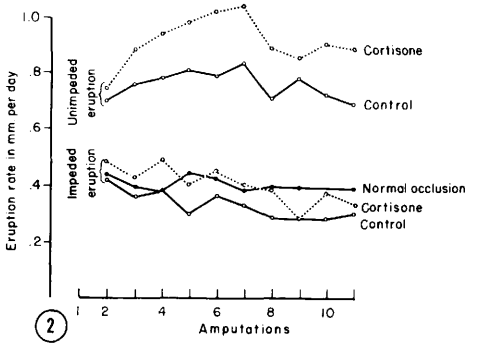

FIG. 2. Effect of cortisone on unimpeded eruption rates of male rats. Unimpeded eruption indicates average eruption rates of left incisors, following release from occlusal contact by amputation, in cortisone-treated and non-treated control rats. Impeded eruption indicates average eruption rates of right incisors in occlusal contact for both groups. Normal occlusion indicates averages of rats in group 3, subjected to the same manipulative procedures but not to incisor apical amputation.

maxillary incisors appears to contradict previous reports(4) of similar observations on mandibular incisors. However, if one examines rat incisors, differences between the maxillary and mandibular incisors become apparent. Particularly evident is the fact that those in the mandible have a smaller diameter and are more mobile. These differences could produce a higher rate of attrition in the normal back and forward masticatory movements of the mandible and explain the higher eruption rate reported(6) for the mandibular incisors.

The effects of occlusal stresses in modifying the eruption rate of incisors have been demonstrated(4). When these stresses are removed by maintaining the incisor free of occlusal contact the rate is accelerated, revealing the true growth potential of the incisor(5). That this growth can be further accelerated by cortisone treatment probably indicates a direct effect on the tissues of the incisor, or the mechanism controlling eruption, in a manner not yet understood.

Summary. Observations on rats maintained on a soft consistency diet or restricted food intake, revealed little if any effect on the eruption rate of the maxillary incisors. However, when these incisors were kept out of occlusal contact by periodic apical cutting, rate of eruption was accelerated. Cortisone treatment brought about a further increase in this accelerated rate indicating a direct effect on the mechanism or mechanisms responsible for incisor eruption or growth.

1. Domm, L. V., Wellband, W. A., PROC. SOC. EXP. BIOL. AND MED., 1960, v104, 582.

2. ———, *ibid.*, 1961, v107, 268.

3. Greenberg, S. M., Aterman, K., *Metab. Clin. and Exper.*, 1955, v4, 264.

4. Taylor, A. C., Butcher, E. O., *J. Exp. Zool.*, 1951, v117, 165.

5. Bryer, L. W., *Intern. Dent. J.*, 1957, v7, 432.

6. Schour, I., Massler, M., *The Rat in Laboratory Investigation,* Lippincott, Philadelphia, 1949, Chap. 6.

Received March 30, 1964. P.S.E.B.M., 1964, v116.

Lipolytic Activity in the Blood After Lipase Ingestion.* (29354)

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The studies of Frazer(1), Becker *et al*(2) Tietz and coworkers(3) suggested the possible importance of a lipolytic enzyme appearing in the blood after lipase ingestion. Blood lipid levels were found to be lowered by ingestion of the pancreatic lipase as a supplement to a fat test meal as measured by either chylomicron or esterified fatty acid levels. Possible explanations advanced for these findings were that an increased fat hydrolysis caused a loss of the fatty acids from the systemic to the portal blood(1,2)

* This study was supported in part by grants from Robert A. Welch Foundation, Division of Research and Development, Office of Surgeon General, and A. H. Robins Company.

or that a pancreatic hormone might be the effective agent(3). A recent report from this laboratory suggested that the lowered blood fat after lipase ingestion was, at least in part, the result of the extension of the absorptive period over a longer time interval (4).

The association of elevated blood lipase levels with the decrease in blood lipids poses the possibility of some lipolytic action in the blood, although it has been reported that the removal of chylomicrons from the blood does not depend upon intravascular hydrolysis(5). Nevertheless, an increased lipolytic activity in the blood after lipase ingestion suggested the desirability of learning more of the nature of the enzyme(s) involved. If a blood lipase plays any role in clearing the fat from the blood, there are at least 3 possible lipases that might be involved. This lipase might be absorbed pancreatic lipase, normal serum lipase, or lipoprotein lipase, known to increase after heparin injection(6).

All the evidence obtained in this study suggests that the enzyme responsible for the increase in lipolytic activity in the blood after pancreatic lipase ingestion has properties resembling more those of the ingested lipase than either serum or lipoprotein lipase.

Materials and methods. After trials with a number of substances which may either inhibit or activate the 3 possible lipases mentioned above, determinations of enzyme activity in presence and absence of cholic acid seemed most promising. Determinations were first made on lipoprotein lipase present in the blood after heparin injection(6). Male albino rats weighing about 300 g were given an intravenous injection of 2 ml of a solution containing 0.8 mg of heparin. Ten minutes later at least 5 ml of blood was drawn from the vena cava for measurement of lipolytic activity.

Similarly, blood was drawn from other rats 3 to 4 hours after being fed 1.5 ml of corn oil by stomach tube, either with or without a pancreatic lipase supplement. The rats receiving the lipase were given 3000 units (Wilson) of high potency lipase(4) initially, and 1500 units at 2 hours and again at 3 hours. To check the efficacy of the supplement in lowering blood lipid levels, esterified fatty acid (EFA) contents(7) were determined on the blood of rats receiving the fat test meal, both with and without the lipase supplement.

The lipolytic activity of the blood plasmas was determined in all cases by measuring the glycerol liberated during a one hour incubation period. The method of Kern *et al* (8) was utilized with slight modifications. The Tris buffer was prepared to contain 10% bovine albumin, and Lipomul-IV was used instead of Ediol. Also, 0.05 ml of 10% sulfuric acid, containing 0.1% of iron salt, was added to the 0.25 ml aliquots used for the tests after the incubation period in order to prevent the cholic acid interfering with the glycerol determination. A concentration of 0.2% cholic acid was employed in some incubation mixtures as a possible inhibitor or activator of the enzyme, depending upon the nature of the enzyme.

For comparative purposes, 0.1 ml of a very dilute aqueous extract of the high potency pancreatic lipase preparation was employed with 0.9 ml of inert plasma in some tests, replacing the 1 ml of test plasma. In all cases the activity of the enzyme was determined both in presence and absence of the bile acid.

Results and discussion. The nature of the enzyme found to be increased in the blood after pancreatic lipase ingestion was studied by measuring the effect of the bile acid in the incubation mixtures containing the known lipases which might be present. The lipolytic activity of an aqueous extract of the high potency pancreatic lipase preparation (4) was found to be markedly increased by the presence of cholic acid in the incubation mixture. Approximately 3 times as much glycerol was freed by the enzyme when bile was included (Table I). This same value was obtained whether differences in amounts of glycerol freed or percentage changes in the two tests were compared.

A quite different effect was obtained when the blood from the heparin injected rats provided the enzyme. Lipoprotein lipase is known to be inhibited by bile salts(9) and in this case, lipolytic activity was decreased

TABLE I. Effect of Cholic Acid upon Lipolytic Activity of Lipases from Different Sources. Also effect of lipase ingestion on blood lipid levels.

Test	No. of rats	Bile supplement†	μg glycerol in incubation mix, min 0″	60″	Actual difference Δ+/Δ−	% changes Actual	% changes Δ%+ > Δ%−
Lipase control		—	369	399		8.1	
	6*	+	383	477	3.13 ± .80	24.6	3.04 ±.71
Heparin given		—	307	595		94.0	
	6	+	431	609	.62 ± .11	41.3	.44 ±.10
No treatment		—	258	294		14.0	
	6	+	321	331	.28 ± .06	3.1	.22 ±.06
Lipase given		—	307	314		2.3	
	8	+	359	397	5.43 ±1.32	10.6	4.61 ±.94
Idem		—	EFA			17.7	
	4	+	μg/ml		.35	6.7	.38

* Lipase control determinations were made on a mixture of a dilute aqueous extract of the pancreatic lipase preparation and an inert plasma.

† + and — signs are used to indicate presence or absence of bile supplement in incubation mixture, respectively. Standard deviations are included with final figures.

to about one-half by the presence of the cholic acid. Similarly, the activity of the serum lipase in the blood 3 to 4 hours after giving only a fat test meal was even more inhibited by the bile acid. Less than one-third as much glycerol was freed from the Lipomul substrate when cholic acid was present in the incubation mixture (Table I).

The activity of the enzyme in the plasma from the rats given the pancreatic lipase supplement was markedly enhanced by the presence of cholic acid in the incubation mixture. There was about a 5-fold increase in enzymic activity as compared with a 3-fold increase in the case of the pancreatic lipase control test. Esterified fatty acid determinations on the blood of half of the rats used in this test were made to be sure that the increased enzyme activity was associated with decreased blood lipid levels. As shown in the last part of Table I, the lipid was lowered to about one-third of that without lipase ingestion.

It is evident from these results that the enzyme found in the blood after pancreatic lipase ingestion more closely resembles that of the ingested lipase than either serum or lipoprotein lipase. The evidence does not prove the ingested pancreatic lipase is absorbed in spite of higher blood lipase levels after its ingestion. However, a protein such as albumin has been shown to be absorbed in part unchanged(10) from the intestine. Hence such absorption with possible appearance in the blood must be considered when lipase is available in excess. If true, then its presence, associated with changes in the rate of fat absorption, might aid in producing the lowered blood lipid levels obtained after lipase ingestion.

The possibility of an increased lipolytic activity in the blood raises the question as to the relative significance of a decrease in rate of absorption as compared with a possible increase in rate of removal of lipid from the blood as a result of the increased lipolysis. When lipoprotein lipase causes a clearing of the blood lipids, there is an increase in the stored fatty acids and less of an increase, but a significant one, in CO_2 elimination(11). If an increased lipolysis occurs in these animals, it is not associated with an increased CO_2 elimination since both the blood lipids and CO_2 formed were equally lowered to about 30% of that without the higher concentration of lipolytic enzymes(4). More information is needed to assess the relative importance of lipolysis in clearing the blood of fat under these conditions.

Summary. The nature of the enzyme appearing in the blood after oral ingestion of pancreatic lipase has been studied by determining the effect of the presence of cholic acid upon the activity of some enzymes which might be present. The activity of lipoprotein and serum lipase were markedly de-

pressed by the bile acid while the enzymic activity of both pancreatic lipase and the enzyme present in the blood after pancreatic lipase ingestion were greatly enhanced by the presence of cholic acid. Although the rate of fat absorption has been shown to be affected by lipase ingestion, the possibility remains that this increased lipolytic activity may play some role in clearing the blood of fat.

The authors wish to acknowledge the technical assistance of Mrs. Patricia Gifford.

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. McPherson, J. C., Moore, W. T., Pope, J. L., Tidwell, H. C., PROC. SOC. EXP. BIOL. AND MED., 1964, v115, 514.
5. Havel, R. J., Fredrickson, D. S., *J. Clin. Invest.*, 1956, v35, 1025.
6. Grossman, M. I., *J. Lab. Clin. Med.*, 1954, v43, 445.
7. Stern, I., Shapiro, B., *J. Clin. Path.*, 1953, v6, 158.
8. Kern, F., Jr., Steinman, L., Sanders, B. B., *J. Lipid Res.*, 1961, v2, 51.
9. Engelberg, H., *J. Biol. Chem.*, 1956, v222, 601.
10. Van Alstyne, E. V. N., Grant, P. A., *J. Med. Res.*, 1911, v25, 399.
11. Becker, G. H., Rall, T. W., Grossman, M. I., *J. Lab. Clin. Med.*, 1955, v45, 786.

Received March 30, 1964. P.S.E.B.M., 1964, v116.

Host-Virus Relationships in Hamsters Inoculated with SV_{40} Virus During the Neonatal Period.* (29355)

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Previous reports(1-3) from this laboratory described the isolation of SV_{40} virus, presented first definitive proof of oncogenic quality of the agent and described the influence of age, virus dose, route of inoculation and other factors on tumorigenesis. The present report describes the findings in continued studies of pathogenesis of SV_{40} virus in hamsters with special reference to viral latency, immunology, and age influence.

Materials and methods. Virus and cell cultures. Prototype strain VA 45-54 of SV_{40} virus was propagated and titrated in cell cultures of grivet monkey kidney (GMK) by methods described previously(2,3). *Hamsters.* Pregnant hamsters were obtained from Lakeview Hamster Colony, Newfield, N. J., and the progeny were inoculated subcutaneously with virus inoculum at the time periods shown in the text. Animal care, clinical observations, and pathologic observations were carried out by previously described methods (2,3). *Animal tissues.* Homogenized whole animals 1 to 7 days of age were assayed for infectious virus whereas pools of various organs were prepared from older animals. Special attention was given to include tests of skin and muscle from the dorsal area of the animals which was the site of injection and the usual site for tumor appearance. The whole animals, tissue pools, and tumors were homogenized for 10 minutes at 16,000 rpm in an Omnimix cup with sufficient Hanks' balanced salt solution to give a 15% suspension. These were clarified by centrifuging at 2200 rpm for 20 minutes in the horizontal head and the supernates were stored frozen at —70°C until assayed for presence and amount of SV_{40} virus. *Virus recovery.* The 15% tissue suspensions were diluted 1:2 and serial 10-fold dilutions were titrated in GMK using 4 tubes per dilution. Preparations which gave negative results were retested, whenever possible, in 20 ml amount (1:2

* This work was supported in part by grant from U. S. Public Health Service.

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