*, beside remains of unchanged dense substance are definite small nuclei. It must surely be obvious that bodies capable of producing nuclei from a previously akaryote state have as much claim to be regarded as representing organisms as others which have nuclei all the time. This has been the basis of my position since 1892 when I found evidence of new nucleus-formation in the olpidiiform bodies in cysts of the ureter and in cancer.

Cytoryctes the cause of vaccinia.—Once the *Cytoryctes* is recognised as a parasite its pathogenic quality cannot be doubted by any who have made a long and careful study of infected corneas on successive days.

Borrel ascribed them to leucocytes, but his own careful drawing, Fig. 48, *2*, *a*, suffices to negative this explanation. His words, "Pour caracteriser un protozoaire il faut un noyau," show that, as it was with the rest of us at that time, the possibility of parasites other than protozoa had not occurred to him.

In his many studies of variola and vaccinia L. Pfeiffer was also handicapped and his work frustrated by the fatal protozoon-idea. The likeness of *Cytoryctes* to some stages of sporozoa is very great. Pfeiffer was the first to point this out, and in 1887 he named the parasite, "*Monocystis epithelialis*," after the gregarines of the earthworm.

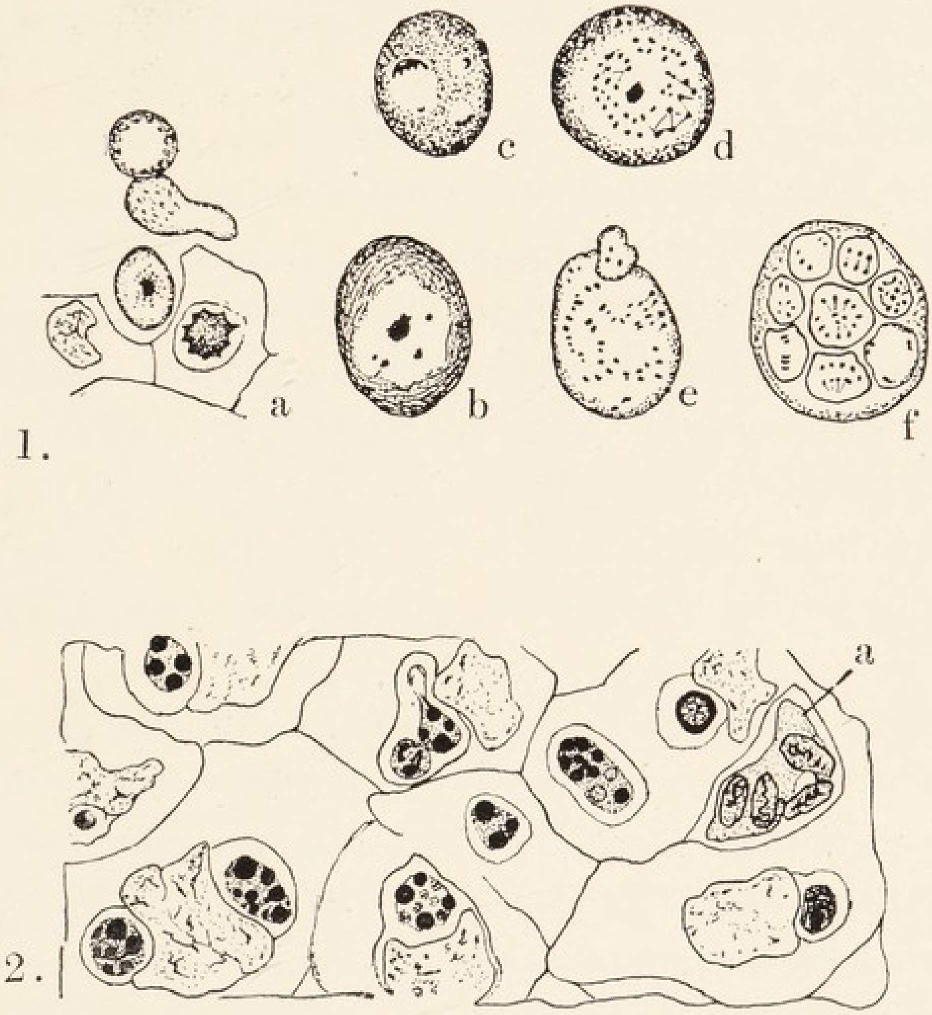

FIG. 48.—ELEMENTS FROM VACCINATED CORNEAS. *1* (from Path. Soc. Trans. 1895), guinea-pig's cornea 3rd day ; *a*, two epithelial cells, one containing a parasite, and three free parasites ; *b*, *Cytoryctes* with central clearing containing chromatin ; *c*, ibid. with three mitoses ; *d*, ibid. with chromatin in nuclear form ; *e*, parasite with scattered chromatin granules and a separated segment ; *f*, ibid. subdivided into nucleated segments. *2* (after A. Borrel, 1903), rabbit's cornea 8th day ; all the epithelial cells contain Borrel's "pseudoparasites," really *Cytoryctes* passing from pycno- to chasmatoplasson state with the appearance of small nuclei ; a leucocyte at *a*.

Spirochaetes in vaccinia.—In the vaccine vesicle spirochaetes have been found by Bonhoff, 1905. Many details remain to be worked out, but enough has been established to show that *Cytoryctes guarnieri* belongs to the same group as *Plassomyxa contagiosa*.

Intranuclear phases of Cytoryctes.—In smallpox about the same time Bosc in France and Councilman and others in America described the intranuclear forms of the parasite, the presence of which explains the difference between the clinical courses of the two modifications of the disease: in vaccinia the stages of *Cytoryctes variolae* are found only in the cytoplasm of host-cells as in Fig. 49, *1* to *5*; in smallpox that stage also occurs and is repeated many times before the distinguishing intranuclear generation appears, Fig. 49, *6* to *9*.

In 1904 Professor Calkins very kindly gave me some sections of smallpox pustules stained by Borrel's indigo-carmine picric acid method; these sections show the intranuclear stages to-day as clearly as when they were given to me. Many of the minute subdivisions have left the nuclei and are scattered in the sections. More observations on fresh material are desirable to find out if they are the heads of flagellates such as were described by Doehle in 1892.

The first interpretation of the whole series of forms was that *Cytoryctes* was a sporozoon; later, after chromidia had been generally recognised, *Cytoryctes* was thought to be a Rhizopod; finally after Dangeard's discovery of Nucleophaga

FIG. 49.—CYTORYCTES VARIOLAE, GUARNIERI. *1*, A cell from a section of a smallpox lesion : three amoebulae are present in the cytoplasm close to the nucleus ; *2*, a cell containing an older amoebula in the cytoplasm ; *3*, a cell containing a large amoeboid parasite in the cytoplasm ; *4*, a cell containing an amoeboid parasite in process of amoebula-formation, and, above, a round dense body, probably a microgametocyte ; *5*, a cell containing an amoeboid parasite in process of gemmule-formation : some of the amoebulae are escaping into the cytoplasm ; *6*, a cell the nucleus of which contains five spores and three secondary sporoblasts developed from spores : there is one spore in the cytoplasm ; *7*, a cell the nucleus of which contains secondary sporoblasts ; *8*, a cell the nucleus of which contains a reticular sporoblast, with spores, some of which have escaped from the meshwork ; *9*, a cell the nucleus of which has been replaced by a large sporoblast, from which some of the spores have escaped into the cytoplasm. Copied from the coloured plates illustrating G. N. Calkin's section of "Studies of the Pathology and on the Etiology of Variola and of Vaccinia," published in the *Journal of Medical Research*, 1904.

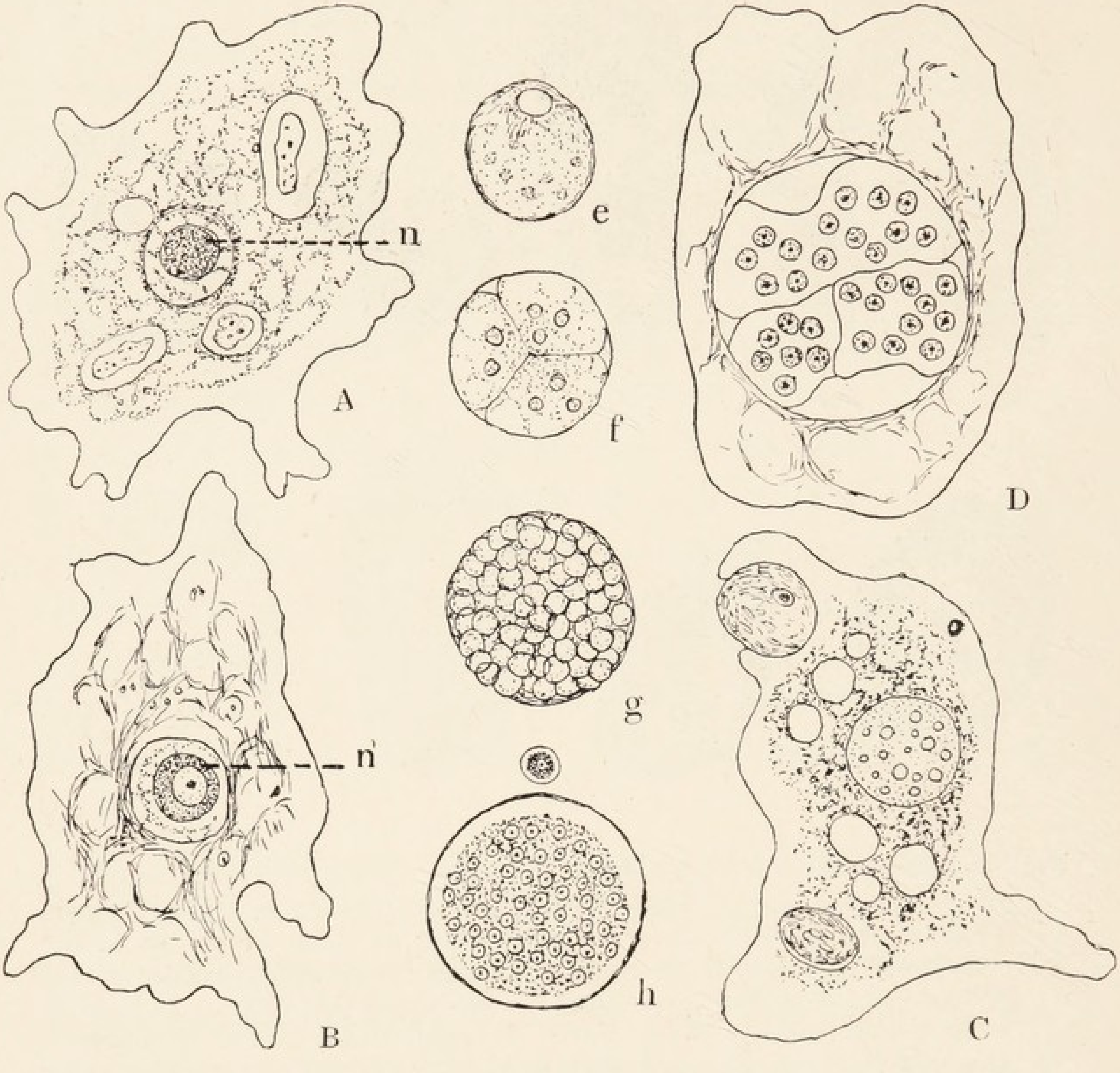

FIG. 50.—NUCLEOPHAGA AMOEBAE. *A*, normal *Amoeba verrucosa*, *n*, nucleolus ; *B*, Amoeba of which the nucleolus, *n′*, contains a *Nucleophaga* which has a central nucleus and a pale cytoplasm ; *C*, Amoeba, the nucleus of which is replaced by a *Nucleophaga*, and which is incepting an encysted *Euglena* ; *D*, Amoeba the nucleus of which is replaced by 3 ripe sporanges of *Nucleophaga* ; *e*, nucleus of *Amoeba verr*. showing the perforation in its membrane by which entered the *Nucleophaga* it contains ; *f*, host-nucleus filled by three young sporanges ; *g*, sporange as seen in the living state ; *h*, sporange stained showing nuclei, one of these on larger scale above. After P.-A. Dangeard.

had been digested in zoology Calkins (1909) wrote of *Cytoryctes* :—" There is no doubt, however, that the parasite is a species of nucleophaga, and the name karyoryctes must go."

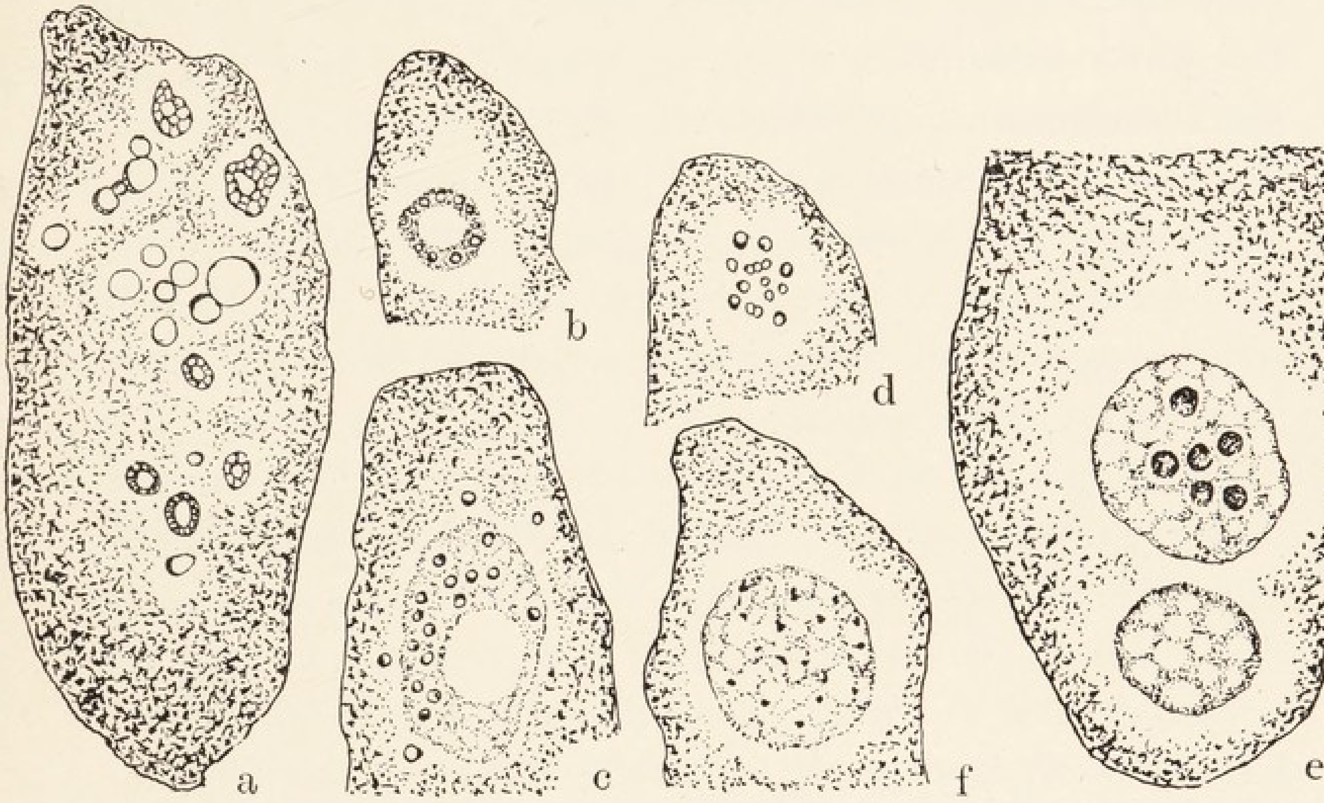

FIG. 51.—KARYORYCTES. *a*, various stages in the macronucleus of a Paramaecium ; *b*, spore-formation begins ; *c*, spores formed, four escaped into host's substance ; *d*, group of spores ; *e*, amoebula-formation, below, " residual body " ; *f*, reticular body containing points of chromatin. After G. N. Calkins.

Nucleophaga and Karyoryctes.—Now we must consider the question left over from Chapter III as to the position of the parasite described by Dangeard in 1895 under the name of *Nucleophaga*, and as to its being homologous or not with the parasite *Karyoryctes* found by Calkins in 1904 in the macronucleus of *Paramaecium aurelia.*

Some of Dangeard's illustrations are shown in Fig. 50. The parasites enter the nucleolus of their host, *Amoeba verrucosa.* They have obvious nuclei from the first. In the grown parasite, *d*, the nuclei are of fungal type and are like those shown in *Olpidiopsis* in Fig. 16, *e*. The spores are liberated by disintegration of the remains of the host-karyoplasm ; this occurs only when the parasite has completed its development. Dangeard concluded that *Nucleophaga* is a vegetable rather than an animal protist because it leaves no food-remains.

Turning now to *Karyoryctes* as shown in Fig. 51, the young parasites as in *c* and *d* have no centrally placed nucleus, but a thickening at the side which gives a " signet-ring " look. The spores as shown at *a* germinate by forming a vacuole and the protoplasm grows into it in plasmodial form, still without nuclei. Another mode of reproduction in *Karyoryctes*, *e*, is not seen in *Nucleophaga*, but is similar to that shown below, Fig. 54, *18*, *19*, in a *Plassomyxa* of a sarcoma. The reticulate body, *f*, was also termed residual, but its points of chromatin recall the chromidial stage of the direct sporangium of *Synchytrium.*

No two related protists could well be less alike than *Nucleophaga* and *Karyoryctes* : the former is a Chytridian, the latter one of the Plassomyxineae.

The accomplished protozoologist has done better than his later thought allows, having discovered in freshwater protozoa a species of vegetable protist, which may be found to play an important part in some human pathological processes. Another organism, *Lymphosporidium truttae*, was also discovered by Calkins (see Part I, pp. 111 to 115). The mode of reproduction seen in Fig. 51, *e*, was the only one found ; this organism also would appear to be a *Plassomyxa.*

The stages of *Karyoryctes* described by Calkins correspond only to the intranuclear stages of *Cytoryctes variolae.* Is there another intracytoplasmic cycle in the same or another animal, or even in man ? Among tumours goître is associated with certain water-courses, and in goîtres intracytoplasmic cell-inclusions resembling *Cytoryctes variolae* occur.

Syphilis

Parasitic forms occurring in Syphilis.—In 1892 flagellates were described by Doehle as they had been found by him in the blood of patients suffering from syphilis, smallpox, vaccinia, measles, and scarlet fever.

My own first aim was to see whether the parasite of syphilis conformed to the type *Cytoryctes variolae.* On the 3rd day after inoculation with the exudation of a chancre I found in the corneal epithelial cells of a rabbit bodies comparable to the early state of Cytoryctes variolae.

Cell-infiltration by leucocytes and young tissue-cells obscured the view in my sections of chancres. At that time, 1894, any spreading sore that resisted the oral administration of mercury was excised. I thought that near the margin of rapidly spreading secondary ulcers the parasites would be found in epithelial cells just in advance of the leucocytes:

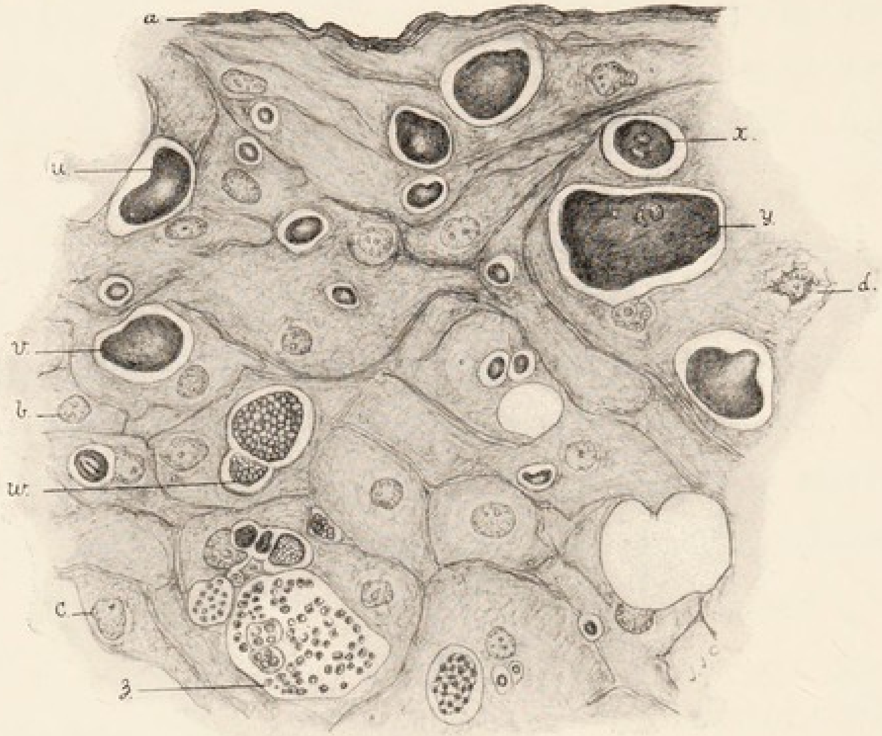

Fig. 52.—Part of the Epidermis in a Section of a Spreading Secondary Syphilitic Ulcer. *a*, Horny layer; *b*, normal nucleus of epidermal cell; *c* and *d*, nuclei of epidermal cells that are breaking up; *u*, *v*, *w*, *x*, *y* and *z*, various stages of the bodies described as protozoa by the author in 1895. Two leucocytes are present among the minute bodies at *z*. Reduced from a drawing made with drawing eye-piece and $\frac{1}{12}$-inch oil-immersion lens. From Part II.

this proved to be the case as shown in Fig. 52. In the section are seen large pycnoplasson parasites; these are linked by stages to others breaking up into minute nucleated elements: only when this subdivision occurs do the parasites attract leucocytes as at *z*.

In 1905 Siegel described flagellates, which attained their maximum number in the second and third weeks of syphilis. His drawings show round and bluntly crescentic bodies about 2μ with a single flagellum; the latter was difficult to discern in fresh preparations. Similar bodies were found in thin (2μ) sections of chancres, where the larger bodies were seen inside, the smaller between the connective-tissue cells. In rabbits and guinea-pigs inoculated with syphilis the same bodies were found in the blood and tissues.

Later in the same year Schaudinn in the course of an investigation of Siegel's observations discovered the mobile form, which he named *Spirochaeta pallida*. His view, stated in Part II, that this was an organism homologous with *Leucocytozoon ziemanni*, a blood-parasite of owls, has proved to have been formed too hastily.

Spirochaetes.—In 1838 Ehrenberg discriminated between the genus *Spirochaeta* with flexible bodies and the genus *Spirillum* with rigid bodies. The best-known spirochaetes, *e.g. S. balbianii* of the oyster's alimentary tract, have blunt ends and an undulating membrane: *S. pallida* on the contrary has taper flagellum-like ends and no membrane; hence Schaudinn altered the name to *Spironema pallida*. This later name will be used here as it was in Part II.

In 1911 Noguchi obtained anaerobic cultures of the spironema in broth containing fresh kidney. Later the medium was changed to white of egg in ascitic fluid broth under paraffin. In some cultures the organisms are thicker than in others, and different strains cause different lesions in the rabbit. Noguchi found the spironemes not to be filtrable, but they grow through a filter in 4 days. Both longitudinal and transverse divisions have been observed in the spironeme.

One can understand how those who study syphilis only by seeking spironemes for diagnosis,[1] and reflecting on the cultural results just mentioned conclude that the spironeme is to syphilis what Koch's bacillus is to tuberculosis.

In order to accept this simple interpretation of syphilis one must ignore the intracellular and other phases mentioned above; indeed many more proved facts than can be explained by accepting Schaudinn's view.

The spironema is not a final phase: like other similar bodies it has been observed to shed granules which appear to be the immediate infecting agents. Of all accounts of granule-shedding the most complete is that given by A. Balfour as he saw it in spirochaetosis of Soudanese fowls: the granules were forced out by repeated contractions of the skin-layer. The question does not concern syphilis alone: spiral forms occur in *Plassomyxa contag.* and in probably the same parasite in flagellate-diphtheria of birds; they have been found in vaccinia, and in some tumours mentioned below. The conditions of cultivation that suit the spironema are uniform; such as would be likely to favour one

[1] Examination by help of the dark-ground illumination microscope is now regularly used in clinical work, and it is of the greatest service: early diagnosis and immediate treatment being now the rule.

phase of a polymorphic organism, in the same way that grape-sugar solutions favour the gemma-stage of *Mucor racemosus*.

The parasite of syphilis and Synchytrium.—It has been shown in Chapter V that bird's-eye bodies occur in *Synchytrium* and in syphilis as well as in cancer. The series of stages seen in Fig. 52 is almost identical with those of the direct sporangium of the thistle-*Synchytrium*, Fig. 23: pycnoplasson, chasmato-plasson, &c. Looking again at Fig. 52, *z*, and comparing it with the stages of *Synchytrium* we should expect the succeeding stage of the parasite of syphilis to be flagellate, and such I have no doubt is the case. In *S. endobioticum* three different flagellate phases occur. It may be that the monoflagellates of Siegel are the zoospores, and the biflagellate spironeme the zygote of the parasite. It seems to me that the most promising line of inquiry to clear up the questions of the relation of the spironeme to the other parasitic forms in syphilis and other diseases is through the study of flagellate-diphtheria of birds, combined with such study of syphilis as was well begun in 1906 by MacLennan.

Gummata.—With regard to the histology of the gumma, in 1895, I stated my opinion that much of the broken-down material consists of parasites in a state of fatty degeneration and compared them to similar bodies in cancer, *e.g.* in the sarcoma of the testis, Fig. 31, in M.G. and S., also Part II, Figs. 41 and 42.

Syphilis and Cancer.—The identity of some intracellular parasitic forms in late syphilis with those of cancer has been shown above in Chapter V, where the production of "cells of endogenous origin" in living parasites of a late syphilitic lesion is described.

Clinical phenomena only too familiar to us express the same fact in another way.

We know that typical syphilitic differ from typical cancerous lesions in certain features, but where syphilitic glossitis is becoming changed into cancer these differences are very gradually acquired, and it is not possible either clinically or pathologically to say at what moment one disease becomes converted into the other. Parasitic protists are equally abundant in both, and they appear to be a series of phases of the same parasite. I do not mean to say that all cancer is a modified form of syphilis, but as far as can be seen it is so in this particular instance. Probably cancer is produced by as many different species of protists as there are different bacteria, etc., that cause chronic granulomas.

Cytoryctes Luis.—I have no doubt that *Cytoryctes luis* (Siegel 1905) is the right name for the causal parasite of syphilis. This designation accords with all the facts ascertained up to the present, and, although when Siegel invented the name he does not appear to have known of my observations (1894), these alone would sufficiently warrant his choice.

Cancer

In pathology the term cancer is applied to a malignant tumour which originates in epithelium such as the epidermis, or mucous membranes such as the lining of the mouth, the stomach, or bowel; or the epithelial part of a gland such as the liver. A sarcoma is a malignant tumour that originates in one or other of the connective-tissues: fibrous tissue, bone, &c. We may think of these two phases of disease as epithelial cancer and connective-tissue cancer respectively.

Cancer affords greater opportunities for investigation than almost any other disease: its dread plenty, long course, and the frequent calls for operation furnish material only too abundant. Added to this the facility with which cancer can be grafted into mice gives those who have sanction opportunity for unlimited observations. Of the countless experiments that have been made with Jensen's mouse cancer the result obtained by Ehrlich and Apolant is the most important: on transplantation epithelial cancer became sarcoma, showing—it cannot otherwise be explained—that the two diseases are one, and that both are caused by the same parasites.

Parasites of a Connective-tissue cancer.—Photographs of a sarcoma of the breast are reproduced in Fig. 53. A detailed account of this tumour having been given in Part II, only the chief features need be noticed here; they demand patient and minute attention.

The photographs were taken from E. H. B.-stained sections as also was the coloured plate, frontispiece to

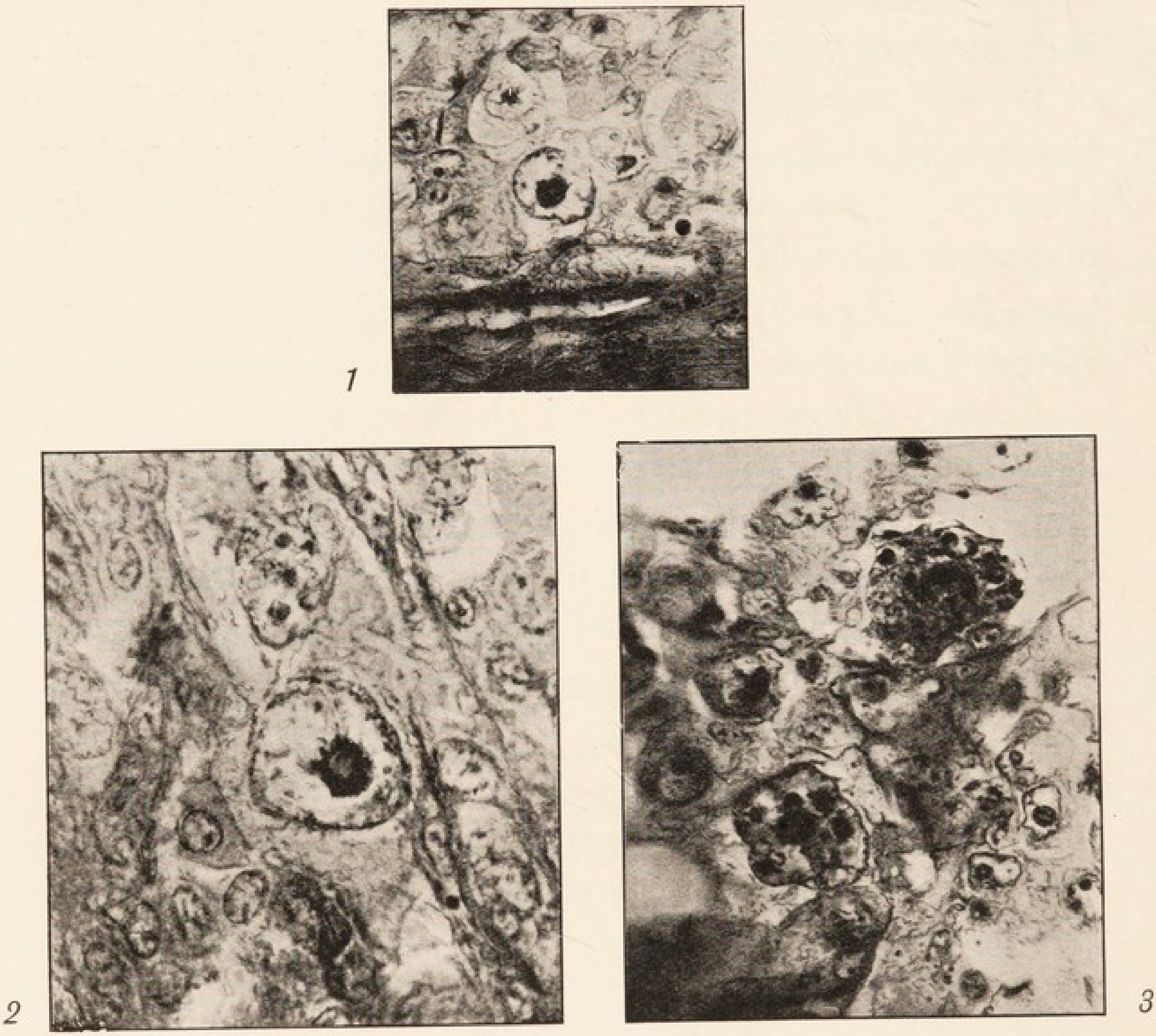

Fig. 53.—Three Microphotographs of an Alveolar Sarcoma of the Breast. In *1* the parasite has absorbed part of the nuclear membrane to which two of its tentacles are attached; *2*, a large intranuclear parasite with knobbed tentacles, one spirally retracted; *3*, a nucleus containing several parasites, and an extranuclear parasite subdividing into stellate amoebulae. *1*, × 400, *2* and *3*, × 800 diams. From Part II.

Part III. The description of the figures will serve for explanation. Sections stained as just mentioned faded quickly, but H. and E.-stained sections are still well stained after 28 years.

It is to be noted that the tissue cells of this tumour are ordinary connective-tissue cells. No epithelial cells were seen in the parts of the gland that were affected by the neoplasm.

The peripheral processes of the intranuclear bodies were seen in the H-E-B.-stained sections better than in sections stained in other ways. They stained a brownish-orange colour, and many of them were seen to possess knobbed "tentacles" like that in Fig. 53, *2*. In the haematoxylin preparations the ends of these processes, being stained like the chromatin, are not so clearly seen.

Chromatin in and subdivision of parasites.—In some parasites, *e.g.* Fig. 54; *5*, *6*, and *13*, chromatin is seen as it had been streaming from the central part of parasites.

In others the chromatin consists of a single central mass, or several such. Spindles of the ordinary characters are present in some of these cells, and simple binary division of the chromatin mass, with or without formation of definite chromosomes, may occur in them, as in similar cells of other sarcomas; but in this tumour the result of this form of nuclear activity was limited to the formation of the small roundish bodies shown in *8* and *16*. The cells *9* to *19* are described beneath Fig. 54. A few words as to the formation

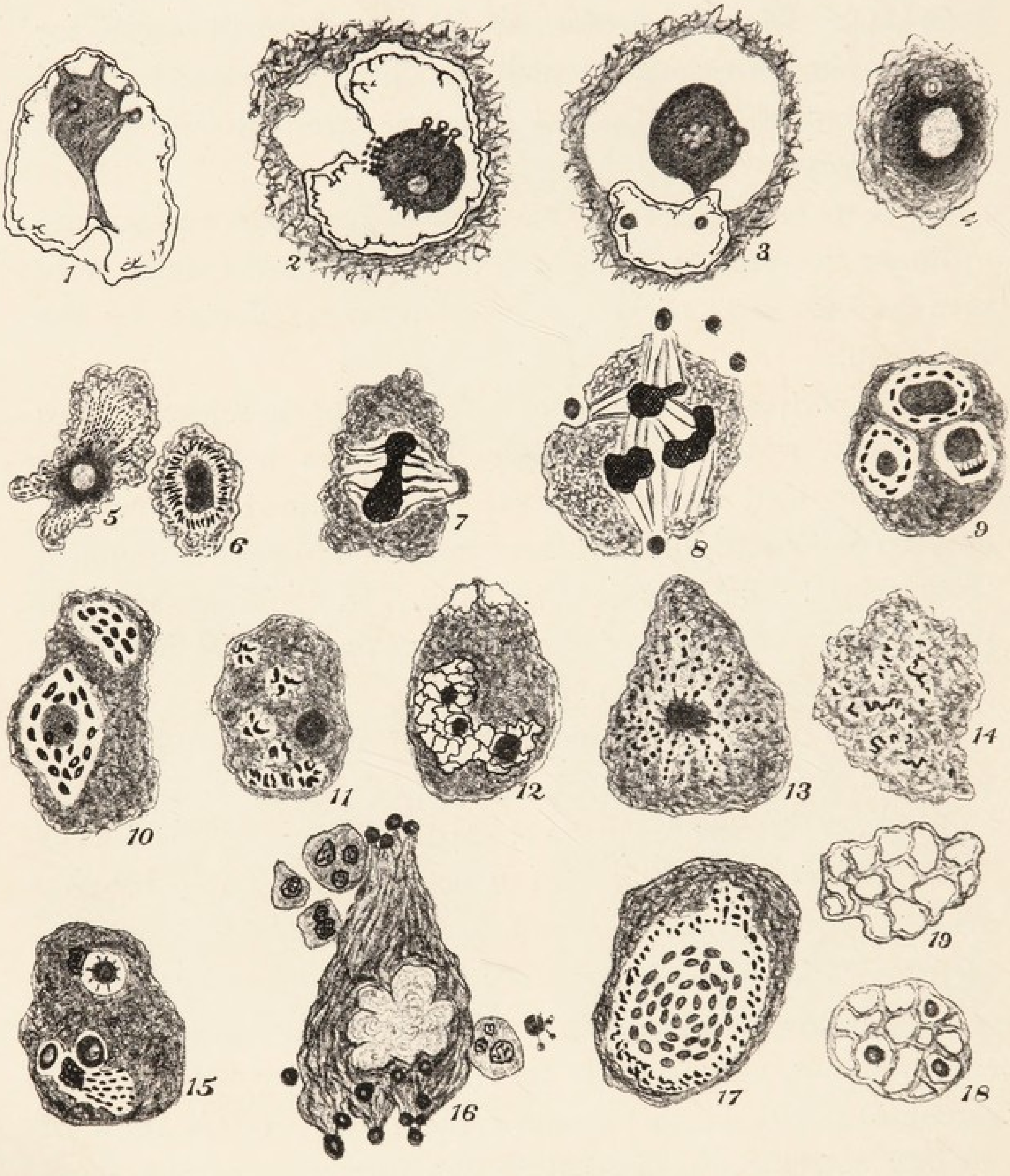

FIG. 54.—ALVEOLAR SARCOMA OF THE BREAST. *1*, Intranuclear parasite attached to the nuclear membrane by a pedicle ; *2*, parasite attached to nuclear membrane by a group of short tentacles ; *3*, parasite in the cytoplasm of a connective-tissue cell ; *4*, a free parasite assuming

of the small rounded bodies, which have already been considered as they arise from the flowing of chromatin matter along the spindle. In *15* is a cell containing two chromatin masses. From the lower one of these a stream of chromatin particles is passing into a limited area of the cytoplasm; and near the same chromatin mass are two rounded bodies. Above is a second chromatin mass adjoining a round body with slender peripheral rays. From long and close study of the cells of this tumour I have no doubt that such round bodies arise from the mixture of a chromatic and a non-chromatic substance, and that they are minute amoebulae, termed gemmules in Part II. Achromatic strands of linin are seen in *8*. Another mode of amoebula-formation is that

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the amoeboid character; *5*, a similar parasite with streaming of chromatin from the chromidial central part; *6*, chromatin appearing as a ring of filaments around a central chromidial mass; *7*, parasite with central mass of chromatin and an irregular spindle—a bud forming to the right; *8*, similar to *7*, but with several chromatin masses and peripheral formation of "gemmules"; *9*, parasite with basiphile chromatin in process of formation around oxyphile masses; *10*, same as *9* below, and a spindle with ordinary chromatin above; *11*, parasite with chromatin in foci somewhat resembling leucocytes; *12*, parasite similar to *9*, but with basiphile chromatin in a network enclosing three oxyphile bodies: such stages in the parasites simulate nuclei of somatic cells; *13*, a parasite with radial moniliform chromatin; *14*, parasite with chromatin in a form resembling an irregular spireme—a "giant mitosis"; *15*, a parasite showing below the formation of an amoebula by chromatin streaming into a localized area, and elsewhere stages in amoebula-formation; *16*, a parasite with multiple amoebula-formation at the surface: also a free amoebula provided with tentacles, and four leucocytes; *17*, a parasite with central amoebula-formation; *18*, a parasite from which gemmules have been formed, and all but three have escaped; *19*, a reticulum from which all the amoebulae have escaped. × 1000 From Part II.

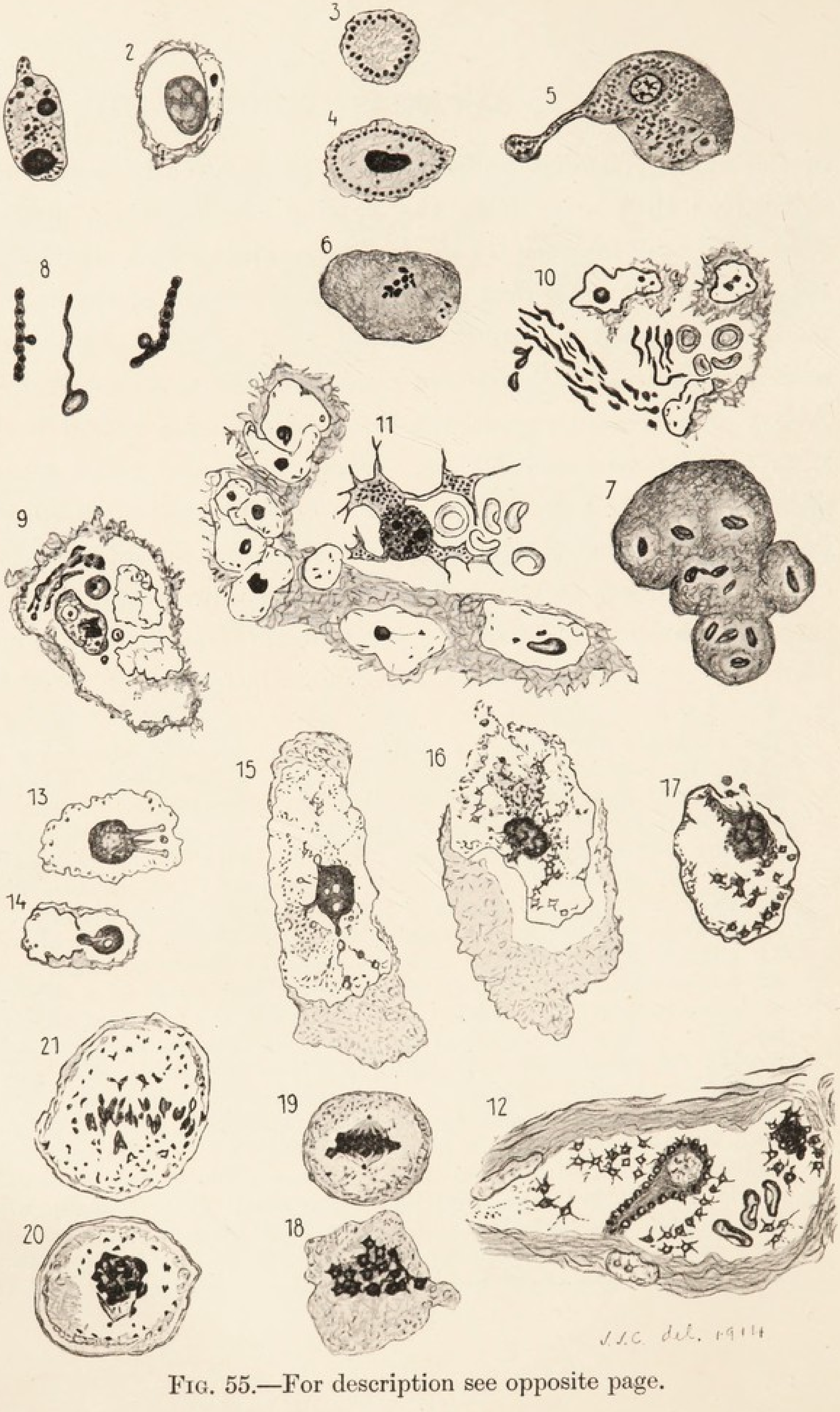

FIG. 55.—For description see opposite page.

shown in *16*. The lobed chromatin body in this case was translucent, probably from the chromatin being very finely divided; the linin system pervades the whole of the cytoplasm, making lines which converge at the points where amoebulae are being formed. Near this cell are a free amoebula and four leucocytes; the latter are quite easily distinguishable, and appear not to have been phagocytic as regards the amoebulae, none of which are seen inside them. Other modes of amoebula-formation are shown in Fig. 54.

To explain modes of subdivision the descriptions of Figs. 54 and 55 will suffice for the average cases, but I invite attention especially to Fig. 55; *12*, and *15* to *19*. In *12* a parasitic mass is breaking-up into stellate amoebulae like those seen in abundance in the mammary sarcoma.

A choriocarcinoma and some parasitic nuclei.—In *15* to *17* are seen parasites whilst still inside the host-nucleus to

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Fig. 55.—Various Structures in the Choriocarcinoma. *1*, Free chromidial parasite; *2*, intracytoplasmic parasite; *3*, *4*, free parasites with peripheral chromatin granules; *5*, free chromidial parasite with a nucleiform chromatin-net; *6*, free parasite with chromatin granules; *7*, ibid., with temporary nucleus-like bodies; *8*, nucleated filaments and a spiral attached to an oval body; *9*, several intracytoplasmic bodies, some assuming spiral form; *10*, plasson masses splitting into spirals; *11*, Leydenia-form free in blood-vessel; *12*, two parasitic masses in a blood-vessel breaking up into stellate amoebulae; *13*, intranuclear parasite with knobbed processes; *14*, similar parasite escaping into cytoplasm; *15*, intranuclear parasite with granular extensions; *16*, ibid., with extensions in the form of stellate amoebulae; *17* ibid.; *18*, end-view of mitosis in a parasite; *19*, ibid., profile; *20*, intranuclear parasite in mitosis with chromidial granules outside the nuclear figure. × 800. From Part IV.

be passing from the pycno- to the chasmatoplasson state, resulting (in *16* and *17*) in the production of stellate amoebulae. In *21* is an early anaphase in an intranuclear parasite with chromidial particles distributed in the plasm: *20*, a similar parasite at metaphase.

The two views of mitosis *18* and *19* show chromosmes quite unlike those of any human cell and quite like those of *Plasmodiophoraceae* and Mycetozoa.

Expansion into and formation of stellate subdivisions and mitotic subdivision are here alternative processes. Fig. 55, *21* shows a mixture of the two. A few instances of subdivision of intranuclear parasites I observed also in the mammary sarcoma described above.

The sections of the choriocarcinoma which Professor Primrose kindly gave me are perfectly stained with iron-haematoxylin, a process which shows certain nuclear features better than does acid haematoxylin.

Conclusions and Comparisons.—If the sarcoma of the breast (Figs. 53 and 54) of which a fuller account is given in Part II, were the only tumour known, and the cytology of granulation-tissue were also known, we should be justified in concluding that the tumour was caused by a parasite which first invades the nuclei of connective-tissue cells, escapes thence into the cytoplasm of the host-cell and thence again into the intercellular spaces. The parasite subdivides either with or without the formation of chromatin into amoebulae which tend to assume a stellate form. When chromatin appears in the parasite it may have the form of nuclei with achromatic linin fibres.

Those who from year to year have numbers of malignant tumours to examine will find some in which the parasitic features are as plain as in this mammary sarcoma. In another growth of the same kind I found the intracellular stages easy to be seen, but other stages were hard to see owing to the parasites being in a more labile state. Fresh teasings are required in such cases. In slow-growing cancers parasites are naturally fewer, but with patience they can be found even in rodent ulcer, one of the slowest and least malignant forms of cancer.

Synchytrium and cancer.—On comparing Fig. 23 with Fig. 54 it is seen how closely the larger intranuclear parasites in this sarcoma resemble the nucleolus of the thistle-*Synchytrium*, presenting club-shaped or more slender knobbed processes from which chromatic substance is differentiated; a conversion of pycno-plasson into chasmatoplasson or into chromidium. The sarcoma parasites in some cases fuse into plasmodia; the latter feature is not so prominent as it is in cysts caused by *Olpidiiforma*, Fig. 46.

In Fig. 24; *a*, the nucleolus of a *Synchytrium* is seen to have become amoeboid; now had such a parasite been reduced to the condition of its nucleolus as is the case in plassomyxines, and had met another in a similar state the two would doubtless have fused into a plasmodium.

In the choriocarcinoma, Fig. 55; *15* to *17* the intranuclear parasites are almost identical with stages of the nucleolus of the thistle-Synchytrium, Fig. 23; *d* and *e*.

In its earlier phases the sarcoma-parasite follows the course of *Plassomyxa contagiosa*, differing from the latter as also do *Cytoryctes variolae* and *C. luis* in that the stages ending in subdivision occur in the host-tissues.

This instance of a typical sarcoma proves Cohnheim to have erred in narrowing the term tumour to exclude infective granulomatous growths.

Epithelial cancer.—To illustrate this variety of malignant tumour I will dwell chiefly on that which arises in stratified squamous epithelium because, as if from synchytrian inheritance, in this habitat the parasites assume their later stages in greater completeness than in less coherent epithelia; but in all spontaneous cancers the pathogenesis is practically the same. With regard to the original site of the parasites in this and other spontaneous cancers it is intranuclear like that of the parasites in the mammary sarcoma described above.

Intranuclear parasites as seen in a section of a breast-cancer are shown in Fig. 56.

The first cancer in which I recognised parasites akin to Sporozoa was a growth arising in that part of the nasal septum which is covered by skin: it had been removed by Scanes Spicer from a man aged 82. The moisture of the cavity in which the tumour grew had allowed parasites, which are usually distorted and disguised in the cell-nests in average specimens of this kind of cancer, to assume their natural shape and to exhibit the process of sporange-formation and subdivision.

The subjoined quotation from Part III (1912) in addition to the description beneath Fig. 57 indicate the more important points.

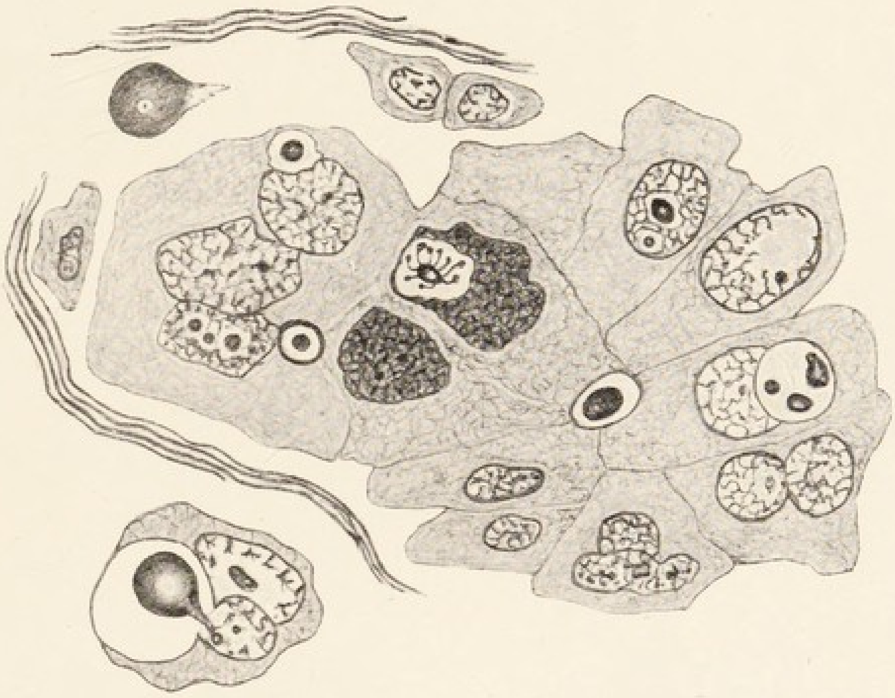

Fig. 56.—Intranuclear, Intracytoplasmic, and Free Parasites in Cancer of the Breast. Drawing eye-piece. × 800 diams. From Part IV.

"I would draw attention to two points, however. First, the occurrence of coccidia-like forms, Fig. 57, *9–12*, in squamous epithelioma similar to those described by Soudakewitch (1892) in cancer of the breast. These forms are brought into series with those shown in *1* to *8* by connecting forms, so they are clearly not coccidia. Insistence on such

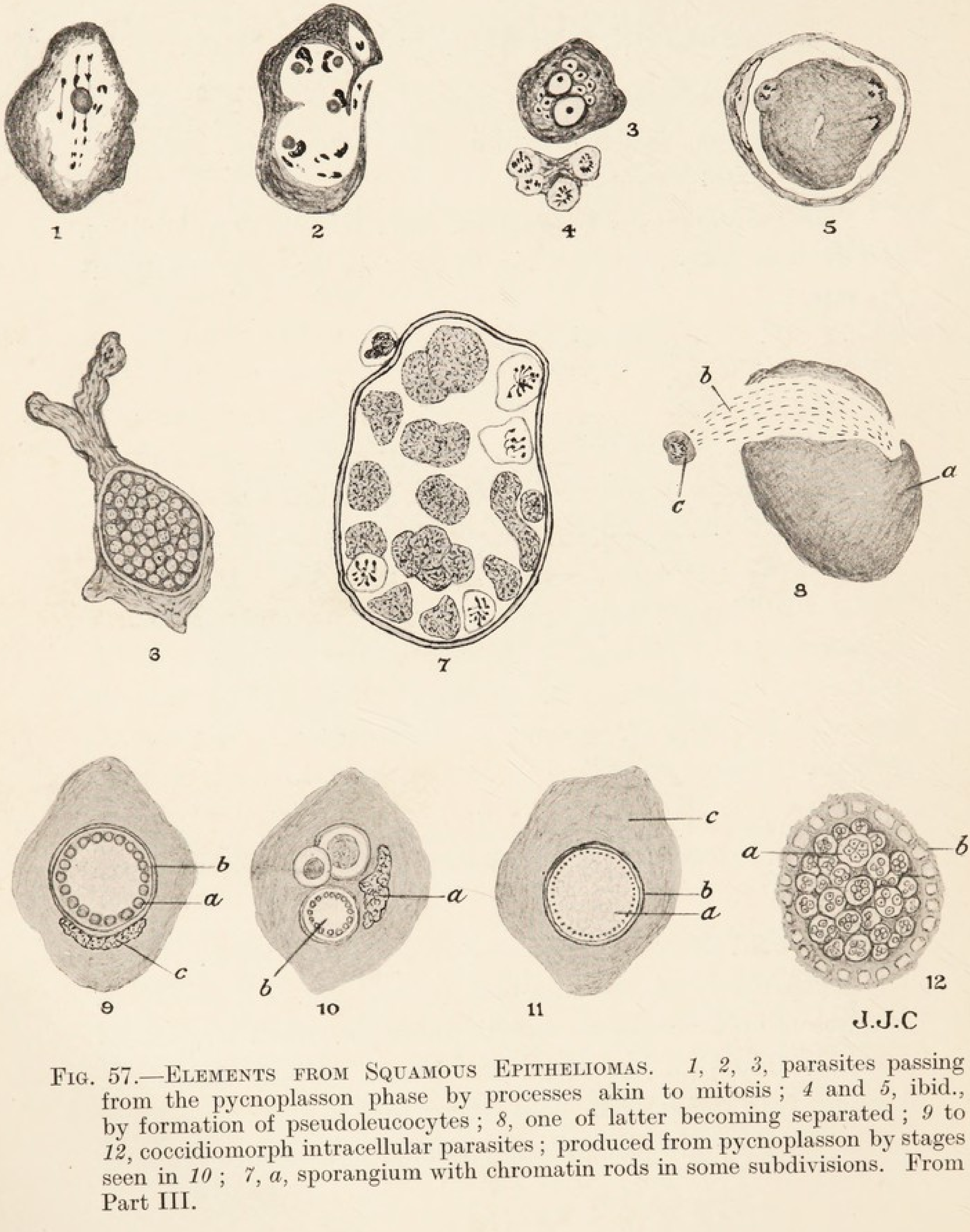

FIG. 57.—ELEMENTS FROM SQUAMOUS EPITHELIOMAS. *1*, *2*, *3*, parasites passing from the pycnoplasson phase by processes akin to mitosis; *4* and *5*, ibid., by formation of pseudoleucocytes; *8*, one of latter becoming separated; *9* to *12*, coccidiomorph intracellular parasites; produced from pycnoplasson by stages seen in *10*; *7*, *a*, sporangium with chromatin rods in some subdivisions. From Part III.

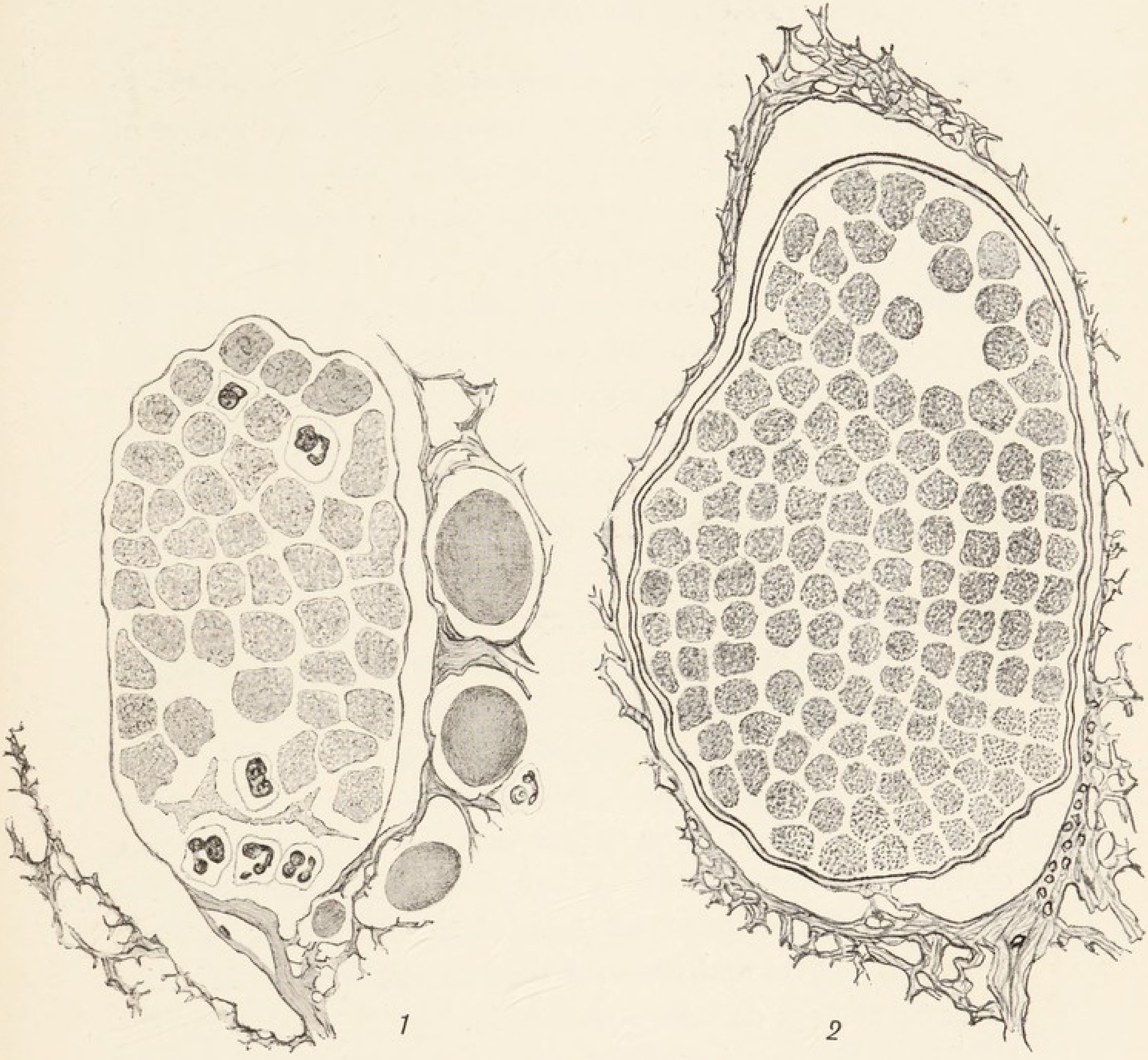

Fig. 58.—Two Sporangia from a Squamous-Celled Cancer of the Septum Nasi
1, A framework contains four parasites ripe for development into sporangia, such as the one in the same framework—which is continuous with its capsule; the latter is poorly developed and six leucocytes have penetrated among the spores; *2*, the large sporangium contains granular spores, its well-formed capsule (=peridium) is continuous at one point with the rest of the framework, which contains some highly refracting granules, probably lime. From Part IV.

and other exclusive forms as the only parasitic protozoa in cancer has really kept back the recognition of the nature of the disease."

Instead of the word "protozoa" in the foregoing extract I should now write "protists."

Sporangium-formation in cancer.—Although seen only occasionally in sections of cancer sporangia are quite unmistakable, as shown in Fig. 58.

Such definitely encapsuled structures do not occur in normal human tissues ; the definite chromatin bars seen in some of the subdivisions of Fig. 57, 7, show that the process is not one of degeneration. By study of even some single sections of this tumour such sporangia were seen to be the end of a series of phases of which the earliest are intranuclear bodies in the plasson state.

Alternative modes of reproduction in cancer-parasites.—In very rapidly-growing squamous-celled cancers the parasites instead of growing for a time in the plasson state, divide by mitosis as soon as they escape from the nucleus in the same way as some of those of the mammary sarcoma described above, and those of the last stage of the infective sarcoma of dogs described in Part II.

To take one instance: At the Hampstead General Hospital a cancerous gland was removed from the left parotid region of a woman aged seventy-one years. The patient had been operated on four months earlier for a growth below the left eyelid, and the pathologist had reported that the growth was a typical squamous-celled cancer.

After removal of the gland, which was of the size of a small walnut, an incision was made into it, revealing a central cavity lined by a layer of yellowish material about 2 mm. in depth, easily separable from the firmer tissue beneath. Sections were made by Dr. Wyatt Wingrave, pathologist, who stained them with pyronin and methyl green.[1]

The section I have is beautifully stained and well fixed. It shows that a layer of healthy parotid was cut away with the tumour, and that the latter is a typical squamous-celled cancer. There are in the younger epithelial cells what appear to be unusually prominent nucleoli. On close inspection some of the latter are seen to have escaped from the nuclei and then to have expanded suddenly, *i.e.* before getting clear of the host-nucleus, and to have become themselves nucleated, and with their nuclei to be dividing by direct cell-division.

The process in this squamous-celled cancer was the same as that of the free parasites of cancer of the uterus described in Part III.

Spirochaetes in cancer.—Spiral forms of parasites consisting almost entirely of chromatin I described in Part II

[1] The following are the details of the method: 3 parts aqueous solution of pyronin; 1 part saturated solution of methyl green. Stain five minutes or longer, transfer to fresh solution of resorcinol (3 grains to watch-glass) for one minute, then to solution of resorcinol in absolute alcohol (0·6 grain to watch-glass) till sufficiently decolorised; then three changes of absolute alcohol, and two of xylene. Mount in dammar-xylene.

in the mammary sarcoma; and Fig. 55, *8* to *10* (chorio-carcinoma), shows other similar forms. Such forms are I think, alternative to the amoebula. Gaylord once met with a definite spirochaete in mouse-cancer. I have no doubt that, if regularly looked for, such instances would be multiplied, but until it is generally recognised that the spirochaete of syphilis is but one of a series of phases of *Cytoryctes luis*, I do not think much can be gained by regular search for spirochaetes in cancer. The infective sarcoma of dogs in which Mettam found spirochaetes might be a useful condition to compare with syphilis.

Cultures of cancer-parasites.—We must imitate nature's ways if we are to succeed in cultivating certain organisms. In the natural process the parasites of molluscum are extruded and fall from the host: we should expect them to develope in water, and so it is found to be by experiment. *Plassomyxa contagiosa* produces broods of new individuals outside their chief host, exogenously as it is termed.

In cancer the parasites breed endogenously passing through repeated cycles which end only with the death of the host.

To imitate nature's way in cancer we must cultivate the parasites in the tissues or fluids of the host and at the temperature of the host. Gaylord has used this method with success.

We have seen, Chapter VI, that the commonest mode of subdivision of *Plassomyxa contagiosa* is by the formation of oil-like spheres, the protoplasm of which may circulate for hours or days.

From a colloid cancer of the peritoneum Gaylord obtained crowds of such greenish bodies, which he found not to consist of fat, and he placed them in tubes in an incubator in the peritoneal fluid. The changes he observed in these bodies were :—1, increase in size with change from a homogeneous to a granular state with loss of the green colour ; 2, Brownian movement of their granules ; 3, formation of pseudopodia ; 4, the formation of nuclei in the previously akaryote bodies. It may be noted that 3 corresponds with the body, Fig. 55, *11*, in a section of a choriocarcinoma, and 4 with a similar appearance of nuclei in *Plassomyxa cont.* Fig. 31, *6* and *7*.

Gaylord injected some of his cultures into the peritoneum of dogs and guinea-pigs, and relates that he obtained in some cases cancerous growths, in others peritonitis.

Besides the endogenous cycles in cancer the parasites would appear also to have an exogenous period in some instances. Such a condition is most likely to be found in epidermal cancer. With material from the secondary growth over the parotid mentioned above I made a water-culture which remained free from bacteria for several days. Water-mounted fragments showed a surprising variety of encapsuled and other protist-like forms, which were entirely concealed in sections. On the 9th day what looked like small zoospores developed from clusters of round homogeneous globules : I hope to repeat this observation.

Plassomyxa forma-maligna.—When we examine a section of a fully-developed molluscum-tumour, such as that shown in Part IV, Plate III, after allowing for the loose parasites that have been detached in mounting the section, we can compute the proportion of parasites to host-tissues as rather more than 1 to 2.

In the section of the mammary sarcoma, Part III, Fig. 32, we see that the proportion of parasites to host-tissue is again something near 1 to 2; and again in the squamous-celled cancer of the nasal septum as illustrated in Plates IV and V, Part III, we see again about the same ratio holds good. As I stated in 1892 about one third of the weight of rapidly-growing cancers is accounted for by the visible parasites present in them.

Can we name the parasite of cancer? We must first consider the transformation of late syphilis into cancer and reflect that in some cases we shall probably be naming *Cytoryctes luis* over again.

By their particular harmony with the host-cells and tissues the parasites produce the anatomical varieties of malignant tumours. Some of these tumours have features so pronounced that a brief inspection by the microscope enables the pathological diagnosis to be made; in others, again, only after careful study can a decision be reached; and, in not a few cases, it is impossible by the microscope alone to distinguish between cancerous and syphilitic or other inflammatory processes.

In general, however, we can recognise a special setting of the parasites in cancer, and without prejudice to future specific recognitions we may name the parasites: *Plassomyxa forma-maligna.*

Imitation-cancer.—If I wished to make an imitation of cancer, I would devise a set of the thinnest knives about 1 mm. long mounted in bunches at the ends of fine steel stems. Pushing with aseptic precautions a bunch just through the skin the knives should be left in place till the epidermal cells had grown beyond their points, which then, increased in number, should again in various directions be pushed deeper. This process repeated for a long time would reach lymph-glands, and a series of ropes of epidermal cells would stretch from skin to glands.

Those, who from day to day are exposed unprotected (as in screen-work) to X-rays, repeat in a way the above imaginary experiment; the connective-tissue-damaging rays taking the place of knives. As is shown in Part IV X-rays epithelioma contains no parasites: it is an imitation-cancer.

The best imitation-cancer I know of was obtained by Lambert Lack, who injected an emulsion of its ovary into a rabbit's peritoneum. Cancer-like growth followed in peritoneum and glands. Mr. Lack kindly gave me two sections. Careful examination showed there were no parasites; it is an imitation-cancer, a very exceptional case of cell-transplantation. It might easily have been a real cancer, if the rabbit had had in it seasoned plassomyxes, which found in the emulsified cells suitable hosts.

In some minds will arise the question—"What about khangri-pots and tar?" Cancers induced by such means should be examined carefully. If they are of the same nature as common spontaneous cancer, they will be found to contain plassomyxes; and the only conclusion that can be drawn will be that these two agents are favourable to the establishment of that particular relationship between tissue cells and parasites that constitutes common human cancer.

And Cohnheim's embryonic rests? Where such rests really exist their cells may function in producing for us internal secretions; when they are the seat of cancer it means that certain plassomyxes have found them to be suitable hosts.

Metastasis.—It is usually assumed that the only way by which a secondary malignant tumour can arise is by metastasis, that is by the detachment of a portion of the original tumour and development of the transplant in another part of the patient's body. In sarcomas as, for example, Fig. 46; *12*, we see many parasites free in small veins, therefore in such tumours secondary growths can arise simply by the parasites finding elsewhere host-cells similar to those of the original tumour.

The mammary sarcoma referred to above is a granuloma, and examination showed that the secondary growths arose in all probability by migration of parasites. That metastasis may occur in sarcoma is also seen in examining in the same tumour the multitude of large amoeboid parasites lying in intercellular spaces could easily detach portions of the growth and, if the host-cells of the latter had been rapidly dividing, metastases would have occurred.

Where a melanotic sarcoma of the choroid of the eye is accompanied by secondary growths in the liver, we assume that metastasis has occurred, and so it may be, but not necessarily; for the parasites may be capable of causing granular pigmentary change in previously unpigmented connective-tissue or endothelial cells.

In epithelial cancer, as for instance where growths secondary to a cancer of the thyroid are present in the skull, metastasis must have occurred and it is easily explained by the movements of the parasites detaching portions of the tumour into lymphatics or veins and so into the blood-stream to find lodgment in parts best suited to growth of the transplants.

Conclusions.—Before one ventures to formulate conclusions the real objective position should be clearly defined. In parasitism by common fungi we saw in Chapter II how vast numbers of the parasites perish. The same feature presents itself in syphilitic gumma and in sarcoma. This fact was emphasised in 1901 by Gaylord:—" All the organs, including the blood, taken from all regions of all cases dying of cancer, including sarcoma and epithelioma, contain large numbers of the organisms." Fresh teasings reveal the presence of parasites in forms that are concealed by routine histological methods. Besides visible forms there are doubtless many in the microhenad phase. Secondary growths are few compared with the number of parasites distributed through the host body.

Cancer being so common we all at some time or another probably have in us potential cancer-parasites. How do they get into us ? It has been shown above and in Part IV that in old-standing syphilis cancer seems to arise in a peculiar relationship of *Cytoryctes luis* to the host-cells. A similar relationship between the parasites of measles and other exanthems may possibly produce cancer : these are problems for future investigation. The frequent presence of *Karyoryctes* in water-protozoa suggests the question whether that or similar plassomyxines may not in some cases become *Plassomyxa forma-maligna* in man or other mammal.

Adenoma of the breast, &c.—The cells which line the younger cysts of this common fibro-cystic tumour are swollen, and contain spherical inclusions of colloid-like (pycnoplasson) aspect, the largest on escaping by breaking-up of the cell become a mass of fine granules in the lumen. These and other features point to these bodies being of the same nature as the plassomyxes described above in smallpox, syphilis and cancer. In the latter disease as might be expected the parasites are in a state of more aggressive vitality than in the simpler adenoma, and until the more demonstrative features of the parasites in cancer are accepted

I do not think much is to be gained by a detailed account of foreign structures met with in benign neoplasms, with the exception of molluscum contagiosum, where they can easily be cultivated. But the appearances in breast adenoma bear one important message, namely that plassomyxes are capable of indefinitely prolonged life and reproduction in the plasson state.

Cell-inclusions are abundant in some adenomas of the thyroid gland, and in the common tumour of the prostate certain features suggest that plassomyxes exist in intimate association with connective-tissue cells.

Hydrophobia.—The incubation of this disease is of very variable duration, between 15 and 60 days as a rule but it may be even longer.

Negri first described corpuscles in cells of the central nervous system of animals killed whilst suffering from hydrophobia. He regarded the bodies as parasites and named the species *Neuroryctes lyssae.*

Negri-bodies are visible in fresh unstained preparations. In dogs that have been killed on account of this disease (street rabies) the bodies may measure 18μ or more; in rabbits that have been inoculated with virus that having been passed through a series of rabbits has acquired a definite virulence (fixed virus), the bodies are small, $\frac{1}{2}\mu$ or less.

By means of Negri's bodies a diagnosis can be made in a few minutes from smears of a suspected animal's brain.

For this purpose Williams and Lowden recommend smears made by placing small pieces cut by scissors from the Cornu Ammonis, the Rolandic area, and the cerebellum. A fragment is laid on a slide leaving room for a label, then a coverslip is laid on the fragment and by even pressure made to move to the opposite end of the slide.

The smears are dried in air and stained either by Giemsa's method after fixation in methyl alcohol, or by Mallory's eosin-methylene blue stain after Zenker's fluid. Not only Negri-bodies but also the swellings on the nerve-fibres, and the collections of lymphoid cells are well shown by this method, which the authors prefer to the section method. Only in animals infected by fixed virus do sections give better results than smears. The bodies were found on the 4th day in fixed virus cases, and on the 7th day in street virus cases.

The elements *a*, and *b*, in Fig. 59 I drew from a section kindly given me by Professor J. H. Ashworth, who had it from the Pasteur Institute, Kasauli, India. It appears to be stained with H. and E. We may note in the cell *b* that the nuclear membrane is broken at the upper end and granules appear to have been passing from nucleus to cytoplasm; below, again, a pyramidal process joins the nucleolus to the nuclear membrane, as if in preparation for a subsequent discharge of granules.

Now it would be a reasonable suggestion that the Negri-bodies resulted from a fusion of such granules. This would hardly account for chromidial forms, *c*, and not at all for well-formed nuclei such as that at *d* being present in some of these bodies: such nuclei recall *Olpidiiforma*, Fig. 46, *3*, and the temporary nuclei in *Plassomyxa contag.*, Fig. 31, *6* and *7*; in syphilis, Fig. 52, *x* and *y*; in cancer, Fig. 55, *5*, &c.

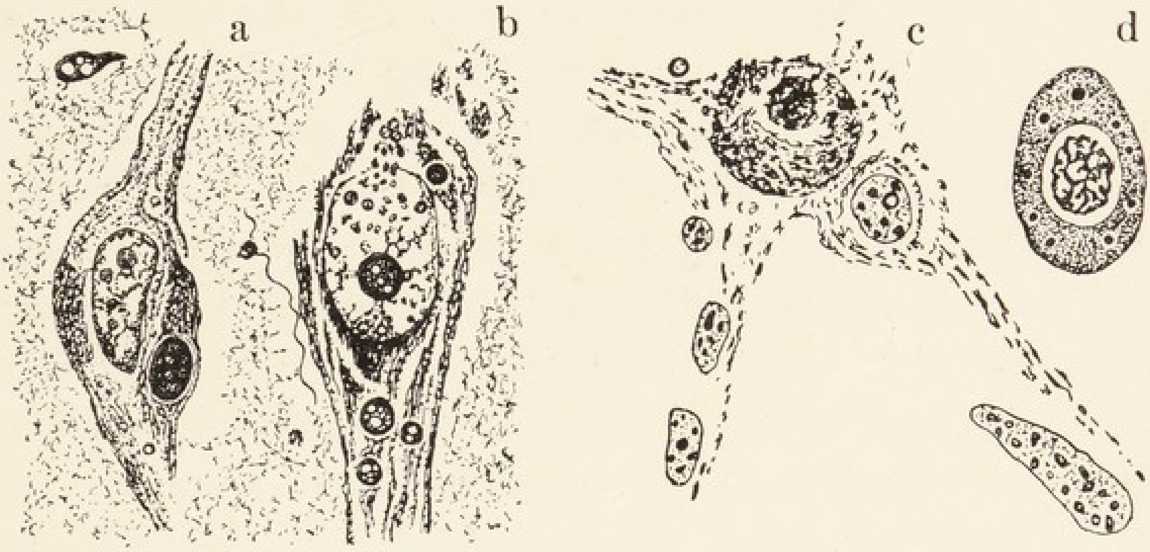

FIG. 59.—NEGRI'S BODIES IN HYDROPHOBIA. *a* and *b*, two adjacent cells in the Cornu Ammonis of a dog; *a* contains a vacuolated Neuroryctes, another is in the brain substance near; *b*, contains three small parasites below the nucleus; *c*, brain-cell of a dog from a smear and five large parasites; *d*, a large parasite with a nucleus. *a* and *b*, × 1000; *c*, after Williams and Lowden; *d*, from Calkins after Negri.

Pasteur.—One cannot leave the subject of hydrophobia without thinking of one name, that of Louis Pasteur, who began his work on hydrophobia in 1880 and treated his first human patient with success in 1885. It may be wholesome for us to reflect that perhaps the greatest name in the history of medicine is not the name of a medical man.

Pasteur's antirabic vaccine is the only true vaccine besides the vaccine of Edward Jenner that is used in human medicine. The first injection is an emulsion of infected rabbit's spinal cord desiccated during 14 days; and, in increasing strength, doses of fixed virus are given for 15 days, the last being of 3-day cord.

Nucleoli.—"The nature of true nucleoli is still imperfectly known" wrote Wilson in 1904. It may well be that study of the large nucleoli of brain cells in hydrophobia will afford some information. The granules escaping from the nucleus at *b* in Fig. 66 may represent the immediate means of defence possessed by the brain-cell. Wilson mentions that the subcutaneous gland-cells of *Pisciola* contain but a single nucleolus, but during growth of the cell the nucleolus fragments into several hundreds of nucleoli, which then appear to migrate into the cytoplasm, leaving but a single one to grow and repeat the process. Writing of chromidia in Part IV I suggested that the specific products of the highly specialised cells of metazoa, for example the secretions of gland-cells, may be chromidial in origin. It seems as though in nuclei that have permanent chromosomes there may be a division of labour, reproduction devolving chiefly on these whilst the nucleolus, in what we call resting nuclei, furnishes secretions of different kinds for diverse purposes.

Vaccines and pro-vaccines.—As stated above there are in human medicine only two vaccines, the Jennerian and the Pasteurian. In general a vaccine is definable as an attenuated culture of a pathogenic organism; *i.e.* it contains living parasites. By appropriate treatment when they are cultivable on artificial media such vaccines can be 'fixed' at any given grade of virulence. Although it is not cultivable artificially the virus of hydrophobia can be fixed as explained above.

In misleading language the term 'vaccine' has been applied to an important class of preparations of dead bacteria. They should be called 'pro-vaccines.' In the late war the immunising pro-vaccine of the typhoid group of bacteria appears to have been successful and its originators deserve our gratitude. If a pro-vaccine could be found to replace *the* vaccine efficiently, such a service to humanity would be greater even than that of Jenner. Unfortunately no sterile substance, whether immunising serum or pro-vaccine, has as yet been found to influence the course of any of the common exanthemata.

Specific remedies for specific fevers ?—A community unvaccinated and never exposed to measles or scarlet fever suffers terribly whenever smallpox or other exanthematous fever appears in it: the healthiest, noblest specimens of manhood and womanhood die, we having no remedy.

Ought we to seek attenuating hosts for the parasites of measles, scarlet fever, &c. as we have the calf to attenuate the virus of smallpox ? Our knowledge of syphilis gives us pause. We know that immunity to syphilis means having the disease :—having the specific parasites living in the tissues of the immune person.

We cannot advise vaccination against syphilis, the parasite of which may lurk in the tissues for a number of decades without evidence of their presence, and then produce some obvious late lesion. Have the common exanthems sequels corresponding to those of the fever of syphilis? Looking through the index of any book on diseases we note in addition to cancer and exanthems others of unknown causation. May not some be caused by the parasites of long-past fevers the causal germs of which still live in us?

We have specific treatment for syphilis. If we had safe and sure specific remedies for the common specific fevers, we might by their use prevent other diseases including possibly some cases of cancer.

Virchow.—In all its epochs pathology has been befogged by more or less mock-knowledge: we have had our own nebula, "parasyphilis," which a little real knowledge has dispelled. Virchow cleared away a great accumulation of such pseudopathology. He found Schwann's merit to consist in the recognition of the cell as the origin of all tissues and physiological properties. His own instruction reads:—"I formulate the doctrine of pathological generation, of neoplasia in the sense of cellular-pathology simply: *omnis cellula a cellulâ.*"

Tumours in present-day pathology.—Having been no more than an amateur in pathology for 26 years past some important advance may have escaped my notice; at any rate I can only think of one accepted change that has been made in the last 30 years respecting the pathology of cancer: what used to be called an alveolar sarcoma is now called a perithelioma. The insistance is on histogenesis; a slight movement on a road well made by Virchow. And what a good road it has been and will continue to be; helping us to decide points in diagnosis and prognosis, and affording reliable indications as to treatment; helping us to trace the primary seat of growth when the tumour that is the first to show itself clinically is found to be composed of tissue foreign to that in which it occurs! Certain types of cell-forms and of structure in tumours we know to be associated with corresponding degrees of malignancy.

Beyond Virchow's is another road, at present a mere track in places. The old road must be patiently traversed before the new one can be reached. Unless this new road is followed patiently and thoughtfully, and all the menacing objects it brings into view are scrutinised critically and tested by experiments planned on natural lines, the causes of the diseases dealt with in this book and of many others must remain unappreciated.

CHAPTER XI

CHROMIDIA AND NUCLEI OF A PROTOZOON

AMONG the vegetable protists noticed above the chytridian *Polyphagus euglenae;* olpidians, such as *Rozella septigena;* and synchytrians present features similar to some found in rhizopods and sporozoa.

Facts stated above indicate that Plassomyxineae are closely related to Synchytriaceae, and hence they too must be considered as belonging to the vegetable protists. Mycetozoa have so many rhizopod features that their animal predominate slightly over their vegetable characteristics. In the present Chapter is given a brief account of chromidial and nuclear features of a definitely animal protist, one of the shelled amoebas. The shelled amoebas have no pathogenic members. Matters relating to amoebas parasitic in man have been defined by Dobell (1919); *Entamoeba histolytica,* the cause of amoebic dysentery has no connection with any free-living organism. It was discovered by Loesch in 1875, and named by him *Amoeba coli,* under which name it is described and illustrated in Part I. It has not been cultivated on artificial media. Extranuclear chromatic structures occur in it and were called chromidia by Schaudinn, but Dobell states that they have nothing in common with true chromidia such as occur in *Arcella.*

It has been recorded in Part IV how complicated the life-cycle of a common amoeba is. Calkins has followed the nuclear process in *Amoeba proteus.* In the case of organisms of indefinite form it is difficult for the student to retain a mental picture of a complicated series of nuclear and chromidial processes, and of cell-divisions. This is easier in the case of the shelled amoebae. Of one of these, *Arcella vulgaris*, the life-history has been worked out with some degree of completeness,[1] so we may take this organism as an example.

Arcella (Ehrenberg) is a common and widely distributed genus. *Arcella vulgaris* occurs chiefly in bogs and other still waters. Seen in surface view it has the appearance shown in Fig. 60 : *1.* Its flattened dome-shaped brown-coloured shell is finely tessellated, and measures from 80μ to 140μ across. In profile view the shell is seen to have a diaphragm with a central perforation, the pylome, less than one-third the width of the shell. A section of the shell shows it to consist of an inner continuous chitinous layer, slightly exaggerated in thickness, *2, b* ; the close-set cuboidal elements of the outer layer are cemented together by the same substance as that which forms the inner layer. From

[1] In order to compile the following sketch I consulted various authors: Delage and Hérouard, 1896; E. A. Minchin, 1901; G. N. Calkins, F. Doflein, and S. J. Hickson, 1909. The chromidial phases were worked out by R. Hertwig, the structure of the shell by Awerinzew, the gametes by Elpetiewsky.

the pylome three or four finger-like pseudopodia project.

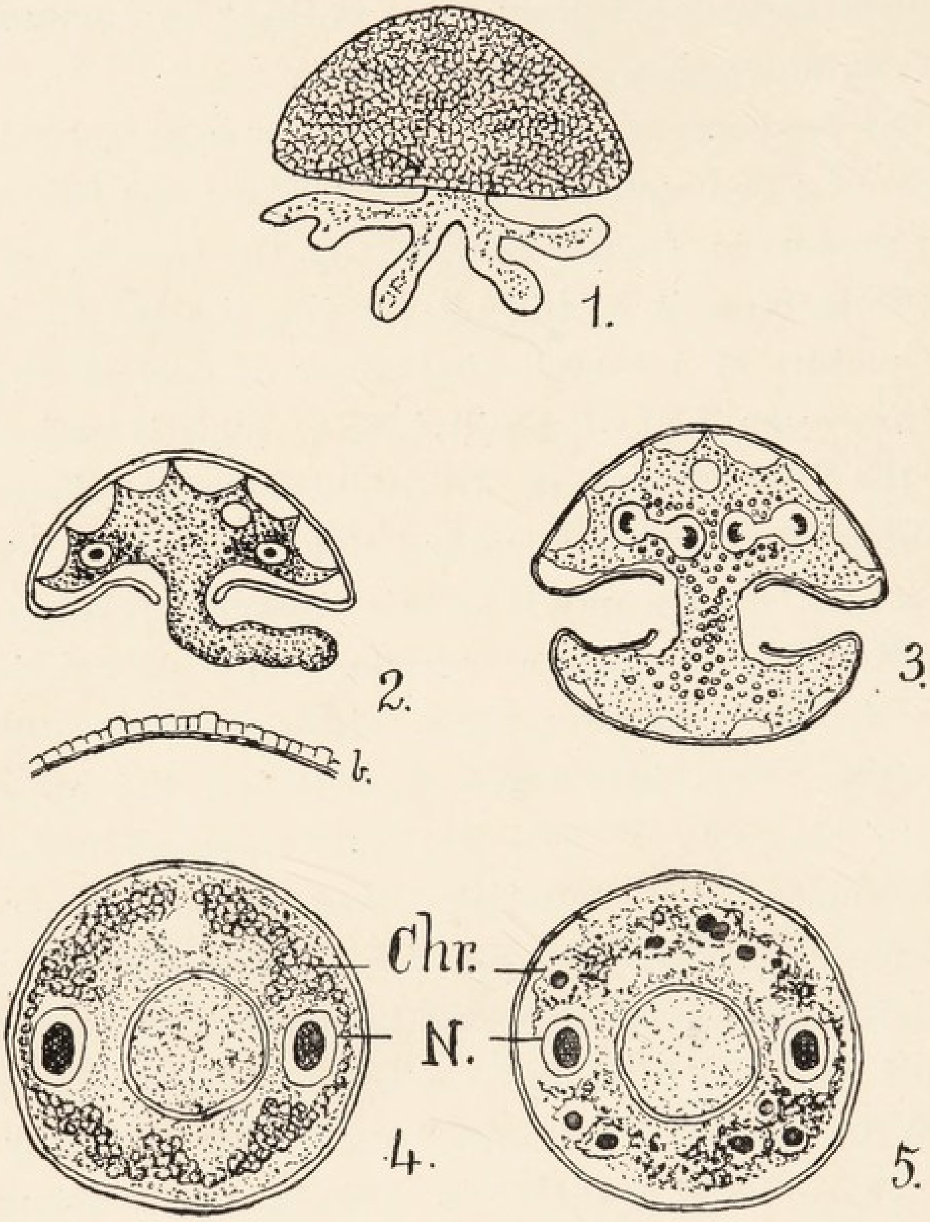

FIG. 60.—ARCELLA VULGARIS. *1*, Surface ; *2*, Profile ; *b*, Section of part of shell ; *3*, Binary division ; *4*, (seen on the flat) Resting state, trophonuclei, N ; and chromidium, Chr. ; *5*, Formation of new nuclei from chromidium. From various sources.

In the vegetative phase, which is assumed in summer when food abounds, the animal has two nuclei, each with a large nucleolus and a nuclear membrane. A circle of gas vacuoles is often seen round the pylome, or they may be united into one eccentric vacuole. One or more contractile vacuoles are also usually present. The animal does not completely fill its shell with which the ectoplasm is in contact only at a number of points.

In the ordinary binary division the two nuclei divide simultaneously by a simple form of mitosis, *3*, after part of the animal's body has been protruded through the pylome. The two new nuclei with other cell-elements pass into the protruded part which secretes a new shell, and soon separates from the parent organism.

When an *Arcella* is viewed from above the outline of the pylome shows as an inner doubly-contoured ring, and another and very important feature is seen—the chromidium, *4* and *5*, *Chr.* In the vegetative state of the animal the chromidium consists of a circle of rather coarse granules, which pass to the outer side of the nuclei with a little space between. In winter the chromidium plays its part in reproduction in various ways. First, the fine granules of chromatin, hardly noticed between the other granules of the resting chromidium, become larger and run together to form new nuclei. The subsequent fate of these is indicated in Fig. 61, *5* to *11*.

In certain conditions *Arcella* encysts, forming a dense cyst-wall which blocks the pylome. Before encystment occurs food-remains (diatom shells) are extruded into the

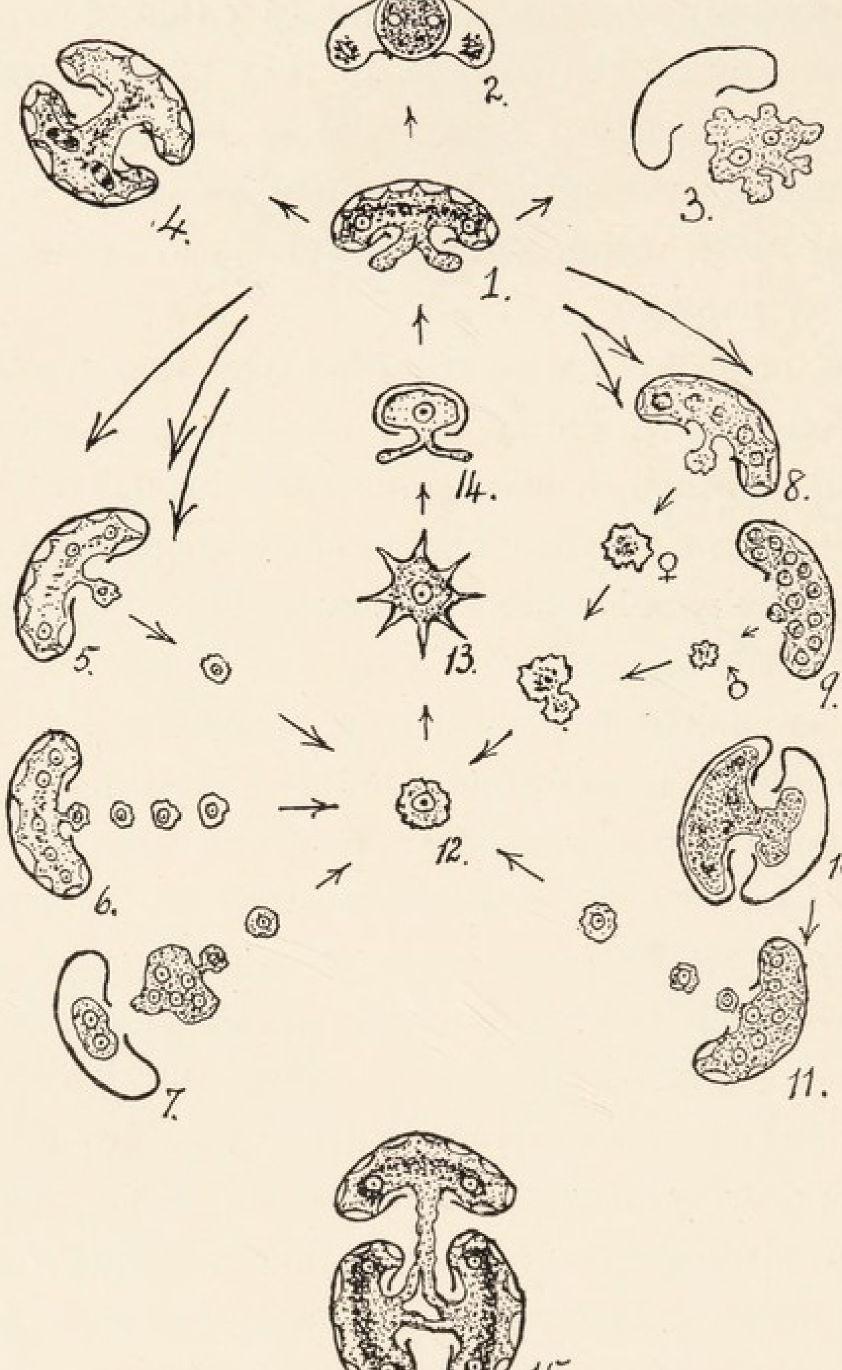

Fig. 61.—Diagram showing the Chief Life-Processes of Arcella vulgaris. From various sources.

empty corners of the shell, Fig. 61 : *2*. At other times the animal leaves its shell, *3*. The chromidial reproductive stages are of two kinds, non-sexual and sexual ; and the sexual processes are again of two kinds, nuclear (karyogamy) and chromidial (chromidiogamy). The asexual chromidial reproduction is shown in Fig. 61 : *5*, *6*, and *7* ; really three degrees of the same process. New nuclei formed from the chromidium, together with the adjacent zone of cytoplasm, become buds, which may be formed slowly, *5*, or more rapidly, *6*, or very rapidly after the animal has left the shell all but the two trophonuclei with a little plasm, *7*. Karyogamy is depicted in *8* and *9*. Many chromidial nuclei, rather larger in the female than in the male gamont, are formed, and they become the nuclei of the male and female sex-cells (gametes), which coalesce.

Chromidiogamy is shown in *10* and *11*. Two *Arcellae*, after adhering by their pseudopodia, come pylome to pylome, and they fuse together, nearly the whole of the substance of one animal passing into the shell of the other and, after mixing, returning again. The animals then separate. The trophonuclei break up and new nuclei are formed from the chromidium to become the nuclei of buds. In whatever way buds are formed they all pass through the same phases before they assume the Arcella-form. These phases are shown in *12*, *13*, and *14* ; *13* is called the *Nuclearia* stage.

Still another vital process common to many protozoa, and called either plastogamy or plasmogamy, is illustrated in *15*. Several *Arcellae* join pseudopodia, protoplasmic currents are interchanged; the animals then separate.

The above brief survey of the salient life-processes in one of the Rhizopoda Lobosa suffices to show that the chromidial phases are essential, and that the sexual reproduction is confined to them. If the summer phases of the organism alone had been studied the most important part of its life-history would be unknown.

If we examine an *Arcella* in the stage shown in Fig. 60: *5*, separate granules stained like chromatin are recognisable in the cytoplasm. The term "chromidiosomes" has been applied to such granules.

The cell-theory adjusted to facts.—As depicted by different observers *Arcella vulgaris* passes through two non-nucleated phases; namely, that immediately preceding the condition shown in Fig. 61, *8* and *9*, and that shown at *10*. These two may be considered as modifications of the same phase.

The distributed nuclear matter seen in some Ciliata such as *Chaenia teres*, and in Cyanophyceae, such as *Chroococcus turgidus*, may, I think, be regarded as true nuclei. The Chroococcaceae were regarded as non-nucleated by Haeckel. It is different with the chromidial phases of organisms such as *Arcella* and *Sorosphaera* (Chapter VII): in these new nuclei arise, not by simple transverse division, but by confluence of chromidial granules.

We may infer from the unqualified acceptance of Flem_ ming's "*omnis nucleus a nucleo*" in even the most recent works on cytology, that the well-known fact just stated has not been duly digested in biology. One effect of this is seen in the otherwise unaccountable grouping of *Karyoryctes cytoryctoides* with *Nucleophaga amoebae* as related in Chapter X.

The confusion just indicated is to be explained only by want of recognition in biology of the plasson state of living matter as seen in the zygotic sporange of *Synchytrium*, Fig. 23, *d* and *e*, and in pathogenic organisms such as are shown in Fig. 29, and Fig. 54, *1* to *4*, &c.

That Borrel did not realise that the appearance of new nuclei in *Cytoryctes variolae*, Fig. 48, *2*, proves this body to be a parasite can only be accounted for by the inadequacy of the cell-theory as at present accepted.

The streaming protoplasm of *Plassomyxa contagiosa* has no recognisable structure, but new nuclei are occasionally formed in molluscum bodies, as they are in the parasites of syphilis and of cancer.

All these instances are exceptions to the "*omnis nucleus a nucleo*," and herein we may see the explanation of the fact that smallpox and other fevers, cancer and other tumours are academic enigmas to-day.

If the cell-theory is adjusted to amply proven facts it must read :—**New nuclei arise not only by division of pre-existing nuclei, but also by free nucleus-formation from chromidia or from plasson.**

ABBREVIATIONS, &c.

M.G. and S. stands for my "Morbid Growths and Sporozoa"; condensed from "Cancer, Sarcoma, and other Morbid Growths Considered in Relation to the Sporozoa," published in 1893.

Parts I, II, III, and IV, stand for the parts of my "Protozoa and Disease," published in 1903, 1908, 1912, and 1915, respectively.

H. and E. means haematoxylin and eosin; *i.e.* acid haematoxylin made according to Ehrlich's formula, and Gruebler's water-soluble eosin; the stains being used in the ordinary way.

E.-B.-H. means Ehrlich-Biondi-Heidenhain stain.

Ehrlich's acid haematoxylin :—*haematoxylin* 2 gm. dissolved in *alcohol abs.* 100 c.c.; add *water dist.* 100 c.c.; and *acetic acid, glac.* 10 c.c.; and alum, to saturation.

The stain must "ripen" by being kept in the light and having the stopper of the bottle removed to admit air from time to time. When ripe it has a dark red colour, the haematoxylin having been oxidised to haematein. The mixture can be kept for years without deterioration. It answers well for staining in bulk. For staining sections it is diluted with distilled water, sections become a brown colour, the blue colour being developed in tap-water.

For counterstain, water-soluble *eosin* (Grübler's) kept in alcoholic solution is mixed with water, a few drops to a watch-glass.

Ehrlich-Biondi-Haidenhain triple stain.—Saturated aqueous solutions of *orange* (100 c.c.), *acid fuchsin* (20 c.c.), *methyl green* (50 c.c.) are mixed in that order with constant stirring. Dehydrate rapidly with alcohol, clear in xylol and mount in xylol-balsam. This stain is brilliant but fades so rapidly that I do not now have it used when a permanent preparation is required.

Foa's solution : 1, solution of mercuric chloride in 0·75 saline solution made by boiling excess of the salt and allowing the solution to cool ; 2, 5 % a solution of bichromate of Potassium. Small portions of tissue are placed in a freshly made mixture of equal parts of 1 and 2 and after 12 hours are transferred to running water, then to 75 % alcohol and then to graduated alcohols to which tincture of iodine is added to remove the mercuric salt from the tissue ; finally to absolute alcohol.

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WILLIAM CLOWES AND SONS, LIMITED, BECCLES, FOR
BAILLIÈRE, TINDALL AND COX.