PROTOPLASMIC LONGEVITY, WITH PARTICULAR REFERENCE TO THE PROTOZOA

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More than two and a half centuries have elapsed since Leeuwenhoek, a Dutch naturalist, discovered an unknown world of microscopic animals—the Protozoa. Many thousands of species have now been described and studied, and their varied forms, structures, and activities never fail to fascinate the observer.

Although Protozoa vary in size from one-half to one twenty-five thousandths of an inch, each is an independent self-perpetuating unit of protoplasm with the characters of a single cell. Few observers allow their imaginations to go back over the past history of any one of these animated bits of living substance, and few realize that its protoplasm has been continuously living since the beginnings of life and, barring accidents, has the ability to live indefinitely in the future. This miraculous phenomenon is what I mean by "protoplasmic longevity" and in the present paper I shall deal mainly with the factors which control this longevity in Protozoa.

What is true of Protozoa is also true of every other type of living thing, including man, for protoplasm always has the same attributes. It is more easily studied in these unicellular animals than in other types and the controlling factors are more obvious.

Before discussing the factors which have to do with longevity, let me consider one matter of great importance, viz. the concept of protoplasmic organization. You all recognize without reflection a star-fish, a sea-urchin, a fish, a frog or toad, a reptile, a bird or mammal. You recognize them at once from their adult structures. You do not stop to reflect that each one of these comes from a minute bit of protoplasm, the egg cell, which is no larger than a protozoon. These adult structures constitute the *derived organization,* i. e. derived through processes of growth and differentiation in the course of development from the single egg cells. The

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protoplasm of an egg cell has what I have called the *fundamental organization* of the species. The fundamental organization gives rise by development to the finished adult with its derived organization. It is quite obvious that the egg cell of a star-fish is as different from the egg cell of a sea-urchin as the star-fish is different from a sea-urchin, and yet the egg cells of these animals are so similar that only a specialist can tell them apart. Furthermore, this fundamental organization of the egg, when subjected to the same series of environmental stimuli, always gives rise to the same type of derived organization, hence the members of the same species of animals are always alike. Change the stimuli and the derived organization is changed, leading to the formation of aberrant structures or even monsters.

The point I wish especially to emphasize is that we find exactly the same difference in fundamental and derived organizations in the single celled Protozoa. For example, Figure 1A represents a cyst of the ciliated protozoon *Stylonychia mytilus*. Like an egg cell, it is a minute ball of apparently homogeneous nucleated protoplasm enclosed in an impermeable capsule. Most other Protozoa have similar cysts and the difference between these cysts in many cases cannot be made out even by the specialist. The homogeneous balls of protoplasm have their fundamental organizations each characteristic of its species, and each of them develops (but without fertilization) after the absorption of water and oxygen, into an actively moving adult organism. From this Stylonychia cyst the adult emerges as shown in Fig. 1B. The derived structures, including the macronucleus, the adoral zone of membranelles, cirri, mouth, contractile vacuole and other structures, are all formed from the fundamental organization contained in the cyst which could give rise to nothing else any

FIG. 1

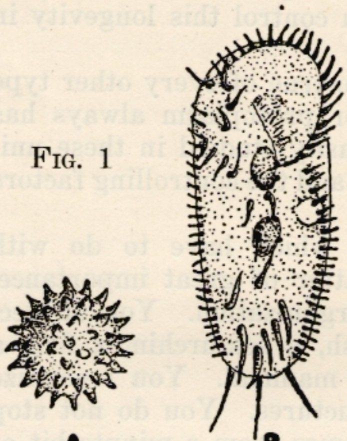

FIGURE 1. *Stylonychia mytilus.* *A*, Fundamental organization encysted. *B*. Adult individual with its derived organization. Two macronuclei, with attached micronuclei in center.

more than the egg cell of a dog could give rise to anything but a dog. In short, fundamental organizations of different species are entirely different although alike for the same species. Now, just as in a metazoon, where the egg cells are germ plasm contained within the derived organization, so in a protozoon the germ plasm or fundamental organization is contained within and is protected and fed by, the derived organization. This is easily demonstrated as follows: Fig. 2A represents a ciliate called *Dileptus anser* and Fig. 2B a *Stentor polymorphus*, representatives of two entirely different groups of ciliates. If we cut out of the center of each a small piece including a portion of the nuclei, these pieces round out into small balls which lose any structures originally possessed. These balls now look exactly like cysts without capsules, both composed of apparently homogeneous protoplasm (Fig. 2 *a* and *b*). Place these in a nutrient medium and one will develop into a Dileptus, the other into a Stentor. As far as the concepts of fundamental and derived organizations are concerned, therefore, Protozoa and Metazoa are alike.

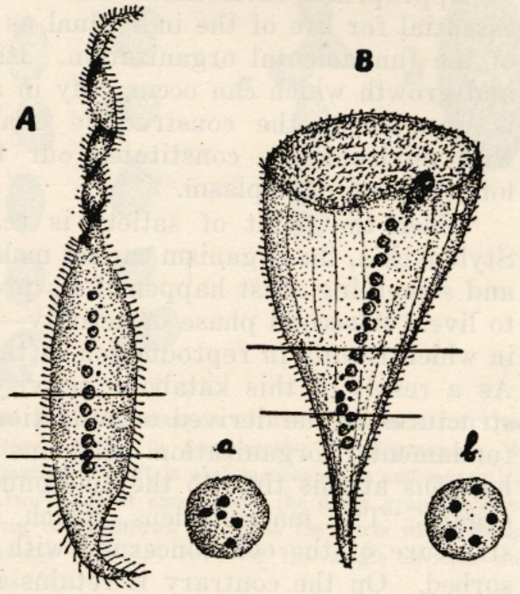

FIGURE 2. *Dileptus anser* (A) and *Stentor polymorphus* (B). *a*, fragment of Dileptus cut from A as indicated by lines. *b*, similar fragment from B; each fragment will develop into its own species.

Let us now turn to the factors controlling longevity. This young Stylonychia which has just come from its cyst reacts to stimuli from the environment, moves about actively, takes in food in the form of bacteria and other minute living things. These are digested in an improvised stomach where they are acted upon by digestive ferments, one resembling pepsin in that it acts in an acid medium, the other, like trypsin, acts in an alkaline medium. The results of digestion in the form of polypeptids and amino acids are distributed throughout the protoplasm, the minute parts of which take all they need for their own regeneration, and the organism grows. When it becomes full sized—and there is a limit of growth for every known organism—a condition ensues which we may speak of as satiety.

Appropriate environment and metabolism are absolutely essential for life of the individual as a temporary custodian of the fundamental organization. Its period of metabolism and growth which can occur only in a suitable environment, is one phase—the constructive anabolic phase—and this, with environment, constitutes our first factor controlling longevity of protoplasm.

When the point of satiety is reached in our example, Stylonychia, the organism cannot make use of anything more and something must happen if its protoplasm is to continue to live. A second phase of activity—a katabolic phase—sets in which results in reproduction of the organism by division. As a result of this katabolic activity practically all of the structures of the derived organization are absorbed into the fundamental organization. Let me briefly describe what happens at this time to the macronucleus and to the motile organs. The macronucleus, which is the most important structure of the cell concerned with metabolism, is not absorbed. On the contrary it retains its integrity but undergoes an independent process of reorganization which varies slightly in different types of ciliates. In *Uroleptus mobilis* there are eight macronuclei, all of which undergo the same type of reorganization. Young macronuclei have characteristic densely staining chromatin granules. After a few hours of metabolic activity, there appear in each nucleus granules of a different kind which do not stain like chromatin and which are hydrolyzed out with the Feulgen nuclear reaction (Fig. 3 *a*). After about eight hours of normal activity, these granules, which I have called the "X granules", come together at the anterior third of each of the eight macronuclei. Here they act as catalysts, and cause a furrow or cleft in the nucleus (Fig. 3 *b*). The portion anterior to this cleft contains chromatin which has a different appearance from that of the nuclear part posterior to the cleft; this anterior portion of each of the eight nuclei is thrown off and absorbed in the cytoplasm. The eight remaining nuclear parts now fuse (Fig. 3 *c*) to form a single division nucleus (Fig. 3 *d*).

FIGURE 3. Reorganization of the macronuclei of *Uroleptus mobilis*. *a*, accumulation of X-granules; *b*, formation of cleft in each macronucleus and separation of two kinds of chromatin; the finely granular chromatin being thrown off while the parts with coarser granules fuse (*c*) to form the single division nucleus (*d*).

This process is undoubtedly one of nuclear reorganization and similar or slightly modified processes occur prior to division in every other ciliate so that the macronucleus undergoes a form of house-cleaning at every period of division and the chromatin granules are restored to a virile condition. In some ciliates the same result is produced by so-called "reorganization bands" which, as catalysts, pass through the macronucleus and bring about the change in chromatin granules from a condition of increasing lassitude to a condition of youthful virility (Fig. 4).

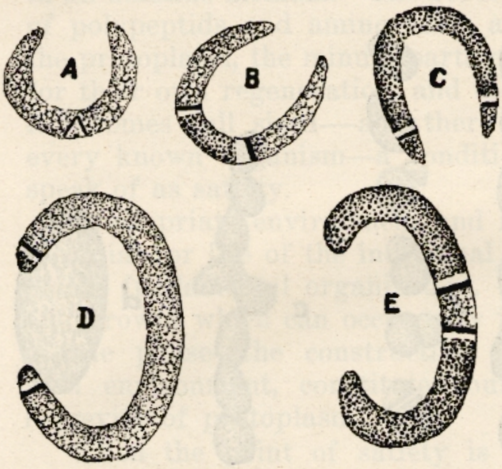

FIGURE 4. Reorganization bands in macronuclei of Aspidisca (A, B, C,) and of Euplotes (D, E,) In the former these bands start in the center and move towards the ends; in the latter they start at the ends and move to the center. The chromatin is changed in character with the passage of the bands.

Similarly with the motile organs. At the base of every one a small bud appears from the fundamental organization. While this bud grows the old motile organ becomes reduced in size by absorption in the protoplasm. The end result is the complete disappearance of the old one while a brand new motile organ, derived from the fundamental organization, takes its place. When these processes are completed the cell divides, and two young cells, each with a new set of motile organs proportionate in size to the young cells and with a reorganized macronucleus, result. Division, then, with catalytic activities, is a second factor in longevity and a most important one, for the protoplasm renews its youth.

Now the question arises—are these two factors, environment with metabolism, and division—adequate for the indefinite maintenance of protozoan protoplasm? Weismann, more than fifty years ago, believed that they are and, for the animal flagellates at least, he was right. But they are not adequate for the vast majority of other Protozoa including Infusoria, Rhizopoda and Sporozoa. In order to determine this matter we have recourse to the method of isolation cultures of which

I shall describe, as briefly as possible, one series of experiments with *Uroleptus mobilis* on which I have worked for many years.

A single individual from a cyst or from conjugation was isolated in a small drop of nutrient medium which was determined by experiment. This drop with the one individual was set aside in a moist chamber. At the end of twenty-four hours there were eight individuals in the drop and five of these were isolated, one each in a container. On the following day one each from these five lines was similarly isolated, while the remainder were placed in a large container like a Syracuse dish with ample nutrient medium, where the organisms divided freely until hundreds were present. This con-

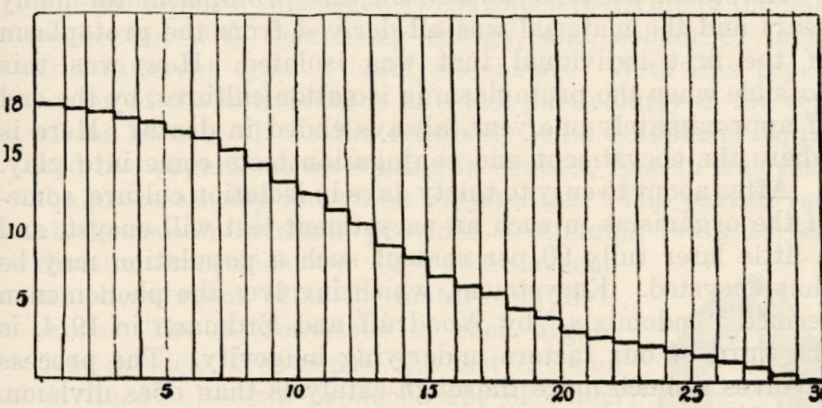

FIGURE 5. Graph of the division rates of one series of *Uroleptus mobilis* showing waning vitality and ultimate death after three hundred days in isolation culture. Ordinates indicate numbers of divisions in ten-day periods; abscissas indicate successive ten-day periods.

stitutes an encystment or a conjugation test about which I shall speak later. This process of daily isolation was continued day after day, month after month, for a period of about a year or until the individual which was originally isolated and its progeny, had divided upwards of 360 times. Now, if division were adequate to keep that protoplasm going, these divisions would certainly have done so. But not at all. Rather, the division rate during this period gradually decreased until it got to nil and the protoplasm died (Fig. 5). The last individuals of such a series were frequently abnormal or undeveloped and the macronucleus was filled with X-granules.

It is very difficult to compare vitality by inspection of the daily records. In order to get such a comparison it is necessary to average the numbers of divisions for unit periods such as ten days. The thirty or more ten-day periods of a typical series of *Uroleptus mobilis* are then plotted to give a curve on which the ordinates are the average numbers of divisions in ten days, and the abscissas the consecutive ten-day periods. Such a graph begins with an average of approximately eighteen divisions per ten days and gradually descends until it ends in zero showing an inevitable waning of vitality of this original bit of protoplasm and the ineffectiveness of division to maintain it (Fig. 5).

As stated above, I worked on this protoplasm for many years and the material was all derived from the protoplasm of the first individual that was isolated. How was this possible when the protoplasm in isolation cultures, by the end of approximately one year, always ended in death? Here is where the encystment and conjugation tests come into play.

After about twenty to thirty days in isolation culture, some of the organisms in such an encystment test will encyst, and a little later fully 90 per cent of such a population may be thus encysted. Encystment, which involves the phenomenon termed "endomixis" by Woodruff and Erdmann in 1914, is the third of our factors underlying longevity. The process involves a much more thorough catalysis than does division. All of the motile organs of the derived organization disappear by absorption while the eight macronuclei break up into small fragments which ultimately likewise disappear by absorption. Water and waste matters are thrown out and only the fundamental organization persists as an apparently homogeneous mass of protoplasm with a nucleus and surrounded by an impervious cyst wall. These cysts may then be dried and may persist in a dry state for months or years. When placed again in a nutrient medium, they give rise to normal individuals. A new set of macronuclei are formed from the micronucleus which is the last stronghold of the fundamental organization, and a completely new derived organization is formed. The result is a young protoplasm with a full potential of vitality and it may now undergo 360 plus or minus divisions before it, in turn, will die. I have kept such a cyst for six months after it was formed in a parent series. After six months it was placed in a nutrient medium which was being used for the parent series. During these six months the vitality of the parent series had waned from seventeen divisions in ten days to two divisions in ten days. The protoplasm derived from the cyst had a vitality represented by twenty divisions in ten days. Yet is was the same protoplasm as the parent series, the only difference being that it had undergone complete reorganization. Here is a third, and a most potent factor controlling longevity and a type in which catalysis affects that most important organ of metabolism, the macronucleus.

The period during which encystment may occur lasts only for a limited length of time in the life history of any series. The protoplasm subsequently continues to divide in the parent series but with decreasing energy, and, sooner or later, a still different type of catalysis ensues. With this type the cortex of the individual is affected, particularly in the region of the mouth. Here the relatively solid ectoplasm liquifies, and two organisms on coming in contact, fuse in this region, the process being known as conjugation. The partial fusion of the two individuals provides a stimulus for a series of division reactions by the micronucleus which we speak of as the maturation divisions. The micronucleus of each organism divides three times, the last division giving rise to two pronuclei one of which remains *in situ,* while the other migrates through the protoplasmic bridge into the other individual and fuses with the resting pronucleus there (Fig. 6). This is fertilization, and it is mutual. Conjugation requires about twenty-four hours and after fusion of the pronuclei, called amphimixis, the two parent individuals separate. A period of three or four days now elapses during which the old macronuclei are broken into fragments and the fragments are absorbed in the cytoplasm. A new macronucleus is formed by division of the fertilization nucleus and the reorganized individual is ready for its first division in about five days.

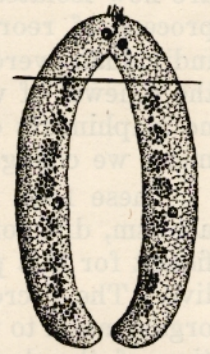

FIGURE 6. *Uroleptus mobilis* in conjugation. The macronuclei are disintegrating; the migrating pronuclei are about to pass at the apex while the quiescent pronuclei are in the centers of the two individuals. The transverse line indicates the plane of cutting.

Conjugation is a fourth factor underlying the phenomenon of longevity. It is about the same in its effects on vitality as endomixis during encystment and again the old protoplasm is completely revivified. The extent of this rejuvenescence may be illustrated by one series in which the protoplasm was dividing at the rate of only one division in forty-three days. Two individuals from this old series conjugated; after conjugation both individuals started to divide at the rate of seventy-five divisions in the same time.

After conjugation involving amphimixis, as with endomixis, we have the same phenomenon of rejuvenescence. The only difference between the two is partial and temporary fusion of two individuals with union of micronuclei in conjugation while no such fusion and union of nuclei occur in endomixis. Even with conjugation amphimixis is not necessary for rejuvenescence. This, with *Uroleptus mobilis,* has been shown repeatedly by cutting apart the two individuals during the early stages of conjugation. Two conjugating individuals, which are united by their anterior ends (Fig. 6), can be cut with a scalpel so that the fused end is completely removed. The operation leaves two freed individuals which are now isolated and cultivated. It is found that the whole process of reorganization goes on just as though the two individuals were still united. Reorganization is perfect and the renewal of vitality likewise is complete. There has been no amphimixis or fusion of pronuclei; hence, by the experiment, we change amphimixis into endomixis.

These four factors—appropriate environment with metabolism, division, endomixis, and conjugation—are now sufficient for this protoplasm to retain indefinitely its power to live. The secret is a constant restoration of the derived organization to the fundamental organization, and a re-formation of the derived organization. This is accomplished in part by division of the cell, more thoroughly by exdomixis and by conjugation.

In conclusion let me briefly compare the Protozoa with the Metazoa. The fundamental organization of the latter in the form of germ plasm in egg and sperm cells is equally immortal, but in most cases they must unite to live. The fertilized egg divides and by a series of successive divisions gives rise to millions of cells which are specialized in ways that are characteristic for each of the higher types of animals. The derived organization, or the adult, like our isolated ciliate, has a more or less limited potential of vitality. When this potential is exhausted the derived organization of the individual dies from old age and the germ plasm or fundamental organization, which is protected and nourished by the derived organization, is killed. In the meantime, however, egg cells have left the body of the individual, and in sons and daughters the fundamental organization is carried on. *Unlike the Protozoa, Metazoa cannot absorb the derived organization into the fundamental and grow out a new one, and here, as I see it, is the most essential difference between* the unicellular animals and the Metazoa. As Weismann maintained more than fifty years ago, natural death of the individual with its special derived organization, is the penalty which Metazoa must pay for the privilege of specialization.