involved in constructing imaging devices that use the various radiations, but we are concerned with the question of their utility as microscopes if such devices should be constructed.
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Regeneration of the Amputated Amphibian Limb: Retardation by Hemicholinium-3

Abstract. *Doses of hemicholinium-3 which partially paralyze the larval salamander profoundly retarded regeneration of the amputated hind limb. The drug also reduced the vascularity and mitotic index of the regenerating tissue. After withdrawal of the drug the rate of regeneration returned to normal. Atropine, in a daily dose of 40 milligrams per kilogram of body weight, did not retard growth. These findings suggest that in the salamander acetylcholine may mediate neurotrophic activity.*

Regeneration of the amputated amphibian limb requires the presence of nerve (*1*). If the nerve is transected after growth has begun, the regenerate gradually breaks down in a distoproximal direction and is resorbed (*2*). Both sensory and motor fibers can initiate and maintain limb regeneration, although in the case of the sensory nerve, the trophic influence is opposite in direction to transmission of impulses from the periphery. Drachman (*3*) has presented convincing evidence that development of striated muscle depends upon release of acetylcholine. He showed that botulinum toxin, hemicholinium-3, and curare prevented development of striated muscle in the growing chick embryo. Fat replaced the muscle mass, a condition usually seen after long-term denervation. The myocardium was unaffected.

Our interest in acetylcholine as a possible trophic factor arose from studies of children with familial dysautonomia. This disorder is characterized by a parasympathetic defect with sensory impairment and subnormal growth (*4*). Since this disorder is probably caused by a single enzyme defect (*5*), the deficiencies ought to be related. We therefore undertook a study of the effects of prolonged cholinergic blockade on tissue of gilled salamanders. The aquatic form of these salamanders was selected because respiration through the gills requires little or no muscle activity.

Ambystoma tigrinum larvae (Lemberger) weighing approximately 15 to 20 g were used in all experiments. Each animal was maintained in a separate vessel (7 by 10 by 4 inches) at room temperature. Water was changed daily. Control groups and treated groups were studied concurrently. Each week the growth rate was determined from measurement of the distance from the point of amputation (knee) to the end of the stump or the tip of the distal phalange. Phalanges did not appear until after the 5th week. The drugs were prepared in modified Ringer (amphibian) solution and injected intraperitoneally with a 25-gauge needle in volumes up to 0.5 ml. Hemicholinium-3 was injected daily or every 2nd or 3rd day, depending on the persistence of paresis.

The mean rate of limb regeneration in seven salamanders treated with hemicholinium-3 in a dose of 1.5 mg/kg was 40 ± 6 percent of the control rate (100 ± 8 percent); in seven salamanders treated with a dose of 3 mg/kg the rate of regeneration was only 34 ± 1.6 percent of the control rate (Fig. 1). These figures represent mean growth measured after 10 weeks. Growth rates during the undifferentiated stump stage and the phalangeal stage were retarded by treatment. As compared with controls, four salamanders treated with atropine in a daily dose of 40 mg/kg showed no decrease in growth rate of the regenerate.

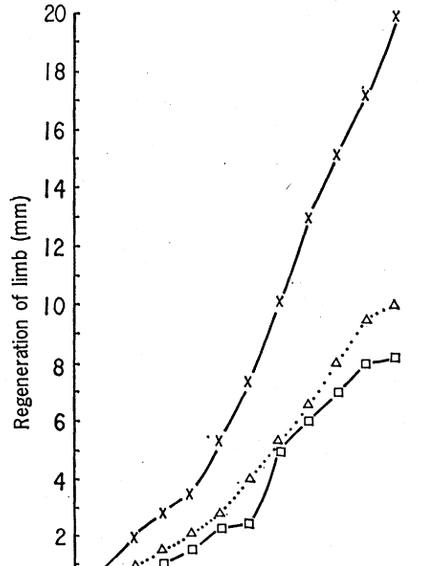

Fig. 1. Effect of injection of hemicholinium on mean growth rates of regenerating salamander limbs. Control, X; hemicholinium (1.5 mg/kg), triangles; hemicholinium (3 mg/kg), squares.

To determine whether salamanders treated with hemicholinium for 11 weeks were permanently poisoned, we amputated the regenerating leg and allowed it to regrow without further administration of a drug. Regeneration was identical to that in controls, indicating that the effect of hemicholinium was reversible. To demonstrate that the initial amputation did not stimulate subsequent regeneration we measured the growth rates 10 weeks after the first amputation and similarly after a reamputation. The first and second rates of regeneration were identical in each of two salamanders studied. We then considered that treatment with hemicholinium might inhibit growth because 25 percent of the body weight was lost during treatment. For that study, the hind limb of an untreated salamander was amputated, but no food was given to the animal during the 10-week regeneration period. Despite a weight loss of 50 percent, the rate of limb growth was not different from that in previous controls.

The diminished growth rate in treated salamanders correlated well with the microscopic findings. Salamanders treated for 5 weeks with hemicholinium showed only about 30 mitotic figures in the median sagittal section of the regenerate. However, more than 400 were seen in similar sections from controls. Colchicine (2 mg/kg) was injected into both groups 12 hours before the salamanders were killed so that mitotic figures would accumulate and could be counted. Evidently hemicholinium did not inhibit regeneration by means of an effect like that of colchicine, for far fewer mitotic figures were seen among treated specimens than among controls. Furthermore, the fact that the mesenchymal cells were not enlarged argues against the possibility that hemicholinium blocks cell division at an earlier stage. The drug also prevented the massive dedifferentiation of muscle tissue normally seen in sections just proximal to the amputation site. In untreated amputees the neurilemmal nuclei increased in number and enlarged with dedifferentiation of the nerve invading more proximal parts, but treated animals showed virtually no nerve changes. Gross and microscopic examinations showed that the vascularity of these regenerating stumps was also strikingly diminished.

Since the salamanders were maintained in a partially paralyzed or paretic state by treatment with hemicholinium, it is possible that disuse of the stump could account for the observed inhibition of growth. However, in one experiment in which triethylcholine (400 mg/kg) was administered, the rate of regeneration was nearly 72 percent of control, despite the fact that this drug paralyzed the animal more effectively than hemicholinium did. Botulinum toxin, which also produces paralysis, has been reported (*6*) not to delay regeneration of the salamander limb. Furthermore several changes were observed which cannot relate to the paralytic effect of drug treatment. After 5 weeks of treatment with hemicholinium the taste buds of the salamander showed regressive and degenerative changes. After 19 weeks of treatment the taste buds disappeared, but they returned when treatment was discontinued. Children with dysautonomia not only show a parasympathetic insufficiency with a growth disturbance but also a lack of lingual taste buds (*7*).

There is much evidence supporting the hypothesis that acetylcholine is a neurotrophic factor (*3*), but there are also compelling reasons against its ready acceptance. Singer (*8*) reports that microinfusion of atropine into the regenerate halts limb growth. On the other hand, infusion of acetylcholine into a denervated limb does not restore its regenerative capacity. However, an infusion cannot duplicate neural activity. A more cogent reason for excluding acetylcholine as the growth mediator is that the concentration of the substance in sensory nerves is negligible compared to that in motor nerves. Yet the sensory nerve is far more capable of influencing regeneration than is the motor component. On the other hand, regenerating tissue contains concentrations of acetylcholine which are greater than normal but which then return toward normal during the period of early differentiation.

As yet we have no way of deciding whether treatment with hemicholinium retards growth primarily by blocking synthesis of acetylcholine or by functioning like curare (*9*). We have attempted to rule out the latter effect by simultaneously administering choline (10:1 molar ratio) and hemicholinium. However, the combination proved toxic. A direct inhibition of mitosis by hemicholinium has not been excluded, but in view of the ability of drug treatment to mimic in the salamander some features of dysautonomia, a disease characterized by a cholinergic defect, hemicholinium appears to act as a cholinolytic agent.

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Polychlorinated Biphenyl: Interaction with Duck Hepatitis Virus

Abstract. *Ten-day-old mallard ducklings fed a polychlorinated biphenyl at concentrations of 25, 50, and 100 parts per million for 10 days suffered no apparent clinical intoxication. Five days later these birds were challenged with duck hepatitis virus, and they suffered significantly higher mortality than birds which were not exposed to the polychlorinated biphenyl.*

Polychlorinated biphenyls (PCB) along with DDE [1,1-dichloro-2,2-bis-(*p*-chlorophenyl)ethane] are reported to be the most abundant of the chlorinated hydrocarbon pollutants in the global ecosystem (*1*). Despite the fact that PCB's have been in wide use since 1930 (*2*), they remained ecologically inconspicuous until 1966 when a Swedish chemist reported their presence in the tissues of pike and other wildlife (*3*). Since then, their presence has been reported in additional wildlife of Europe and North America (*1*, *4*). These compounds are interesting because they are an important source of interference in the chemical detection of DDT and its metabolites (*5*) and