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Received May 21, 1958. P.S.E.B.M., 1958, v99.

Lipemia in the Rabbit Following Injection of Pituitary Extract.* (24276)

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It has been recognized that the pituitary gland plays a role in regulation of lipid metabolism. Injection of pituitary substances into experimental animals has been reported to produce the following changes: (A) Decrease in fat content of carcass and adipose tissue, and increase in fat content of liver(1, 2); (B) Increase of ketone bodies in blood and urine(2,3,4); (C) Depression of respiratory quotient(5); (D) Increase in plasma content of unesterified fatty acids(6). These effects appear to result from mobilization of lipid from fat depots, with subsequent deposition of fat in the liver, and increased oxidation of fatty acids associated with accumulation of ketone bodies(2,7,8). Increase in liver fat content has been observed following injection of anterior pituitary extract, and of various anterior pituitary fractions, including adrenotropic, growth, and thyrotropic preparations (9,10). Increase in plasma unesterified fatty acids has been observed after injection of growth hormone(6). A further effect of pituitary material upon circulating lipids has been described. Evans(11), Munoz(12), Baumann(13), and Campbell(14) observed lipemia in dogs and rabbits made diabetic by repeated injections of anterior pituitary extract or of purified growth hormone. Greenbaum(15) described a transitory increase in plasma triglyceride of rats receiving daily injections of growth hormone. Seifter(16) reported that a dialyzable material obtained from posterior lobe of hog pituitary is inhibitor of post-heparin plasma clearing factor and causes an increase in plasma lipids of animals with liver damage, or of animals fasted for 20 hours. The present study was undertaken to determine the effect of injections of crude pituitary extract and of various purified pituitary fractions, upon serum lipid pattern of the rabbit.

Methods. Male rabbits weighing 2.5 to 4 kg were used. They were fed Purina rabbit chow with the exception that in experiments where blood sugar determinations were made

* This investigation was supported by research grants from N. Y. Heart Assn. and Nat. Heart Inst., N.I.H.

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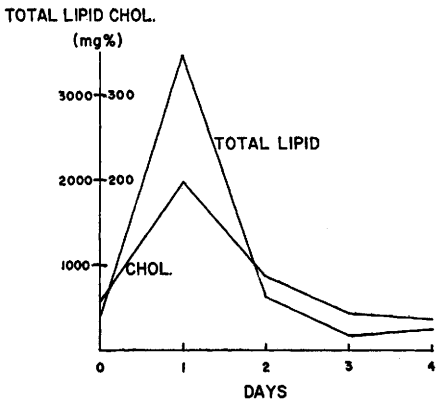

FIG. 1. Time curve of lipemic response following single subcut. inj. of extract of 100 mg lyophilized whole hog pituitary gland.

the food was withheld 3 hours before bleeding. Analytical methods are described in the following references: serum cholesterol(17), serum total lipid(18), blood sugar(19), plasma unesterified fatty acids(20), paper electrophoresis of serum(21). Samples of lyophilized or acetone-desiccated whole pituitary gland were pulverized in mortar, then extracted for 5 hours at 5°C with M/10 Na_2HPO_4 (125 ml for 1 g of pituitary powder). The suspension was clarified by centrifugation and filtration. Approximately 40% of the pituitary material is extracted by alkaline solution.‡

Results. A single subcutaneous injection of the extract from 100 mg of lyophilized whole hog pituitary caused gross lipemia in 16 of 20 rabbits. The increase in serum cholesterol and total lipid values was apparent 6 hours after injection. It reached a maximum in 18 hours and usually disappeared within 48 hours (Fig. 1). Electrophoretic study of serum in 5 rabbits following a single subcutaneous injection of lyophilized hog pituitary extract showed that most of the newly appearing serum lipid had little or no mobility and thus consisted largely of chylomicra (Fig. 2). In 5 other rabbits which received a daily injection of pituitary extract for 3 days, the lipemia was maintained during entire period and on the 4th day electrophore-

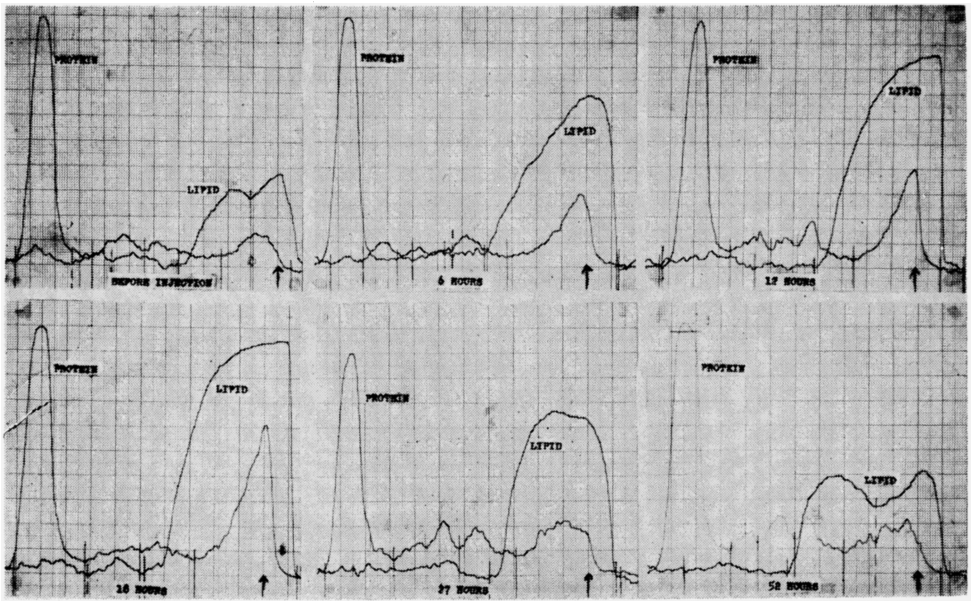

FIG. 2. Electrophoretic patterns of sera obtained during lipemic response following single subcut. inj. of extract of 100 mg of lyophilized whole hog pituitary gland. Serum was obtained before inj., and 6, 12, 18, 27 and 52 hr after inj. Arrows indicate origin in each tracing.

‡ Recent experiments show that more efficient extraction is obtained with 0.1N NaOH for 15 minutes at 5°C.

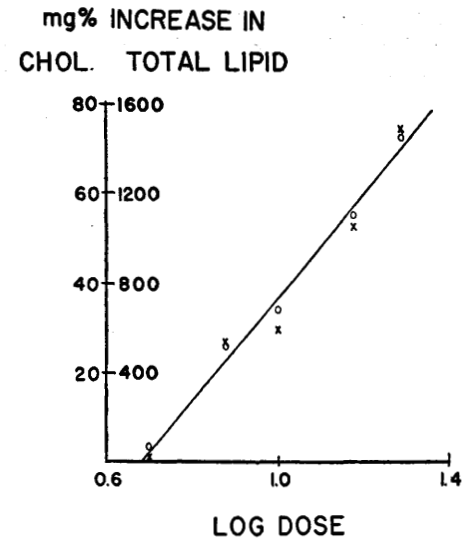

FIG. 3. Relationship of response to dose. Vertical axis: increase in total lipid and cholesterol values of serum 18 hr after single subcut. inj. of extract of lyophilized whole hog pituitary gland. Horizontal axis: logarithm of dose (mg gland/kg body wt). Each point represents avg increase in serum total lipid or serum cholesterol of 6 rabbits. Crosses represent increase in total lipid. Open circles represent increase in cholesterol.

sis showed a prominent beta-lipoprotein peak in addition to the chylomicron peak. A change in alpha lipoprotein was not observed. An additional finding in 3 of 5 rabbits during the first 24 hours after injection, was an increase in a protein component which had no electrophoretic mobility (Fig. 2). This may represent gamma-globulin, or a protein associated with the chylomicra. A 2 to 3 fold increase in serum unesterified fatty acids was found in 4 rabbits whose unesterified fatty acids were determined before, and 18 hours after, a single injection of hog pituitary extract (Table I). Blood sugar levels were determined in 20 rabbits during control and lipemic periods and showed no significant change. In 5 of these rabbits, in which intravenous glucose tolerance tests (0.5 g glucose/kg body weight) were done during control and lipemic periods, there was no detectable change in glucose tolerance curve during the lipemic period.

Dose-response relationship was studied in 5 groups of 6 rabbits which received doses of lyophilized whole hog pituitary extract representing 5, 7.5, 10, 15 and 20 mg of gland/kg body weight respectively. The response, R, (increase in serum cholesterol or serum total lipid 18 hours after a single injection), showed a linear relationship to the logarithm of dose, D, (mg of pituitary gland/weight of rabbit in kg). This relationship is shown in Fig. 3. The dose-response equations were: $R_{\text{cholesterol}} = 120\ \text{LOG}_{10}\ D - 85$; $R_{\text{total lipid}} = 18\ (120\ \text{LOG}_{10}\ D - 85)$.

Dose-response curves were also obtained with several other pituitary samples. Lipemia-producing activity of lyophilized hog whole pituitary gland, and of lyophilized hog anterior pituitary gland, was 3 to 4 times greater than that of acetone-desiccated preparations of whole hog, sheep or beef pituitary. Frozen human pituitary glands obtained at autopsy, through the courtesy of Dr. Sigmund L. Wilens, had an activity comparable to that of lyophilized whole hog pituitary glands. Dose-response curves obtained with samples of lyophilized anterior and posterior hog pituitary lobes indicate that the activity of a single anterior lobe is about 7 times greater than that of a single posterior lobe.

Crude anterior pituitary extract is known to contain 6 fractions with distinct hormonal activities. The lipemia-producing activity of

TABLE I. Effect of Hog Pituitary Gland Extract upon Serum Unesterified Fatty Acids. Each rabbit received a single injection of extract representing 60 mg of lyophilized whole hog pituitary.

	Unesterified fatty acids (μeq/l)		Serum cholesterol (mg %)		Serum total lipid (mg %)	
Rabbit	Before inj.	18 hr after inj.	Before inj.	18 hr after inj.	Before inj.	18 hr after inj.
CAL.	190	630	56	162	400	2080
RA.	220	600	55	116	380	2220
K.	180	670	62	152	410	2180
ILL.	150	390	66	128	440	1180

TABLE II. Effect of Crude Pituitary Extract and of Various Anterior Pituitary Fractions upon Serum Lipids of Rabbit. Serum total lipid was measured before, and 18 hours after, a single subcutaneous injection of each preparation.

Preparation	No. of rabbits	Dosage	Change in serum total lipid (mg %)
Oxycel ACTH (Armour R216-170A)	3	120 I.U. (4.8 mg)	−10‡ (+20, +220, −265)§
$a_1 + a_2$ corticotropin (Lederle S1079-31)	5	270 " (3 ")	0 (+110, 0, +10, −50, −80)
$\gamma_1 + \gamma_2$ corticotropin (Lederle S1079-45)	4	270 " (3 ")	+10 (+40, +130, −120, −20)
Growth hormone (Armour R50109)	5	12.5 mg	+10 (−120, +160, −30, +110, −70)
Idem	4	25 "	+250 (+110, +60, +30, +790)
Lactogenic hormone (Armour R10109)	4	12.5 "	+30 (+50, +160, +10, −120)
Thyrotropic hormone (Armour S3112)	3	10 I.U.	+10 (+100, −80, +15)
Follicle-stimulating hormone (Armour 19911)	4	25 mg	+30 (+20, +110, −60, +30)
Luteinizing hormone (Armour S10407)	4	12.5 "	+40 (+80, −50, +115, +10)
Lyophilized whole hog pituitary extr.	6	9 " *	+540 (+570, +380, +1180, +1150, −30, −40)
Idem	5	24 " †	+1450 (+1680, +1810, +740, +1790, +1250)

* Represents soluble pituitary material extracted from 23 mg of lyophilized pituitary gland.
† *Idem* 60 mg
‡ Avg serum total lipid change of group.
§ Total lipid change of each rabbit in group.

these purified anterior pituitary fractions was compared with activity of crude extract (Table II). The extract derived from 60 mg of lyophilized whole hog pituitary gland contains 24 mg of soluble pituitary material; a single injection of this amount of crude extract consistently produces lipemia in the rabbit (Table II). Each of the 6 purified anterior pituitary fractions was tested at a dosage level which is 5 or more times greater than the amount of hormone which is contained in 60 mg of whole hog pituitary gland. Follicle-stimulating, luteinizing, lactogenic, and thyrotropic hormones had no effect upon rabbits' serum lipids at corresponding dosage of 10 mg, 12.5 mg, 12.5 mg, and 10 International Units. Growth hormone had no effect at dose of 12.5 mg, but at dose of 25 mg caused a significant increase in serum total lipid content in 1 of 4 rabbits. Oxycel-purified ACTH had no effect in a dosage of 120 I.U. "Peptide" ACTH preparations, furnished by Dr. Paul H. Bell of Lederle Laboratories, had no detectable effect upon the rabbits' serum lipids in dosages containing 270 I.U. of ACTH activity.

The lipemia-producing material in the extract of lyophilized whole hog pituitary gland showed these properties: (1) Activity was unchanged when the extract was stored 1 week at −15°C. (2) Activity was destroyed by heating at 100°C for 30 minutes, at pH 4.5 or 7.0. (3) Extracts which had been dialyzed 48 hours against running tap water showed no detectable loss of activity. No activity was present in the dialysate obtained by equilibrating a suspension of 300 mg of powdered gland through viscose tubing against 30 ml M/10 Na_2HPO_4 at 4°C for 24 hours. (4) All activity of the crude extract could be recovered from the precipitate obtained by addition of 2 volumes of saturated ammonium sulphate at pH 6.5. (5) When spun in ultracentrifuge at 40,000 rpm for 20 hours, the active material did not sediment or float at specific gravity 1.22. All activity was concentrated in the lower fraction after ultracentrifugation at 40,000 rpm for 20 hours at specific gravity 1.005. (6) The active ma-

terial was completely adsorbed by calcium phosphate gel at pH 4.0. It was eluted from the gel by M/10 sodium phosphate buffer at pH 10. These properties are consistent with the hypothesis that the active material is a protein.

Discussion. The results indicate that crude extracts of lyophilized whole or anterior pituitary glands, derived from men, hogs, sheep, or cattle, contain a substance which causes lipemia in the rabbit. The relationship of the lipemia effect to increase in liver fat content and to increase in production of ketone bodies, described by other investigators, following injections of pituitary preparations, remains to be studied. All 3 effects may result from release of stored fat from fat depots.

Six different hormones are present in the anterior pituitary gland: *viz.* adrenotropic, growth, lactogenic, thyrotropic, follicle-stimulating, and luteinizing hormones. Purified preparations of these hormones had no significant effect upon serum lipids of the rabbit at dosages which contained at least 5 times the amount of hormone present in a lipemia-producing dose of the crude extract. These findings suggest either that the crude extract of the gland contains a lipemia-producing substance which is distinct from the recognized pituitary hormones, or that 2 or more pituitary hormones act in a synergistic manner upon the rabbit's serum lipids.

Summary. An extract prepared from lyophilized hog anterior pituitary gland produces lipemia in rabbits. A similar effect is produced by whole pituitary glands from men, hogs, sheep, and cattle. Activity of the crude extract of lyophilized glands is considerably greater than can be accounted for by the presence of any of the purified anterior pituitary fractions so far tested. This suggests that the lipemia-producing substance is distinct from the recognized pituitary hormones, or that 2 or more pituitary hormones act in a synergistic manner upon the rabbit's serum lipids.

The authors are grateful for the help by colleagues, A. Myers, H. Kalinsky, and Drs. F. E. Kendall, L. L. Abell, A. R. Wertheim, and S. Bern. Pituitary samples were generously provided by Dr. C. E. Graham (Wilson Labs.), Dr. D. A. McGinty (Parke-Davis and Co.), E. G. King and I. Bunding (Armour Labs.), Dr. G. A. McVicar (Univ. of Toronto) and Dr. S. L. Wilens (N. Y. Univ.). Dr. P. H. Bell (Lederle Labs.) made available samples of α- and γ-corticotropin.

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