Effect of Lipase Ingestion on Blood Lipid Levels.* (28955)

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Frazer(1) reported that the chylomicron levels of the blood were markedly depressed when a fat meal was supplemented with pancreatic lipase. In agreement with this report, Becker *et al*(2) found the ingestion of a whole pancreatic preparation with a test fat meal to be associated with greatly decreased chylomicron levels in the blood of aged persons with low fat tolerances. Young subjects with more normal fat tolerances were reported to be unaffected. These findings were attributed to a significant diminution in pancreatic and serum lipase in aged individuals, with less hydrolyzed fat transported in the portal blood.

More recently Tietz(3) found the concentration of the total esterified fatty acids (EFA) of the serum to be lowered and blood lipase levels usually elevated after administration of a pancreatic preparation with the fat meal. These investigators suggested that the increase in fat tolerance of patients receiving the pancreatic extract might point to the importance of the lipolytic enzyme to fat metabolism. The possibility was advanced that the increase in serum lipase values might not be caused by absorption of the lipase but rather by the stimulating effect of a pancreatic hormone in the preparation administered.

The above investigations suggested the desirability of further studies on mechanisms associated with the effect of lipase ingestion on the blood lipid level during postprandial lipemia and in hyperlipemic subjects. Two possible explanations for the effect on blood lipid levels may be that of an effect upon rate of fat absorption or an increased lipolytic action of some form of lipase in the blood. This study was also prompted by the availability of a stable, highly purified, high potency lipase preparation† (16,000 to 24,000 lipase units per gram).

In the human subjects included in the study, blood lipid levels were found to be decreased and serum lipase levels elevated after ingestion of the high potency lipase. The overall lowered blood lipid levels appear to be the result of an effect of the ingested lipase upon rate of fat absorption.

Materials and methods. It was first determined whether the ingestion of the high potency lipase preparation employed was effective in changing blood lipid or serum lipase levels. After an overnight fast all subjects were given an homogenized fat test meal, containing 10% corn oil, 0.06 ml of Tween 40 per 100 ml of mixture, 40% skim milk powder, and sugar and flavoring to taste. The human subjects were given a sufficient amount of the test meal to include 0.5 g of fat per kg body weight while the dogs received 1 g fat per kg.

Blood samples were collected in all cases before and at 3, 6 and 9 hours after ingestion of the test meal. One week later the experiment was repeated. Three enteric coated lipase tablets (1,000 lipase units each) were ingested with the meal and an additional tablet after a 3-hour interval. Later the test was repeated with 20,000 units on 2 of the normal subjects not responding to the smaller intake.

Three different methods were employed to measure changes in blood lipid levels. Optical densities (OD) were obtained on the undiluted plasmas except that of the hyperlipemic patient in which a 1 to 20 dilution was required. Esterified fatty acid content was determined by the method of Stern and Shapiro(4), while that of the cholesterol was obtained by the method of Zak(5). The EFA values were compared in several cases with those of the total triglycerides of the serum.

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TABLE I. Changes in O.D., EFA, Lipase and Cholesterol Levels of Serum as Affected by Lipase Ingestion.

Subject	Optical densities: Fasting value	Optical densities: Decrease, %	EFA decrease, %	Serum lipase increase, %	Cholesterol changes,‡ %
HW*	.415	28	29	17	+5
JC*	.448	40	28	29	−3
DS	.547	25	22	9	−1
JL	.662	34	28	19	−4
JM	.398†	18	20	14	+2
Dog 1	.155	20	—	—	+2
" 2	.131	35	—	—	−1
" 3	.137	24	—	—	0

* These subjects given 20,000 units since the 4,000 units given the others were not effective.
† Hyperlipemic serum diluted 1 to 20 for O.D. determinations.
‡ The + and − signs indicate increased or decreased amounts, respectively.

The glyceride method of Blankenhorn *et al* (6) was modified by measuring the fatty acid esters by the Stern and Shapiro technique (5). Serum lipase values were obtained according to Tietz *et al*(7), using Tris buffer during the incubation period and an electrometrical determination of the end point at pH 10.0.

To determine the effect of lipase ingestion upon rate of fat absorption, 6 rats were given test meals of 0.2 ml per dm^2 body surface (8) of corn oil containing labeled tripalmitin 1-C^{14} (28 μc per 0.2 ml of fat mixture). The test meal was supplemented with 2 ml of a 6% starch solution containing either 2500 units of lipase or an equal weight of egg albumin in alternate experiments.

Hourly samples of blood were collected from the tail of the rat over a 6-hour period, and activity determinations were made as previously described(9). Corrections were made for residual activity on the alternate experiments. As an added check upon rate of fat absorption, the activity of 30 min collections of expired CO_2 was also determined as described previously(10-11).

Results and discussion. Optical density measurements were made on the oxalated plasmas of all subjects as a relative measure of the particulate blood lipids (Table I). All values shown in the Table are those of the areas under curves obtained from test period data. It is of interest that after ingestion of 4000 units of lipase, only in the 2 subjects with average fasting OD values less than .500 (.415 and .448) were the OD values not decreased. All others with fasting averages above .500 were markedly depressed. However, the plasma OD of these 2 subjects was similarly decreased after ingestion of a 20,000 unit lipase supplement, approximately the same as that given by Becker *et al*(2). Although the dogs had relatively low average fasting levels, the average decrease in OD of the dog plasmas was about the same as that in the human subjects (29%). However, the dogs had received a lipase supplement similar to the larger one given the human subjects or about 265 units per kg body weight.

To supplement the OD determinations of plasma as relative measures of the particulate matter in the blood, such as chylomicrons, esterified fatty acids (EFA) and triglyceride content were also determined. The changes in EFA content in mEq/l (Table I) are in good agreement with the changes in OD determined on the same plasmas. Triglyceride measurements gave values which were quite comparable to those obtained from EFA determinations. The average decrease in EFA was 25% as compared with a 30% decrease in OD. These results are in excellent agreement with those of the investigators cited above. The findings are especially interesting when one considers the report of Brown *et al*(12) that, similarly, heparin had a much greater depressing effect on blood lipid levels, postprandially, of subjects with initially higher fasting lipid levels.

In Table I is also shown the effect of lipase ingestion upon serum lipase values. Serum lipase content was increased in all cases when

TABLE II. Percentage Changes in Areas Under the Curves of Blood and Expired Carbon Dioxide Activity Values as Affected by Ingestion of Lipase with Labeled Triglyceride.

Supplement		Blood 0-2 hr	CO_2 0-2 hr	Blood 4-6 hr	CO_2 4-6 hr	Blood 0-6 hr	CO_2 0-6 hr
None	Activ.	45	2315	498	24,710	517	37,642
Lipase	"	60	2956	278	11,461	374	26,227
% change with lipase*	"	+33	+28	-45	-54	-28	-30

* The + and - signs indicate an increase or decrease in values, respectively.

OD or EFA values were decreased after lipase ingestion. Although removal of chylomicrons from blood plasma has been reported not to depend to significant extent upon intravascular hydrolysis(13), the data of Tietz and coworkers(3) and of this study suggest the possible importance of the finding of an average increase of about 17% in serum lipase associated in each of the human subjects with a decrease in blood lipid content.

As anticipated, serum cholesterol levels of all subjects were unaffected during the 5-hour period following lipase ingestion. Also, the fasting blood cholesterol level in the lipemic subject was not changed by ingestion of 5,000 units of lipase daily over a 2-week period, nor by 10,000 units daily for another 2 weeks. However, none of the subjects studied, including the hyperlipemic individual, were hypercholesterolemic.

The effect upon rate of fat absorption following supplementation of a fat test meal with high potency lipase is shown in Table II. Areas under the curves were calculated for various time periods from the activity values of the blood and CO_2 samples collected. The values obtained suggest an increased rate of absorption of approximately 30% during the first 2 hours after lipase ingestion. The activity found in the blood and CO_2 appear to be decreased around 50% during the 4- to 6-hour interval, with both values decreased about 30% over the entire 6-hour period. This earlier appearance of lipid in the blood and the decreased elevation later on, associated with a decreased total absorption during the entire period can easily account for overall lower blood lipid levels as measured by OD and EFA values. This is evident if the absorptive period was extended over a longer period as indicated by the earlier increased rate of fat absorption and the decreased total amount absorbed over the entire test period.

The principal objective of this investigation was to study the effect of ingestion of a high potency lipase upon blood lipids in order that possible mechanisms involved might be further investigated. The evidence obtained indicates the desirability of additional studies, now in progress, on the mechanisms involved in the entrance or removal of lipids from the blood, and in the nature of the lipolytic enzymes of the blood associated with the increase in serum lipase.

Summary. Supplements of lipase were given with test fat meals to human subjects and dogs to determine the effect on blood lipid levels. Optical densities and hence lipid levels of the blood plasmas were lowered in all subjects when sufficient lipase was ingested. EFA and triglyceride determinations confirmed this measurement of the decrease in blood lipid levels. Human subjects with lower fasting OD values and EFA levels appeared to require more lipase to obtain this effect. Blood cholesterol values of normal subjects were not affected, nor was that of an hyperlipemic subject during a 4-week test period. The lowered blood lipid levels after lipase ingestion may be accounted for in part by an effect on rate of fat absorption. The lowered levels appear to be the result of extension of the absorptive period over a longer time interval. In all the human subjects, serum lipase values were elevated when blood lipid levels were lowered. The association of elevated blood lipase levels with decreased blood lipids suggests the possibility of some lipolytic action in the blood.

1. Frazer, S. C., *J. Physiol.*, 1943, v102, 329.

2. Becker, G. H., Meyer, J., Necheles, H., *Gastroenterology*, 1950, v14, 80.

3. Tietz, N. W., Borden, T. A., Hamilton, J. C.,

Murphy, N. L., *Circulation Res.*, 1960, v8, 214.
4. Stern, I., Shapiro, B., *J. Clin. Path.*, 1953, v6, 158.
5. Zak, B., *Am. J. Clin. Path.*, 1957, v27, 583.
6. Blankenhorn, D. H., Rouser, G., Weimer, T. J., *J. Lipid Res.*, 1961, v2, 281.
7. Tietz, N. W., Borden, T., Stepleton, J. D., *Am. J. Clin. Path.*, 1959, v31, 148.
8. Deuel, H. J., Jr., *The Lipids*, Interscience Publishers, Inc., New York, 1955, vII, 128.
9. Burr, W. W., Jr., Dunkelberg, C., McPherson, J. C., Tidwell, H. C., *J. Biol. Chem.*, 1954, v210, 531.
10. Tidwell, H. C., Dunkelberg, C., Wood, W. A., Burr, W. W., Jr., *ibid.*, 1956, v220, 733.
11. Harlan, J. W., *Atomlight*, 1961, v19, 8.
12. Brown, D. F., Heslin, A. S., Doyle, J. T., *New England J. Med.*, 1961, v264, 733.
13. Havel, R. J., Fredrickson, D. S., *J. Clin. Invest.*, 1956, v35, 1025.

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Effects of L- and D-Thyroxine on Cholesterol Synthesis and Turnover in the Chick.* (28956)

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The recent interest in cholesterol-lowering agents and the well-known alterations in serum cholesterol with L-thyroxine have stimulated a search for a calorigenically less active analog with similar effects on blood cholesterol levels. It has been postulated that L-thyroxine stimulates hepatic cholesterol synthesis and also the rates of biliary excretion and/or degradation, the net result being a reduction in serum level(1). D-thyroxine, by various animal assays estimated to be about 20% as active physiologically as the L-form (2), has been reported to have liver and serum cholesterol-lowering effects in the rat (1), produces marked reduction of liver fat in the chick(3), and has minimal effect on basal metabolic rate or on the electrocardiogram in myxedematous patients(4). Acetate carbons have been shown to be utilized in the biosynthesis of cholesterol(1). The liver, being the major site of cholesterol synthesis (5), has been the tissue studied by various investigators of mammalian cholesterol synthesis. The present studies were undertaken to compare the pharmacological effects of L- and D-thyroxine on rate of cholesterol synthesis from acetate-2-C^{14} both *in vivo* and *in vitro* and the disappearance of cholesterol-4-C^{14} in radioiodine thyroidectomized chicks.

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Materials and methods. Day-old White Leghorn cockerels were divided into 2 groups of 24 animals each and were fed chick grower ration.† Control and experimental groups received this ration throughout the experimental period. Birds were housed in individual cages with feed and water *ad libitum*. The chicks of the experimental group received radioiodine (350 μc) at the end of the first and third week to ablate the thyroid. Prior to each radioiodine dose the chicks were injected intraperitoneally daily for 5 days, with 0.1 unit of beef thyrotropin (TSH)‡ to increase thyroidal uptake. Thyroidal uptake (48 hours) ranged from 12 to 25% after the first I^{131} dose, while after the second dose, uptake was 1 to 7%. At 6 weeks of age the chicks were judged to be thyroidectomized by the lack of comb growth, rise in serum cholesterol, and lack of thyroid tissue in the neck in a few sacrificed at this time. The serum protein-bound iodine level dropped from a range of 1 to 1.5 μg per 100 ml to 0.6 to 1.0, and the serum cholesterol levels had risen from a range of 150-175 mg per 100 ml serum to 200-235 mg.

† The chick grower ration contained 5% hydrolyzed animal fats, 23% protein; with additional iodide salts omitted, iodine content averaged 1 mg per kg.

‡ Thytropar (Armour).