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)

TABLE I. Elevation of Plasma Triglycerides and Inhibition of Post-Heparin Lipoprotein Lipase Activity after Staphylococcal Toxin Pancreatitis.

	Triglycerides*				
	Control level	Maximal elevation†	Time‡ of maximal elevation	Maximal LPL inhibition, %	Time‡ of maximal inhibition
Mean (10 rabbits)	1.2	4.4	2.9	34	2.7
S. D. ±	.3	1.1	1.6	15	.6

* Triglycerides in meq/l.
† Maximal elevation over indicated control level.
‡ Time in hr after inj. of staphylococcal toxin.

the method of Van Handel and Zilversmit (8) and free fatty acids (FFA) by the method of Dole(9). Plasma lipolytic activity was measured by the amount of FFA released from an activated triglyceride (Lipomul)† substrate during one hour of incubation at 37°C. The triglyceride substrate consisted of 8 parts 10% bovine serum albumin in phosphate buffer (0.16 M) titrated with hydroxide to pH 8.7, 1 part fresh serum and 1 part 3% cottonseed oil (obtained by dilution of Lipomul in the phosphate buffer). The substrate was activated by incubation at 37°C for 30 minutes. Equal amounts of plasma and substrate were mixed and incubated for one hour with frequent shaking. The FFA were determined in the pre-incubated and the incubated plasma/substrate mixture. Lipolytic activity was expressed as μeq of FFA released by 1 ml of plasma during 1 hour of incubation (μeq/ml/hr).

Inhibition of LPL by plasma obtained after induction of pancreatitis was determined by the following procedure: Post-heparin plasma with high lipolytic activity obtained 15 minutes after intravenous injection of heparin (0.75 mg/kg) in normal rabbits was mixed with an equal amount of plasma obtained before and at various intervals following induction of pancreatitis. Addition of normal plasma to post-heparin plasma produced a decrease in the original lipolytic activity equal to the effect of dilution. The final lipolytic activity of the post-heparin/normal plasma mixtures was taken as 100% activity, *i.e.*, 0% inhibition. The lipolytic activity of each mixture was determined as described before and percent inhibition was calculated from the difference of the final lipolytic activity of the mixtures.

† Upjohn Co., Kalamazoo, Mich.

Results. Intraductal injection of staphylococcal toxin to rabbits resulted in extensive pancreatitis within several hours. The pancreatic tissue showed severe edema, necrosis, hemorrhage and diffuse interstitial inflammation. The pancreatitis was invariably fatal and all the animals died within 8-12 hours.

Injection of the toxin also produced a rapid rise of the plasma amylase. Average values were in the range of 250-300 units compared to initial control values of 80-100 units. In 2 of the experiments the amylase activity exceeded 500 units. Degree of amylase elevation was not correlated with severity of pancreatitis nor with the changes in plasma triglycerides.

The staphylococcal pancreatitis resulted in an elevation of plasma triglycerides in all rabbits studied (Table I). Maximal elevation was noted in 2 rabbits (5.8 meq/l and 6.1 meq/l) in whom mild lactescence of the plasma was also noted. The maximal triglyceride elevation occurred in rabbit 3 two hours and in rabbit 9 three hours after administration of the staphylococcal toxin.

Intraductal injection of toxin conferred a marked inhibitory effect on the activity of post-heparin LPL, which was observed in 9 of the 10 experiments. The inhibition varied between 27-55%. When inhibition of LPL activity was demonstrated, elevation of the plasma triglycerides was also present. However, maximal LPL inhibition and maximal triglyceride elevation were not parallel.

No significant changes of plasma FFA were demonstrated throughout the experiments.

Discussion. Pancreatitis induced by injection of potent staphylococcal toxin into the pancreatic duct resulted, in addition to the previously described lipid changes(6), in the appearance in the plasma of the property of

inhibiting the activity of post-heparin LPL. The activity of plasma pancreatic lipase, which is known to be modestly elevated in pancreatitis, was not determined. However, elevation of plasma pancreatic lipase activity occurs late in the course of pancreatitis and cannot be related to the early appearance of LPL inhibition, which was demonstrated as early as 25 minutes after injection of staphylococcal toxin. Even though some small elevation of plasma pancreatic lipase cannot be ruled out, its action on the substrate employed would rather tend to decrease the amount of demonstrated LPL inhibition. Whether the LPL inhibitor(s) can also inhibit the activity of pancreatic lipase cannot be determined by our results.

We have previously obtained evidence for the presence of LPL inhibitor(s) in pancreatic tissue, as well as in pancreatic juice(10). The possibility that damage of the pancreas may result in release of the pancreatic inhibitor(s) into the circulation is thus very tempting. Meng and Hardley(11) have shown that the intact pancreas is essential for the normal post-heparin release of LPL. Furthermore, a deficient LPL response to heparin injection has also been reported in a patient with acute pancreatitis accompanied by hyperlipemia(12).

It is thus possible that the decreased LPL response to heparin in pancreatitis may in part be due to liberation of circulating LPL inhibitor(s) by the damaged pancreas. The finding of LPL inhibition in experimental pancreatitis falls in line with previous reports on circulating LPL inhibitors in several other states including essential hyperlipemia, also accompanied by elevations of plasma triglycerides.

Whether the presence of LPL inhibitors in these states is related to the elevated plasma triglycerides is not clear since the role of LPL in the normal physiology of lipemia clearing has not been definitely demonstrated.

Summary. The plasma of 9 of 10 rabbits with staphylococcal toxin pancreatitis inhibited the activity of post-heparin LPL. The pancreatitis resulted also in a significant elevation of plasma triglyceride level. The maximal triglyceride elevation, however, was not related to the maximal inhibition of LPL. The possibility that a circulating inhibitor(s) of LPL was liberated from the necrotic pancreatic tissue is discussed.

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