Effects of Actinomycin D on the Distal End Regeneration in *Hydra vulgaris* Pallas

The process of hypostome and basal disc regeneration in hydra involves activation, migration, multiplication and transformation of cells[1]. MORGAN[2] has proposed epimorphosis and morphallaxis as the mechanism of restitution in hydra. This was later supported by authors like SANYAL[3] and SANYAL and MOOKERJEE[4]. In order to see the effects of inhibitors of energy metabolism on this dynamic process, several antimetabolites were used under different conditions without much effect on the initial regeneration of the distal end in *Hydra vulgaris*[5]. Recently CLARKSON[6] studied in detail DNA, RNA and protein synthesis during first hour of distal end regeneration of decapitated *Hydra littoralis*. He found no appreciable increase in DNA synthesis, a large increase in RNA synthesis and a slight increse in protein synthesis. He further studied effects of Actinomycin D(AD) on RNA and protein synthesis in hypostome regenerating hydra and obtained substantial suppression of RNA synthesis without much effect on protein synthesis[7]. Hypostome formation was not inhibited completely under these conditions. In the present study AD at various concentrations applied at different hours before and after amputation was tested with a view to studying its effect on the distal end regeneration.

Material and method. *Hydra vulgaris* Pallas were cultured following the method of LOOMIS and LENHOFF[8]. 3 different experimental conditions were set up. The animals were cut at the subhypostomal level and immediately transferred to AD at 5 and 10 µg/ml in hydra solution. In the second set of experiments, amputation was done after several hours of pretreatment with AD solution and kept in the same solution for observation. In the third set of experiments the actinomycin treated animals after amputation were left in normal hydra solution. Controlled animals were maintained with each set of experiments. When 75% of the animals in each set-up regenerated, the results were considered as positive. The increase in length of the tentacles was considered as the rate of regeneration in this study. The length of each tentacle was measured in relaxed state on the screen of a Reichert Visopan Microscope. The mean of the total length of all the tentacles at a particular hour was divided by the magnification value to obtain the real length, and is shown in the Figure.

Results and discussion. Normally a decapitated *Hydra vulgaris* takes about 24 h to reconstitute hypostome with tentacle rudiments. When hydras were kept in 10 µg/ml AD solution immediately after amputation, the reconstitution time was delayed by an average of 6 h and the rate of regeneration appreciably decreased during the first 72 h of differentiation, after which cytolysis followed and the animals disintegrated. In 5 µg/ml series only the rate of regeneration decelerated (Figure).

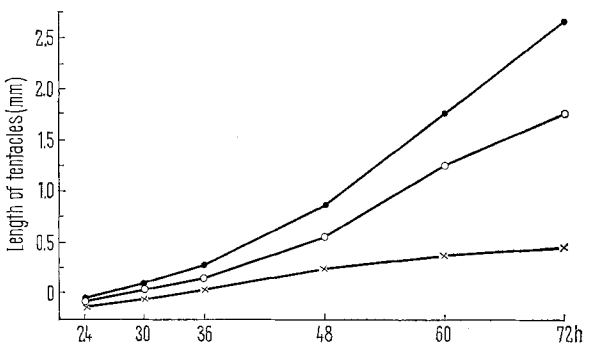

Fig. 1. Showing the rate of increase of the length of tentacles after Actinomycin-D treatment. ●—●, control; ○—○, 5 µg/ml; ×—×, 10 µg/ml.

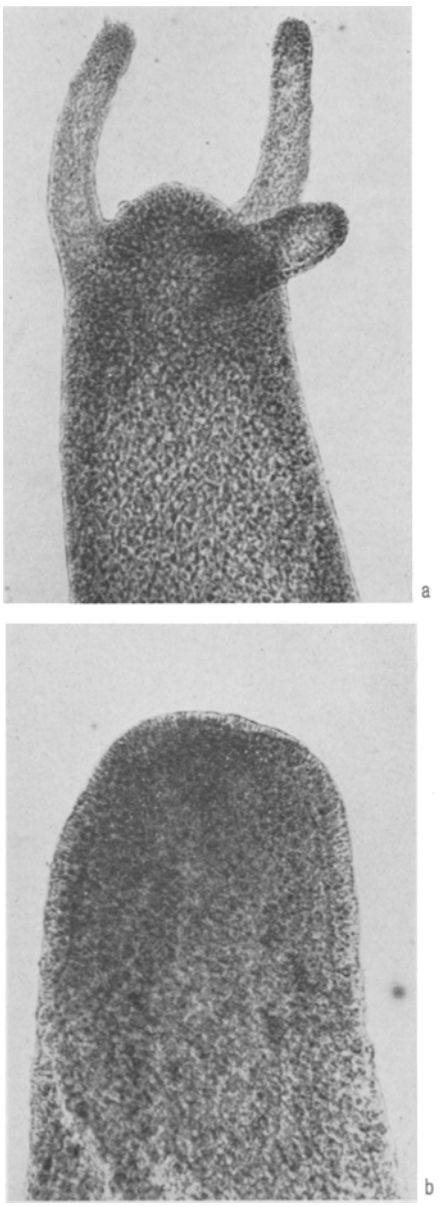

Fig. 2. a) Hydras pretreated in Actinomycin-D for 48 h regenerated after 72 h b). Pretreated for more than 48 h, failed to regenerate.

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Whole hydras were pretreated in 10 µg/ml AD separately for 24 and 48 h and subsequently were amputated. In the former case, a definite rate of regeneration up to 48 h was observed, followed by gradual disintegration. In the latter case, there was no differentiation at all and by 96 h all had disintegrated. Hydras treated in 5 µg/ml AD did not disintegrate till 10 days.

When 24 h pretreated animals in 10 µg/ml AD brought back to normal solution after amputation, differentiation started after 48 h. The 48 h pretreated animals differentiated after 72 h (Figure 2, a). The 72 h pretreated animals did not differentiate but continued to live for 10 days or more (Figure 2, b) and no regeneration occurred in 96 h pretreated animals and all disintegrated beyond 4 days.

Decapitated hydras treated with AD at 10 µg/ml up to 48 h could not totally suppress the initial reconstitution process, but animals pretreated more than 48 h before amputation failed to regenerate. This indicates that although there is a burst of RNA synthesis at the early hours of hypostome determination[6], it is not really essential for the initial reconstitution process. The initial differentiation of the proximal end of hydra cut at the subhypostomal level is perhaps accomplished by structural proteins, synthesized with the help of a stable variety of messanger RNA. The existence of a stable mRNA or a masked templet material for initial hypostome determination in hydra has been respectively suggested by CLARKSON[7] and DATTA[5]. A masked RNA has also been reported to be responsible for the AD resistant protein synthesis in *Arbacia* egg[9]. From the above results it was revealed that the differentiation could only be suppressed if treatment with AD was done more than 48 h before amputation. This could perhaps be the time required for total turnover of the preexisting stable mRNA associated with the initial determination process. Treatment for more than 48 h in 10 µg/ml AD possibly inflicts permanent damage on the metabolic activities of the cell, as a result of which no regeneration took place[10].

Zusammenfassung. Actinomycin D-Wirkung auf die Mund-Tentakel-Regeneration bei *Hydra vulgaris*. Auch später absterbende Tiere beginnen die Regeneration, was als Folge einer stabilen RNS angesehen wird.

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Department of Zoology, Presidency College, Calcutta (India), 17 February 1970.

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[10] The authors are indebted to Prof. S. MOOKERJEE, head of the Department of Zoology, Presidency College for lending laboratory facilities.

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The Effects of Passive muscle Stretching on the Discharge of Individual Phrenic Motoneurones

Two mechanisms are discussed for the rapid change of ventilation at the start of muscular excercise: 1. direct stimulation of the respiratory centers by impulses from the brain during active excercise, 2. indirect reflex-induced stimulation of the respiratory centers attributed to the action of receptors of the limbs[1]. The purpose of this work was to evaluate the effect of stimulating muscle spindles and tendon organs on respiratory activity, while avoiding secundary effects induced by respiratory changes. We therefore investigated the response of phrenic motoneurones on passive muscle stretching in anaesthetized and paralyzed dogs under constant artificial respiration.

Methods. 5 dogs were anaesthetized with pentobarbitone sodium (Nembutal Abbott) 30 mg/kg i.v. After insertion of an endotracheal tube, spontaneous ventilation was abolished by continuous infusion of Gallamine triethiodide (Flaxedil Abbott). Artificial ventilation with pure oxygen was maintained by a respirator. Both vagi had been cut. The roots of the phrenic nerves on the two sides were dissected free in the neck and cut. Thin filaments were separated from the cut central end of the phrenic nerve on one side, subdivision usually beeing carried out until only a single responding motor unit was present. For stretching the muscles gastrocnemius and flexor digitorum superficialis, their tendons were cut from the calcaneal tuber and connected to the piston-rod of an airmotor (Bellows Valvair Corp.) with steel wire. The stretch-lenght was 35 mm in all experiments.

Results. Figure 1 shows some characteristic features of the response of phrenic motoneurones before, during and after stretching the calf muscles. During the whole procedure the arterial PCO_2 was kept at 37.8 Torr constantly. Before the onset of stimulus (upper row of the figure) one can see recurrent trains of 2 units separated by silent periods, with the smaller unit firing at the end of the inspiratory phase. At the onset of stimulation-indicated by the incline of the lower trace of the record the recruitment of an additional unit in the first poststimulus discharge is evident along with an increase of the discharge of the smaller unit.

With prolongation of the stimulus (second row), the frequency of cycles increases and the duration of discharges are shortened. Soon afterwards the frequency of the cycles decreases and the duration of discharges is again longer. By the end of stimulus (third row), the number of impulses in both the additional unit and the smaller unit is diminished. With the offset of the stimulus-indicated by the decline of the lower trace, there is again brief increase in the activity of phrenic motoneurones. Immediately afterwards the activity subsides and only one unit remains firing. In Figure 2 the frequency of all impulses per cycle and the duration of each discharge phase of the above experiment are plotted against time. In the relaxation phase at the beginning the average value of impulse frequency is about $15 \times \text{sec}^{-1}$, meanwhile the discharge phase lasted about 2 sec. The respiratory rate at this time was $12 \times \text{min}^{-1}$. After stretching the muscles one can differentiate 2 phases of a response of the phrenic motoneurones to the peripheral stimulus. In the first phase with the onset of stimulus – pointed

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