THE EFFECT OF A FALL OF THE TEMPERATURE ON REGENERATION IN THE AMPHIBIA AND ON THE RESISTANCE OF THE REGENERATING TISSUE UNDER THESE CONDITIONS TO THE CYTOTAXIC ACTION OF NOVEMBICHIN AND TEM

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(Received May 29, 1958. Presented by Active Member AMN SSSR V. N. Chernigovskii)

A marked fall in the activity of metabolic processes in tissues in a state of hypothermia is known to occur, so that this state has been defined as one of rest from the energetic and functional activities of the cell or animal.

A characteristic feature of poikilothermic animals is their adaptation to a wide range of temperatures, although this refers only to their basal metabolism. So far as processes of growth, proliferation and regeneration are concerned, demanding a more intensive metabolism and, in particular, an increase in processes of synthesis, these are always adapted to a narrow range of specific positive temparatures [9].

On the basis of work on the production of synchronous division in cultures of protozoa [13] and bacteria [11], and also in tissue cultures [7], using the method of so-called temperature shock with subsequent return to the optimum temperature, it follows that before cell-division can take place, certain processes must be carried out which are sensitive to changes of temperature and which are artificially inhibited by such changes until such time as the optimal temperature is restored.

The object of the present investigation was to ascertain if it was possible to inhibit growth in regenerating organs of amphibia by means of slight hypothermia, and to study the cutostatic effect of compounds such as colchicine [10], novembichin and TEM [2] while the energetic and functional activities of the animals were thus in a slightly depressed state.

EXPERIMENTAL METHOD

The objects investigated were the regenerating tails of tadpoles (Rana temporaria and Rana ridibunda) of stages II and IIIa (Blacher). The experiments were performed during June - July, 1957.

In order to determine the mean rate of growth of the regenerating tail at a particular temperature, one fifth the length of the tail was amputated in 25 tadpoles, after which the length of the regenerated portion was measured every 4 days by means of an ocular micrometer under a loupe (ocular 10, objective 1.75). The experiment continued for 14 days. The arithmetic means of each of the three measurements were calculated and used to construct a curve of growth of the regenerating tail.

Each experimental study of the effect of the cytostatic drugs was also carried out on 25 tadpoles, from which the tip of the tail was amputated immediately after a single injection of the drug. The control series (amputation of the tail, but no drug administered) consisted of 25 tadpoles. The regenerating tails were measured just as in the previous experiments.

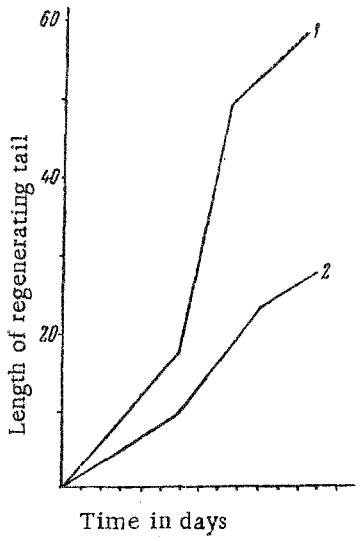

Fig. 1. Rate of growth of the regenerating tails.
1) At optimal temperature;
2) at a lowered temperature.
Abscissae – time in days from the moment of amputation.
Ordinates – length of regenerating tail in divisions of the ocular micrometer (one division equivalent to 0.087 mm).

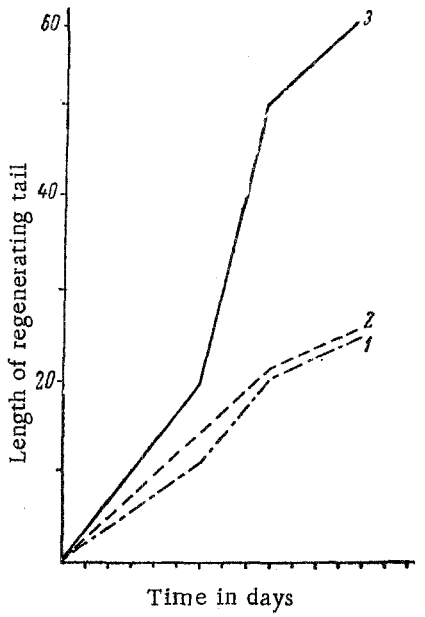

Fig. 2. Rate of growth of regenerating tail at optimal temperature with the action of novembichin (1), TEM (2) and control (3).

The method of keeping the tadpoles for predetermined periods of time in solutions of the test substances is very unsuitable, for it does not allow the dose of the drug ingested by the tadpole to be measured and also many of the drugs are rapidly hydrolyzed in aqueous solution.

Intraperitoneal injection of the tadpoles was thought to be impossible in view of their very high intraperitoneal pressure and the feeble development of the musculature of their abdominal wall.

We used a simple method of injection of the solutions into the peritoneal cavity of the tadpole. The injection needle must first be passed through the musculature of the tail a short distance from its base, and its tip is then pushed through into the peritoneal cavity. The rapid contraction of the muscles of the tail after withdrawal of the needle prevents escape of the injected fluid back along the puncture wound. This method of injection is especially convenient in the larger tadpole of Rana ridibunda. Doses are calculated per kg body weight.

Since tadpoles of the same species and stage, taken from the same tank, are sufficiently homogeneous experimental material, as a preliminary measure we weighed 5-6 specimens taken at random and determined the mean weight for this particular stage from which we calculated the dose. Altogether 10 experiments were carried out. The preparations were injected in physiological saline (0.65% solution of sodium chloride).

EXPERIMENTAL RESULTS

A mean daily temperature of the external environment of 18-20° is the optimum at which rapid growth of the regenerating tail was observed (Fig. 1, 1). When the water temperature was lowered by 2-4°, i.e. at an average daily temperature of 14-16°, an obvious fall in the rate of growth of the regenerating tail was observed (Fig. 1, 2).

Subsequently, the regenerating tails growing intensively at optimal temperature will be referred to as type A, and those whose retarded development was adapted to lowered temperature conditions, as type B. If the mean length of the rapidly growing type A regenerating tail on the 14th day was taken as 100%, then the difference between it and the length of the type B tail, expressed as a percentage of the length of type A, was 60%.

It could thus be concluded that a fall in the average optimal temperature pf 2-4° inhibits the regeneration of the tail of the tadpole by 60%.

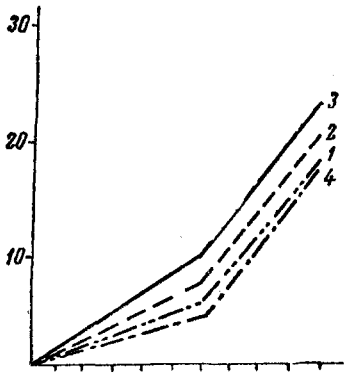

Fig. 3. Rate of growth of the regenerating tails at a lowered temperature and under the action of novembichin (1), TEM (2), control (3) and of a double dose of TEM (4) (16 mg/kg).

The mitotic coefficient for the type A regenerating tail, estimated for 10,000 cells of the epidermis of the regenerating tail and of the adjoining 5 mm of the residual organ, was 2.5% on the 9th day after amputation. The mitotic coefficient for the type B regenerating tail under the same conditions of counting was 0.8%.

Preliminary experiments to determine the toxicity of the tested compounds to tadpoles showed that the LD 100% (the dose lethal to 100% of animals) of novembichin and TEM for tadpoles of stage II and IIIa per kg of body weight was 10-12 times as high as the LD 100% for rats. A single dose of TEM of 8 mg/kg, and of novembichin of 10 mg/kg was well tolerated by the tadpoles, i.e. if the LD 100% of these drugs for rats, which has been well studied, was taken as n, the single dose tolerated by tadpoles was approximately 4n. Hence the cytostatic action of these two compounds could be compared if the dose used was equivalent to 4n.

In respect of colchicine, however, the LD 100% for tadpoles only exceeds the LD 100% for rats per kg body weight by $1^1/_2$ times. For this reason colchicine was given in a dose of 0.6 mg/kg, i.e. a dose equal to n. The action of novembichin and TEM in equivalent doses on the type A regenerating tail took the form of considerable inhibition of growth of the tail (Fig. 2, 1 and 2).

If the degree of inhibition of growth is represented as the difference between the lengths of the regenerating tail in the experimental and control groups, expressed as percentages of the control values, the inhibition of type A regeneration by novembichin and TEM would be about 60% (57 and 54%). The inhibitory action of colchicine on type A regeneration was significantly weaker – 23% in all.

The type B process of regeneration was more resistant to the action of novembichin and TEM (Fig. 3, 1, 2), for hardly any inhibition of growth of the regenerating tail was observed (13 and 17%). Doubling of the dose of novembichin (20 mg/kg) caused practically no increase in the effect. Doubling the dose of TEM (16 mg/kg) led to some intensification of inhibition of growth of the regenerating tail (up to 23%) (Fig. 3, 4). On the other hand, colchicine had an equally inhibitory effect on both type B and type A regeneration (27%). In a lethal dose (1 mg per kg) colchicine caused total inhibition of both type A and type B regeneration, and the animals died on the 7th-8th day.

Certain phases of synthesis possibly exist in the life cycle of the cell which are particularly sensitive to some injurious agents, one of which may block these phases and produce a certain degree of resistance of the cells to the subsequent action of other injurious factors, i.e. may produce nonspecific resistance of the cell to several injurious factors. M. N. Meisel' and his co-workers [4], for example, established that yeast cells, exposed to the toxic action of berberine, thioflavine or ethyl alcohol, became resistant to the action of ionizing radiation.

On the other hand, it has been found in plants and cultures of protozoa [1, 5] that if cells acquire resistance to a superoptimal temperature they will also be resistant to a lowered temperature and to the action of certain noxious chemical agents In such cases signs of weakening of the processes of synthesis are observed, namely: retarded growth [6] and lowering of enzyme activity [12] of tumors resistant to the chloroethylamines, slow growth of regenerating tissues resistant to TEM and novembichin (in our experiments), depression of photosynthesis in the leaves of plants resistant to a raised temperature [3] and so on.

On the basis of the information in the literature on this subject [1, 8, 12] it can be assumed that the fall in metabolism to a certain extent, mainly at the expense of depression of oxidative processes, is an important condition for the acquisition by the cells of resistance to the action of certain injurious factors.

The resistance of type B regeneration to the action of novembichin and TEM in our experiments is presumably also due to a fall in the metabolic activity of the tissues concerned.

SUMMARY

The average velocity of growth of the tail regenerate in Rana temporaria and R. ridibunda tadpoles of the II and III stage (according to Blacher) was studied at various temperatures. It was demonstrated that the average 24-hours temperature of 18-20° C is the optimal for the growth of the regenerate. By decreasing the water temperature by 2-4° C the velocity of the regenerate's growth is retarded by 60% (in comparison with the growth at the optimal temperature). The author studied the effect of colchicin, novembichin and 2, 4, 6 triethyleneimio 1, 3, 5-triazine (TEM)on the regeneration of the tail at optimal temperature (type A), as well as in its regeneration at decreased temperatures (type B). The technique of administration of these preparations into the tadpole body cavity was developed and LD 100% determined. The process of regeneration in type B revealed the resistance to the effect of novembichin and TEM.

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