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*A Mechanism of Action of Neurotransmitters on the Regeneration of the Planarian Worm *Dugesia tigrina*. Role of Acetylcholine as a Negative Feed-back*

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Abstract: By means of histochemical and pharmacological analysis it is shown that acetylcholine has an inhibitive effect on regeneration in the planaria *Dugesia tigrina*.

It seems that serotonin and adrenaline on the one hand, and acetylcholine on the other hand, play a role in a mechanism of regulation in which the latter plays a role in a negative feed-back process.

Introduction

On the basis of histochemical and pharmacological studies it was possible to suggest a mechanism of regeneration in which serotonin or N-adrenaline plays a promotive role in the presence of acid mucopolysaccharides and collagen (Lenicque 1973, 1974). The aim of the present work is to find out whether or not acetylcholine is also involved.

Material and Methods

Small pieces of *D. tigrina* were isolated from the antepharyngeal part of the worms. These fragments are able to regenerate the head within 4-5 days after the operation.

Although it is not possible to detect acetylcholine histochemically, the activity of the corresponding enzyme - acetylcholinesterase - can be visualised on frozen sections (20 μ m) by the acetyl- and butyrylthiocholine method of Koelle: A dark brown precipitate could be located along the nervous system and in the subepithelial layer of the ventral side. Control sections treated with eserine 10 \cdot 5 M or heated or incubated without substrate were uncoloured, cf. Pearse (1968).

The pharmacological analyses were performed on populations of 20 pieces of animals cultivated during a week in a Petri dish in 50 ml of tap water (controls), or in the solutions of the agents to be tested. Each series of experiments was repeated three times. For diagnosis the progress of healing, the adhesiveness of the animals on the substrate,

their motility and behaviour, and the development of the pigmented eyes were observed. The following neuropharmaca were used: d-tubocurarine, atropine, d camm thonium, pempidine and eserine. The duration of the treatments was three days.

Results

1) *Acetylcholinesterasic activity during regeneration.* In the normal animal a tremendous acetyl- and pseudo-acetylcholinesterase activity is located in the cerebral ganglia and in the nerves. Another site where activity can be detected is the subepithelial layer of the ventral side. This activity diminishes slightly during the first day after operation and more quickly during the second one. The third day specimens are characterized by a nearly total lack of activity. However, on the fourth day the activity reappears at the anterior part of the planaria where the brain and the eyes have just regenerated. On the fifth day the normal acetylcholinesterasic activity has returned (Figs. 1 and 2).

It must be pointed out that during all this time, even when no enzyme activity can be detected by the method of Koelle, the behaviour of the animals is quite normal; they thus react normally to light or to tactil excitations, which indicates that the nervous integrity remains. Addition of eserine to the water on the other hand brings about paralysis. One may therefore ask about the significance of the disappearance

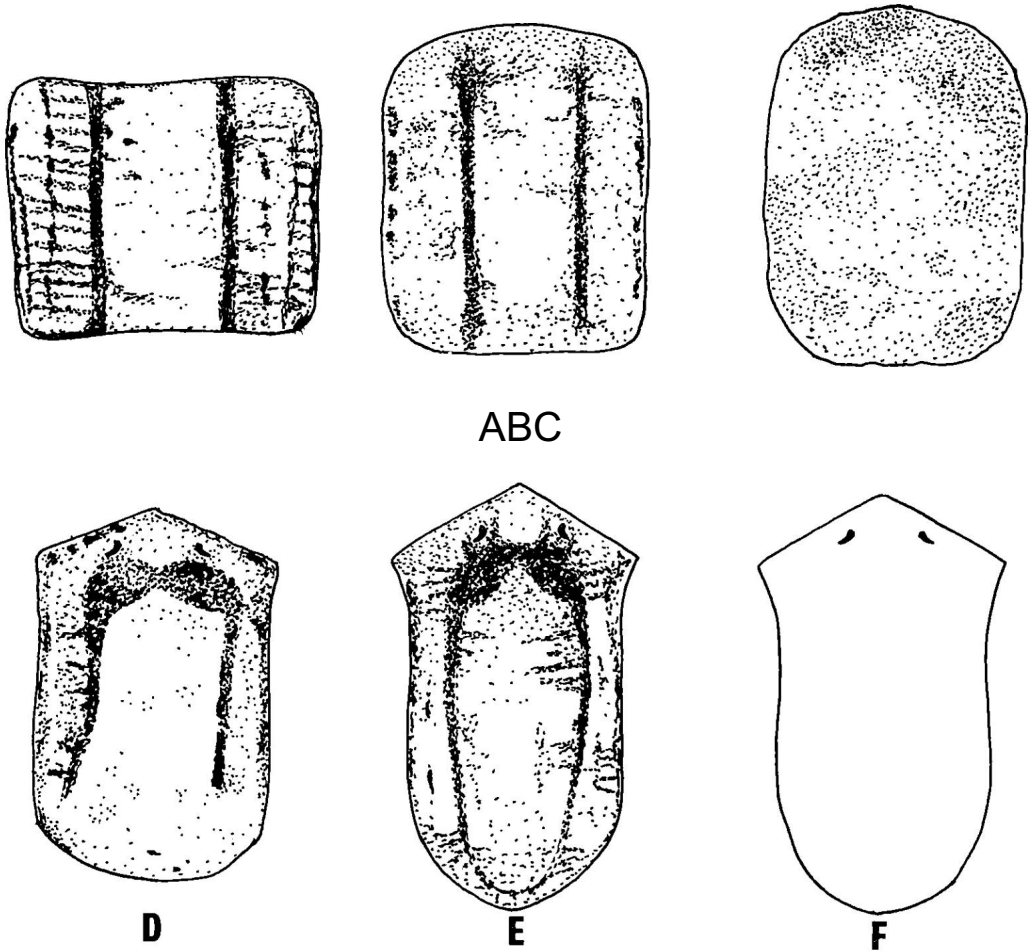


Fig. 1. Acetyl- and pseudo-acetylcholinesterase activities in 20 μm thick frozen sections of regenerating antephenaryngial pieces of *Dugesia tigrina*. **A.** Immediately after the operation, **B.** 24 hours, **C.** 3 days, **D.** 5 days, **E.** 10 days. **F.** Control of E treated with eserine. The enzymatic activity follows the course of the nerves. Note some activity at the anterior part of the piece in C. It is important to point out that even at stage C the animals have a normal behaviour. (Schema drawn on the basis of series of sections.)

of the cholinesterase activity during the period of high regenerative activity. The pharmacological study seems to give the answer to this question.

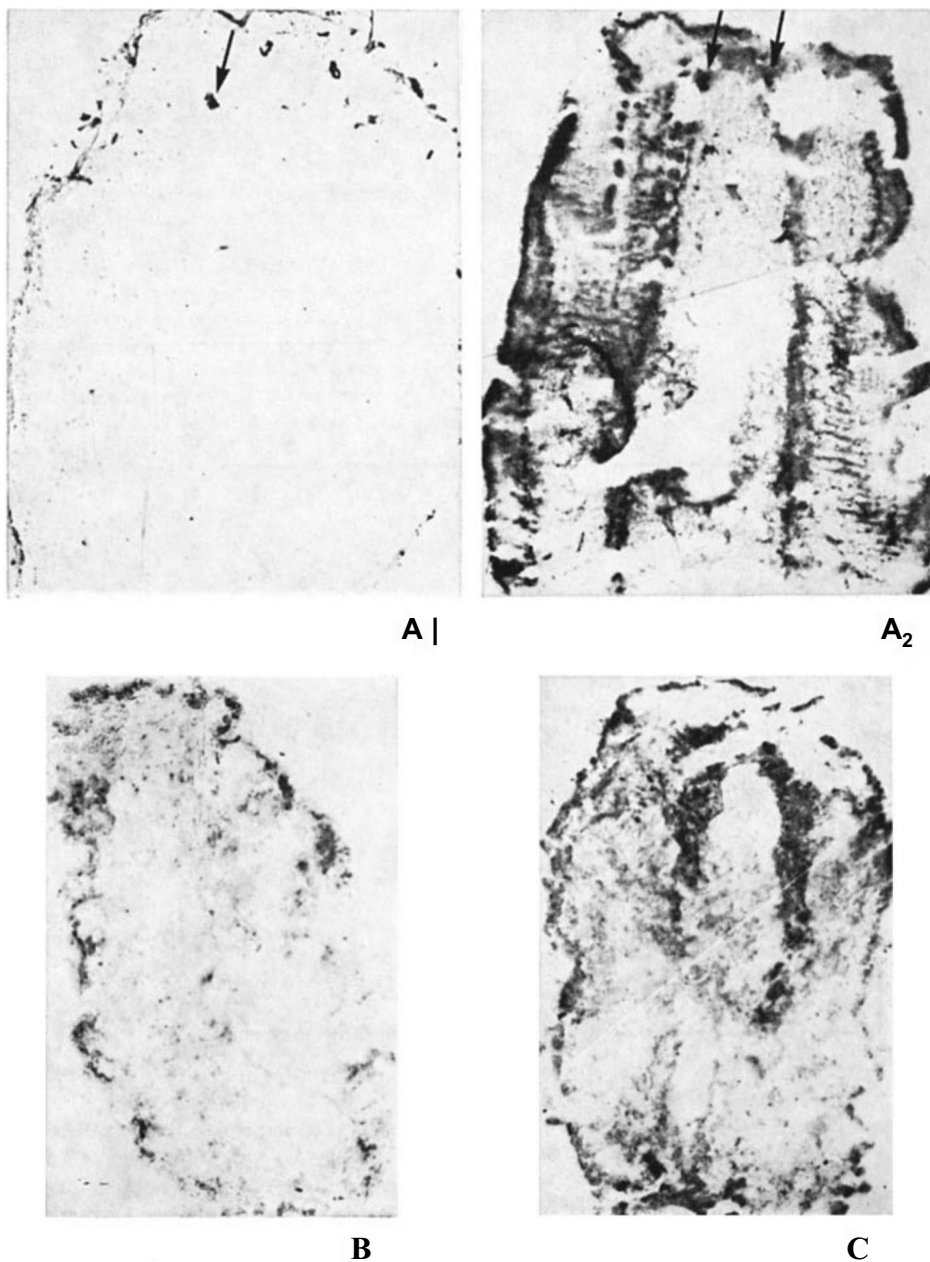
2) *Effect of acetylcholine on regeneration.* Accumulation of acetylcholine in the tissues is brought about by the treatment with eserine 10^{-5} M. It is evident from curves B (Figs. 3 and 4) that eserine

inhibits the capacity of regeneration, while d-tubocurarine or atropine have a protective effect in eserine treated specimens (curves A). In fact these antagonists of acetylcholine have also a slight promotive effect on the speed of regeneration and this also holds for d cam thonium and pempidine (Lenicque and Jacobson 1969).

Discussion and Conclusion

This study indicates that acetylcholine has an inhibitive effect on regeneration. If the disappearance of the corresponding enzyme also means a disappearance of the whole cholinergic system one may think that this disappearance is a prerequisite for regeneration to occur during this period.

In our previous papers (Lenicque 1969, 1971,



A1

A₂

B

C

1972, 1973, 1974) we have shown that the biogenic amines 5-hydroxytryptamine, serotonin), adrenaline and their precursors—hydroxytryptophan respectively DOPA—have a promotive effect on regeneration. The present work indicates that acetylcholine has an inhibitive effect. It seems that these neurotransmitters are involved in a mechanism of regulation of regeneration and that

Fig. 2. Photographs of sections treated by the method of Koelle. A₁. Control to A₂ (inhibition by eserine). A₂. Note enzymatic activity along the nerves and in the sub-epithelial layer; the arrows show the eyes. B. Animal at three days after the operation; no enzymatic activity. C. the acetylcholinesterase activity in nerves and brain ganglia five days after the operation.

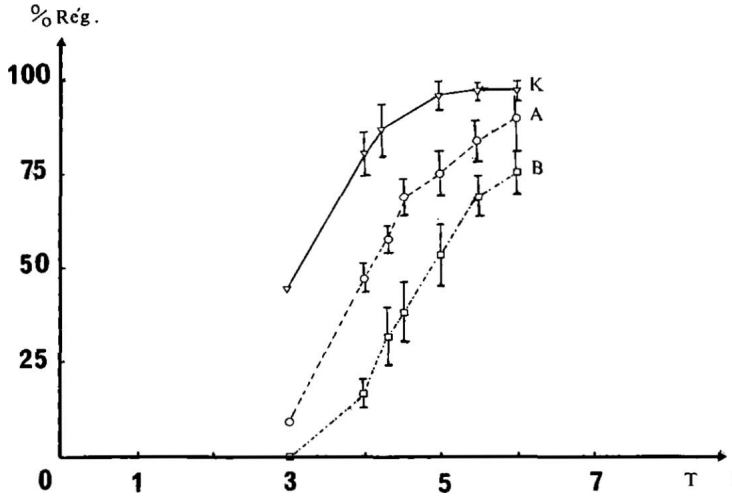
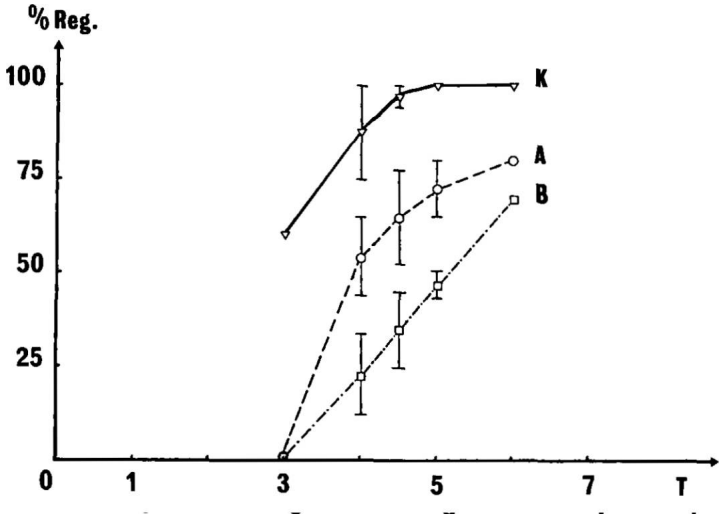


Fig. 3. Effect of eserine 10^{-5} M on the regeneration and protection by *d*-tubocurarine 10^{-5} M. The test is the formation of the dark pigmented eyes. Reg. percentage of animals with eyes, T time in days, K controls, A eserine + *d*-tubocurarine, B eserine. N = 180 animals.

Fig. 4. Effect of eserine 10^{-5} M on the regeneration and protection by atropine. A eserine + atropine, B eserine. Same symbols as in Fig. 3. N = 180 animals.

in pharmacological experiments or if corresponds to a physiological mechanism. The reason for our reservation is that it is impossible to detect any cholinesterase activity in the tissues of this animal. However, it seems that similar mechanisms of regulation of regeneration may be involved in the two categories of animals although the neurotransmitters may be different: a biogenic amine and a still unknown agent in *M. senile*, serotonin and acetylcholine in the case of *Dugesia tigrina*.

acetylcholine may play a role in a negative feedback process.

A similar study was performed on the sea anemone *Metridium senile* (Lenicque, unpublished), but it is difficult to know whether the inhibitive effect of acetylcholine is only revealed

Acknowledgements

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