Discussion. The present findings confirm previous observations of Wiesner and Yudkin (7), and of Didcock and Jackson(3) of the toxicity of P and PT to the fetus *in utero*. An explanation of the action might be found in the work of Baker, Miller, Davison and Smith (1), who reported inhibition of respiration of rat organ homogenates, especially those of the thymus, lymph nodes and spleen. In the present experiments, the absences of gross malformations was striking, even in fetuses from mothers treated from the 7th to the 21st g.d. This indicates that processes vital for the fetuses are affected by P or PT, terminating life. This is in contrast to other compounds which affect less important biochemical systems in the fetus, permitting the embryos to survive but inducing malformations.

Summary. The single LD_{50} for the adult rat was, for P or PT, approximately 15 mg/kg i.p. Toxicity for the rat fetus was tested in groups of pregnant rats injected with varying doses and at different gestation days. The compounds were found to be more toxic to the fetuses than to the mothers, with a peak of fetus sensitivity around midterm (11th and 12th g.d.). Doses of 5 mg/kg i.p., administered on the 11th and 12th g.d. destroyed more than 90% of all fetuses. Doses of 0.5 mg/kg or 1 mg/kg i.p., given daily over longer periods of gestation, led to a loss of 18% to 75% of all fetuses. General stunting, but no gross malformation, was observed in surviving fetuses.

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Lipoprotein Lipase Activity in the Dog Pancreas and Pancreatic Juice.* (28297)

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Although the role of circulating lipoprotein lipase (LPL) "clearing factor" in the normal physiology of lipemia-clearing is subject to much controversy, there is increasing evidence that a variety of pathologic hyperlipemic states are associated with either a deficiency or an inhibitor of LPL(1-4). The hyperlipemia of acute pancreatitis in man and experimental animals(5) is well known; however no satisfactory explanation for this phenomenon has been suggested. Recently we have obtained evidence for inhibition of LPL by the plasma of rabbits with experimental acute pancreatitis, in which an elevation of triglycerides was also observed(6). In addition, Meng and Goldfarb(7) have shown that the intact pancreas is necessary for normal mobilization of LPL after heparin injection, and LeVeen, Giordone and Zinberg(8) have suggested that the pancreas may be one of the sources of LPL. Thus a disturbance in LPL metabolism might account for the hyperlipemia seen in pancreatitis. In an effort to clarify such a possible relationship between the lipid aberration of pancreatitis and LPL, canine pancreatic juice and pancreatic tissue were examined for presence of LPL activity.

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Methods. Uncantaminated pancreatic juice was obtained from 3 healthy female mongrel dogs (20-25 kg) by cannulating the pancreatic duct directly through a Thomas fistula (9). The juice was collected in ice chilled tubes at various intervals during the post-absorptive state, before and after stimulation with secretin‡ (100 clinical units, i.v.) or pancreozymin‡ (100 clinical units, i.v.) or both.

Lipolytic activity was determined by incubation of pancreatic juice with a triglyceride substrate prepared by diluting cottonseed oil (Lipomul§) in phosphate buffer (0.1 M). The complete substrate designed for determination of the activity of LPL(10) consisted of 8 parts 10% bovine serum albumin‖ in phosphate buffer titrated with ammonium hydroxide to pH 8.7, one part 5% (final dilution) cottonseed oil and one part fresh human serum. activated by incubation for 30 minutes at 37°C. To determine the specificity of the substrate in some experiments albumin or serum was omitted from the incubation mixture. Equal amounts of pancreatic juice and substrate were mixed (final pH 8-8.5) and incubated at 37°C with frequent shaking for one hour. Lipolytic activity was expressed as milliequivalent of free fatty acids (FFA) liberated by one liter of pancreatic juice during one hour incubation (mEq/l/hr). FFA were determined by the method of Dole(11) except that Nile Blue A was used as the titration indicator.

The lipolytic activity was also determined after substances having differential inhibitory properties on the activity of LPL and pancreatic lipase (1M NaCl, 20 mg/ml protamine sulfate, 0.2 M NaF, 50 mg/ml sodium taurocholate) were added to the incubation mixture. The inhibitory substances were added in such a manner that the final volume, substrate concentration and pH were the same in all incubation media. In all the experiments where lipoprotein lipase activity was determined the complete activated triglyceride-lipoprotein substrate was mixed with pancreatic juice in the presence of sodium fluoride (0.2 M), a specific inhibitor of pancreatic lipase.

Pancreatic juice was subjected to calcium phosphate gel adsorption and elution with sodium citrate according to the method of Nikkila for partial purification of post-heparin LPL(12). Lipolytic activity of the untreated pancreatic juice, the supernatant after calcium phosphate gel adsorption, and the eluate was determined as described before. In addition the lipolytic activity of the eluate was determined in the presence of protamine sulfate and of triglyceride substrate from which serum (and hence lipoprotein) was omitted.

Fresh pancreatic tissue obtained immediately after sacrificing a dog by exsanguination was studied for its lipolytic properties. Pancreatic slices, weighing approximately 1 g, were sectioned into multiple thin slices, each weighing 50 mg or less. They were placed in 25 ml Erlenmeyer flasks, each containing 1 ml of Krebs-Ringer phosphate buffer (pH 7.4) per 100 mg wet weight of tissue and incubated under air at 37°C with gentle agitation for one hour. The effect of added heparin (30 μg/ml) to the incubation medium was also determined. Lipolytic activity of aliquots of the incubation medium was determined as described above and expressed in milliequivalents of FFA liberated by one gram of tissue during one hour of incubation (mEq/g/hr). The effect of complete and incomplete substrate, inhibitors, and adsorption on calcium phosphate gel on lipolytic activity was also studied. All determinations were performed in duplicate.

Results. I. Lipoprotein lipase activity of pancreatic juice. High levels of lipoprotein lipase activity were found in pancreatic juice collected during the postabsorptive state or after stimulation with secretin and pancreozymin, when incubated with the complete triglyceride-lipoprotein substrate in presence of sodium fluoride. The lipoprotein lipase activity varied from 8.0 to 38.0 mEq/l/hr (Table I) depending mainly on volume rate of flow and type of stimulation. Most of the studies were performed on pancreatic juice collected after

‡ Boots Pure Drug Co. Ltd., Nottingham, England.

§ Upjohn Co., Kalamazoo, Mich.

‖ Bovine Serum Albumin, Fraction V, Armour Pharmaceutical Co., Kankakee, Ill.

TABLE I. Lipoprotein Lipase Activity of Pancreatic Juice.

	Lipolytic activity (mEq/l/hr FFA*)
Postabsorptive state	13.0
	19.0
	16.5
Secretin	15.0
	10.8
	8.1
Pancreozymin	37.0
Secretin and pancreozymin	38.0
	26.1
	20.3

* Each value represents a single determination performed on samples obtained from 3 different dogs.

stimulation with secretin and pancreozymin to insure high rates of flow and maximal enzyme concentration.

II. Identity of lipolytic activity. 1. Substrate specificity. While the conventional pancreatic lipase can hydrolyze simple triglycerides as well as the triglycerides associated with lipoproteins, LPL exhibits its effect specifically only on the triglycerides of chylomicrons and other low density lipoproteins. Thus the effect of omitting serum, and hence lipoproteins, from the triglyceride substrate was studied (Table II).

Lipolytic activity of pancreatic juice in the mixtures without serum was always lower than the activity determined with the complete triglyceride substrate, and sodium fluoride almost completely inhibited this residual activity. Protamine sulfate, on the other hand, exerted little or no inhibition on lipolysis of simple triglyceride. The effect of protamine sulfate on lipolysis of the complete triglyceride-lipoprotein substrate was more pronounced, although very rarely was the inhibition as complete as that of sodium fluoride on the lipolysis of simple triglyceride. However, addition of fluoride and protamine sulfate to the complete triglyceride substrate invariably produced inhibition of almost 100%.

In all experiments albumin was added in high concentration, since it acts as an acceptor of the liberated FFA during the lipolytic reaction(14). Thus, the unbound FFA which tend to inhibit the lipolytic reaction(15) are removed from the medium. The amount of albumin in the incubation mixture was not a limiting factor, since even in the experiments with very high activity, addition of more albumin after completion of the incubation was not followed by additional lipolysis.

2. Differential inhibition studies. The effect of adding inhibitory substances on lipolytic activity of pancreatic juice in the presence of complete triglyceride substrate is summarized in Table III.

Protamine sulfate and 1 M NaCl, which have been shown to be inhibitors of LPL(13), significantly inhibited lipolytic activity of pancreatic juice assayed in the presence of complete triglyceride lipoprotein substrate. Sodium fluoride, on the other hand, which has no effect on LPL but is a strong inhibitor of pancreatic lipase, exerted a less pronounced and variable inhibitory effect in this substrate. Sodium taurocholate, a known activator of classical pancreatic lipase, has been shown to be also an inhibitor of LPL(13). When this substance was added to the incubation mixture an inhibitory effect was invariably noted.

TABLE II. Substrate Specificity and Differential Inhibition of Pancreatic Juice Lipolytic Activity.*

	Complete triglyceride substrate	Effect of protamine sulfate (20 mg/ml)	Triglyceride substrate without serum	Effect of NaF (0.2 M)
Dog No.	Lipolytic activity (mEq/l/hr)	% inhibition	Lipolytic activity (mEq/l/hr)	% inhibition
1	42.5	46	28.3	86
2	36.4	56	12.3	74
3	18.9	34	10.2	78
3	25.1	28	19.3	88
2	26.3	35	14.2	68

* Each value represents a single determination on samples obtained on different occasions from dogs 1, 2 and 3 respectively.

TABLE III. Differential Inhibition of Lipoprotein Lipase Activity of Pancreatic Juice.*

Dog No.	Original activity (mEq/l/hr)	Inhibition % NaCl (1 M)	Protamine sulfate (20 mg/ml)	Sodium taurocholate (50 mg/ml)	NaF (.2 M)
3	38.0	11.5	68.0		25.0
1	8.1	50.0	59.6		20.0
3	26.1		49.0	74.0	-6.0
2	20.3	34.0	26.5	66.0	.9
3	43.3	12.1	44.0		8.5
1	10.4	25.0	34.0	73.5	13.0

* Each value represents a single determination on samples obtained on different occasions from dogs 1, 2 and 3 respectively.

3. Calcium phosphate gel adsorption. When pancreatic juice was subjected to calcium phosphate gel adsorption and subsequent elution with sodium citrate, a method which partially purifies LPL, significant recovery of the original activity was obtained (Table IV). Furthermore, lipolytic activity of the eluate was strongly inhibited (88%) by protamine sulfate.

III. Effect of secretin and pancreozymin. Pancreozymin, which has a stimulating effect on the pancreatic secretion of enzymes, had a similar effect on lipoprotein lipase activity (Fig. 1). The effect of secretin is shown in Fig. 2. Here is seen a decrease of enzymatic activity in the face of a marked increase of volume rate of flow, reflecting the effect of secretin in increasing fluid but not enzyme output. When secretin and pancreozymin were injected together, high rates of flow and enzyme concentration were noted (Fig. 3). No effect on the plasma LPL activity was observed after intravenous injection of these substances.

Intravenous injection of heparin (0.75 mg/kg) was without effect on volume rate of flow or on lipoprotein lipase activity of pancreatic juice, but resulted in liberation of high levels of lipolytic activity in the plasma.

TABLE IV. $Ca_3(PO_4)_2$ Gel Adsorption of Lipoprotein Lipase Activity of Pancreatic Juice.

Dog No.	Original activity* (mEq/l/hr)	LPL activity of eluate (mEq/l/hr)	% inhibition of LPL activity of eluate by protamine (20 mg/ml)
3	86.0	2.0	
3	68.2	19.0	
1	20.3	4.8	88

* Each value represents a single determination on samples obtained from dogs 1 and 3 respectively.

IV. Dilution studies. Pancreatic juice was examined for its lipoprotein lipase activity after serial dilutions in saline and simultaneously subjected to protamine sulfate inhibition. Equal amounts of each dilution of pancreatic juice were mixed with activated triglyceride-lipoprotein substrate, without and with protamine sulfate, and final lipolytic activity was assayed as described above. The final volume, pH and substrate concentration were the same in all incubation media. With increasing dilutions progressive increase in enzymatic activity was observed (Table V). The degree of inhibition with protamine also increases with dilution. However, the increment in inhibition and the increase in activity were not related in a parallel fashion.

V. Lipoprotein lipase activity of pancreatic tissue. Incubation of slices of pancreatic tissue resulted in appearance of high LPL activity in the incubation medium. When heparin was

TABLE V. Effect of Dilution on LPL Activity of Pancreatic Juice.

Original activity (mEq/l/hr)	Dilution	LPL activity after dilution* (mEq/l/hr)	%inhibition of LPL activity by protamine (20 mg/ml)
20.7			24.0
	1:2	55.2	25.5
	1:4	110.4	33.0
	1:8	256.9	65.0
	1:32	389.5	85.0
	1:64	271.3	100.0

* Each dilution of pancreatic juice was mixed with equal amounts of activated triglyceride-lipoprotein substrate and final volume, pH and substrate concentration were the same in all incubation media.

added to the incubation medium no further increase in liberation of LPL activity was noted. This probably represents maximal release of enzyme by autolysis of the pancreatic tissue during the incubation.

Differential inhibition studies on lipolytic activity of the incubation medium showed results similar to those obtained from pancreatic juice. Calcium phosphate gel also adsorbed a significant degree of the original activity released from pancreatic tissue. The eluted activity was also inhibited by protamine. Dilution studies gave results similar to those seen after dilution of pancreatic juice.

Discussion. There is evidence that the prompt appearance of LPL in the circulation after injection of heparin or other polyanions (13) might be due to release of the enzyme from the vascular endothelium(16) or from adipose or myocardial tissue which contain the enzyme in highest concentration(13). Despite this, an increasing number of observations suggest that the intact pancreas may play a role in post-heparin mobilization of circulating LPL. Meng and Goldfarb(7) have shown that pancreatectomy reduced the lipemia-clearing property of the post-heparin plasma by more than 80%. Similarly, a significantly impaired post-heparin liberation of LPL was observed in patients with cystic fibrosis of the pancreas(17). More convincing evidence was presented by LeVeen *et al.* (8) who concluded that one of the sources of LPL might be the pancreas itself.

The present experiments clearly demonstrate the presence of LPL activity in the pancreas, as well as in its exocrine secretion before and during hormonal stimulation. Whether this lipoprotein lipase activity is a property of the conventional pancreatic lipase or a separate enzyme cannot be answered by the presented results. The partial concentration of the enzyme by calcium phosphate gel adsorption and its almost complete inhibition by protamine cannot be taken as evidence for 2 separate enzymes since this method is not specific for LPL. In addition, the possibility that the origin of at least part of the concentrated enzyme might be the adipose tissue of the pancreas cannot be excluded.

Of particular interest is the effect of heparin on the circulating LPL and of pancreozymin on the LPL activity of pancreatic se-

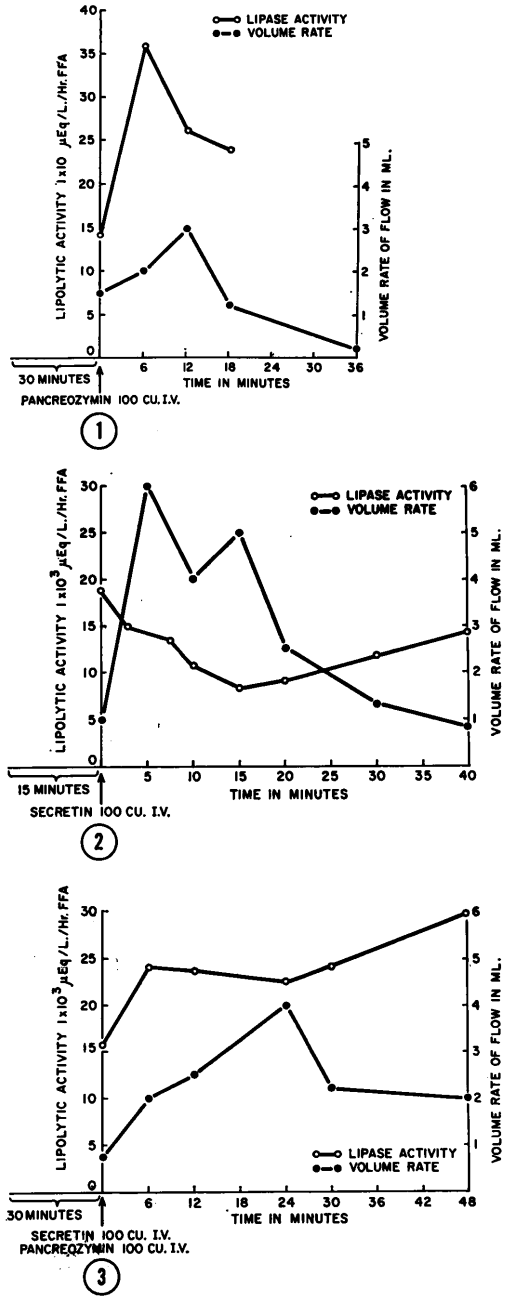

FIG. 1. Effect of pancreozymin on LPL activity and volume rate of flow.

FIG. 2. Effect of secretin on LPL activity and volume rate of flow.

FIG. 3. Effect of pancreozymin and secretin injected together on LPL activity and volume rate of flow.

cretion. Our results show that while intravenously administered heparin was without effect on the LPL activity of pancreatic juice, the usual effect on the plasma was noted. Pancreozymin, on the other hand, was without effect on plasma lipolytic activity, but it invariably produced a marked elevation of the LPL activity of pancreatic juice. One can postulate that heparin releases pancreatic LPL directly into the bloodstream, whereas pancreozymin augments its secretion in the pancreatic juice.

The increment in LPL activity of pancreatic juice with progressive dilution suggests that an inhibitory substance(s) is also present. With increasing serial dilution of pancreatic juice and concomitant dilution of the inhibitor, the opportunity for exogenous inhibition is enhanced. Indeed, there was a progressive increase in LPL inhibition by protamine with increasing dilutions of pancreatic juice.

In the light of these findings a mechanism to account for the hyperlipemia of pancreatitis may be suggested. It is possible that necrosis of the pancreas results in removal of a source of LPL or in release of inhibitor(s) of LPL into the circulation, thereby interfering with the normal clearing reaction. Recently we had obtained evidence for LPL inhibition in plasma of rabbits with experimental pancreatitis in whom elevation of the circulating triglycerides was also noted(6). In addition, Orvais and Evans(18) have demonstrated inhibition of post-heparin LPL activity by plasma obtained from a patient with pancreatitis accompanied by lipemia. Similarly, in an unpublished study of the hyperlipemia of a patient with pancreatitis we have observed the presence of an inhibitor of post-heparin LPL in the serum.

Summary. 1. Lipoprotein lipase (LPL) activity was demonstrated in canine pancreatic juice and pancreas. 2. The LPL activity of the pancreatic juice was augmented by stimulation with secretin and pancreozymin, the known hormonal stimulants of exocrine secretion of the pancreas. 3. An inhibitor(s) of the pancreatic LPL activity was suggested by dilution studies in pancreatic juice and in the pancreas. 4. The possible relationship of the pancreatic LPL activity and its inhibitor(s) to the lipemia accompanying acute pancreatitis is discussed.

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