sensitized to recipient tissues into normal adult mice was capable of inducing runt disease. The recipient mice showed the characteristic clinical and pathologic sequelae described in this condition when induced in immunologically incompetent hosts. Severity of the disease was influenced by number and strain of transferred cells. A strain cells elicited a more intense clinical and pathological reaction against their CBA hosts than was seen in the A host transfused with CBA cells. During the period of runting, homologous skin grafts of the opposite strain were tolerated far beyond the usual rejection time of untreated homografted A and CBA mice.

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Studies on the 5′ Adenylic Acid Deaminase of the Myofibrills.* (27408)

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The enzyme 5′ adenylic acid deaminase, first discovered in muscle by Schmidt(1), occurs in high concentration in this tissue in close association with myosin though its biological function is unknown. The enzyme has been purified in various ways and extensive studies of muscle 5′ adenylic acid deaminase have been published(2,3,4). Since there is some disagreement in results obtained with the "purified" enzymatic preparations, it was thought desirable to study some of the properties of this enzyme in the highly organized myofibrills. The myofibrillar suspension is readily prepared and while containing mainly actomyosin, also exhibits strong 5′ adenylic acid deaminase activity. Its use for the enzyme studies has the advantage of having the deaminase in a form less subject to modification from steps necessary for its chemical isolation and more akin to its possible structure in the intact muscle.

Materials and method. ATP, ADP, AMP 5′, AMP2′3′, GMP, IMP and ITP† were purchased from the Sigma Co., St. Louis, Mo. Plaquenyl hydroxychloroquine, atabrine and rivanol were kindly provided by Winthrop Laboratories, New York City. Quinidine sulfate was purchased from Merck & Co., Rahway, N. J. Camoquine dihydrochloride was a gift of Parke, Davis and Co. and proquanyl was donated by Ayerst Laboratories. Quinine hydrochloride was a product of Eli Lilly & Co. Acroflavin and acridine orange were products of National Aniline Co.

0.1 M succinate buffer was prepared from succinic acid and NaOH and 0.1 M citrate buffer from citric acid and NaOH.

The myofibrillar suspension was prepared in a 0.05 M, pH 7.0 borate buffer as previously described(5). The incubation mixture usually contained about 0.3 mg myofibrillar protein and 50.0 μM buffer in a 1.0 ml volume. The incubation was carried out at room temperature (23-25°) for 10 minutes and the reaction terminated by addition of 0.25 ml of 25% TCA solution. By means of controls run with each set of experiments it was found that changes due to temperature variation were negligible. Determination of the ammonia formed was performed with the Conway microdiffusion method(6) followed by Nesslerization.

Results. The maximal velocity of the reaction was obtained at 5.0 mM AMP con-

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† Abbreviations used: AMP = Adenylic acid (5′). AMP (2′3′) = A mixture of 2′ and 3′ adenylic acid. IMP = Inosinic acid (5′). GMP = Guanylic acid (5′). ADP = Adenosine diphosphate, ATP = Adenosine triphosphate, ITP = Inosine triphosphate.

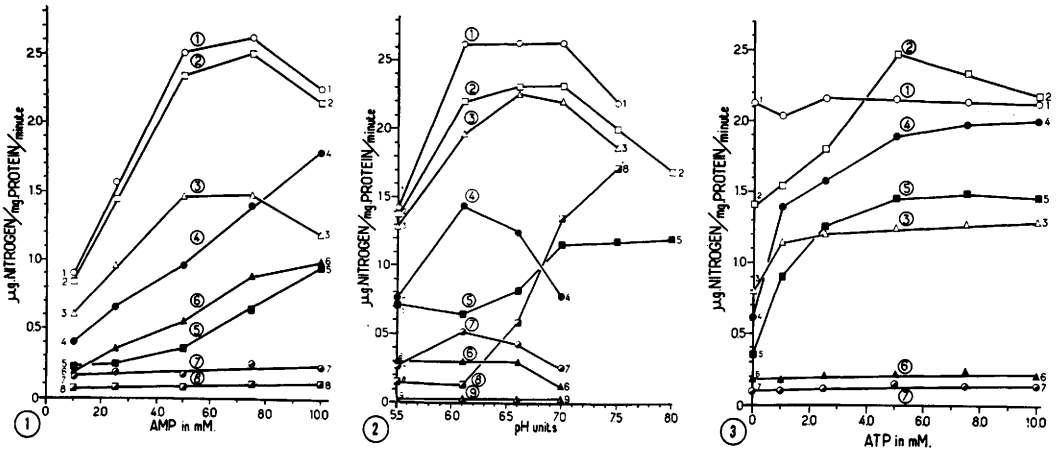

FIG. 1. Ammonia splitting of myofibrills at pH 6.1 as a function of substrate concentration. Each tube contained about 0.3 mg myofibrillar protein to which was added: *curve 1,* 50 μM citrate; *curve 2,* 50 μM citrate and 10 μM plaquenyl; *curve 3,* 50 μM succinate; *curve 4,* 50 μM citrate and 5 μM quinidine sulphate; *curve 5,* 50 μM succinate and 10 μM plaquenyl; *curve 6,* 50 μM succinate and 5 μM quinidine sulphate; *curve 7,* 50 μM citrate and 0.5 μM sodium fluoride and *curve 8,* 50 μM succinate and 0.5 μM sodium fluoride.

FIG. 2. Ammonia splitting of myofibrills at various pH's. Each tube contained about 3 mg myofibrillar protein and 5 μM AMP to which was added: *curve 1,* 50 μM succinate and 5 μM ATP; *curve 2,* 50 μM citrate; *curve 3,* 50 μM citrate and 10 μM plaquenyl; *curve 4,* 50 μM succinate; *curve 5,* 50 μM citrate and 5 μM quinidine sulphate; *curve 6,* 50 μM succinate and 10 μM plaquenyl; *curve 7,* 50 μM succinate and 5 μM quinidine sulphate; *curve 8,* 50 μM citrate and 0.5 μM sodium fluoride and *curve 9,* 50 μM succinate and 0.5 μM sodium fluoride.

FIG. 3. Ammonia splitting of myofibrills at various ATP concentrations. Each tube contained 0.3 mg myofibrillar protein and 5 μM AMP to which was added: *curve 1,* 50 μM succinate; *curve 2,* 50 μM citrate; *curve 3,* 50 μM succinate and 10 μM plaquenyl; *curve 4,* 50 μM succinate and 5 μM quinidine sulphate; *curve 5,* 50 μM citrate and 5 μM quinidine sulphate; *curve 6,* 50 μM citrate and 0.5 μM sodium fluoride and *curve 7,* 50 μM succinate and 0.5 μM sodium fluoride.

centration in succinate buffer and at 7.5 mM AMP concentration in citrate buffer (Fig. 1). The higher velocity of deamination in citrate as compared to succinate was true over the entire pH range investigated (Fig. 2).

The effect of several nucleotides on deaminase activity was studied. ATP, ADP, AMP2′3′ and GMP were not deaminated by the myofibrills. ATP, ADP and ITP were found to enhance the deamination of the substrate in succinate buffer, but not in citrate buffer. The increase of enzyme activity at pH 6.1 was about 80-100% and ATP, ADP and ITP were about equally effective. No such effect was obtained if IMP, GMP or AMP2′3′ were added to the incubation mixture (Table I). The activating effect of the ATP was then studied in some detail. In succinate buffer the ATP effect was independent of substrate concentration (2.5 to 7.5 mM AMP). In citrate buffer, no enhancement was demonstrated at ATP concentrations of less than 10 mM (Fig. 3).

Further experiments were then performed in search for materials which interfere with the enzyme activity. For that purpose the effect of several compounds known as antimalarial drugs was studied. Acridine orange, acroflavine, rivanol, atabrine, proquanyl, camoquine and quinine HCl did not exert significant effect on deaminase activity in concentrations of 2.0 mM for acridine orange and 5.0 mM in the case of the other compounds. Quinidine sulfate, however, inhibited the activity of the enzyme markedly in succinate and citrate buffers. By increasing the substrate concentration the inhibitory action of the quinidine was reduced markedly. This effect was demonstrable in both citrate and succinate buffer (Fig. 1).

ATP, ADP and ITP reversed completely the inhibitory effect of quinidine in succinate buffer, but IMP, GMP and AMP2′3′ did not influence the quinidine inhibition (Table I). In connection with these findings the effect of the ATP was studied more extensively. In

TABLE I. Effect of Some Nucleotides and Drugs on the 5′Adenylic Acid Deaminase of the Myofibrills.

Incubation mixture*		μgN/min/ mg prot.	% activity
		1.50	100
+ 5 μM ATP		3.01	200
+ 5 " ADP		3.03	200
+ 5 " ITP		2.97	200
+ 5 " AMP (2′3′)		1.48	100
+ 5 " GMP		1.53	100
+ 5 " IMP		1.48	100
+ 5 " Quinidine		.22	15
+ 5 " "	+ 5 μM ATP	1.53	100
+ 5 " "	+ 5 " ADP	1.57	101
+ 5 " "	+ 5 " ITP	1.54	101
+ 5 " "	+ 5 " AMP (2′3′)	.30	20
+ 5 " "	+ 5 " GMP	.35	23
+ 5 " "	+ 5 " IMP	.25	17

* The incubation was performed at pH 6.1 for 10 min. Incubation mixture consisted of 0.3 mg myofibrillar protein + 5 μM AMP + 50 μM succinate to which was added the substances listed in first column.

succinate buffer 5.0 mM ATP completely reversed the inhibition caused by 5.0 mM quinidine but the activating effect of ATP was absent even at 10.0 mM ATP concentration (Fig. 3).

Plaquenyl was inhibitory only in succinate but not in citrate buffer. By increasing substrate concentration, about 65 to 75% of the inhibition was reversed (Fig. 1). ATP, ADP and ITP were again effective in reversing the inhibition caused by plaquenyl, and GMP, IMP and AMP2′3′ again were without effect. If 1.0 mM ATP was added the inhibition caused by 10.0 mM plaquenyl was reversed. At 10.0 mM ATP concentration the activating effect of ATP was about the same as without plaquenyl (Fig. 3).

5×10^{-4}M fluoride caused 88% inhibition in succinate buffer within the pH range of 5.5 and 7.0. The same amount of fluoride caused 80% inhibition in pH 6.1 citrate buffer which gradually diminished to 20% at pH 7.5 (Fig. 2). The inhibitory action of fluoride was not influenced by either increased substrate concentration or by ATP (Fig. 1 and 3).

1.0 to 5.0 mM Ca^{++} or Mg^{++} and 0.1 to 1.0 mM Zn^{++} or Cd^{++} did not interfere with the ammonia splitting of the enzyme nor did they influence the activating effect of ATP.

Discussion. The substrate specificity and pH dependence of the 5′ adenylic acid deaminase of the myofibrills was similar to that described by Schmidt(1) and Ya-Pin Lee(3) (Table I).

Nikiforuk and Colowick(2) found that citrate ions increased the activity of the 5′ adenylic acid deaminase of the muscle, prepared by their methods. Ya-Pin Lee(3), however, found no effect by citrate in the case of a highly active enzyme, purified according to his method. Both authors however agree that phosphate and pyrophosphate ions in high concentration inhibit the activity of their enzyme preparations.

Lyubimova and Matlina(4) reported that ATP and ADP activated their 5′ adenylic acid deaminase. A similar effect was found by Weil-Malherbe(7) using 5′ adenylic acid deaminase prepared from brain tissue.

In our experiments citrate, ATP, ADP and ITP potentiated the enzyme activity. All of these compounds extended the optimal pH range of enzyme activity towards the alkaline side (Fig. 2). The potentiating effect of these nucleotides, however, was not demonstrated in citrate buffer. The effect of citrate, phosphate and pyrophosphate on enzyme activity suggests that 5′ adenylic acid deaminase of the muscle is anion sensitive. The potentiation effect of ATP, ADP and ITP is much less than that of a specific activator and the concentration requirement to elicit maximal stimulation is higher. Because of these considerations, we believe that the activating effect of the ATP, ADP and ITP is probably the consequence of the assumed anion sensitivity of the enzyme.

Two drugs, quinidine sulfate and plaquenyl hydroxychloroquine, were found to inhibit enzyme activity in concentrations comparable to that of the substrate. By increasing the substrate concentration, about 75% of the inhibition was reversible. ATP, ADP and ITP also reversed the inhibition caused by quinidine, but the activating effect of these nucleotides did not occur in presence of quinidine. In the case of the plaquenyl inhibition, ATP, ADP, ITP and citrate were effective in restoring fully the potentiated activity of the enzyme. Since AMP2′3′, GMP and IMP

were not able to reverse the inhibition caused by these 2 drugs, the probability of unspecific complex formation between the drugs and nucleotides as the cause of the inhibition is diminished. We believe that the mechanism of inhibition of these drugs can be best explained by a dual interaction, that is, competition between the substrate and the drug coupled with competition between some buffer anions and the drug for enzyme binding sites. The former effect probably plays the leading role in quinidine inhibition and the latter in plaquenyl inhibition.

In contrast to the inhibition of the drugs, fluoride inhibition was not influenced by substrate concentration or by the presence of other nucleotides. It was, however, diminished markedly by increasing the pH of the citrate buffer which confirms the findings of Nikiforuk and Colowick(2). The inhibition produced by quinidine and plaquenyl did not show a marked pH dependence although the inhibitory power of the quinidine did slightly diminish in the alkaline range. In agreement with the results of Nikiforuk and Colowick (2) and Ya-Pin Lee(3), divalent cations did not interfere with the enzyme activity.

Summary. The potentiating effect of citrate ions, ATP, ADP and ITP on the 5′ adenylic acid deaminase activity of the myofibrills was explained as a consequence of anionic sensitivity of the enzyme. Quinidine sulfate and plaquenyl hydroxychloroquine were found to inhibit the enzyme in a concentration comparable to that of the substrate. As to the mechanism of the inhibition, a dual competition between the drugs and the substrate and/or some buffer anions was proposed and discussed.

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Lipoprotein Lipase Inhibition in Rabbits with Experimental Pancreatitis. (27409)

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Although elevation of plasma triglycerides has often been observed during spontaneous and experimental pancreatitis(1,2) the mechanism responsible for this phenomenon has not been explained. On the other hand, in many other states also accompanied by hyperlipemia deficient mobilization of postheparin lipoprotein lipase (LPL) or the presence of a circulating inhibitor of LPL has been demonstrated(3,4,5).

This study was accordingly undertaken in an attempt to demonstrate whether experimental pancreatic injury confers on the plasma the property of inhibiting postheparin LPL.

Methods. Pancreatitis was induced in 10 male albino rabbits by intraductal injection of 2 ml of diluted (1:80) staphylococcal toxin*(6). Blood samples were drawn from the exposed femoral arteries before and at varied intervals after injection of the toxin. Serum amylase activity was determined by the method of Somogyi(7), triglycerides by

* Supplied by Lederle Co., Pearl River, N. Y. Batch #42925-140 in a preservative of phenyl mercuric borate (1:50,000)