Proceedings of the Iowa Academy of Science

Volume 63 | Annual Issue Article 71

1956

# Some Observations on Regeneration in Dileptus Anser

Paul A. Meglitsch
*Drake University*

Thomas Johnson
*Drake University*

# Some Observations on Regeneration in *Dileptus Anser*

By Paul A. Meglitsch and Thomas Johnson

One of the most interesting capacities of protozoans is their ability to replace lost parts following injury. Although they are structurally the equivalent of cells they are functional organisms, and a study of their behavior makes it possible to bring together concepts usually applied in the cellular field with those applied in the analysis of whole organisms. The same factors that operate to evoke a particular form in the whole organism must act in a small regenerating piece of a protozoan. Whether these factors are nuclear genes or protoplasmic organization, they act rapidly in the regenerating animal, regulating the form of the piece. Unlike the metazoan material, the morphogenetic activities are not a matter of differential growth rates, but are rather redistribution and reorganization of materials present in the regenerating piece. In this sense the repair of injury inprotozoans more closely resembles cellular differentiation than organogeny.

A long series of studies on protozoan regeneration was begun in 1891 with Balbiani's work on Stentor. Lillie (1896), Morgan (1901), Calkins (1911), Young (1922), among others, described regeneration in various protozoan species and carried out experiments in an attempt to discover some of the factors responsible for controlling the process. The most extensive study of regeneration in Dileptus was that of Sokoloff (1922,1924). He found that a piece of *Dileptus anser*, representing about 1/70th of the anterior end, 1/72nd of the middle part, and 1/73rd of the posterior part, would regenerate completely. He describes a direct correlation between the size of the piece, the region of the body from which the piece was taken, and regeneration rate. The larger pieces and the more anterior pieces were found to regenerate more rapidly. The range of times for complete regeneration was given as from one hour and twenty minutes to nine hours without any particular change of media.

Although other investigators have studied regeneration in protozoa, none have used *Dileptus anser*. Weisz (1949), in a series of papers, found that in both *Stentor* and in *Blepharisma*, the rate of regeneration varied with the body region from which the piece was taken. He feels that there is a polarity of the macronucleus which is, at least in part, responsible for controlling regeneration. The actual stimulus for regeneration, however, seems to be the cutting of kinety one, the "dominant" ciliary row.

These investigations have been carried out on *Dileptus anser* collected from Lake Whitmer, a small artificial lake in the city of Des Moines. The organisms were maintained in the laboratory in a medium of 2% split pea, inoculated with *Escherichia coli* and *Chilomonas paramecium*. In later cultures traces of thyroid were added. *Dileptus* grew best at a pH between 7.0 and 7.5.

Single organisms were distributed in drops of culture medium on cover slips and cut with a fine glass filament. The merozoans were kept in hanging drop mounts in culture fluid until regeneration was complete. This species, reaching a maximal size of 500 to 700$\mu$ in optimal conditions in the laboratory, is characterized by a prominent contractile proboscis, a large open cytostome, a long somewhat flattened body, with a distinct pointed tail. The proboscis is equipped with a row of trichocysts, and long cilia along the oral surface. The remainder of the body has a uniform ciliation. Studies of material fixed in Zenker's or Kleinenberg's fluids, and stained with Harris's hematoxylin, Heidenhain's hematoxylin and Feulgen's nuclear reaction revealed a macronucleus composed of numerous (typically in the neighborhood of 150) particles and a smaller number of micronuclei. Jones (1951) found a minimum of sixteen micronuclei per organism. Since not all of its traits can be seen in living organisms, the following criteria were chosen as defining complete regeneration: (a) formation of a complete cytostome, (b) development of a moving proboscis, and (c) development of a well-formed, clearly defined tail.

The organisms were cut to determine whether the region from which the piece was taken correlated with capacity to regenerate, or rate of regeneration; to determine whether the size of the piece was correlated with ability to regenerate or rate of regeneration; and also, to determine whether a freshly regenerated organism could regenerate a second time.

Fifty organisms, not in division were cut into three pieces, and the regeneration of anterior, middle, and posterior pieces followed. Since cutting was done by hand, it was impossible to obtain identical levels of cut for each specimen. Anterior pieces which consisted of less than about half of the proboscis plasmolyzed immediately or within a few minutes. Pieces composed of over half of the proboscis, but which were cut anterior to the cytostome were never found to complete their regeneration, although they did not ordinarily plasmolyze immediately. These pieces would live for various periods of time, from about twenty minutes to an hour or so. Most of the anterior pieces were cut just behind the cytostome. These pieces regenerated completely. Plasmolysis was never observed. Complete regeneration was achieved in 25 to 75 minutes, averaging 53 minutes. The middle pieces often disintegrate at the time that the second cut is made. This appears to be a purely mechanical prob-

lem, resulting from the delicacy of the plasmalemma at the cut edge. When cut quickly and cleanly most of the middle pieces persisted. The middle pieces were capable of regenerating completely, and regenerated in very nearly the same length of time as the other pieces. The times for regeneration varied from 40 minutes to 80 minutes, averaging 58 minutes.

The posterior pieces also regenerated completely, unless only the tail region was removed. Very short posterior pieces consisting almost entirely of tail regions promptly plasmolyzed. Most of the pieces consisted of approximately the posterior third of the body. These posterior pieces very rarely failed to complete regeneration. In only a couple of instances did they plasmolyze. The rate of regeneration of posterior pieces was found to be slightly longer than the anterior pieces and middle pieces. The time range from 55 minutes to 80 minutes, averaging 65 minutes.

It is evident that there is similarity in the rate of regeneration in anterior, middle and posterior pieces, although there is a slight variation in time. The difference, however, is much less than that reported by Sokoloff. Viability differed somewhat in various sections. The anterior pieces that include ony proboscis material cannot regenerate. Assuming that the pieces are of approximately equal size, representing about 1/3 of the body length, the anterior pieces appears to have the greatest viability. Failures of the middle and posterior pieces do not appear to indicate lack of regenerative capacity, but reflect somewhat greater loss due to mechanical factors. In the failing posterior pieces, it appeared that the position of the contractile vacuole in relation to the line of cut was of some importance in leading to plasmolysis.

Pieces cut free-hand necessarily vary in size. As the foregoing discussion indicates, the very small anterior and posterior pieces were unable to regenerate fully, and plasmolyzed, usually very soon after cutting. Very small pieces from the middle region of the body, if they did not plasmolyze, were capable of regeneration. One extremely small piece, cut by accident, and not included in the data summarized above, regulated its form, produced a proboscis and tail, and then plasmolyzed. This piece was so small that it could not have contained more than one or two macronuclear particles. No accurate measurements of volume was available, but this piece was considerably less than the 1/70 of the body volume quoted by Sokoloff as minimal for regeneration. It should be noted that its regeneration was not completely successful. This very small piece completed its regeneration in very little more time than larger pieces from the middle region, but may not have had a completely formed cytostome. In general, it was evident that the larger pieces regenerated a little faster than the smaller pieces. The extent of changes in rate correlated with differences in size of piece was quite

small, representing at the most about 15 to 20% of the total time required. Since the pieces were regenerating in culture fluid, some of this difference observed may be traceable to differences in the fluid rather than size of piece. We can only conclude that regeneration rate is but little affected by size of piece, and that little may fall into the realm of experimental error.

Preliminary observations on organisms cut a second time, immediately after regeneration is completed, can continue the regenerating process if they do not plasmolyze. A somewhat higher percentage plasmolyze at the time of cutting. Their relatively smaller size with respect to the cutting filament may be responsible. The pieces that do not plasmolyze regenerate promptly, and in times that are no longer than those for organisms cut for the first time.

It is apparent at once that the results of this investigation are almost at complete variance with the findings of Sokoloff, except that in both instances a high regenerative capacity is found. The difference is rather simple to explain, however, since Sokoloff's material was not identical with ours. Although Sokoloff identified his species as *Dileptus anser,* his diagrams indicate that the macronucleus of his organism was in the form of a long, curved, beaded strand. Of the various species of Dileptus described by Kahl (1935), Sokoloff's *Dileptus* appears to most closely resemble *Dileptus gigas,* although it differs in some ways from this species, also. Prior to the time of Kahl's monograph there was considerable taxonomic confusion in many of the ciliate genera.

It would appear that where the macronucleus consists of a long beaded strand, the regenerative capacities of different body levels differs materially.

This kind of observation has been made using *Stentor,* and *Blepharisma,* as well as *Dileptus* and *Spirostomum.* In *Dileptus anser,* the macronucleus consists of scattered particles, and no morphological basis for polarity of the macronuclear material exists. It is probable that the macronuclear particles are carried about by cytoplasmic movement, and that all of the particles have essentially similar potency.

The importance of nuclear material in making regeneration possible is indicated by the failure of small pieces from the anterior and posterior parts of the body to reorganize. The macronuclear material does not extend into the proboscis or tail. Small pieces from these regions, therefore, were lacking in nuclear material, and were incapable of regenerating, although small pieces from the middle region of the body could regenerate.

Further studies are required to determine to what extent the environment of the organism helps to determine regenerative rates, and to define more precisely the effect of piece size on regenerative ability.

## Literature Cited

Calkins, G. N., (1911) (a), "Regeneration and Cell Division in Uronychia", Journal of Experimental Zoology, Vol. 10, pp. 95-116.

Calkins, G. N., (1911) (b), "Effects Produced by Cutting Paramecium Cells", Biological Bulletin, Vol. 21, pp. 36-72.

Jones, E. E., (1951), "Encystment, & the Nuclear Cycle in the Ciliate *Dileptus anser*", Journal, Elisha Mitchell Sci. Soc., Vol. 67 (2), 205-217, Illus.

Kahl, A., (1935), "Urtiere oder Protozoa I. Wimpertiers oder Ciliata (Infusoria) in Dahl's Tierwelt", Deutschlands, Jena, Fischer.

Lillie, F. R., (1896), "On the Smallest Parts of Stentor Capable of Regeneration", Journal of Morphology, Vol. 12, pp. 239-249.

Morgan, T. H., (1901), "Regeneration of Proportionate Structures in Stentor", Biological Bulletin, Vol. 2, pp. 311-328.

Sokoloff, B., (1922), "Le Noyau est-il indispensable a la regeneration des Protozoaires?", C. R. Soc. Biol., Paris, Vol. 87, pp. 1144-1147.

Sokoloff, B., (1924), "Das Regenerationsproblem bei Protozoen", Arch. Protistenk, Vol. 47, pp. 143-252.

Weisz, Paul B., (1949), (a), "On the Growth of Regenerating Fragments in Stentor coeruleus", Journal of Experimental Zoology, Vol. 109 (3) pp. 427-437.

Weisz, Paul B., (1949), (b), "Regeneration in Stentor and the Gradient Theory", Journal of Experimental Zoology, Vol. 109 (3), pp. 439-449.

Young, D. B., (1922), "A Contribution to the Morphology and Physiology of the Genus Uronychia", Journal of Experimental Zoology, Vol. 36, pp. 353-390.

Department of Biology
Drake University
Des Moins, Iowa