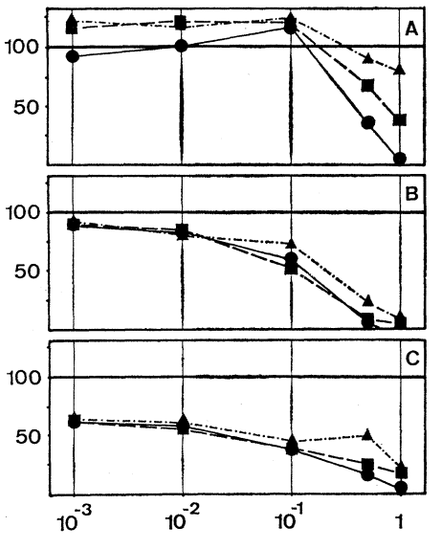

Fig. 2. Inhibition of plasmin (A), plasmin-streptokinase activator (B), and urokinase (C) by caprolactone (———), valerolactone (– – –), and butyrolactone (– · – · –) on normal human fibrin plates. Abscissa: molar concentration of lactone; ordinate: lysed area in percent of control without lactone.

on the proteolytic action of human plasmin which we had obtained by spontaneous activation with an activity of ten casein units per milligram of nitrogen (*5*). All lactones inhibited proteolysis by competition; caprolactone was the strongest inhibitor (Fig. 1). Comparison with ε-aminocaproic acid revealed that the inhibitory effect of 0.5*M* caprolactone corresponded to that of 0.3*M* ε-aminocaproic acid.

Use of the fibrin-plate method (*6*) permitted evaluation of the effect of lactone on the fibrinolytic activity of human plasmin (Fig. 2A). We first added the respective concentration of lactone to the human fibrinogen solution (90 percent coagulability) in the petri dish; then the fibrin plate was prepared by admixture of thrombin (Topostasin Roche). The presence of lactone in the native human fibrin plate (from 0.1*M* upward) caused increasing inhibition of the plasmin effect. The relation of the inhibitory potencies of lactones was caprolactone > valerolactone > butyrolactone. What seems to be potentiation of the fibrinolytic effect at low concentrations of lactone —a phenomenon known from experiments with low concentrations of other fibrinolytic inhibitors (*1*)—might also be explained otherwise. We observed during the preparation of our fibrin plates that, at high lactone concentrations, the coagulability of fibrinogen was impaired. Therefore, we cannot overlook the fact that lactones begin to interact with the fibrinogen molecules at low concentrations in a manner which renders the fibrin plate so prepared a substrate of increased sensitivity toward plasmin.

The fibrin-plate method also enabled us to test the influence of lactones on the activation of plasminogen through specific activators due to the fact that native human fibrin contains plasminogen. We used human plasmin-streptokinase (*7*) and human urokinase [activity 2700 CTA (Committee on Thrombolytic Agents) units per milligram of protein (*8*)] as activators. Our human fibrin plates contained various concentrations of lactones that inhibited these activators in proportion to their concentrations (Fig. 2, B and C). The inhibitory effect upon urokinase is considerable, even at lactone concentrations as low as 0.001*M*. The difference between the effects of the three lactones is not very great, but their regression curves in the diagrams indicate that the relation of potencies is identical to that found in inhibition experiments with plasmin, namely, caprolactone > valerolactone > butyrolactone.

Aliphatic carboxylic acids need an amino group to become inhibitors of fibrinolysis, and certain cyclic fibrinolytic inhibitors, such as the lactams, have amino groups. Similarly configurated cyclic compounds without amino groups, the internal esters of carboxylic acids, can act as potent inhibitors of the fibrinolytic system through their specific electrostatic and hydrophobic bonding to the components of this system alone.

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18 April 1967

Sodium- and Potassium-Activated Adenosine Triphosphatase of Gills: Role in Adaptation of Teleosts to Salt Water

Abstract. *The activity of adenosine triphosphatase activated by sodium and potassium ions is greatly increased in the gill and pseudobranch of the euryhaline killifish,* Fundulus heteroclitus, *after its adaptation to seawater. Adenosine triphosphatase activity in gills of fish in salt water is reduced by hypophysectomy. The data suggest that this enzyme is involved in the excretion of sodium ions by the gill and that the adaptive increase which occurs in seawater is influenced by the hypophysis.*

An enzymatic property of cell fragments that accelerates the hydrolysis of adenosine triphosphate in the presence of sodium and potassium has been described in many tissues (*1*). Substantial evidence suggests that the Na^+- and K^+-activated adenosine triphosphatase of erythrocyte membranes is connected with the simultaneous active transfer of sodium out of red blood cells and of potassium into the cells (*2*). There has been little direct evidence to link this enzyme with the active unidirectional transport of sodium across epithelial cell membranes such as the renal tubule, the toad bladder, and the teleost gill. Recently an adaptive increase in the activity of Na^+- and K^+-activated adenosine triphosphatase has been reported to accompany large increases in sodium reabsorption induced in mammalian kidneys (*3*).

Our studies show that adaptation to seawater by a euryhaline teleost, *Fundulus heteroclitus*, is associated with a considerable increase in the Na^+- and K^+-activated adenosine triphosphatase activity of its gills. The changes in enzymatic activity thus parallel the alterations in active transport of ions through the gill which are known to occur as part of the adjustment of this species to changes in osmotic stress. Hypophysectomy reduces the activity of

Table 1. Enzymatic activities in gills of fish adapted to fresh water and to salt water. Values are the means and ranges. The number of separate determinations is given in parentheses below the mean. The units for adenosine triphosphatase activity are micromoles of P_i per hour per milligram of protein; for glutaminase, micromoles of NH_3 per hour per milligram of protein. For succinic dehydrogenase, arbitrary units are given as change in optical density per hour per milligram of protein.

System	Adenosine triphosphatase		Glutaminase	Succinic dehydrogenase
	Na^+- and K^+ activated	Residual		
		Fresh water		
Homogenate	3.6 (1.1–5.2) (6)	14.6 (12.6–17.8) (6)	12.3 (10.1–14.4) (2)	0.49 (0.48–0.49) (2)
Microsomes	12.4 (9.9–14.9) (4)	23.6 (19.8–27.3) (2)		
		Salt water		
Homogenate	20.1 (17.9–23.8) (4)	18.6 (16.3–21.3) (4)	17.4 (16.8–17.9) (2)	.26 (0.25–0.26) (2)
Microsomes	84.4 (66.0–102.8) (6)	31.5 (28.7–34.2) (4)		
		Hypophysectomized and in salt water		
Homogenate	11.2 (9.2–12.5) (4)	14.2 (13.1–15.2) (4)	13.1 (11.0–15.2) (2)	.34 (0.32–0.35) (2)
Microsomes	37.0 (32.0–48.0) (4)	28.5 (18.0–40.0) (4)		

this enzyme in the gills of fish kept in salt water, an indication that induction of the enzyme may be dependent in part on the hypophysis.

Male *Fundulus heteroclitus* 7 to 8 cm long were captured in the vicinity of New Haven, Connecticut, in August 1966. One-third of the fish were maintained in running, dechlorinated city water at 20°C. The remainder were kept in recirculating water from Long Island Sound (salinity approximately 2.6 percent) at the same temperature. Half of the salt-water fish were hypophysectomized in October 1966. For 3 weeks before autopsy in February 1967, all fish were "trained" to accustom them to being caught and handled (*4*). They were anesthetized with tricaine methane sulfonate (MS 222, Sandoz).

The gills from 24 fish in each group were pooled in two or more portions and were homogenized in the proportion of 100 mg of tissue to 1 ml of an ice-cold solution, *p*H 6.8, containing 0.25 mole of sucrose, 5 mmole of sodium ethylenediaminetetraacetate, 1 g of sodium deoxycholate, and 30 mmole of histidine buffer per liter. Homogenization was carried out with a Teflon pestle at 1725 rev/min using 15 strokes. The homogenate was squeezed through a double layer of gauze. A portion was immediately assayed for enzyme activities, and the remainder was centrifuged at 10,000*g* for 30 minutes to remove cell debris, nuclei, and mitochondria. The supernatant fluid was then centrifuged at 105,000*g* for 60 minutes, and the sediment was gently resuspended with a Vortex mixer in the original homogenizing solution without deoxycholate. Samples (0.1 ml) of the whole homogenate and of the microsomal suspension were assayed for adenosine triphosphatase, as outlined by Epstein and Whittam (*5*), in 5 ml of a medium containing 10 mmole of imidazole buffer, *p*H 7.8, 5 mmole of disodium adenosine triphosphate, 5 mmole of $MgCl_2$, and either 100 mmole of NaCl and 20 mmole of KCl, or 120 mmole of NaCl per liter. Incubation was carried out in a shaking water bath at 37°C for 5 minutes. The reaction was started by the addition of adenosine triphosphate and stopped by the addition of 1.0 ml of 35 percent trichloroacetic acid. The activity of Na^+- and K^+-activated adenosine triphosphatase was derived from the difference between the amount of inorganic phosphate (P_i) released from adenosine triphosphate in the medium containing K^+ and that released in medium containing no K^+; it is expressed as micromoles of P_i released per hour per milligram of protein. Breakdown of adenosine triphosphate in the medium containing no K^+ is referred to as "residual adenosine triphosphatase." The concentration of protein in the tissue suspensions was measured by the technique of Lowry *et al.* (*6*). The activities of succinic dehydrogenase (*7*) and glutaminase (*8*) were also measured in the whole homogenate of gill tissue.

The activity of Na^+- and K^+-activated adenosine triphosphatase per milligram of tissue protein was approximately six times higher in gill homogenates prepared from *Fundulus* adapted to seawater than it was in gills from their freshwater counterparts. The specific activity of this enzyme was higher in the microsomal fraction than it was in the whole homogenate, and its activity in gill microsomes of fish kept in salt water greatly exceeded that of microsomal preparations from fish adapted to fresh water. The importance of these changes is emphasized by the fact that they were not paralleled by changes in the activities of residual adenosine triphosphatase, succinic dehydrogenase, or glutaminase (Table 1).

Hypophysectomy was found to depress the Na^+- and K^+-activated adenosine triphosphatase of the gills of fish kept in seawater. Other enzymes were not greatly affected. The adenosine triphosphatase activity of such preparations is intermediate between that of intact fish adapted to seawater and that of fish living in fresh water (Table 1). These differences are consistent with changes in electrolyte transport through the gill produced by hypophysectomy. Although a hypophysectomized *Fundulus* is able to live in salt water, the outward flux of sodium across its gills is decreased relative to that of intact fish in the same environment (*9*). The adaptive increases in sodium transport and in Na^+- and K^+-activated adenosine triphosphatase observed in the gills of fish placed in salt water therefore depend in part upon the presence of the hypophysis.

It has been suggested for many years that the specialized cells in teleost gills described by Keys and Willmer (*10*) are related to the excretion of salt by the gill (*11*). These cells change in configuration and fine structure when euryhaline fish are transferred from fresh- to seawater (*12*). The pseudobranch, a modified derivative of the first gill enclosed, in this species, in a pocket in the roof of the mouth, consists almost exclusively of Keys-Willmer cells (*13*). It was therefore of special interest to examine the content of Na^+- and K^+-activated adenosine triphosphatase of the pseudobranch of fish adapted to fresh water and to salt water. Because this organ is small, adenosine triphosphatase was assayed only in the whole homogenate of pooled pseudobranch tissue from all the freshwater fish or from all the saltwater fish. The activity of Na^+- and K^+-activated adenosine triphosphatase was ten times higher in the pseudobranch of *Fundulus* adapted to seawater than

it was in the organ of fish accustomed to freshwater.

The transport function of the gill changes dramatically when euryhaline teleosts accustomed to fresh water enter seawater. In fresh water the bidirectional flux of sodium and chloride is low, and the gill actively absorbs small amounts of Na^+ and Cl^- from the dilute external medium (*14*). In salt water (*9*, *15*), the flux of sodium across the gills is increased by several orders of magnitude. The gills of *Fundulus* must transfer 4 to 5 percent of the body's exchangeable Na^+ per hour (an amount of Na^+ equal to that contained in the seawater drunk) into the hypertonic external medium against an electrochemical gradient (*9*). The impressive augmentation of Na^+- and K^+-activated adenosine triphosphatase activity that accompanies the increase in active sodium outflux strongly suggests that this enzyme plays an important role in the active transport of Na^+ across the gill.

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6 March 1967

Food Intake Controlled by a Blood Factor

Abstract. *Intake of food by hungry rats was reduced 50 percent below normal after their blood had been mixed with that of satiated rats. Intake of food by deprived rats was not reduced when the donor rat was deprived of food for 24 hours prior to mixing of the blood or when a deprived donor was fed to satiety immediately before such transfusion.*

Blood factors have been of prime importance in many theories of the control of food intake. The glucostat (*1*), lipostat (*2*), and aminostat (*3*) theories of hunger assume monitoring (by the central nervous system) of glucose, lipid, or protein reserves of the body, respectively. When these reserves are low, quantities of these substances in the blood or products of their metabolism are assumed to be low, thereby activating a feeding center or inhibiting a satiety center. However, we are aware of no evidence in the literature indicating that blood-borne factors control food intake.

If the blood of satiated and food-deprived animals differs in hunger or satiety factors, the crossing of blood between satiated and deprived animals should affect their subsequent food intake; the hungry animal would then be expected to eat less than normal, and the satiated animal would be expected to eat more than normal.

Techniques of crossing blood by using parabiotic animals or by transfusion under anesthesia were unsuitable for our study. With parabiotic rats it is impossible to control the blood composition of the two members of the pair independently; in techniques requiring anesthesia, tests of food intake cannot be made until the effects of anesthesia have disappeared. We used a technique that avoids these problems (*4*). Cannulas were permanently implanted in the right external jugular vein of a pair of rats 2 days prior to blood mixing. The cannula, which terminated in the superior vena cava, extended subcutaneously from the point of entry into the vein along the ventral midline up the side and emerged, through an incision on the back, 2 or 3 cm below the neck of the animal. Immediately before the transfusion, both animals received heparin intravenously (0.15 ml, 1000 unit/ml) through the cannulas. The rats were then placed side by side in restraining tubes. The distal ends of their cannulas were connected to a valve system that permitted blood to be withdrawn from each animal and to be injected into the other animal of the pair without exposing the blood to air. This blood was withdrawn in 2-ml portions crossed and reinjected until a total of 26 ml had been transferred; this is an adequate amount to insure thorough mixing of the blood of rats weighing 350 to 400 g (*5*). At the completion of transfusion, 50 percent of the blood in each animal was his own, and 50 percent was his partner's.

Sprague-Dawley albino male rats (16 pairs, 90 to 120 days old) were used. One member of each pair was given free access to a milk diet (sweetened condensed milk diluted, 2 parts milk to 1 part water); the other had access to this diet for only 30 minutes of each 24 hours for 10 days of adaptation. All animals had continuous access to water except during transfusions. During this adaptation period, we recorded milk intake of both groups of animals. By the 5th day, the daily intake had stabilized.

On the experimental day, the blood of a pair of rats, one deprived and one satiated, was mixed 30 minutes prior to the time the deprived animal was usually fed. Immediately after this procedure, both members of the pair were given access to the diluted sweetened condensed milk for 30 minutes, and the amount ingested was recorded.

The mean intake and standard deviation of the distribution of intakes of the 16 deprived animals in the 30-minute feeding period on the day prior to blood mixing were 15.8 and 5.2 ml, respectively; in the 30-minute feeding period immediately after blood mixing, the mean and standard deviation were 8.0 and 5.9 ml, respectively (Fig. 1). The difference in the mean intake on these two successive days, representing a reduction of 50 percent, is statistically significant ($t = 4.01$, $P < .001$).

The intake of the satiated animals immediately after blood mixing with the hungry animals was not increased.