TABLE II. Order of Relative Potencies of Corticoids: Roman Numerals Refer to Compounds in Table I.

Decreasing order of potency	Activity		
	Anti-granuloma	Thymus involution	Liver glycogen deposition
1	XI	XI	XI
2	XII	XII	VIII
3	XIV	XIV	XIV
4	IX	XIII	IX
5	XIII	VIII	XV
6	VIII	IX	XII
7	XV	XV	X
8	X	X	VI
9	VI	VI	XIII
10	V	IV	VII
11	IV	VII	V
12	VII	V	IV
13	III	I	III
14	I	III	I
15	II	II	II

tive potencies in the anti-granuloma and thymolytic assays, and 9α-fluoro-16α-hydroxyprednisolone acetonide which had a greater relative potency in the liver glycogen deposition assay.

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In vitro Activation of Lipolytic Activity in the Serum of Dogs by Bile Salts.* (29256)

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Bile salts have been reported to inhibit the lipolytic activity of heparin-induced clearing factor lipase (CFL)(1). However, in a series of characterization studies of CFL we found that sodium cholate and its conjugates increased the lipolytic activity of dog postheparin plasma. These compounds were found to initiate *in vitro* lipolytic activity in serum from normal, untreated dogs. The present report extends these observations and relates the characteristics of this cholate activated lipase (CAL).

Methods. Lipolytic activity was assayed in citrated plasma or serum from dogs, cats, rabbits, rats, goats and humans. All samples were centrifuged in the cold at 4000 *g* for 10 minutes and maintained at 0°C until assayed for lipolytic activity.

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TABLE I. Hydrolysis of a Triglyceride Substrate by Cholate Activated Lipase of Dog Serum or Citrated Plasma.*

Test material	FFA acceptor	Lipolytic activity, mEq/l/45 min‡
Serum (14)†	HSA	.15 ± .33 (0 – 1.2)
" (15)	$CaCl_2$	.21 ± .34 (0 – 1.16)
Na cholate (5)	HSA	.18 ± .25 (0 – .64)
Na taurocholate (7)	$CaCl_2$	.45 ± .49 (0 – 1.16)
Serum and Na cholate (11)	HSA	17.01 ± 3.72 (9.72–24.92)
Serum and Na glycocholate (1)	HSA	8.29
Serum and Na glycotaurocholate (1)	HSA	7.15
Serum and Na taurocholate (3)	HSA	4.43 (1.76– 6.56)
Serum and Na taurocholate (22)	$CaCl_2$	20.51 ± 8.54 (6.16–33.58)
Citrated plasma and Na cholate (5)	HSA	24.42 ± 6.87 (15.20–33.87)
Citrated plasma and Na taurocholate (3)	$CaCl_2$	22.42 (17.40–32.29)

* Values are given as mean ± 1 standard deviation with range in parentheses.
† No. of determinations.
‡ Incubation for 45 min at 37°C.

Lipolytic activity was determined by measuring the amount of free fatty acid (FFA) released from a triglyceride substrate (Ediol, Schenley) during 45 minutes' incubation at 37°C. Each ml of the incubation mixture contained 0.01 ml of Ediol (5 mg coconut oil), 0.2 ml test serum or plasma, 0.10 ml of 20% bile salt solution and either 0.4 ml of human serum albumin (100 mg albumin) or 0.1 ml of 0.5 M calcium chloride as fatty acid acceptor. When albumin was used as fatty acid acceptor, each ml of the incubation mixture contained 0.29 ml of either 0.066 M phosphate buffer pH 7.9 (final concentration of buffer 0.019 M) or 0.1 M Tris buffer pH 8.2 (final concentration 0.03 M). When calcium chloride was used as fatty acid acceptor, each ml of the incubation mixture contained 0.59 ml of either 0.1 M Tris buffer pH 8.2 (final concentration 0.059 M), or 0.2 M Tris buffer pH 8.2 (final concentration 0.118 M) or glycine buffer pH 8.2 (final concentration 0.118 M). A total volume of 5.0 ml of incubation mixture was usually employed. Since sodium cholate and sodium glycocholate react with calcium to form insoluble precipitates, sodium taurocholate was used exclusively in the system containing calcium chloride as fatty acid acceptor. All systems used supported linear kinetics for 45 minutes.

One ml test plasma was preincubated 30-60 minutes with 0.5 ml of 20.0% solution of bile salt at 0°C before mixing with the substrate. The final concentration of bile salt was 20 mg/ml of incubation mixture unless otherwise indicated. The final concentration of each potential inhibitor in the incubation mixture will be indicated in the results. Initial and terminal samples were withdrawn from the incubation mixture for extraction and titration of long chain fatty acids by the method of Dole(2). Nile blue A was used as the indicator.

Materials. Bile salts tested were: sodium cholate, sodium glycocholate, sodium taurocholate and sodium glycotaurocholate (Nutritional Biochemicals Corp.), and sodium cholate (Mann Research Laboratories). Substances tested for inhibition of CAL activity were: DL alpha tocopherol, progesterone, eserine sulfate and hexestrol (Nutritional Biochemicals Corp.), cortisone acetate (Merck, Sharp and Dohme), sodium fluoride (Mallenkrodt), cholesterol (Difco Laboratories), Triton WR-1339 (Winthrop Laboratories), sodium chloride (A.C.S., Allied Chemical); (ethylenedinitrilo) tetraacetic acid, tetrasodium salt (EDTA, Matheson, Coleman and Bell) and protamine sulfate (Eli Lilly). Other materials used during the course of these experiments were pancreatic lipase (Steapsin, Nutritional Biochemicals Corp.), tributyrin (Mann Research Laboratories) and heparin (heparin sodium, Abbott).

Results. Demonstration of cholate activated lipase (CAL). Forty-eight serum or citrated plasma samples from 19 dogs were tested for CAL. In Table I it can be seen that addition of sodium cholate, sodium glycocholate, sodium taurocholate or sodium glycotaurocholate to fresh dog serum or citrated plasma resulted in the appearance of a

TABLE II. Effects of Inhibitors on Lipolysis by CAL,* Postheparin Plasma Lipase (CFL),† and Pancreatic Lipase.

Potential inhibitor	mg/ml incubation mixture	% inhibition		
		CAL	CFL	Pancreatic lipase
Eserine	5.0	96.8 (3)	55.0 (1)	−16.4 (1)
Hexestrol	10.0	93.4 (3)	82.0 (1)	8.2 (1)
Triton WR-1339	10.0	67.9 (3)	71.0 (2)	95.3 (1)
Cortisone acetate	2.5	33.3 (1)	—	—
DL Alpha tocopherol	10.0	33.2 (3)	−3.5 (1)	28.2 (1)
Sodium chloride	29.2	32.7 (3)	23.8 (2)	−64.6 (1)
EDTA	19.0	30.9 (1)	27.7 (1)	−102.7 (1)
Sodium fluoride	2.1	22.7 (3)	71.6 (2)	−32.9 (1)
Protamine sulfate	1.0	20.6 (2)	55.0 (1)	−23.5 (1)
Progesterone	10.0	2.5 (1)	—	—
Cholesterol	10.0	0	—	—

Values are means where possible. No. of determinations in parentheses. $CaCl_2$ used as FFA acceptor.

* CAL obtained by preincubating dog serum with Na taurocholate.

† Dog plasma, 10 min postheparin.

substance capable of causing the release of FFA from a triglyceride substrate. Neither dog serum nor bile salts possessed this ability when tested independently. There was no striking difference in CAL potential between serum and citrated plasma.

Different bile salts are not equally effective in their ability to activate this lipolytic system (Table I). However, the small number of determinations using various combinations of bile salt and fatty acid acceptor makes it impossible to establish clearly this point.

Sera from other species were tested for CAL. No CAL activity was obtained in sera from 3 rabbits, 2 rats, one goat and 6 humans. However, a mixture of sodium cholate and fresh cat serum caused release of 7.95 mEq/l/45 minutes FFA.

CAL was also found in gall bladder bile from the dog. In one instance, bile, reported to be free of lipolytic enzymes(3), effected the release of 1.72 mEq/l/45 minutes FFA. Addition of sodium taurocholate to this bile resulted in the development of lipolytic activity sufficient to effect the release of 4.88 mEq/l/45 minutes FFA.

Some properties of CAL. Preincubation of serum at 56°C for 5 minutes destroyed its CAL potential. The activated enzyme was also found to be heat-labile. Serum stored in the cold (0-3°C) for several weeks retained approximately two-thirds of its original activity. Overnight dialysis of serum in 0.9% NaCl resulted in no significant loss of CAL potential. The pH optimum of CAL was found to be 8.0-8.3. Activity fell slowly on the acidic side of the optimum, but rapidly on the alkaline side.

Studies have indicated that CAL has the ability to hydrolyze triglycerides other than those in coconut oil. Using tributyrin as the substrate (0.01 ml/ml of incubation mixture) and calcium chloride as fatty acid acceptor, release of 16.93 mEq/l/45 minutes FFA was effected by serum preincubated with sodium taurocholate. When chylomicra were used as substrate under similar conditions, 5.72 mEq/l/45 minutes FFA was released.

Activation concentration of sodium taurocholate. To find the bile salt concentration resulting in maximum activation of CAL, varying concentrations of sodium taurocholate in the incubation mixture were tested. Calcium chloride was used as fatty acid acceptor and the same serum sample was used. A concentration of sodium taurocholate below 1.2 mg/ml of incubation mixture effected no release of FFA. Four mg/ml of incubation mixture provided maximum activation. At higher concentrations of sodium taurocholate up to 40 mg/ml, the amount of FFA released remained constant.

Inhibition of CAL activity. Various potential inhibitors of lipolysis by CAL were tested and compared to their effect on lipolysis by pancreatic lipase and CFL. In Table II potential inhibitors are listed in order of their inhibitory effect on CAL. No correlation was

observed between the effects of these substances on lipolytic enzymes from 3 different sources. Eserine and hexestrol were the most effective inhibitors of CAL activity. Hexestrol, triton and sodium fluoride were the most effective inhibitors of CFL activity. Triton was the only compound tested which was an effective inhibitor of pancreatic lipase; the majority of materials tested actually increased its lipolytic activity.

Source of CAL. Serum from a depancreatized dog was tested daily for CAL activity for one week following operation. The operation, diet and insulin therapy were carried out according to Markowitz(4) and the common bile duct was not disturbed. Within 48 hours after pancreatectomy, serum CAL activity fell from 18.2 to 13.3 mEq/l/45 minutes FFA and remained unchanged for 7 days. As serum CAL activity with this system (sodium cholate and human serum albumin) ranged normally from 9.72 to 24.92 (Table I), this decrease was not considered significant.

In one experiment the common bile duct in a dog was tied above the level of the pancreatic duct and below the cystic duct. Serum was tested for spontaneous lipolytic activity as well as CAL activity for 55 days following the duct ligation. No spontaneous lipolytic activity was demonstrated at any time although the icteric index rose to 100. Serum CAL activity decreased gradually to 70% over the first 25 days following the operation but afterwards despite marked daily fluctuations no further decrease occurred.

Discussion. The data presented have demonstrated an inactive precursor of a lipolytic enzyme in the circulating blood of the dog and cat and in bile from the dog. This enzyme can be activated *in vitro* by various bile salts and has therefore been referred to as cholate activated lipase (CAL). The data indicate that the properties of this lipolytic enzyme are different from those of either pancreatic lipase or postheparin plasma lipase (CFL).

The strong inhibitory effects of eserine on CAL are interesting. Eserine is a known cholinesterase inhibitor(5). However, CAL differs from cholinesterase in that it rapidly hydrolyzes triglycerides of long chain fatty acids. Also, cholinesterases are reported to be inhibited by bile salts(6), upon which CAL activity is dependent.

The source of the inactive precursor, the site of action and the physiological significance of CAL are unknown. The pancreas does not appear to be a primary source and the transient fall in serum CAL activity following ligation of the common bile duct does not necessarily implicate the liver as its source. Derangements in liver functions incidental to the operation could have depressed its formation at some extrahepatic site.

The dependence of CAL activity upon the presence of a high concentration of bile salts suggests the liver cell as the site of action. Normal blood levels of cholate are reported to be .002 to .03 mg/ml in the systemic circulation(7). This is far below the critical level of cholate required to activate serum *in vitro*. It could be argued that less bile salt might be required for *in vivo* than *in vitro* activation of the precursor. However, even the level of serum cholate reached in obstructive jaundice was insufficient.

It is of interest that the two species in which the enzyme could be demonstrated are both carnivores and that no activity was elicited in the sera of herbivores and humans.

Summary. The inactive precursor of a lipolytic enzyme which requires bile salts for activation *in vitro* has been identified in serum from dogs and cats, and bile from dogs. It is absent from the serum of humans, rabbits, rats and goats. The activated enzyme has been referred to as cholate activated lipase(CAL). Both CAL and its inactive precursor are heat labile. The pH optimum of CAL is 8.0-8.3. CAL rapidly hydrolyzes long and short chain fatty acid triglyceride. Eserine and hexestrol are inhibitors of CAL. Comparison of the effect of various potential inhibitors on CAL with their effects on pancreatic lipase and postheparin lipase suggest that the lipolytic enzymes from these three sources are not identical.

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Effects of Increased Left Ventricular Resistance Loads on Flow and Composition of Cardiac Lymph in the Dog.* (29257)

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Studies from our laboratory(1), and by others(2), have shown that the dog heart has a substantial lymph flow under normal conditions. Rates of cardiac lymph flow have been found to be increased by hypoxia(1,3), epinephrine and ephedrine(2), and by acute plasmapheresis(2).

The present study was undertaken to determine whether increased resistance to left ventricular emptying alters the flow of cardiac lymph or its composition. The resistance to emptying was produced by 1) stenosis of the aorta, 2) intravenous norepinephrine and 3) intravenous angiotensin II.

Methods. Stock dogs were anesthetized with intravenous sodium pentobarbital. The heart was then exposed through a left lateral chest incision in the 4th interspace. Positive pressure respiration was maintained with a mixture of 95% oxygen and 5% carbon dioxide *via* a tracheal catheter. A small amount of T 1824 dye (about 0.2 ml of a 1% solution) was injected into the ventricular myocardium to visualize the mediastinal lymphatic channels draining the heart.

Details of our technique of visualizing the mediastinal lymphatics draining the heart(4) and of cannulation of one of them have been described(1). After successful cannulation of a lymphatic, all other lymphatics ascending from the heart were ligated. Dressings moistened with Ringer's solution were then placed across the chest wound. An intravenous infusion of Ringer's solution (10 to 20 drops per minute) was maintained throughout each experiment, except when solutions of norepinephrine or angiotensin II were being administered.

Stenosis of the descending thoracic aorta was accomplished by tightening a screw clamp placed distal to the left brachiocephalic artery. The aortic clamp was tightened fairly rapidly, to raise the proximal diastolic arterial blood pressure between 20 and 30 mm Hg. At the end of a one-hour collection period the clamp was loosened slowly. Norepinephrine solutions were made up in a concentration of 16 μg of norepinephrine base per ml; angiotensin II solutions were made in a concentration of 2.5 μg per ml. These pressor agents were infused intravenously at a rate sufficient to raise the diastolic blood pressure 20 to 30 mm of Hg. The infusion rate to accomplish this was invariably less for angiotensin II than for norepinephrine.

Because of technical problems it was not possible to test all 3 methods of augmenting the resistance load in each animal successfully cannulated. However, in 3 dogs it was possible to compare the effects of norepinephrine with those of angiotensin II, and in 2 of them a second one-hour control period was obtained before administration of the second drug. A total of 9 dogs were successfully cannulated. In 2 of these animals 2 initial control periods were obtained.

The initial collection of lymph specimens was started after a "flush" period of 15 min-

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