



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 Cyclase 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)

(3). Cyclic AMP participates in the regulation of several metabolic systems (1), and its concentrations in cells are controlled not only by hormone-stimulated adenyl cyclase but also by a specific cyclic 3',5'-nucleotide phosphodiesterase (cyclic phosphodiesterase) which hydrolyzes cyclic AMP to adenosine monophosphate (4). Cyclic phosphodiesterase is inhibited by methyl xanthines, such as theophylline and caffeine, and has been found both in the soluble fractions of tissue homogenates and in association with membranes (4).

One approach to proving that hormone-sensitive adenyl cyclase resides in plasma membrane has been to isolate fractions rich in plasma membranes from mammalian cells and to demonstrate greater adenyl cyclase activity in these fractions relative to other cellular fractions. However, plasma membrane preparations used previously for this purpose either have been grossly contaminated with other cell organelles or the purity has not been investigated (5, 6). Despite the high specific activity—that is, adenyl cyclase activity per unit protein—of these preparations, the possibility remained that the enzyme was present only in a contaminant. Furthermore, since adenyl cyclase activity is not always proportional to the amount of protein used for assay of the enzyme (3, 7), the specific activity of adenyl cyclase could not be used as a criterion of purity of these preparations. For these reasons, the present studies were performed to determine the presence of a hormone-responsive adenyl cyclase system in a plasma membrane preparation of well-established purity.

Plasma membranes from parenchymal cells were isolated from rat livers by the procedure of Neville (8). The absence of contamination of this preparation by other cell organelles has been established on both morphologic and enzymatic grounds by Neville (8), Emmelot *et al.* (9), and Barclay *et al.* (10). Liver homogenates were prepared by grinding whole liver slices suspended in 20 volumes of chilled 1 mM KHCO₃ in a Dounce homogenizer.

Adenyl cyclase was measured by the conversion of ATP labeled with ³²P in the alpha position to labeled cyclic AMP as described elsewhere (5), with the following modifications. The final concentrations of the components of the incubation medium were 3.2 mM tris(hydroxymethyl)aminomethane (tris)-ATP- α -³²P (specific activity, 20 to 30

Table 1. Adenyl cyclase activity of pure plasma membrane from rat liver parenchymal cells. Results shown are the mean \pm one standard deviation of nine observations in three experiments. Adenyl cyclase activity is expressed as nanomoles of cyclic AMP per 10 minutes per milligram of protein.

Addition	Conc.	Adenyl cyclase
None		0.20 \pm .12
ACTH	10 μ g/ml	0.16 \pm .06
Epinephrine	10 μ g/ml	0.19 \pm .11
Glucagon	10 μ g/ml	2.03 \pm .10
NaF	10 mM	1.94 \pm .10
Theophylline	10 mM	0.07 \pm .06
Theophylline and NaF	10 mM	2.07 \pm .12

count/min pmole, 5 mM MgCl₂, 25 mM tris-HCl (pH 7.4), and an ATP-regenerating system consisting of 100 mM creatine phosphate and creatine kinase, 1 mg/ml (Sigma, 44 unit/ml). Reactions were initiated by the addition of 20 to 35 μ g of membrane protein suspended in 1 mM KHCO₃ to complete a reaction volume of 0.05 ml which was incubated for 10 minutes at 30°C.

Cyclic phosphodiesterase activity was measured by the release of inorganic phosphate with snake venom as a source of 5'-nucleotidase (4). The incubation medium contained 1 mM cyclic AMP, 5 mM MgCl₂, 25 mM tris-HCl (at pH 7.4), and 0.1 mg/ml of snake (*Ophiophagus hannah*) venom (Ross Allen Reptile Institute). The venom readily hydrolyzed AMP and was shown to be free of measurable cyclic phosphodiesterase activity. Reactions were initiated by the addition of approximately 1 mg of enzyme protein suspended in 1 mM KHCO₃ (total reaction volume, 1.2 ml) and were allowed to proceed for 30 minutes at 30°C. Inorganic phosphate was measured by the method of Fiske and SubbaRow (11), and protein was determined by the method of Lowry *et al.* (12).

The effect on adenyl cyclase activity of the addition of glucagon, epinephrine, ACTH, sodium fluoride, and theophylline to the incubation medium is shown in Table 1. The marked stimulation by glucagon is consistent with the known effects of this hormone on cyclic AMP concentrations in liver (1). The absence of stimulation by epinephrine is noteworthy in view of the well-established cyclic AMP-mediated effects of catecholamines on liver metabolism (1). Bitensky *et al.* (7) have provided evidence for the existence of indepen-

dent adenyl cyclase systems responsive to glucagon and epinephrine in rat liver homogenates but have also shown that the adenyl cyclase response to epinephrine is preferentially destroyed by a variety of gentle treatments. Thus, the absence of an epinephrine response in parenchymal cell plasma membrane may be ascribed either to its location in another cell type or cell structure or to selective destruction in the process of isolation. Adrenocorticotropin, a potent activator of adenyl cyclase in adrenal gland (1) and fat cells (5), failed to stimulate adenyl cyclase in liver plasma membrane.

The plasma membrane preparation had a cyclic phosphodiesterase activity (μ mole of P_i per 30 minutes per milligram of protein) of 0.07, while the activity in the rat liver homogenate was 0.15. However, since the plasma membrane probably contains less than 1 percent of the whole cell protein (13), this activity represents only a small fraction of the total liver cyclic phosphodiesterase. In any event, it is possible to measure adenyl cyclase response to hormones in our preparation in the absence of theophylline (Table 1).

We conclude that the plasma membrane of rat liver parenchymal cells contains an adenyl cyclase system which is specifically activated by glucagon.

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