erly conclude that their modes of action are physiologically independent within the ranges of concentration of the chemicals used. This information could be available but obscured by the use of the physiologists' definition.

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24 January 1969; revised 3 March 1969

Fissioning in Planarians: Control by the Brain

Abstract. *Reduced population densities lead to increased rates of fissioning in planarians whereas higher population densities suppress fissioning. This effect is not primarily due to mucus deposition or substances secreted into the water. Experiments are presented which show a system of population feedback control. In the presence of other planarians, the brain exerts an influence (probably neurohormonal) to suppress fissioning. This influence becomes attenuated with axial distance from the brain.*

Planarians of the species *Dugesia dorotocephala* and *Dugesia tigrina* usually reproduce asexually in laboratory cultures. In such reproduction, the anterior portion of the planarian tears away from the posterior region. Each fission fragment then regenerates the appropriate missing part to yield complete but smaller worms. No morphologically differentiated plane of fracture is evident before the onset of fissioning to account for the fissioning or for the point of rupture. Observation of the fissioning process suggests that it is a behavioral or neuromotor event and thus, most probably, mediated by the central nervous system. Previous results (*1*) indicated that a higher incidence of fissioning occurred with low population densities whereas higher population densities suppressed it. Preconditioning of the water and test container by putting in a number of planarians and removing them did not inhibit the fissioning of planarians subsequently isolated in such test habitats, although it did delay its onset by about a day. Since there is some evidence to suggest that the cephalic ganglion (the brain) of the planarian acts as a morphogenetic organizer (*2*) we conjectured (i) that, in the presence of other planarians, it exerted some influence—probably through the ventral cords which arise from the neuropil (*3*)—to suppress fissioning, and (ii) that this influence becomes attenuated with the distance away from the head. The experiments described here test these conjectures.

Our experiments were independently conceived and executed, although we later discovered that Child (*4*) had inferred a cephalic suppression of fissioning. However, we do not consider that his theoretical or experimental basis for doing so were very compelling. His rationale was based on the concept of an axial metabolic gradient. Child did not recognize the obligatory role of population density in the production of this cephalic suppression, and he made no attempt to control or manipulate this factor. Absence of any surgical controls constituted another serious technical defect in his experimental design.

All of the planaria used in our experiments were *Dugesia dorotocephala* maintained for more than 18 months in the laboratory at 21° ± 1°C in enameled dishpans containing aged tap water. They were fed twice weekly on raw beef liver. After each feeding, the pans were cleaned and the water was changed. Each pan contained 600 to 900 worms kept in the dark except during cleaning time when there was dim illumination. The bowl was processed ("preslimed") in the following way. (i) Nonexperimental planarians were placed in a clean bowl or dish a number of hours before the experiment; (ii) they were allowed to deposit a coat of slime; and (iii) they were then emptied along with the water; (iv) the bowl was gently rinsed without rubbing; and (v) it was then refilled with clean aged tap water.

In the first experiment we used 180 planarians. Sixty were selected in each of three different length classes, (i) 6 to 8 mm, (ii) 10 to 12 mm, and (iii) 15 to 17 mm. Each planarian was isolated into a clean petri dish, 40 mm in diameter, in aged tap water at zero time. Unfissioned planarians were transferred each day to a clean petri dish and water. They were observed at 9:00 a.m. and 3:00 p.m. each day to ascertain whether fissioning had occurred. This experiment was performed in three balanced blocks with 30 subjects in each size class in the first block and 15 in each size class in the second and third blocks. After each fissioning in the second and third blocks the fission fragments were anesthetized with 0.15 percent Chloretone, to relax them in the extended position, and were measured.

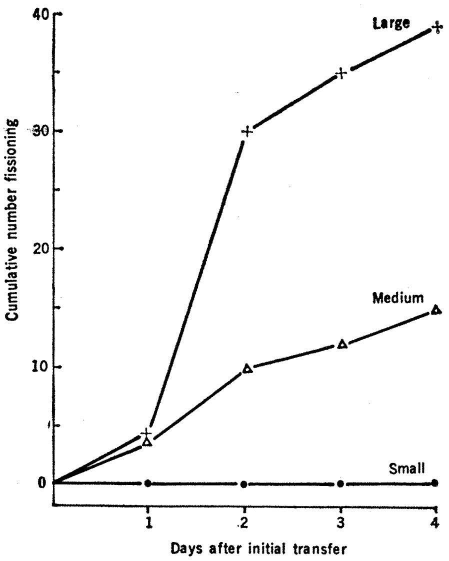

Fig. 1. Cumulative number of fissionings as a function of time after isolation into a new unslimed habitat for three different size classes (15 to 17 mm, 10 to 12 mm, and 6 to 8 mm) of *Dugesia dorotocephala*; 60 planarians were used in each size class.

The cumulative number of fissionings in each size class as a function of the time after the initial transfer is shown in Fig. 1. The lengths of the anterior fragments for the medium and long subjects were 6.1 ± .4 and 7.8 ±

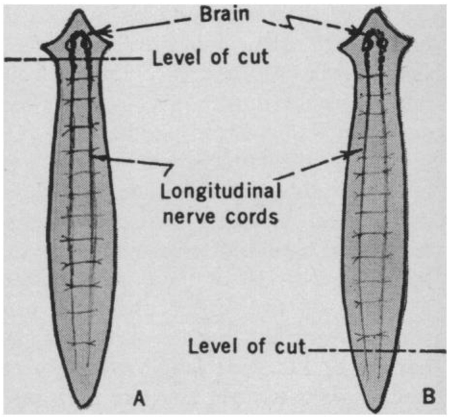

Fig. 2. Schematic diagram of *Dugesia dorotocephala* showing the relation of the nervous system to the levels of surgical transection.

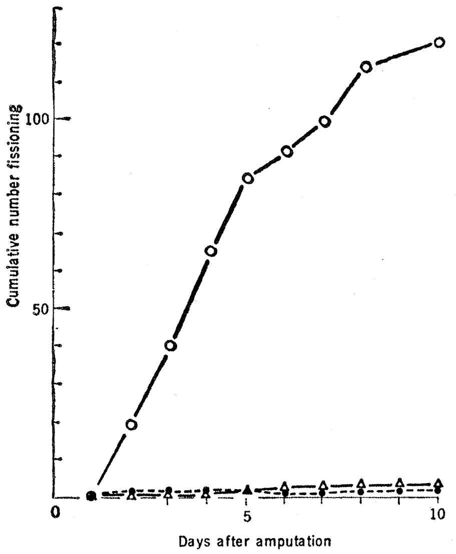

Fig. 3. Cumulative number of fissionings as a function of time in *Dugesia dorotocephala* from which the head was removed, from which the tail was removed, and which were uncut. There were 180 subjects in each treatment group maintained in subgroups of 30 subjects per bowl during the period of the experiment. (Open circles) Head amputees; (triangles) normal planarians; and (solid circles) tail amputees.

.7 mm, respectively. No fissioning occurred in the short animals. Thus, the probability of fissioning per unit time is greater for longer than shorter subjects. The lengths of the anterior fragments within each size class are fairly constant, showing a standard deviation of less than 10 percent. There is a slight but significant increase in the lengths of the head fragments derived from the longer subjects.

In a second experiment, 120 planarians 12 to 15 mm long were decapitated just behind the auricles (Fig. 2A). Thirty of these were placed in each of two clean and two preslimed glass fingerbowls (100 mm in diameter) containing approximately 150 ml of clean aged tap water. From another 120 planarians of the same length we removed approximately the same amount of tissue as that removed from the decapitated ones by amputating the end of the tail (Fig. 2B); 30 of these were placed in each of two clean and two preslimed fingerbowls. Thirty uncut planarians of the same length were placed in each of two clean and two preslimed fingerbowls. Because rates of fissioning in clean and preslimed bowls were not significantly different, the results for these groups were pooled.

The decapitated subjects exhibit a much higher rate of fissioning than those with amputated tail tips or uncut controls, neither of which show any appreciable incidence of fissioning. These results, with those of the first experiment, support our previously stated hypothesis. The slightly longer anterior fragments of the longer group is consistent with the fact that they have slightly larger heads and brains. Whether the suppression of fissioning by the cephalic ganglion is mediated by axial transport of some substance, for example, a neurohormone, or by emanation of nerve impulses cannot be determined decisively from these data. There is, nevertheless, some circumstantial evidence which leads us to favor the former mechanism. Neurosecretory granules are produced by neurosecretory cells in the brain of these planarians and are transported through axons into the neuropil (*5*). In addition, the spatial and temporal pattern of onset of the fissioning agrees well with the results to be anticipated from the diffusion of a substance of relatively small molecular weight along the ventral nerve cords (*6*).

The fission rate for the intact medium and large subjects of the first experiment is markedly greater than that for the intact ones of comparable size in the second experiment. This result reconfirms the finding of previous studies (*1*) that population density and, to a lesser extent, presliming tend to suppress fissioning in this species. The reason presliming had no effect in the second experiment is that 30 subjects per bowl are capable of generating their own slime coat on the inner surface of the bowl.

Insofar as Child's (*4*) experiments are comparable to our own they gave similar results. As in our experiment, his decapitated planarians yielded the highest incidence of fissioning, but he obtained many more fissionings among his uncut controls than we did, so the difference was not as clear as in our study. This fissioning in his uncut control group probably arose from variations in population density which, as mentioned earlier, were unspecified in his study.

The primary functional significance of the suppression of fissioning by the brain resides in the fact that this suppression is contingent on population density and thus is a part of a feedback control system for adjusting the rate of reproduction to population density.

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Glucagon-Sensitive Adenyl Cylase in Plasma Membrane of Hepatic Parenchymal Cells

Abstract. *The plasma membrane of hepatic parenchymal cells contains an adenyl cyclase system that is stimulated by glucagon. Adrenocorticotropin and epinephrine do not stimulate this adenyl cyclase, and very little cyclic phosphodiesterase activity is present in the membrane. These findings support the concept that glucagon exerts its regulatory action in the liver by stimulating adenyl cyclase activity in the plasma membrane.*

Current theories regarding the interaction between certain hormones, such as glucagon, epinephrine, and adrenocorticotropin (ACTH), and their target cells place the hormone-specific site, or "receptor," in the plasma membrane (*1*, *2*). One effect of this interaction is known to be stimulation of the activity of adenyl cyclase, an enzyme system which catalyzes the conversion of adenosine triphosphate (ATP) to adenosine 3′,5′-monophosphate (cyclic AMP)