CHAPTER II

Effect of temperature on the rate of regeneration and fission in Homalozoon vermiculare Stokes and Urostyla sp.
Regeneration Rate of Homalozoon vermiculare (Stokes) and Urostyla Species with Reference to Temperature.

INTRODUCTION

Regeneration is a process by means of which the lost or missing parts are formed. Weisz defines three phases in the normal course of regeneration: (1) Preparative (biochemical), (2) Formative (synthetic) and (3) a regulatory (differentiation) phase. The first and second stages include to levels of information transfer, namely DNA-dependent RNA synthesis and RNA-dependent protein synthesis. The process of regeneration can be slowed down, accelerated or completely retarded by changing the normal environmental conditions. Changes in the environment like alteration in temperature, osmolality, osmotic pressure, ionic imbalance, pH, chemicals, metabolic inhibitors and radiations have been studied and have provided information on the process of morphogenesis under stress.

Regeneration studies on Protozoa show that temperature is an important factor which influences the rate of regeneration (16). The rate of regeneration, like the rate of other growth processes, decreases with a fall in temperature. Increase in temperature increases the rate of regeneration up to a limit beyond which a further rise in temperature decreases the rate of regeneration. Regeneration has been extensively studied in Blepharisma (10, 24) and in other protozoa (1, 25, 35) with attention focused on morphogenetic processes. A few studies have appeared on the effect of various factors upon regeneration, with the exception of those on the effects of certain chemicals (20, 26) and of radiations (15, 12, 13, 19).

This chapter records the rate of regeneration and fission of Homalozoon vermiculare (Stokes) and Urostyla sp. with reference to temperature.

MATERIALS AND METHODS

Cultures of Homalozoon vermiculare (Stokes) were grown in hay infusion between 18°C - 20°C. The cultures were fed daily on Paramecium aurelia grown separately in hay infusion fortified with horlicks. Tests at room temperature were performed in the constant-temperature BOD incubators. Culture medium and dishes were equilibrated at the test temperature for several hours before the ciliates were transferred to assure immediate exposure of the ciliates to the temperature designated. Temperature accuracy was ± 1°C. Pipettes, microneedles and the cavity dishes were sterilized before use.
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Healthy, non-dividing ciliates were isolated on to a slide and were cut into equal halves with a fine needle. The anterior and posterior halves, ten in number, were inoculated into separate cavity dishes in 10 ml of culture, fed with equal number of Paramecia. The regenerating ciliates were observed every hour and the time taken for 50% of the individuals to regenerate was noted. The rate of regeneration was studied at 14°, 16°, 18°, 20°, 22°, 24°, 26°, 28° and 30°C. The experiments were run in triplicate at all temperatures.

Cultures of Urostyla sp. were grown in hay infusion fortified with horlicks at room temperature 26°C±1°C. Before cutting, the ciliates were narcotized with 1.5% solution of methyl cellulose and the cut ciliates were transferred immediately into fresh culture medium. The experimental procedure was similar to that of Homalozoon.

The rate of fission of the two ciliates was also studied at the same temperatures. 4 ciliates were inoculated in 10 ml of culture and the experiment was run in triplicate and observations were recorded for eight days.

Observations

From the data obtained at each temperature it is seen that regeneration is sensitive to temperature and is temperature-dependent, a fall in temperature slowing regeneration, a rise accelerating it. The anterior and posterior halves show different rates of regeneration, the anterior regenerating faster than the posterior. This difference is noticed at all temperatures studied.

Homalozoons are cultured at 18°C, a rise in temperature from 18°C—26°C accelerated regeneration. However, this increased rate of regeneration decreases with further rise in temperature and is completely blocked at 30°C. A temperature below 18°C delays the process, the rate of regeneration decreasing with decreased temperatures and finally stopping at 10°C. Regeneration time for 50% of the individuals for the anterior and posterior halves at 26°C was 5½ and 7½ hours respectively. At 14°C the time taken for regeneration is nearly twice that at 26°C.

Urostyla sp grown at 26°C±1°C shows acceleration of the process till 30°C, beyond which it slows down. Temperature below 20°C do not favour the process of regeneration. There is a block at 18°C as well as at temperatures below 18°C. Regeneration time for 50% of the individuals for the anterior and posterior halves at 30°C was 4 hours and 4.5 hours respectively. At 20°C the time taken was twice that at 30°C.
The rate of fission of the two ciliate species at the different temperatures was in accordance with that of the rate of regeneration. (Graph I, II, III, IV).

**DISCUSSION**

The term "reconstitution" often is used to refer to all regenerative processes in the invertebrates and vertebrates. Reconstitution may be subdivided into:

1) Regeneration by outgrowth of new tissues from the wound surface called epimorphosis.

2) Reorganisation or remodelling of internal parts called morphollaxis.

Studies on regeneration have shown that these two processes are sensitive to various environmental factors.

Two factors which have been found to account for variability in regeneration time of Blepharisma and Stentor are the state of nutrition and the interdivisional status (27, 33, 34). Suzuki (24) has worked on the effect of time in the inter-divisional cycle upon regeneration in Blepharisma undulans japonicus. Regeneration is readily affected by the state of nutrition of the cells, starved ones regenerate more slowly than well fed ones (18).

Continuous exposure to high concentrations of actinomycin-D (250 mg/ml) completely inhibits oral regeneration in amputated tail pieces of Stentor coeruleus. Once oral regeneration is initiated the same concentration of actinomycin-D does not inhibit normal regeneration. Ribonuclease (1 mg/ml) is known to inhibit normal oral regeneration in posterior pieces exposed continuously to this enzyme (36). Ellwood and Cowden (11) have reported that both actinomycin-D and 6-fluorouracil were initially ineffective in suppressing regeneration in Stentors, but after exposure for relatively long periods, regeneration was inhibited. Very high levels (15-25γ/ml) of actinomycin-D inhibited regeneration in Stentor if added before the achievement of stage four. RNAase at 0.6 mg/ml also prevented regeneration in Stentor coeruleus.

The reactions essential for regeneration after transection of a cell may be ascertained by eliminating individual processes occurring in the cell and determining whether regeneration may still continue at the same rate as in the control. Inhibitors of aerobic processes such as cyanide and azide have little effect on Blepharisma during regeneration.
Dinitrophenol is also without specific effect. Iodoacetic acid and urethane affect anaerobic metabolic reactions. Choramphenicol and RNA ase retard regeneration. DNA ase was found to have no action. Colchicine retards regeneration as it causes generalized injury to the cell. Mercaptoethanol has a powerful inhibitory effect on regeneration, strychnine sulphate produces a generalized injury—removal of the pellicle. (16).

The osmolality and differences in pH, ionic imbalance and osmotic pressure are known to affect the regeneration rate of Blepharisma and Condylostoma magnum (14, 17 30). High concentrations of balanced salt or sucrose solutions block regeneration. NaCl and KCl solutions block regeneration at lower osmolar concentrations. High concentrations of EDTA retard regeneration and very often cause death. A pH range of 5.5 to 7.7 does not affect regeneration, while with pH 4.7 regeneration is retarded for several hours (17). Blepharisma acclimated to solutions of high osmolality do not tolerate low osmolality as indicated by the retarded regeneration in such solutions (18). Regeneration of Blepharisma in high salt concentrations is slow (14). Tartar (30) reported that no regeneration occurred in Condylostoma magnum at 5% salinity.

The sensitivity of regeneration to different types of radiations, has been discussed in the previous chapter.

The rate of regeneration, like other growth processes, decreases with a fall in temperature, and increases with a rise in temperature up to a certain point beyond which it is decreased or stopped. Earlier studies on Blepharisma have shown that regeneration fails at the lower end of the biokinetic zone because of lack of energy liberation. Regeneration in Blepharisma is accelerated by maintaining the cells at 30°C but retarded at 35°C. Even brief exposures to 40°C retarded regeneration. Regeneration is slow when maintained at temperatures below 25°C and stopped at 10°C. Between 13°C - 30°C the rate of regeneration increases with rise in temperature (16). Our observations with Homalozoon and Urostyla have shown a decrease in regeneration rate at low temperatures. A temperature above 28°C retards regeneration in Homalozoon while in Urostyla it is retarded at 32°C. A further rise in these two temperatures respectively blocks regeneration in the two species. This may be due to the denaturation of some enzymes necessary for normal growth and differentiation. If action of temperature is selective upon the enzymes, some enzymes may continue to synthesize cell materials, while others have ceased leading to unbalanced growth. Morgan (23) found that low temperatures block regeneration. 10°C was found to block regeneration in Blepharisma (18). The effect of temperature on nuclear changes during regeneration have been worked on Blepharisma (18) in Stentor (8, 9, 28, 29). Thermal injury to the protozoans has been observed at 38°C in Blepharisma (18) and in other protozoa (22).
Regenerating organisms have a definite polarity. Experiments by Child and others have demonstrated a proximodistal gradient passing from high intensity at the anterior end to a low intensity at the base. Stentor exhibits reparative and physiological regeneration (1, 29). During reparative regeneration, the oral structures regenerate from the cut surfaces of the posterior piece and foot parts regenerate from the cut surface of the anterior piece. There is clearly a head to foot polarity. Uhlig (32) demonstrated in Stentor coerulesus an axial gradient in foot formation that was highest at the posterior end and also proved strongest on the ventral side, where the oral anlage is formed. The same results have been reported in Stentor coerulesus by Tartar (27, 28, 29). This was later confirmed by Uhlig (33). Eberhardt (10) working on Spirostomum ambiguum noted that the oral differentiation was closely related to the anal pole. Welsz (34) noted that the presence of the holdfast (a posterior terminal structure) also hastens oral differentiation in Stentor, since middle fragments regenerate more slowly than posterior ones. Child (6) attributed this difference to the fact that middle fragments must carry out regeneration of both head and tail. Tartar (29) does not agree with this and pointed out: "If the presence of a holdfast hastens oral differentiation, then stentors from which the head only is excised should regenerate sooner than animals from which head and tail are removed, but they do not."

In this investigation the axial gradient theory is confirmed by the difference in the regeneration rate of the anterior and posterior halves. The anterior half regenerating faster than the posterior half at all temperatures which favour regeneration.

It has been shown that the reproductive rate in protozoa is temperature-dependent (4, 18, 21, 31). Thormar (31) found that cell division was blocked at 33.5°C in Tetrahymena pyriformis. Buetow (4) found that growth of Euglena ceases beyond 30°C and fission is blocked at 32°C. In Trypanosoma there is a block in reproduction at 31°C (21).

The effect of temperature on the rate of fission has been found to be similar to that of regeneration in Blepharisma (18). Raising the temperature increases the rate of division of Blepharisma only up to a point, after which division rate declines and finally stops. Approximately the same upper limits have been observed and curves relating temperature and regeneration and temperature and fission rate are similar (18). Our observations on the rate of fission and regeneration at the same temperatures on the two unrelated ciliates, Homalozoon and Urostyla with different macronuclear organization, are similar to that of Blepharisma. This suggests that the processes which lead to the two phenomena-fission and regeneration may be similar in the different groups of ciliates.
| 5. Child, C. M. | 1941 | Patterns and problems of development. Chicago, Univ. of Chicago Press. |


14. Giese, A. C. and Benedetti, T. 1971 Reacclimation to low salt levels of Blepharisma acclimated to high salt levels, as measured by rate of regeneration. Physiol. Zool 44: (1) 1-8.


Graph 1 Rate of regeneration of *Homalozoon vermiculare* exposed to different temperatures immediately after cutting.

A — Anterior

P — Posterior
Graph II  Rate of regeneration of Urostyla sp. exposed to different temperatures immediately after cutting.

A — Anterior

P — Posterior
TEMPERATURE (°C) vs. HOURS TO 50% REGENERATION
Graph III  Rate of fission of *Homalozoon vermiculare* at different temperatures.
Graph IV Rate of fission of *Urostyla* sp. at different temperatures.