Table 1. Specific rate constants for the hydrolysis of *p*-nitrophenyl esters by mixed micelles of *N*-α-myristoyl-L-histidine (NMH) and cetyltrimethylammonium bromide (CTAB). Conditions are the same as in Fig. 1. At all concentrations of II and III studied, the surface tension of the solutions gave values of 34 to 37 dyne/cm.

| NMH ($10^{-5}M$) | CTAB ($10^{-4}M$) | $k_{obs}$* ($min^{-1}$) | $k_c$† (liter $mole^{-1}$ $min^{-1}$) |
|---|---|---|---|
| p-*Nitrophenyl acetate (3.45 to 6.91* × *10⁻⁶*M) | | | |
| 4 | 8 | $2.41 \times 10^{-2}$ | 602 |
| 5 | 10 | $3.02 \times 10^{-2}$ | 604 |
| 6 | 12 | $3.42 \times 10^{-2}$ | 570 |
| 7 | 14 | $3.94 \times 10^{-2}$ | 563 |
| 8 | 16 | $4.49 \times 10^{-2}$ | 561 |
| 10 | 20 | $5.17 \times 10^{-2}$ | 517 |
| 20 | 40 | $9.12 \times 10^{-2}$ | 456 |
| 30 | 60 | $12.81 \times 10^{-2}$ | 427 |
| 40 | 80 | $15.34 \times 10^{-2}$ | 384 |
| 50 | 100 | $18.94 \times 10^{-2}$ | 379 |
| p-*Nitrophenyl caprylate (9.0* × *10⁻⁶*M) | | | |
| 4 | 0.8 | 1.26 | $315 \times 10^2$ |
| 5 | 1.0 | 1.36 | $272 \times 10^2$ |
| 6 | 1.2 | 1.51 | $252 \times 10^2$ |
| 7 | 1.4 | 1.63 | $233 \times 10^2$ |
| 8 | 1.6 | 1.64 | $205 \times 10^2$ |
| 10 | 2.0 | 1.78 | $178 \times 10^2$ |

* $k_{obs}$ was corrected for the rate of hydrolysis observed in the presence of cetyltrimethylammonium bromide alone. † $k_c = k_{obs}$/concentration of *N*-α-myristoyl-L-histidine (*9*).

catalyst, when NPA is used as substrate, the rate of ester hydrolysis as the concentration of II is increased appears to be proportional to the catalyst concentration; at higher concentrations, the rate is independent of catalyst (Table 1). These results can be explained if it is assumed that the initial step in the reaction sequence involves the formation of an adsorption complex between the ester and the mixed micelle. Then under conditions where [III] ≫ [ester] (square brackets indicating concentration), the substrate should become saturated with respect to catalyst (*13*). In the case of the NPC hydrolysis, this ester appears to have a higher affinity for the mixed micellar catalyst so that the limiting value is reached at lower catalyst concentrations. This would be expected because of the longer, more hydrophobic acyl chain present.

If the reciprocal of $k_{obs}$ is plotted against the reciprocal of the concentration of the catalyst from the values shown in Table 1, a straight line is obtained for both esters, a further indication that the kinetics of the reactions studied show saturation phenomena. The $k_c$ values (Table 1) for NPA hydrolysis are higher than those reported (*8*) for histidine hydrochloride ($k_c = 6$), imidazole ($k_c = 20$) or histidyl-peptides ($k_c = 15$ to 147).

The liberation of *p*-nitrophenol is apparently accompanied by the simultaneous formation of an *N*-imidazole-acyl- *N*-α-myristoyl-L-histidine intermediate, and the subsequent hydrolysis of this intermediate is rate-limiting in the overall hydrolysis of the esters. Under conditions where the [ester] ≫ [II], approximately 1 mole of *p*-nitrophenol per mole of II is liberated instantaneously (*14*).

The kinetic behavior of our model makes it quite similar to enzyme-catalyzed reactions.

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11. Prepared by reacting myristoyl chloride with L-histidine in a basic aqueous medium (m.p. solid $\xrightarrow{158°C}$ liquid cryst. I, $\xrightarrow{182°C}$ liquid cryst. II $\xrightarrow{192°C}$ decomp.). Analyses: calculated for $C_{20}H_{35}O_3N_3$: C, 65.75; H, 9.59; O, 13.15; N, 11.51. Found: C, 65.84; H, 9.60; O, 13.20; N, 11.36. (Analyses by Mikroanalytische Laboratorium, Bonn, Germany.)
12. The rate of ester hydrolysis in 50 m*M* tris-HCl buffer *p*H 7.2 decreased markedly in the presence of micelles of cetyltrimethylammonium bromide. With NPA, the rate decreased about 50 percent, while with NPC the decrease was in the order of 90 percent. These results agree with those of S. Riegelman, *J. Amer. Pharm. Assoc. Sci. Ed.* **49**, 339 (1960), and A. G. Mitchell, *J. Pharm. Pharmacol.* **14**, 172 (1962).
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## Lactones as Inhibitors of the Fibrinolytic System

Abstract. *Caprolactone, valerolactone, and butyrolactone inhibit proteolytic and fibrinolytic activities of human plasmin. In very low concentrations, they also inhibit activation of plasminogen through plasmin-streptokinase activator and human urokinase. The degree of inhibitory potency depends upon the number of carbon atoms in the lactone.*

Aliphatic aminocarboxylic acids act as inhibitors of the fibrinolytic system. Their effect upon the activation process of plasminogen is stronger than their action upon plasmin itself. The activity of these compounds is maximum, when there are six carbon atoms in the chain and the amino group is located in terminal position (*1*). Lactams also inhibit fibrinolysis competitively, and caprolactam exerts the strongest inhibitory action (*2*).

We studied lactones to determine whether the simultaneous presence of carboxylic and amino groups in both the aliphatic and cyclic compounds causes inhibition, or whether the specific polarity of the cyclic arrangement alone is of importance for the inhibitory effect. Lactones are the internal esters of carboxylic acids, which correspond to lactams in their cyclic configuration, but which do not have the amino group; they also exert inhibitory effects in other biological systems (*3*).

By means of the casein test (*4*), we investigated the influence of caprolactone, valerolactone, and butyrolactone

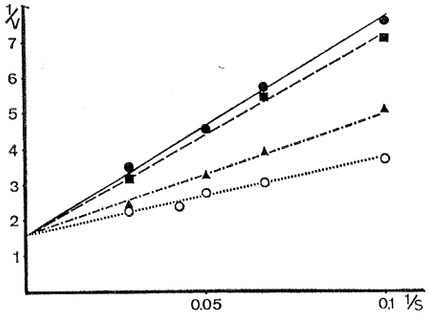


Fig. 1. Competitive inhibition of human plasmin in the casein test by 0.5*M* caprolactone (———), valerolactone (– – –), and butyrolactone (– · – · –). Control or action of plasmin without lactone (. . . . . ). S, concentration of substrate in milligrams of casein per milliliter; V, extinction at 280 mμ of supernatant, that is, the fraction soluble in chloroacetic acid.

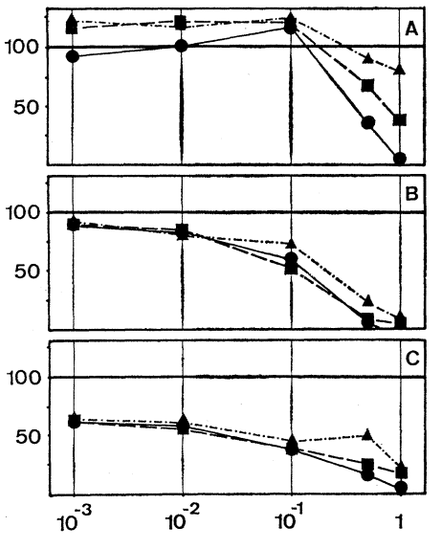


Fig. 2. Inhibition of plasmin (A), plasmin-streptokinase activator (B), and urokinase (C) by caprolactone (———), valerolactone (– – –), and butyrolactone (– · – · –) on normal human fibrin plates. Abscissa: molar concentration of lactone; ordinate: lysed area in percent of control without lactone.

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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## 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