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THE LIFE CYCLE OF THE PROTOZOA[1]

THE obligation which this occasion brings offers a challenge to the zoologist whose pleasant privilege it is to address you, to summon from his experience his ripest judgment upon the problems of greatest interest in his field of investigation. It affords him the opportunity to round up his vagrant ideas, to corral his scattered observations and to brand them with the symbols which we all recognize. He must therefore leave the specialized field in which he ranges and come into the arena of our common and central problems.

The Protozoa, according to our generally accepted view of the evolution of the animal kingdom, stands at the base of the animal tree of life. From them have sprung, perhaps in a polyphyletic fashion, the other phyla. The structure of the collar cells of the Porifera suggests the origin of this phylum from the Choanoflagellata. The occurrence of nematocysts, tentacles and eyespots in the Dinoflagellata turns our attention from them to the Cœlenterata, while some ciliates and the platyhelminthes have much in common. Be this as it may, the Protozoa are found in the oldest fossiliferous rocks, and the genera of Radiolaria therein conform rather closely to genera living to-day, while the fossil Dinoflagellata of the flints of Delitzsch are scarcely distinguishable from species living in the modern seas. The striking similarities of the most ancient fossil Protozoa to recent ones afford some ground for the inference that the Protozoa living to-day differ but little from those when life was young. We may therefore turn to this group with some confidence that the phenomena which we discover therein to-day are

[1] Vice-presidential address before Section F—Zoology of the American Association for the Advancement of Science and presidential address before the American Society of Zoologists at Boston, December 27, 1922.

both significant and instructive as to form, structure and function, when the waters of the primitive seas formed the first balanced aquarium.

The Protozoa appear to have emerged at that level in the evolution of living substances in which the nucleus was being established, superseding the phase of distributed chromatin and substituting a permanent organization of chromatin within a nuclear membrane for the temporary aggregation of chromatin granules at certain critical stages in the life cycle, such as division. While it is as yet too early in the period of the cytological exploration of the Protozoa to conclude that the evolution of chromosomes first took place in this group of animals, it does seem highly probable that this is true and quite probable that most Protozoa have definite nuclei and that they have chromosomes and that they conform in principle to the basic features of mitosis in the Metazoa and Metaphyta.

The seeming exceptions to this conclusion which we find in accounts of the *de novo* origin of nuclei from chromidia lack adequate cytological evidence and are in my opinion based upon false interpretations of cytoplasmic contents and conditions resulting from the cycle of metabolism in part, and, in a few reported instances, are based upon undetected parasitic infections. Our confidence in the conformity of the Protozoa to the integrity of and descent of nuclei and in the individuality of chromosomes which we find in the Metazoa, increases steadily as sound cytological investigation of the group progresses. We may therefore infer that the Protozoa have a cellular organization and are equipped with the essential structural basis for the mechanism of heredity, in so far at least as the possession of chromosomes, mitosis and even chromomeres and also genes are concerned.

With the presence of this equipment established in the group we may well ask in how far these supposedly simple organisms have also taken on the complicated life history of the Metazoa and Metaphyta. It is to several aspects of this question that we will turn our attention more closely this evening.

The searcher for the origins of biological phenomena will find the Protozoa a fertile but perplexing field for their discovery. Here have arisen all the fundamental types of symmetry, spiral, both leiotropic and dexiotropic, radial, bilateral and a host of modifications of these. Here also are several distinct types of mitosis, different locations of the centrosome, extraordinary derivatives of this organ ranging from the nematocysts of the dinoflagellates to the complicated neuromotor systems of the trichonoymphid flagellates. Sex and sexual dimorphism, ranging from slightly different staining reactions between gametocytes to profound sexual dimorphism of gametes, have had their origin here. The Protozoa have provided the arena in which the primitive pageant of life made its first parade before it settled down into the more humdrum and less interesting mediocrity of metazoan and metaphytic conformity.

Let us then turn to the fundamental problem of the life cycle. Within the phylum of the Protozoa there have been evolved certain developmental processes which seem to have a rather precise correspondence with processes which constitute the fundamental parts of the life cycle of the Metazoa. These have attained their fullest development only in the highest representatives within the several classes of Protozoa, such as the ciliates, flagellates, Sarcodina and Sporozoa. They appear to have evolved independently in the several groups. Their origin thus seems to be polyphyletic. These points of agreement are:

1. The elaboration of the mechanism of heredity up to the point of nuclei with chromosomes whose individuality, continuity and diversification appear to be comparable with that attained in the metazoan nuclei.

2. The development of sex, of sexual reproduction—perhaps of the sex complex of chromosomes, and coincident with these the phenomena of gametogenesis and fertilization comparable in essentials of chromosome individuality and segregation with the conditions in the Metazoa.

3. The universal occurrence of the phenomenon of asexual reproduction, not to be confused with the attendant phenomenon of mitosis of unicellular organisms. Mitosis is important because of the unicellular state in some Protozoa.

4. The development of a multicellular stage following fertilization or following its possible equivalent; this multicellular stage I shall

designate as a somatella. In it there is generally no progress to the point of division of labor and differentiation of tissues, although the differentiation of sexual and somatic cells occurs in some instances, as in *Paramœcium* and *Volvox* and in the pansporoblast of the Sporozoa, and histological differentiation of the somatic cells is clearly arrived at also in the pansporoblast.

5. The occurrence of death as a biological phenomenon as normal to the Protozoa as it is to the Metazoa.

In addition to these unifying features which tend to bring into one biological scheme the Metazoa and the higher Protozoa, we have the outstanding fact to which biological thinking of the time has given too little attention; namely, that in many, possibly most, of the species of most of the Protozoa, and indeed in the still wider group of the Protista as well, sexual reproduction has never been found and probably does not occur in some species in which it has been reported. Nevertheless, there prevails among these primitive organisms the phenomenon of evolutionary divergence, or speciation, and the origin of specific distinctions. These distinctions seem to be indistinguishable in scope and kind from those of the Metazoa when tested by genetic studies, by morphological and cytological analyses, and by the more precise refinements of biochemical relations as revealed in serological reactions. Let us now turn to a fuller consideration of these points which I have enumerated as having been evolved in the group of Protozoa.

Prior to taking these up let us remind ourselves of the fundamentals of the life cycle of the living substance which constitutes so important a feature of this aggregate of matter and energy as over against that which constitutes the non-living world. The essential elements of such a cycle, be it in the plant or animal, in the Protozoa or the Metazoa, the Protophyta or the Metaphyta, appear to be the following:

We begin the cycle with the zygote, carrying two sets of ancestral genes contributed to the individual by the haploid gametes, thus establishing a diploid state in the individual. This diploid condition of the nucleus of the zygote is then transmitted to all nuclei of all cells of the subsequent subdivisions of the zygote until segregation takes place in gametogenesis.

The interaction between nuclei containing these genes and the cytoplasm in which they are found presumably controls by chemical action the subsequent processes of growth and differentiation and thus determines the volume and nature of new cytoplasm and its contents and the rate of its production. This control persists throughout the whole of the life of the zygote and of all of its subdivisions. We may designate the sum total of living substance thus controlled in all of the succession of functional individuals as the zygotic or Huxleyan individual.

The first step in the development of the zygote is cleavage and the formation of a multicellular complex which, in higher forms, becomes differentiated by histogenesis and organogenesis into complex and specialized organs of the individual.

The subdivision of the zygotic individual into varying numbers of functioning individuals by various processes of asexual reproduction is made possible by the persistence of undifferentiated tissues or by processes of dedifferentiation. The initial genetic equipment of the zygote thus continues its control throughout the life of all of these subdivisions resulting from asexual reproduction of different patterns. The genetic type of organization established by the fusion of the gametes maintains its integrity throughout this process, although the various bodies which may carry it yield to the assaults of the environment.

The universality of this phenomenon of asexual reproduction is one of the striking features of the living substance, especially in the Protozoa, where we see it manifested in binary and in multiple fission and sporulation of various types. We find it in the Metazoa in the budding processes of hydroids and medusæ, transverse segmentations of planaria and of the strobila of the tapeworm, in the various types of gemmules, statoblasts and brown bodies of Bryozoa, in the embryonic fission of midges and of the armadillo, and in identical twins in man. It may also appear in teratomata and embryomata of vertebrates and perhaps bears some relation to the potentialities of the living substance to afflict itself with malign neoplasms. The fact seems to be that the living substance of the individual is constitutionally unable, even in its highest levels of evolution, to wholly shake off the deeply rooted tendency to reproduce others of its kind or other kinds, or to give rise to some other phase of a life cycle by some form of asexual reproduction. Broadly viewed, the whole evolutionary process in plants and animals appears to have progressed by the suppression of asexual reproduction and increasing emphasis on the differentiation of the body of the zygote as first produced.

It is therefore perhaps not without significance that the process of asexual reproduction is so deeply entrenched among the protists and antedates, in the process of evolution, the origin of sexual reproduction itself. In spite, however, of this tendency for the multiplication of functional units by the process of asexual reproduction, we find that the living substance sooner or later arrives at a stage both in ontogeny and in phylogeny in which gametogenesis ensues and by this method provision is made for the origin of new life cycles, starting with a variety of types limited only by the possible permutations in the ancestral genes and the viability and survival values of these combinations. With the accomplishment of gametogenesis the processes of senescence are hastened and the death of the individual or individuals ensues. We may summarize, then, the essential features of the life cycle as syngamy, or sexual reproduction; cleavage of the zygote; histogenesis and organogenesis and the division of labor; asexual reproduction resulting in the multiplication of functioning individuals; gametogenesis; senescence and death.

There has been a tendency in some quarters to set aside the Protozoa, as exempt from the program involved in this life cycle as manifested in the Metazoa and Metaphyta. Indeed, some have even denied the cellular character of the Protozoa while others have intimated that the phenomenon of heredity was of necessity of a different type in this primitive group and even that the Protozoa are immortal. In addition to these restrictions on the point of view there have arisen in the course of the investigations of the Protozoa certain interpretations of the developmental phenomena discovered therein which seem to militate against the possibility of the existence of one or other of these fundamental stages of the life cycle. Let us therefore turn to the Protozoa and consider in some detail in how far, in reality, they tend to conform to the common scheme of the life cycle which has been so widely adopted and prevails so universally in the Metazoa and the Metaphyta.

The first point to which our attention is turned is naturally the existence of the mechanism of heredity in the Protozoa. We are confronted at once by the statement that the Protozoa are not purely cellular; they are rather organisms. This question appears, however, to be purely a formal one and is met by the definition of a cell from the dynamic point of view, to wit: that a cell is not only a nucleus with a surrounding cytoplasm, but that it is also a chemical engine for the flux of matter and the flow of energy. Cell boundaries are not essentially a part of the definition of a cell. From the dynamic point of view the Protozoan is as surely a cell or a group of cells as is an egg or a blastula, or a man. The fact that it is also a complete organism within, it may be, the confines of one cell, merely complicates its structure by necessitating in many Protozoa the existence, within the cytoplasmic domain of a single nucleus, the presence of the organelles of the individual and the performance of the several different functions of life within the boundaries of the one domain. It is apparent to one who scans the widespread occurrence of multicellular states among the Protozoa that it is clearly a matter of entire indifference to the functional efficiency of the Protozoan as to how many cells there may be in its make-up. It is more a matter of importance that the quantity of the nuclear and cytoplasmic substances should be properly adjusted to each other and to the volume of matter and amount of energy in the process of transformation. There is such a suitable adjustment which Hertwig has long since emphasized which finds many illustrations in any comparative study of any considerable group of Protozoa. A huge radiolarian with the enormously complex skeletal structure composed of thousands of elements integrated in a common and complex pattern may have a single nucleus of huge dimensions with as many as 1,600 chromosomes. Two species of flagellates living in the same environment in the digestive tract of a termite may have in one instance a single large nucleus with its attendant intricate neuromotor system of many complex elements, while the other of approximately the same size may have 2, 4, 8, or it may be 50 nuclei. But these will have in a general way a correspondingly small quantity of chromatin in each nucleus and the total in all of the nuclei will approximate in quantity that in the single nucleus of the accompanying species of like size.

Our conceptions of the nucleus in Protozoa have been thrown into confusion and to some extent therefore excluded from the category of metazoan and metaphytan nuclei by the accounts of protozoologists of the occurrence among Protozoa of certain cytological phenomena which appear to undermine and to render impossible our modern concepts of the mechanism of heredity in that they seem to invalidate the continuity and individuality of the chromosomes.

The complicating concepts are; namely, the occurrence of amitosis, of the formation of nuclei *de novo* from chromidia; chromidiogamy or the formation of gametes from chromidia; and of multiple nuclear division, in which a single nucleus parts at once into numerous nuclei. Added to this group of perplexing phenomena is the persistent proposal that Protozoa reproduce by the process of autogamy emanating from the Schaudinn-Hartmann school at Berlin and its followers.

Without attempting at this time to go into the cytological details necessary to refute any or all of these seemingly unusual and complicating cytological phenomena, I will pass them by with the statement that in the course of more than ten years of intensive work on the cytological processes of flagellates and rhizopods, I have been wholly unable to find any satisfactory cytological evidence to support any view that the nuclear phenomena of the Protozoa differ fundamentally from those of the Metazoa.

In the first place amitosis as described in the Protozoa is either a pathological or degenerative process, as it is in the Metazoa, or it is based on a partial account of the normal process of mitosis in which the nuclear membrane remains intact throughout the whole process, as it does in flagellates and rhizopods, and in its anaphases presents a superficial resemblance to pathological amitosis. The persistence of the nuclear membrane in no way interferes with the occurrence of chromosomes constant in number and kind. In other words, the doctrine of chromosome continuity, insofar as amitosis is concerned, is no more affected in the Protozoa than it is in the Metazoa.

We have had accounts of the *de novo* origin of nuclei and of chromidiogamy by an association of chromidia in the cytoplasm into new nuclei while at the same time the old nuclei disappear. These accounts of this process in *Arcella, Actinosphærium,* and in certain flagellates are, in our observation, the results of the failure to apprehend the fact that the process of metabolism in the protozoan individual brings about in the protozoan cell the formation of intracytoplasmic substances which, on the one hand, provide for the rapid multiplication of cells, as does the yolk of the egg after fertilization, and, on the other hand, obscure, by the very fact of their stainability, the actual process of mitosis in nuclei. The observer is led to give a false interpretation of degeneration to the condition in which the nucleus of the protozoan fades as a result of the process of metabolism, not unlike that in the growing egg during yolk formation. In this process it loses much of its stainability, appears to be degenerated and is hidden in the mass of stainable substance, the so-called chromidia, in the cytoplasm. It then undergoes rapidly a succession of cell divisions, during which period the chromidia are reduced in mass, the new nuclei show increased stainability, but the observer has mistakenly attributed their origin to the disappearing chromidia.

The *de novo* origin of nuclei in *Amœba proteus* described recently by Carter is, we believe, entirely of this nature. Similar accounts of *de novo* formation of nuclei and of chromidiogamy in *Arcella* appear to me to be of a similar nature, and it is possible there is also a confusion here by some investigators of moribund stages of individuals and of parasitized individuals. Some instances of the *de novo* origin of nuclei in flagellates appear to be instances of parasitism rather than *de novo* origin.

In like manner some at least of the so-called cases of multiple nuclear division are to be attributed to a process of rapid mitosis rather than multiple, coincident fragmentation of a single nucleus. In any event, the cytological evidence upon which the processes of chromidial organization of nuclei, chromidiogamy and multiple nuclear division are based are undoubtedly wholly inadequate to establish the occurrence in the Protozoa of these biological processes so fundamentally unusual and so subversive to the more adequately founded concept of the mechanism of heredity. It appears that they should be dismissed entirely as wholly unproven in fact and be regarded as inadequate interpretations of other processes, normal, pathological or parasitic in nature.

The occurrence of autogamy in the Protozoa presents far less theoretical difficulties. Its incorporation in any system of interpretation of the operation of the mechanism of heredity involves no greater difficulties than does hermaphroditism and self-fertilization in the cestode or cleistogamy in the violet. The main difference lies in the fact that owing to the small size and limited number of cells in the protozoan cycle the uniting autogamous gametes are genetically nearer their common cellular ancestor than they are in the tapeworm or the violet. Some, however, of the processes of autogamy, as, for example, that of *Endamœba coli,* described by Schaudinn and by some other investigators in the parasitic Protozoa, rest upon misinterpretations of the process of cleavage in the encysted organism, and upon the mixture by investigators of the life cycles of several different parasites as parts of the cycle of one. We may conclude, then, that insofar as the fundamental nature of nucleus, chromosomes and mitosis is concerned, Protozoa conform in essential features to the nuclear mechanism of the Metazoa and that autogamy, if present, is not peculiar to Protozoa and offers no serious obstacle to the modern conception of the mechanism of heredity.

The existence of sexual phenomena in the Protozoa has been a matter of frequent record and of repeated observation in various types of Protozoa. It was the result not only of the brilliant series of interpretative papers by Schaudinn, foremost leader in modern protozoology, but also of those by his many followers. In a series of brilliantly conceived, rapidly developed and handsomely illustrated monographs, Schaudinn demonstrated the existence of sexual reproduction as a part of an elaborate life cycle in a series of Protozoa from rhizopods to the malarial parasite, thus giving rise to the general belief that sexual reproduction was probably universal in the Protozoa and that it was only a question of skillful interpretation and fortunately selected material for any investigator to be able to demonstrate its occurrence in any species of the group.

Some doubt has arisen as to the validity of some of Schaudinn's conclusions, and doubt also as to the accuracy of some of his observations. This has arisen in part from his well-known mistakes in his account of the life cycle of the trypanosomes in which he confused in one life cycle those of no less than three different organisms. The elaborate and critical work of Minchin and of others who have worked with trypanosomes has failed in the slightest to confirm Schaudinn's conception of the sexual reproduction of these organisms.

It would not be fitting for one to pass criticism on any of the other accounts which this investigator has given us unless one had himself painstakingly worked over similar material. I wish to take this occasion, however, to state that prolonged research with the protomonad, polymastigote, dinoflagellate and euglenoid orders of the Mastigophora and in the Rhizopoda during the past ten years by my collaborators, students and myself has thus far failed to give us the slightest critical evidence of the occurrence of sexual reproduction in any of these orders. It is possible to find in one's material and to picture, as Goldschmidt and others have done, a sequence of fixed and stained stages which will simulate, in the most striking degree, the successive steps in the process of sexual reproduction if one neglects chromosomes. But over against these facts one must place his observations that dividing flagellates may within the brief time of a single minute assume positions simulating in their space relations final stages of plasmotomy and to exhibit later the appearance of the initial stages of conjugation owing to the great facility with which the neuromotor organelles shift the positions of the parting sister cells. One should, therefore, utilize such material with utmost caution. Throughout all of our work we have failed to find in the groups named the least evidence of gametogenesis and of syngamy based upon cytological evidence involving chromosome number and behavior. We are, therefore, highly skeptical of all accounts of sexual reproduction in the flagellates other than those in the Volvocidæ, where differentiated gametes, as in the case of *Volvox,* occur.

In the same way our recent investigations in the mitotic process occurring in the cysts of intestinal rhizopods leads us to reject entirely Schaudinn's account of sexual reproduction in this group, and has increased our skepticism in the account of its occurrence in the free-living rhizopods. It is a matter of prime consequence that protozoologists and others accept no account of sexual reproduction as adequately established which does not rest upon essential cytological features of gametogenesis and fertilization, and the clear cut, unquestionable establishment of the haploid and diploid sequence in these processes.

The seeming absence of sexual reproduction in the bacteria and in many of the protophytes combined with its seeming absence in the lower Protozoa does not militate against the fact of its establishment in the higher forms, but does bring us face to face with the fact that it is probably within the group of Protozoa and perhaps independently in its several orders that the phenomenon of sexual reproduction has had its evolutionary origin. Insofar as this fact bears upon our thesis that the life cycle of the Protozoa and the Metazoa are essentially similar we should have to admit that the similarity inheres only in the higher representatives of the several orders of the Protozoa, or at the most in the Sporozoa and Ciliata and in a number of species perhaps of the other orders.

This conclusion, if true, brings us to a consideration of the possibility that there may occur also in these primitive forms which now represent the earlier steps of the evolutionary process of the phenomenon of reproduction a periodic nuclear reorganization of the genes that is not primarily sexual. In its morphological features and in its relationship to the permutations of genes and to their origin in the life cycle such reorganization may play a part in the mechanism of heredity not unlike that accomplished by the phenomenon of sexual reproduction in the Metazoa. Whether there is such a nuclear phenomenon and the manner in which it operates are both matters upon which we have as yet little or no light. The phenomenon of endomixis appears to be somewhat more like that of parthenogenesis than a more primitive form of nuclear reorganization. In the absence of full knowledge of the behavior of chromosomes during endomixis in *Paramœcium* and other ciliates final judgment must be suspended as to the identity of endomixis and parthenogenesis.

It is an undeniable fact that in the Metazoa and Metaphyta the gametocytes go through a long period within which cell division is suspended and metabolic and histogenetic processes of considerable importance in the development of the egg and sperm proceed within and without the nucleus of the cell; thus, for example, the oocyte acquires its load of yolk and the spermatocyte prepares for the elaborate histogenesis of the resulting gamete. It is during this period of suspended mitotic activity that synapsis and cross-over which makes possible the segregation and new alignments of genes take place.

Let us also remind ourselves that following activation by fertilization, the egg, by reason of its food reserve in yolk, proceeds at once with a series of rapidly succeeding cell divisions. This phenomenon of cleavage is fundamentally and essentially similar in all Metazoa and Metaphyta. It is therefore unquestionably a fundamental phenomenon of the living substance, but it appears in these groups to be a consequence of the activation resulting from fertilization or its equivalent. This equivalent is found in some cases in the stimulation of ova to development by mechanical, chemical or physical external stimuli. These facts suffice to raise the query in our minds as to whether or not sexual reproduction is absolutely an essential feature even in those organisms in which the process of evolution proceeds by periodic reorganization of the genetic constitution by syngamy. Might it not be possible that there is a process in primitive organisms even more fundamental than sexual reproduction, in which the genes of the chromosomes are subjected to periodic readjustments of an unknown kind during a period prior to the occurrence of renewed mitotic activity?

This inquiry directs our attention to the fact that in those Protozoa in which sexual reproduction does not occur, or at least has never been found, there occurs nevertheless the same type of periodic rest found in gametocytes followed by rapid cell division, resulting in the formation of a temporary somatella similar to that which is found following activation resulting from fertilization. I refer to the period of encystment so frequent in Protozoa which occurs in the presence of an abundance of food and appears to be primarily a cessation of motility, a period of elaboration of reserve food, and is followed by a period of rapid cell division.

Our attention has already been directed to the fact in the life cycle of human intestinal Protozoa. During our prolonged search among the encysted phases of these organisms for evidences of maturation and fertilization we have been wholly unable to detect the least traces of any critical evidence of the occurrence of either of these processes. We do find, however, that all of the six species known in man and some of the flagellates also found in human stools tend to encyst, and then to proceed with rapid cell division, building up a 4-, 8-, 16- or more-celled body within the cyst. Free living rhizopods and flagellates are known to exhibit the same phenomena.

The successive steps in this process are well illustrated in the species *Councilmania lafleuri*, the largest of the amœbæ of man. This amœba, which in its active stage feeds upon bacteria, red blood corpuscles and on the active and encysted stages of other protozoan parasites of man, prepares for encystment by the ejection of the contents of its food vacuoles and consequent reduction in volume. It then seals itself within an extraordinarily impervious membrane or cyst wall and immediately elaborates within the substance of its cytoplasm a centrally located and relatively very large glycogen vacuole. This substance exhibits the typical reactions to iodine and to Best's carmine which characterize the glycogen of mammalian cells. This glycogen vacuole may fill as much as one half or more of the volume of the cyst. It crowds the nucleus into the peripheral cytoplasm. Unfortunately, owing to lack of material, we have been unable to get at the nuclear modifications during this earliest phase by careful cytological studies. The stages available for examination have been mainly those subsequent to the formation of the glycogen. The nuclei during this period of greatest glycogen abundance are often very much enlarged and the chromatin which is usually gathered in the central karyosome may be spread out in expanded, greatly elaborated condition. The next step in the process is an elaboration within the outer cytoplasmic film and in immediate contiguity to the glycogen of numerous small chips or splinter-like chromatoidal bodies. As these increase in number the glycogen diminishes in volume until ultimately it entirely disappears. During the period of its decrease and the origin of these chromatoidal bodies, the nuclei begin their mitotic divisions, and generally proceed through the first and second of these divisions. When the glycogen has entirely disappeared, the chromatoidal bodies are generally found in a bundle lying near the center of the cyst, and reaching nearly from side to side. This cluster of bodies stains intensively with hæmatoxylin and may be the most prominent feature within the cytoplasm.

In the meantime the nuclei have passed through a third and fourth mitosis, resulting in 8 and 16 nuclei within the cytoplasm. At the termination of this process there then ensues a peculiar form of growth in which the 16-nucleated somatella which shows absolutely no trace of subdivisions proceeds to give rise to a series of budding amœbulæ which one after another are dropped off from the parent mass. This process bears a peculiar and significant relationship to the substance elaborated in the chromatoidal bundle and takes place with special reference to its location within the cyst.

In the first place a definite and minute pore is opened through the cyst wall directly opposite one end of the chromatoidal bundle. Prior to the opening of this pore there generally appears in the periphery of the cytoplasm a well-marked deeply staining strand of material in the form of a single arc or of a tripartite one. The substance of these structures is deeply chromophile and is even directly continuous with the end of the chromatoidal bundle which appears to be reduced gradually in volume as the ridges are formed and the amœbulæ emerge. The chromophile substance from the ridges and from the end of the chromatoidal body seems to be drawn upon definitely by the cytoplasm emerging from the pore into the bud, so that the buds are noticeably more deeply stained than the cytoplasm of the parent somatella, and to correspond in stainability to the chromophile substance which antedates in its formation their emergence. Generally at some time before the budding process is completed the entire amount of chromophile material in the chromatoidal bodies entirely disappears.

The sequence of events within the cyst involving elaboration of glycogen and its disappearance, the formation of the chromatoidal substance and its relation to the growth processes, are suggestive of the sequence in the egg and the relationship of specific yolk substances to cleavage and differentiation in the metazoan egg.

It is obvious to one who studies the process of encystment so widely prevalent among the Protozoa that this feature of their life is not merely a device for survival during periods of drought and a means of protection which favors dispersal of the species into new localities. The process occurs in the presence of abundance of food and appears to be more clearly related to metabolism than to any protective necessity or impending danger of desiccation. It is certainly useful as a means for survival, but its origin and primary function appear to be fundamentally metabolic and related to growth. In view of this fact and of its intimate relationship to rapid mitosis and the formation of a multicellular body, one is led to infer that the period during which the glycogen is elaborated is one of profound cytological significance and one, perhaps, in which it may be possible for a nuclear reorganization to take place. Moreover, it may be that this is a reorganization of the genetic complex of its chromosomes. It is on this point precisely that we lack any cytological evidence at present. It is, however, to this period in the life cycle that I wish to direct the attention of protozoologists, geneticists and cytologists, with the hope that some one may find an organism in which this stage may be studied to advantage, because of the size of the nucleus, number of chromosomes, and ease of control of material.

Certain features of the protozoan nucleus appear to lend support to the possibility that there may be a more fundamental and primitive form of nuclear reorganization than that which sexual reproduction offers. These features are: first, the fact that in some flagellates only a relatively very small amount of the chromatin within the nucleus takes part in mitosis. In the second place, there appears in nuclei of the dinoflagellates to be a persistent organization of beaded chromosomes with subparallel or even spiral arrangements within the nucleus. This parallelism (though not synaptic) might offer a basis for cross-over, or its equivalent, during a period of syniezesis. Should such a phase in the cycle of the cell make possible such reorganization, we might find in them a mechanism for genetic modifications. The consequence of such permutations of the genetic substance might not be unlike those resulting from synapsis in sexual reproduction and thus provide for evolution in the absence of syngamy.

In this connection attention should be directed to the findings of Jameson upon the exact location in the life cycle of the period at which maturation occurs in the Sporozoa studied by him. He has found that reduction division follows immediately upon fertilization with the result that the haploid condition in these Protozoa persists throughout the natural period of growth and recurrent asexual reproductions which prevail during the life of the parasite within the host, while the diploid lasts but one cell generation. It thus appears that while in the Metazoa the haploid condition of the organism is limited to the second maturation division only of the spermatocytes and oocytes of the second order and thus to a very brief period in the total life cycle, in some Protozoa this diploid-haploid relationship may be reversed in relative duration.

In the light of the fact that many of the Protozoa appear to have no sexual reproduction, and yet nevertheless to have accomplished an evolutionary progress of no small magnitude, these observations of Jameson are significant. They lend weight to the possibility that sexual reproduction originates within the Protozoa, and, as in some of the cryptogams, establishes a diploid condition controlling only a part and apparently only a very small part of the prolonged life cycle. It gives us occasion to consider whether or not sexual reproduction may not have been elaborated gradually and independently within different groups in the Protista, and subsequently in them and in higher forms of life the diploid state has extended its domain more and more throughout the life cycle of the organism. Indeed, the very fact that the Protozoa run with a six cylinder instead of a twelve cylinder engine, granting the universality of Jameson's observations for the sake of argument, may be the very reason why they have not been successful in building up a differentiated body. They run the multicellular part of their course with only a brief-lived and relatively inefficient somatella instead of a specialized soma.

It was stated in the introduction to this discussion that the Protozoa are multicellular as truly as the Metazoa. The difference between Protozoa and Metazoa lies not in the fact of occurrence, but in the degree to which multicellularity progresses. In the Protozoa the multicellular phase which results from cleavage is limited to few mitoses and small number of cells. In amœbæ, as far as we at present know, it does not get beyond a sixteen-celled stage. In most flagellates eight seems to be the stopping point. In *Paramœcium* likewise the eight-celled stage is the limit of the development of the somatella.

Furthermore, the length of life of the individual in the multicellular phase is generally a brief one, for example, the sixteen-cell stage of *Councilmania* proceeds rather quickly to asexual reproduction by budding, and in the most of the polymastigote flagellates the eight-celled somatella rather quickly disappears by successive plasmotomy into unicellular units. However, in both of these classes of Protozoa there are multicellular species which live for prolonged periods in the multicellular phase, as, for example, in the Foraminifera, in the pansporoblasts of the Sporozoa, in the colonial Volvocidæ, in some polymastigotes, and in not a few of the ciliates.

Some of the somatellas of the Protozoa proceed in the direction of histogenesis and the division of labor by the segregation of sexual and somatic cells, as, for example, in some of the Volvocidæ where the gametocytes form only a part of the body or in the pansporoblast of the sporozoans in which we have one or two sex cells enclosed within a body of four or more other cells and the body itself may be differentiated into cells of two kinds. In the case of the ciliates, we find, as in *Paramœcium,* the eight-cell stage of the somatella formed by cleavage differentiated into four somatic nuclei and four sexual nuclei—three of which degenerate, and the body thus formed proceeds by asexual reproduction to reduce the number of nuclei to the normal condition of a single trophic macronucleus and a single sexual micronucleus, a condition perpetuated by subsequent asexual binary fission.

It will perhaps facilitate our conception of the fundamental similarity of the life processes of the life cycle of the Protozoa and the Metazoa if we turn our attention to the familiar life cycle of the malarial parasite in which Schaudinn distinguished a cycle separated into two phases based upon the host in which the parasite lives. It is in this sequence that this cycle is treated in the text-books and charts which base their account upon his investigations. All start the life cycle with the invasion of a red blood corpuscle by the sporozoite introduced by the mosquito into the blood of man.

Looking at this problem purely as a biological rather than an anthropomorphic phenomenon one is forced naturally to begin the life cycle not from the point of importance to man, but at the most significant phase of the organism itself, namely, the zygote. This, the so-called ookinete, is formed in the stomach of the mosquito by the fusion of the slender flagella-like macrogamete or spermatozoan with the larger spheroidal, passive, immobile macrogamete or egg. It is in reality a fertilized egg which creeps out of the stomach of the mosquito, encysts in the wall of this organ, and undergoes rapid cell divisions giving rise to a multicellular plasmodium-like sporoblast, having hundreds and perhaps thousands of cells in a common protoplasmic body at first spheroidal in form and retained within the encapsuling membrane. There is no histogenesis in this body. Then follows an asexual reproduction in which the body falls apart into its constituent cells, comparable in kind to the shaking apart of the blastomeres in the egg of *Amphioxus*. These separated blastomeres then differentiate in the unicellular sporozoite which may be transferred by the mosquito into the blood of man. Here they find a favorable field for their growth in or upon the red blood corpuscles and undergo a succession of mitoses giving rise to a small multicellular body which in turn undergoes asexual reproduction by simultaneous plasmotomy into its constituent cells, a process again similar to that of the breaking apart of the blastomeres of the egg.

This may be repeated again and again in the course of the cycle in the human host. There comes a time, however, in the life cycle in which certain of the merozoites instead of proceeding to the formation of new somatellas form instead gametocytes, and in the favoring environment of the mosquito's stomach proceed to the maturation of gametes. We have, then, in this protozoan, as in the case of the Metazoa and Metaphyta, the fundamental processes of fertilization, cleavage, asexual reproduction and gametogenesis with the gametocytes and gametes sexually differentiated. The parallelism is precise in every important respect except in the fact that histogenesis and organ differentiation do not appear even though the number of cells in the somatella on the wall of the mosquito's stomach may reach into the thousands.

It is also obvious that death is a normal part of this cycle as truly as it is in the case of a navel orange tree, the earthworm or man. It results, in the case of the navel orange tree, when any part of the original zygote disintegrates and is never fully accomplished until all its parts have disintegrated or have been transformed into gametes. The cycle of the individual extends from the zygote to the gametes. This individual is, of course, the Huxleyan individual and it dies when any of its parts which we may call functioning individuals die of disease or are destroyed by accident, or die of old age. But the Huxleyan individual does not entirely disappear until all of its parts reproduced asexually either perish singly or are transformed into gametes. Senescence and death in the navel orange tree are not unlike that in the malarial parasite. The original zygote which formed the navel orange is now dispersed in thousands and hundreds of thousands of navel orange trees throughout the orange orchards of the world. Some of these may produce new gametes, but in time they are destroyed by man and the environment, or grow old and perish, unless continued by asexual processes.

In the same way the malarial parasite dies with the formation of its gametes and is continued by asexual processes. In the case of *Paramœcium* the zygote undergoes cleavage, forms an eight-celled body which by asexual reproduction is reduced to the binucleate condition with the single trophic and a single sexual nucleus. This is continued indefinitely with the aid of endomixis, or at least the limits are not yet known to which lines of descent may progress by asexual reproduction.

There comes a time in the life of some of the asexually produced individuals in which the trophic nucleus or soma breaks down, its genetic control over the cytoplasm in which it has lived ceases. The sexual nucleus by the process of maturation takes over this body which in a dedifferentiated condition becomes that of the gamete, and ultimately that of the new zygote. We have here, then, in this and many other Protozoa, the rather exceptional phenomenon of the entire body of the parent controlled by the genetic complex of the macronucleus, growing old and its nucleus disintegrating and resorbed in the cytoplasm of the gametocyte and gamete. The gamete thus eats one of its parents. The only difference between this function and that of the hydroids or of a man lies in the fact that the amount of parental cytoplasm appropriated differs. *Paramœcium* takes it all, the human egg only a minute part. In both cases the ancestral genes which once controlled the parental cytoplasm are alike doomed to senescence and death, and a new régime is established in the new gamete and zygote.

We find also that there is a parallelism of deep significance between the relation of asexual reproduction in the Protozoa and in the Metazoa to the phenomenon of senescence and death. The tendency of asexual reproduction in all organisms is to prolong the chances of survival of the original zygotic complex in one or another functional individual. In every case senescence follows and death is the price of sexual reproduction. The length of time during which asexual reproduction may prolong the existence of functional individuals carrying original zygotic constituents is a matter to be determined only by experiment and observation. It is not essentially different in the Protozoa and the Metazoa.

When we turn to those primitive forms of life in which sexual reproduction has not as yet made itself apparent, we are faced by the fact that it would appear at first sight that death can not possibly be a phenomenon where sexual reproduction is unknown. This is true, however, only if the original genetic complex remains unchanged. Should there, however, occur in the life cycle of the Protozoa and other primitive organisms, including the bacteria, a periodic and recurrent reorganization of the nuclear structure involving permutations in the genes, we will be forced to admit that every such organization carries with it inevitably the seeds of death of the previous individual. The genetic complex which existed prior to the reorganization vanishes when that reorganization ensues and a new individual in every sense of the word comes into control of the organization.

I have brought forward this concept of the normality of senescence and death among the most primitive organisms as one which may be helpful in directing discussion and research into the nature, structure and periodical changes in the mechanism of heredity and the mechanism of organismal control of these simplest forms of life. It may perhaps be helpful and serve to facilitate progress if we emphasize the similarities of organisms and seek to find the common processes underlying them all rather than to emphasize their differences and thus obscure our vision of the more fundamental problems of life.

C. A. Kofoid

PRUNING THE ACADEMIC TREE[1]

There is little reason to offer excuse for discussing the method of presenting a scientific subject, if one but remembers that each science is rapidly expanding and as it does so the subject matter and pedagogical methods must be modified. We can not be content to teach as our forefathers taught even though they in their time were successful. Not so long ago there were naturalists who studied and taught all phases of science and the student obtained a certain advantage which is lost by our present system of special teachers. The intimate inter-relationships of the sciences are largely lost in the maze of special detail. The broad, inaccurate naturalists' teaching of earlier days had to give way to the closely analytic, specialized method of the last decade, but now the immense accumulation of data must be synthesized into the truly general, fundamental principles so as to free the students' time for further specialized study.

We all have been inclined, on the whole, to teach as though our students were going to be specialists in our particular subjects instead of realizing that this, in all likelihood, will be the one time in their course when they will come in contact with our subject. There is much that a specialist must know which but fogs the issue for one gaining general knowledge or training. We too often lose sight of the college ideal of education and attempt to turn out ambulating encyclopedias rather than individuals who are trained to organize and apply.

Each increase of knowledge has seemed to call for the addition of a requirement for graduation without greatly affecting the curricula already prescribed. The result packs the students' hours from entrance to graduation with one continuous procession of informational courses until, should the student by chance have a free hour in his program, he really feels guilty. Well might be inscribed over the gates of many of our colleges "Leave thought behind all ye who enter here" for small time is left for such intellectual processes.

We must cut the Gordian knot of required subjects for every hour of college life whenever possible in order that opportunity for individual selection may enter. We must recognize that in general elementary courses only the principles and not the details stick or are of ultimate value. Our work must be so planned that the student's life shall not be one

[1] Read before the North Dakota Academy of Science, May 5, 1922.