INHIBITION OF LIPOPROTEIN LIPASE ACTIVITY FOLLOWING INJECTION OF PITUITARY EXTRACTS INTO RABBITS*

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Rabbits injected subcutaneously with alkaline extracts of bovine, hog, or human anterior pituitary glands develop a marked hyperlipemia within 12 to 24 hours, as has been shown recently by Rudman and Seidman (1). During the course of experiments here reported which were designed to learn more about the pathogenesis of this hyperlipemia, it was found that injection of the pituitary extracts was regularly accompanied by a striking inhibition of the lipemia-clearing activity that normally occurs in the plasma following the intravenous injection of heparin (2). Moreover, relatively small amounts of serum from rabbits given pituitary extracts were capable of retarding or abolishing completely the ability of postheparin plasma obtained from normal animals to clear lipid emulsions. The inhibition of clearing paralleled closely the severity of the hyperlipemia and in a number of instances preceded the onset of hyperlipemia, the findings suggesting a possible causal connection between the two phenomena.

Materials and Methods

Rabbits were given a single subcutaneous injection of an extract of either bovine, hog, or human anterior pituitary glands. At varying intervals of time following the injection the animals were given heparin intravenously and plasma obtained 15 minutes later was tested for its ability to clear a synthetic lipid emulsion. In other experiments, serum obtained following the injection of pituitary extracts was added to postheparin plasma obtained from normal animals, and the ability of such mixtures to clear a synthetic lipid emulsion was assayed. In most cases, lipemia clearing activity was measured by reduction in optical density; in some instances production of glycerol or non-esterified fatty acids was also determined.

Pituitary Extracts.—Bovine pituitary glands were obtained frozen from the slaughter house and were kept at −20°C. until used. Just prior to use, the glands were thawed, trimmed of

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attached dura, bone, and brain, and the posterior lobe was removed. The glands were then quickly frozen in a mixture of alcohol and solid carbon dioxide, and dried *in vacuo* from the frozen state. The glands were ground to a powder in a mortar and stored in a desiccator at 4°C.

The dried pituitary powder was extracted with 0.1 N sodium hydroxide, usually in the proportion of 100 mg. of powder to 10 ml. of sodium hydroxide. Extraction was carried out at 4°C. for 30 minutes with frequent shaking. The pH of the extract was adjusted to 7.5 with 6 N hydrochloric acid, and the insoluble residue was removed by centrifugation. The supernates were stored at 4°C. until used, usually for 1 or 2 days. In a few experiments pituitary glands from human beings obtained from the postmortem service of The New York Hospital and, in more recent experiments, lyophilized swine pituitary glands[1] were used. The results with both these preparations were comparable to those observed with the bovine glands.

Rabbits.—Market-bought adult rabbits of mixed breeds and both sexes were employed. The animals were fed a diet of Rockland rabbit pellets supplemented twice weekly with lettuce and carrots. This diet is low in fat and free of cholesterol. The pituitary extracts were injected subcutaneously between the shoulder blades in 10 or 15 ml. amounts with little or no visible immediate reaction on the part of the animals. The injection sites when examined 6 to 72 hours later showed only very slight local erythema and no other evidence of inflammatory reaction.

Lipemia Clearing Activity.—Clearing factor was produced in rabbits by the intravenous injection of heparin,[2] 7.5 mg. per kilo. Fifteen minutes after injection the animals were bled from the marginal ear vein into conical centrifuge tubes containing 3.2 per cent sodium citrate, in a ratio of 9 parts blood to 1 part citrate. The tubes were inverted several times and centrifuged at 4°C. for 10 minutes at a speed of 3,000 R.P.M. The supernatant plasma (henceforth referred to as postheparin plasma or PHP)[3] was separated and stored in an ice bath until used. In several experiments clearing factor was obtained from human beings following an intravenous injection of heparin in a dose of 1 mg. per kilo.

Lipemia-clearing activity was assayed by reduction in optical density according to the method of Grossman *et al.* (3). 0.1 ml. of a 1–100 dilution in saline of a coconut oil emulsion (ediol[4]) was placed in a Coleman microcuvette together with 0.5 ml. of imidazole buffer at pH 7.4, both having been previously warmed to 37°C. 0.4 ml. of postheparin plasma was then added and the contents quickly mixed by inversion and the optical density read in a Coleman Junior spectrophotometer at a wave length of 700 mμ. The tubes were incubated in a water bath at 37°C. and optical density recorded at intervals of 15, 30, 60, and 90 minutes. In some experiments lipemia-clearing activity or its inhibition was also followed by changes in the liberation of glycerol according to the method of Lambert and Neish (4), and by the release of non-esterified fatty acids using the method of Dole (5).

EXPERIMENTAL

Hyperlipemia Induced in Rabbits by Pituitary Extracts

An experiment was first done to confirm the findings of Rudman and Seidman that the injection of crude pituitary extracts into rabbits results in the development of hyperlipemia (1), and also to learn whether extracts of other tissues could produce similar changes.

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[2] The heparin used in these studies was generously supplied by Dr. George H. Berryman of the Abbott Company, Chicago.

[3] PHP, postheparin plasma.

[4] Obtained from SchenLabs Pharmaceuticals, Inc., New York.

An extract of lyophilized beef anterior pituitary glands was prepared so that each milliliter of extract represented 10 mg. of dried gland. Similar extracts were prepared from lyophilized beef liver, brain, pancreas, and heart, each milliliter of extract in these cases, however, being equivalent to 30 mg. of the dried material. Eight rabbits were each given a single injection of 15 ml. of beef pituitary extract. In control studies four rabbits were each given 15 ml. of an extract of either beef liver, pancreas or brain, and two received 15 ml. of beef heart extract

TABLE I

Changes in Optical Density of Blood Serum Following Injection into Rabbits of Extracts from Beef Pituitary, Liver, Pancreas, Brain, and Heart

Rabbit No.	Extract of	Optical density	
		Initial	At peak lipemia (16–24 hrs.)
1	Beef pituitary*	0.019	0.031
2	" "	0.022	0.095
3	" "	0.029	0.188
4	" "	0.020	0.280
5	" "	0.025	0.290
6	" "	0.020	0.454
7	" "	0.022	0.628
8	" "	0.023	1.300
9	Beef liver‡	0.023	0.022
1-0	" "	0.022	0.029
1-1	" "	0.020	0.016
1-2	" "	0.016	0.016
1-3	Beef brain‡	0.035	0.028
1-4	" "	0.052	0.060
1-5	" "	0.057	0.028
1-6	" "	0.022	0.030
1-7	Beef pancreas‡	0.027	0.030
1-8	" "	0.047	0.030
1-9	" "	0.047	0.023
2-0	" "	0.021	0.025
2-1	Beef heart‡	0.037	0.037
2-2	" "	0.040	0.036

* Each rabbit given 15 ml. of extract subcutaneously. Each milliliter of extract equivalent to 10 mg. of lyophilized pituitary gland.

‡ Each rabbit given 15 ml. of extract subcutaneously. Each milliliter of extract equivalent to 30 mg. of lyophilized tissue.

subcutaneously. The animals were bled just prior to injection and at periodic intervals thereafter for 36 to 48 hours. Serum from the animals given pituitary extract began to be visibly lipemic 6 to 12 hours after injection, and reached a peak of lipemia between 16 and 24 hours. The sera then returned to their initial clarity within another 18 to 24 hours. The degree of visible lipemia varied from moderate opalescence in some cases to striking milkiness in others (see Table I). It is of interest that in many similar experiments comprising all told more than 100 rabbits about one-quarter of the animals failed to develop visible lipemia following injection of pituitary extract, and some failed to respond even after four injections. None of the

control animals given either beef liver, pancreas, brain, or heart showed any visible lipemia nor any significant increase in the optical density of the blood serum (Table I).

Similar findings, the development of visible lipemia and a sharp increase in the optical density of the serum, were observed following the injection into rabbits of extracts of hog and human pituitary glands. In a number of experiments serum total lipid and total cholesterol levels were also determined. In virtually all cases there was a three- to eightfold increase in total lipids paralleling the increase in optical density, and a slight to moderate increase in total cholesterol. A typical experiment in which a rabbit was given 15 ml. of an extract of bovine anterior pituitary gland is illustrated in Fig. 1.

Many of the animals appeared to be obviously sick following injection of the pituitary extracts. Within 3 hours after the injection they became weak and lethargic and did not eat or drink. The weakness grew progressively worse, particularly in rabbits developing severe hyperlipemia, to the point where in some cases the paws were splayed out laterally and these animals had considerable difficulty holding their heads up. They were tachypneic and appeared to be in shock, for little or no blood could be obtained even when the central artery of the ear was severed and the few drops that did flow were dark and viscid. As the hyperlipemia waned after 24 to 48 hours, these symptoms gradually subsided; the animals began to eat and drink and gradually recovered completely. With some batches of the pituitary extract, however, as many as one-third of the injected animals sickened and died. Death occurred between 3 and 24 hours after injection, in some cases prior to the onset of hyperlipemia. Postmortem examination failed to reveal any anatomical changes that could account for the symptoms observed or death. No toxic symptoms were observed in the animals given extracts of beef liver, brain, pancreas, or heart, and none died.

It was evident from these experiments that extracts of pituitary glands from beef, swine, and human beings when injected into rabbits brought about a marked increase in blood lipids. Extracts of liver, pancreas, brain, and heart had no such effect. Also, the development of hyperlipemia was followed in a considerable number of instances by a sequence of toxic symptoms and occasionally even by death of the animal for which no satisfactory explanation was readily available. Attention was then directed to the possible pathogenesis of this pituitary-induced hyperlipemia.

Inhibition of Lipoprotein Lipase Activity Following Injection of Pituitary Extracts

The injection of heparin into the circulation results in the appearance of an enzyme in the blood which hydrolyzes the triglycerides of chylomicrons, or those of synthetic lipid emulsions, to glycerol and non-esterified fatty acids (2). This heparin-induced enzyme, referred to as clearing factor or lipoprotein lipase, is thought to be one of the mechanisms responsible normally for the removal of lipids from the blood stream, and there is evidence at hand both in experimental animals and in human beings that interference with this enzyme system results in the development of hyperlipemia (6, 7). Studies were therefore

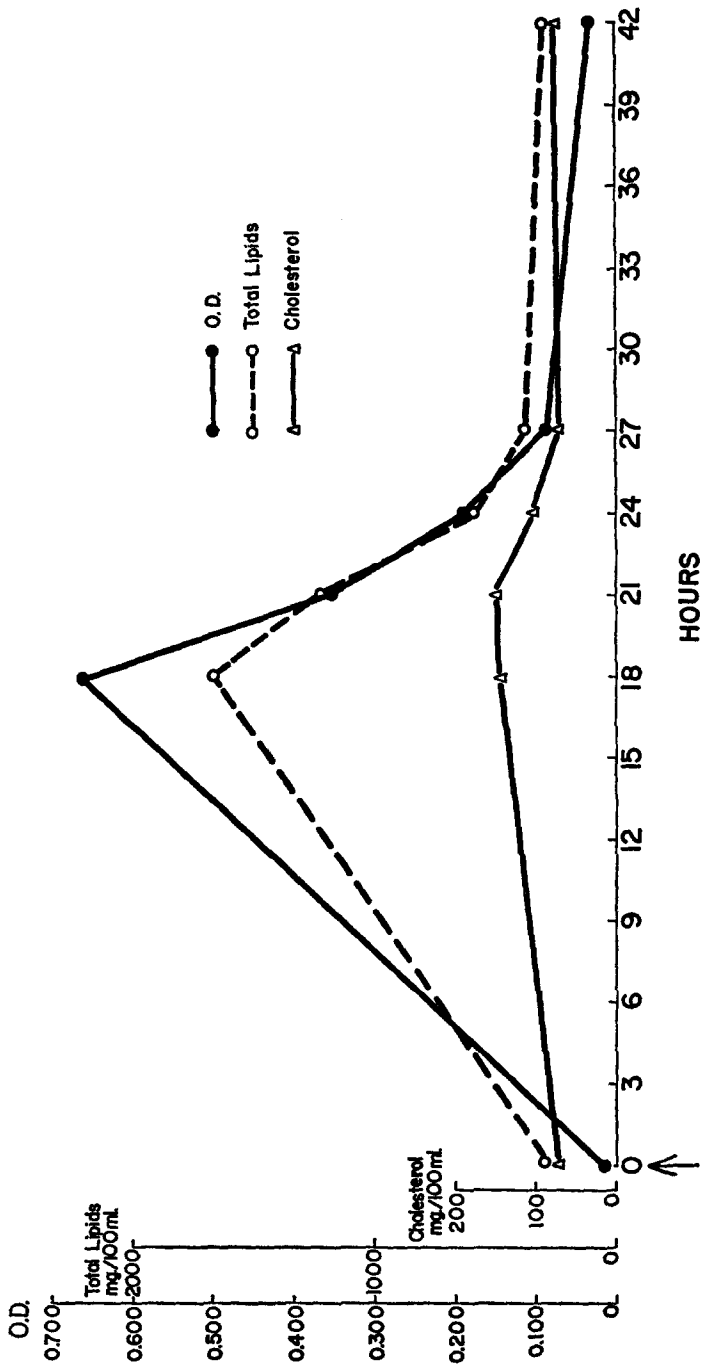

FIG. 1. Changes in serum optical density (O.D.), serum cholesterol, and total lipids following a single subcutaneous injection of bovine pituitary extract into a rabbit.

done to learn what effect, if any, the injection of pituitary extracts had on the heparin-activated lipemia clearing system.

In a series of experiments 16 rabbits were each given a single injection of 15 ml. of a beef pituitary extract subcutaneously. At varying intervals of time thereafter the animals were given heparin intravenously, 7.5 mg. per kilo, and bled 15 minutes later into citrate. The postheparin plasmas obtained were then assayed for clearing activity. The PHP specimens obtained 1 hour after the injection of pituitary extract had no diminution of their clearing activity. Those obtained between 3 hours and 18 hours, however, all showed partial to complete inhibition of clearing, as illustrated in Fig. 2. At 24 and 30 hours, three out of four samples of PHP had normal clearing activity again. In general, the degree of inhibition of clearing activity paralleled the height of the lipemia. Thus, rabbit 30-90 in Fig. 2, which showed partial

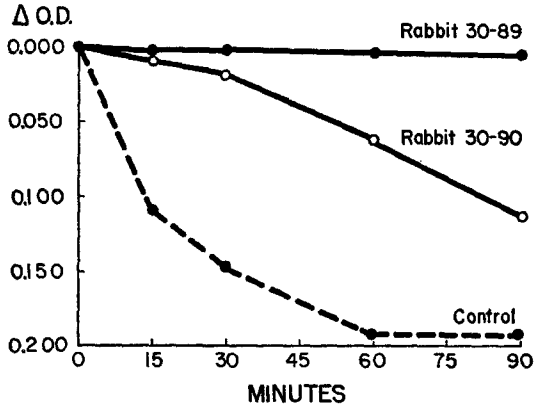

FIG. 2. Inhibition of lipoprotein lipase production following injection of bovine pituitary extract.

Rabbits 30-89 and 30-90—postheparin plasma obtained 12 hours after injection of pituitary extract.

Control–postheparin plasma obtained from a normal rabbit.

inhibition of clearing at 12 hours, had at the time a serum optical density of 0.072 while the serum of rabbit 30-89 with complete inhibition of clearing at 12 hours had an optical density of 0.292. It is of interest that in this and in other experiments the inhibition of lipemia-clearing activity often preceded by several hours the development of visible lipemia. Comparable findings were observed in studies using pituitary extract of swine and human origin.

The end products of the hydrolysis of the triglycerides of chylomicrons by lipoprotein lipase are unesterified fatty acids and glycerol; the reduction in optical density is a secondary manifestation of the resulting decrease in particle size. The possibility was considered that the lipolytic action of the enzyme might proceed normally following the injection of pituitary extract though not reflected in a reduction in optical density. An experiment was therefore done in which the clearing reaction in rabbits given pituitary extracts was measured by glycerol liberation as well as by change in optical density. Control postheparin plasma was obtained from three rabbits and the animals were then each given 15 ml. of beef pituitary extract. Injection of heparin was repeated 12 hours later in the case of two of the rabbits and 18 hours later in the case of the third; PHP was collected and assayed for clearing factor by measuring

changes in optical density and also the amount of glycerol produced. The results of this experiment are illustrated in Table II. The control PHP's obtained just prior to injection of the pituitary extract caused a sharp decline in optical density and at the end of 2 hours produced 41 to 45 micrograms of glycerol. The PHP specimens obtained 12 hours after injection cleared the lipid emulsion poorly and only 11 and 17 micrograms of glycerol, respectively, were liberated. The PHP from the third rabbit, bled at the height of lipemia, 18 hours after injection of pituitary extract, cleared hardly at all and liberated only 6 micrograms of glycerol. Similar results were obtained in other experiments in which the lipolytic activity of the clearing enzyme was measured by release of non-esterified fatty acids. It was plain from these experiments that the production of lipoprotein lipase was inhibited whether the lipolytic action was measured by changes in optical density or by liberation of glycerol or non-esterified fatty acids. To make certain that failure to detect clearing activity was not due to the presence of excessive

TABLE II

Inhibition of Lipoprotein Lipase Activity Following Injection of Beef Pituitary Extracts

Rabbit No.	Time PHP obtained	Clearing activity*	Glycerol produced
			μg.
1	Control	0.250	45
	12 hrs.	0.122	17
2	Control	0.243	44
	12 hrs.	0.156	11
3	Control	0.226	41
	18 hrs.	0.021	6

10 ml. beef pituitary extract given subcutaneously to each of 3 rabbits.

PHP—postheparin plasma obtained 15 minutes after heparin, 7.5 mg. per kilo intravenously. Heparin given and PHP obtained twice from each rabbit; once just prior to injection of pituitary extract (control) and again 12 or 18 hours later.

The clearing system contained 0.4 ml. PHP, 0.1 ml. ediol 1-100, and 0.5 ml. buffer. Optical density and glycerol production determined at 0 and at 2 hours.

* Clearing activity measured by reduction in optical density.

amounts of free fatty acids connected with the developing hyperlipemia, amounts of free fatty acid equivalent to those present in the lipemic serum were added to PHP from a normal rabbit, and only slight impairment of clearing activity resulted. In a further control experiment, bovine serum albumin was added to the PHP from lipemic rabbits in order to provide additional fatty acid acceptors, and no enhancement of clearing was observed.

Control Studies With Extracts of Beef Liver, Brain, Pancreas, and Heart.—Extracts of beef liver, brain, pancreas, and heart in doses three to five times as great as those of pituitary extract were injected subcutaneously into groups of four rabbits each. The animals were given heparin intravenously at intervals of 3 to 24 hours after injection and the PHP assayed for clearing activity. No inhibition of clearing was demonstrable in any of these studies.

These experiments demonstrated that the injection of pituitary extracts into rabbits interfered in some manner with the ability of the animals to elaborate active clearing factor in response to the intravenous injection of heparin.

Moreover, the impairment of the formation of clearing factor appeared to be associated with the development of visible lipemia, and often preceded it. An experiment was next done to learn whether serum from rabbits given pituitary extracts could inhibit *in vitro* the clearing activity of postheparin plasma from normal rabbits.

Inhibition of Clearing Activity in Vitro By Lipemic Serum from Rabbits Given Pituitary Extracts.—Lipemic serum from a rabbit given pituitary extract 18 hours previously was added in amounts of 0.01, 0.02, 0.05, and 0.1 ml., respectively, to clearing mixtures containing 0.4 ml. PHP from a normal rabbit and 0.1 ml. of diluted ediol. The total volume of each clearing mixture was brought to 1.0 ml. with buffer solution. The control clearing mixture contained 0.1 ml. of normal rabbit serum but no lipemic serum. The results of this ex-

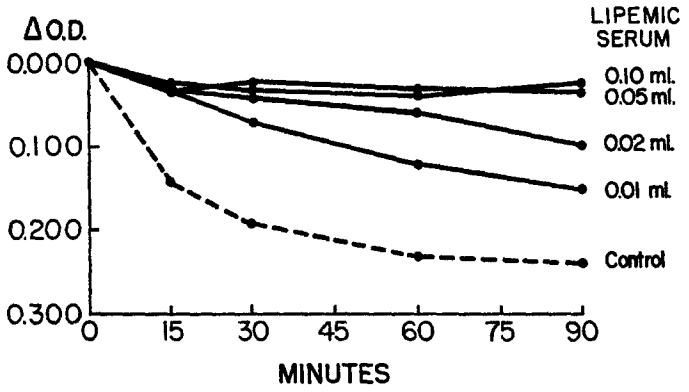

FIG. 3. Inhibition of clearing activity of postheparin plasma from a normal rabbit by means of lipemic serum obtained from a rabbit given pituitary extract.

periment, shown in Fig. 3, demonstrate that clearing was almost completely inhibited by 0.05 and 0.1 ml. of lipemic serum, and significantly though only partially inhibited by as little as 0.01 ml. of lipemic serum. In further studies the development of inhibitor to lipoprotein lipase in the serum of injected rabbits was found to parallel closely the changes in the turbidity of the serum (see Fig. 4). In other experiments it was found that the lipemia clearing activity of postheparin plasma from human beings was also readily inhibited *in vitro* by small amounts of lipemic serum obtained from rabbits given pituitary extracts.

Failure of Beef Pituitary Extract to Inhibit the Clearing Reaction in Vitro.—An extract of beef pituitary glands, previously shown to be capable of inducing lipemia and inhibition of clearing activity when injected into rabbits, was added *in vitro* in varying amounts to a clearing system containing postheparin plasma from a normal rabbit. As shown in Table III, the

pituitary extract itself in amounts up to 0.1 ml. failed to inhibit clearing. This indicated strongly that inhibition of clearing was not due to something contained in the pituitary extract itself but rather to something produced *in vivo* following injection of the extract.

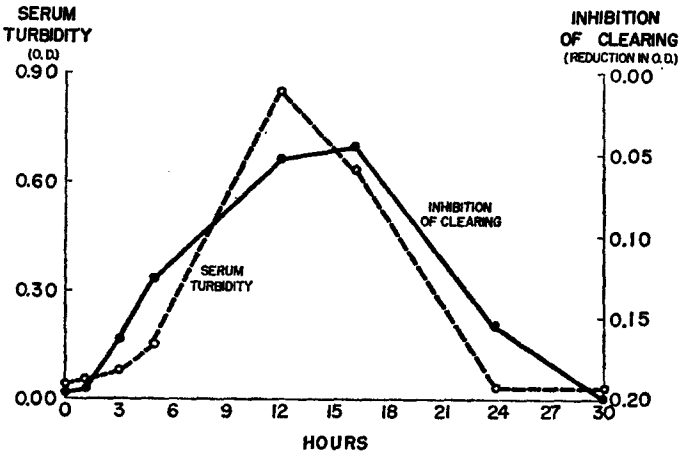

FIG. 4. Changes in turbidity and the development of an inhibitor to clearing in the serum of a rabbit given pituitary extract. Inhibition of clearing was measured by adding 0.2 ml. of serum from the test animal to a mixture containing 0.4 ml. of postheparin plasma from a normal rabbit, 0.1 ml. of ediol, and 0.3 ml. of buffer.

TABLE III

Failure of Beef Pituitary Extracts to Inhibit Lipoprotein Lipase Activity in Vitro

Time	Optical density				
	Beef pituitary extract				
	0	0.04 ml.	0.06 ml.	0.08 ml.	0.1 ml.
min.					
0	0.240	0.219	0.230	0.229	0.245
15	0.085	0.063	0.080	0.085	0.133
30	0.043	0.041	0.050	0.045	0.064
60	0.030	0.030	0.040	0.039	0.047
90	0.030	0.027	0.039	0.033	0.032

Each clearing mixture contained 0.4 ml. postheparin plasma from a normal rabbit, 0.1 ml. ediol 1–100, the stated amount of beef pituitary extract, and buffer to bring the volume to 1.0 ml.

Taken together, these findings indicate that an inhibitor of lipoprotein lipase activity appears in the blood of rabbits a few hours after the injection of pituitary extracts. This inhibitor prevents the elaboration of active clearing factor when heparin is injected into the animal and also suppresses *in vitro* the activity of clearing enzyme obtained from normal animals or human beings.

Failure of Lipemic Serum to Inhibit the Anticoagulant Effect of Heparin.—Lipoprotein lipase is thought to contain heparin as an integral part of its structure, and agents such as protamine sulfate or heparinase which antagonize or destroy heparin also abolish the lipolytic action of the enzyme (8). In view of this, the possibility was considered that the inhibition of lipoprotein lipase activity herein described could be due to the presence of a heparin antagonist in the serum of rabbits given pituitary extracts. To explore this possibility further, a number of experiments were done in which lipemic serums capable of inhibiting completely the lipolytic action of clearing factor were assayed for their effect on the anticoagulant action of heparin.

TABLE IV

Recalcification Time of Dilutions of Heparin Incubated with Normal Rabbit Serum and with Lipemic Serum from a Rabbit Given Pituitary Extract

Dilution of heparin	Recalcification time (*min.:sec.*)	
	Normal serum	Lipemic serum
1–100	30:00	30:00
1–200	30:00	30:00
1–300	9:31	9:02
1–400	7:16	10:39
1–500	4:06	7:27
1–750	3:26	4:13
1–1000	2:35	2:57

0.1 ml. of normal serum or lipemic serum incubated with 0.1 ml. of each dilution of heparin for 15 minutes at 37°C. 0.4 ml. of normal citrated rabbit plasma and 0.25 ml. of 0.05 M $CaCl_2$ added to each tube for determination of recalcification time.

In these experiments, 0.1 ml. of lipemic serum from a rabbit given pituitary extract was incubated for 15 minutes at 37°C. with 0.1 ml. of each of a series of dilutions of heparin from 1:100 to 1:1000. A parallel control series contained 0.1 ml. of normal rabbit serum incubated with the heparin dilutions. 0.4 ml. of normal citrated rabbit plasma and 0.25 ml. of 0.05 M $CaCl_2$ were added to each tube and the recalcification time determined. The data from several such experiments, one of which is illustrated in Table IV, demonstrated no significant difference between the recalcification times of heparin dilutions incubated with normal serum and those incubated with lipemic serum.

It was evident from these findings that the serum of rabbits given pituitary extracts did not alter the anticoagulant effect of heparin *in vitro*, indicating that the ability of such sera to inhibit lipoprotein lipase was not due to the presence of heparin antagonists.

Characterization of the Inhibitor.—Preliminary studies of the inhibitor detected in the serum of rabbits given pituitary extracts revealed it to have the following characteristics. Dialysis against running tap water overnight had no

effect on its ability to inhibit lipoprotein lipase. Its potency was unimpaired by storage at 4°C. for 3 weeks or heating at 70°C. for 30 minutes. It was resistant to enzymatic degradation by trypsin, papain, and pepsin. Absorption with barium sulfate did not diminish the inhibitory activity. After centrifugation of lipemic serum at 30,000 R.P.M. for 1 hour, virtually all the inhibitor was present in the supernatant fat layer. Extraction of lipemic serum with chloroform or petroleum ether failed to remove the inhibitor. Taken together, these findings point to the inhibitor as being a stable, large molecular substance of relatively low density, probably a lipoprotein.

DISCUSSION

The findings herein described confirm fully the observation made by Rudman and Seidman that the injection of crude pituitary extracts into rabbits leads to the development of a striking hyperlipemia (1). Subsequent studies by these investigators have indicated that the active material in the extracts is a protein separate from the known hormones of the anterior pituitary and probably represents a new pituitary hormone (9).

The initial reports of Rudman and Seidman make no mention of toxic symptoms in their animals following injection of the pituitary extracts. In the experiments reported in this paper approximately one-third of the animals sickened within a few hours after injection and not a few died. Postmortem examinations failed to reveal any lesions that could explain the sickness or death of these animals, and no evidences of sickness and no deaths were observed in numerous control animals given injections of extracts made from tissues other than the pituitary. A possible explanation for these toxic sequelae was the lowering of tissue oxygen tension attendant upon the rapidly developing lipemia, as has been shown recently by Joyner *et al.* (10). However, some of the animals sickened and died prior to the onset of lipemia, and in other studies in which rabbits were given triton intravenously, comparable lipemia ensued without evidence of toxicity (11). A more likely explanation is an interference with the ability of the animals to utilize fatty acids and glucose for energy purposes following the injection of pituitary extracts. In this connection, it is of interest that a sharp increase in the plasma non-esterified fatty acids followed regularly within 1 hour after the injection of pituitary extract into rabbits; the fatty acids reached peak levels, often 10 to 20 times the baseline, at 3 to 5 hours, and returned to the normal range usually within 12 hours (12). A block in the utilization of fatty acids at the cellular level may perhaps be the initial event mediated by the pituitary extracts, resulting in the rapid accumulation of non-esterified fatty acids in the plasma and the eventual development of lactescence and hyperlipemia. In many animals there was also a concomitant increase in blood glucose, the hyperglycemia occasionally persisting for as long as 48 to 72 hours. Furthermore, studies on the metabolism of isolated

tissues removed from animals given various pituitary preparations have demonstrated a significant reduction in glucose uptake and impairment of fatty acid catabolism (13, 14). Since fatty acids and glucose represent the major if not the sole sources of energy available to the animal, the interposition of a metabolic block hampering their conversion into energy could result in profound weakness, shock, and even death.

It is clear from these studies that the pituitary extracts employed contained an agent or agents, the nature of which is still undetermined, which upon injection into the animal body caused the liberation of a substance that inhibits the enzymatic action of lipoprotein lipase. An inhibitor of the clearing system has been previously described by Seifter and Baeder (15) in the serum of rats after experimental induction of the nephrotic syndrome and after cortisone injections; the inhibitor in those cases, however, was dialyzable and that here reported is not. An inhibitor has also been isolated from normal dog serum by Hollett and Meng (16) and found to be a glycoprotein. The inhibitor found in rabbit serum following injection of pituitary extracts appears to be a lipoprotein. Moreover, extraction of such rabbit serum known to contain inhibitor of lipoprotein lipase using the procedure described by Hollett and Meng failed to yield any inhibitor.

There appeared to be a very close relationship between the development of hyperlipemia and the appearance of inhibitor to lipoprotein lipase. In every instance in which hyperlipemia followed injection of pituitary extracts, the inhibitor could also be readily demonstrated in the serum. Conversely, in those animals in which lipemia did not ensue, little or no inhibitor was present in the serum. The appearance and disappearance of visible lipemia paralleled closely the rise and fall of inhibitor activity, and those cases with the most striking lipemia generally also had the highest titer of inhibitor. The fact that in some instances the inhibitor appeared prior to the development of hyperlipemia suggested that the inhibitor might be responsible for the increase in serum lipids. A causal connection between inhibition of lipoprotein lipase activity and the development of lipemia has been shown by Bragdon and Havel (6) in rats given protamine and has also been reported in some cases of essential hyperlipemia in human beings (7). However, the fact that the inhibitor did not regularly appear before the onset of visible lipemia makes the causal relationship between the two uncertain for the present. Also, the question as to whether both phenomena result from the same or from different factors present in the pituitary extracts cannot be decided until further purification of the extracts has been accomplished.

SUMMARY

Rabbits given a single subcutaneous injection of an alkaline extract of hog, bovine, or human anterior pituitary glands developed marked hyperlipemia

within 12 to 24 hours. The injections in some instances were followed by sickening and death of the animal, though no anatomical changes responsible for these consequences could be determined. No such sequelae were observed in animals given much larger injections of comparable extracts made from other tissues.

An inhibitor to lipoprotein lipase appeared regularly in the serum of the injected animals in association with the hyperlipemia. The injection of heparin into such animals failed to result in the elaboration of clearing factor, and serum from these animals inhibited *in vitro* the hydrolysis of lipid emulsions by active lipoprotein lipase obtained from normal rabbits or human beings. The inhibitor was produced only *in vivo* by the pituitary extracts. It did not antagonize the anticoagulant action of heparin, and is probably a lipoprotein.

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