compares satisfactorily with published data in other small mammals. Inulin clearance is independent of plasma concentration over a wide range of U/P ratios and independent of urine flow except at very low flow rates, when it is depressed. 2. A ratio of endogenous creatinine to inulin clearances of 0.95 indicates that in the guinea pig, endogenous creatinine clearance is a measure of glomerular filtration rate. 3. The average ratio of urea to inulin clearance obtained here (0.61) agrees well with the established figure for most mammals. 4. Inulin and urea clearances were consistently reduced by prior administration of creatinine. The mechanism of this reduction is beyond the scope of this study. 5. The clearance of exogenous creatinine is greater than that of endogenous creatinine. This, with the foregoing, suggests that in this species there is a tubular component in excretion of exogenous creatinine.

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Corticotropin-Induced Hyperlipemia in Rabbits.* (27760)

KENNETH R. WOODS, ELIZABETH B. FREEMAN, AND AARON KELLNER
Department of Pathology, The New York Hospital-Cornell Medical Center, New York City

The injection into rabbits of crude pituitary extracts or of certain peptides derived from pituitary glands results in a prompt increase in circulating non-esterified fatty acids (NEFA) and in development of hyperlipemia (1-5). Corticotropin, and to a lesser extent several others of the presently recognized pituitary hormones, have been shown to stimulate release of NEFA from rat adipose tissue when incubated *in vitro,* but demonstrations of corresponding *in vivo* effects of ACTH have hitherto not been conclusive(6). In the present experiments, purified corticotropin A_1 was administered to rabbits by repeated subcutaneous injection or by intravenous infusion. There ensued the following sequence of events: prompt and striking increases in circulating NEFA, increase in lipid content of the liver and kidneys, hyperlipemia, and appearance in the plasma of an inhibitor to lipoprotein lipase activity.

Materials and methods. Corticotropin A_1 was purified by chromatography on IRC 50 according to the procedure of Li(7). The chromatographically homogenous and well resolved effluent peak corresponding to A_1 was

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combined and neutralized with HCl. Commercially available ACTH (Armour) was employed in some experiments, and in others, a highly purified preparation of corticotropin A_1 separated by IRC 50 chromatography, electrophoretically homogeneous in starch gel, and possessing an activity of 120 units/mg was kindly provided by Dr. Arthur I. Cohen of the Boston Dispensary. The effects on lipid metabolism, to be described herein, were essentially the same with all 3 preparations. Aliquots were either injected subcutaneously or infused *via* an indwelling plastic catheter into the jugular vein or into a marginal ear vein by means of a constant rate infusion pump** at rates of .02-.08 ml/min. Blood was collected in heparin from the marginal ear vein or from a jugular vein catheter at various intervals and was promptly centrifuged in the cold to separate the plasma. NEFA content was determined by the method of Dole and Meinertz(8). The degree of lipemia was assessed by photometric absorbance of the plasma at 700 mμ. Total lipids were determined gravimetrically in plasma by hot alcohol-ether extraction(9), and in liver and kidney by chloroform-methanol extraction(10).

Market-bought rabbits of mixed breeds and both sexes varying in weight from 2 to 5 kg were used. During the period of infusion the rabbits were kept in a close-fitting wire and sheet metal housing.† Neither their heads nor limbs were restrained, but movement was somewhat limited. Rabbits receiving saline for prolonged periods under the same circumstances exhibited no significant modification of plasma NEFA levels. *Pituitary adipotropic peptides.* A fraction of hog anterior pituitary glands was prepared from the acetic acid extract of defatted glands after oxycel extraction of ACTH.‡ This fluid, usually discarded during production of ACTH, was neutralized and the resultant dense precipitate was removed by centrifugation. The supernate consisted of at least 12 electrophoretically separable components,§ 2 of which demonstrated lipid mobilizing properties when incubated with rabbit or rat adipose tissue *in vitro*. Further purification, to be described elsewhere, has yielded peptides similar in purity and fat-mobilizing activity to Astwood's pituitary peptides I and II(4) and Rudman's pituitary fraction H(3). Because of their common source, lack of established hormonal activities,‖ and comparable effects on adipose tissue, these peptides are here referred to generically as pituitary adipotropic peptides or PAP.

Results. Single injection. The subcutaneous injection into rabbits of a single dose of a partially purified preparation of PAP containing approximately 10 mg peptide nitrogen and little or no cortcotropin¶ resulted in a prompt and sustained rise in plasma NEFA followed by increasing hyperlipemia (Fig. 1). Fatty infiltration of the liver and kidneys was evident in the gross at postmortem examination. A single subcutaneous injection of large doses of corticotropin A_1 caused sharp and substantial increases in plasma NEFA levels of rabbits, but the response, though intense, was not prolonged. NEFA began to decline within one hour and approached normal values within 4 hours, even after administration of a very large dose (100 units) of the purified corticotropin. Hyperlipemia was not observed, and postmortem examination revealed no apparent fatty change in the liver and kidneys. The transitory NEFA response and the failure to develop hyperlipemia and fatty liver differed markedly from the effects of a single subcutaneous injection of PAP and suggested that ACTH was removed or inactivated at a more rapid rate than was PAP.

**Harvard Apparatus Co., Dover, Mass., Infusion Pump Model 600.

† Acme Metal Products, Inc., Chicago, Ill.

‡ Kindly provided by Dr. Harold Upjohn, The Upjohn Co., Kalamazoo, Mich.

§ Zone electrophoresis in polyacrylamide gel by method of L. Ornstein and B. J. Davis, Cell Research Laboratory, Mt. Sinai Hospital, New York City.

‖ Melanophore expanding activity(11) has been found in association with each of the adipotropic peptides isolated in this laboratory.

¶ Corticotropin assay *in vitro*(12) showed no detectable activity, while assay *in vivo* by measurement of corticosterone release into adrenal vein blood of hypophysectomized rats determined by H_2SO_4 fluorescence(13) was less than 4 mU/mg.

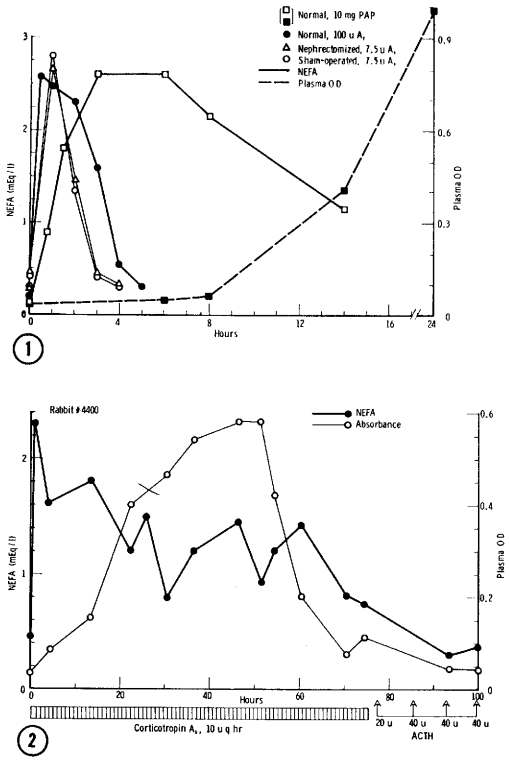

FIG. 1. Plasma NEFA and optical density following a single subcut. inj. of pituitary adipotropic peptides (PAP) or of corticotropin A_1 into normal, nephrectomized, and sham-operated rabbits.

FIG. 2. Plasma NEFA and optical density of a rabbit given multiple injections of corticotropin A_1.

Since the kidney is known to extract a considerable amount of injected ACTH(14), 2 rabbits were subjected to bilateral nephrectomy and then injected with purified corticotropin. Absence of the kidneys failed to alter the extent or duration of the response significantly (Fig. 1). *Repeated injections.* The injection of purified corticotropin A_1 at hourly intervals resulted in a more prolonged though regressive elevation of NEFA levels (Fig. 2). In addition, increasing plasma optical density and a 5-to-6-fold increase in plasma total lipids was apparent within 14 hours. This hyperlipemia continued during the first 50 hours after which it began to decline rather abruptly. After 72 hours, the plasma regained its normal degree of optical clarity and NEFA levels approached normal values despite continued injections. *Continuous infusion.* It is probable that the dosages employed in the foregoing experiments were considerably beyond the rabbits' normal capacity for corticotropin production(15). To approach the physiological levels which might be operative in plasma NEFA control, experiments were next performed in which corticotropin A_1 (assay 120 units/mg) or Armour ACTH (lot #7608C) was slowly infused intravenously into 18 rabbits. Comparable results were obtained with both preparations. Infusion rates of 0.15 milliunit/min/kg or lower produced no significant NEFA response. Infusion of 1 mU/min/kg or more caused prompt increases in plasma NEFA levels and the NEFA increases were prolonged. The plasma became visibly opalescent within 5 hours and assumed a strikingly milky appearance as the infusion continued (Fig. 3); plasma total lipids rose to levels of 3,000 to 4,000 mg per 100 ml. In association with the hyperlipemia, an inhibitor of lipoprotein lipase activity was demonstrable in the plasma similar to that described following the injection of crude pituitary extracts (2). At postmortem examination of animals sacrificed during the development of lipemia, the liver and kidneys were distinctly yellow and Sudan IV stain disclosed increased lipids in the hepatic parenchymal cells and in the tubular epithelium of the kidney. Gravimetric determinations of total fat content showed an increase from a normal of 3.5-4% to 8-12% of the wet weight in the case of the liver, and from 3.5-4% to 5-7% in the case of the kidney.

To determine whether the action of corticotropin is directly on adipose tissue or by way of adrenocortical stimulation, 2 rabbits

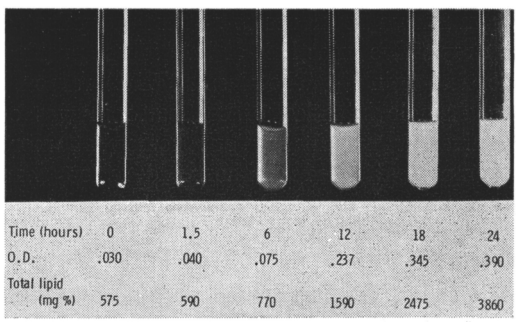

FIG. 3. Plasma of rabbit at intervals during intravenous infusion of corticotropin A_1.

were subjected to bilateral adrenalectomy. They were given 1 mg of the synthetic steroid dexamethasone 21-phosphate postoperatively and drank salt water during 20 hours prior to corticotropin infusion. These animals both exhibited sharp rises in NEFA level upon corticotropin infusion. Though they failed to survive longer than 4 hours, one of them developed a slight but definite hyperlipemia (plasma optical density 0.090). Further evidence that the effects of corticotropin administration on lipid metabolism in rabbits are not mediated by the adrenal was provided by experiments in which dexamethasone was infused intravenously for 24 hours at the rate of 0.5 mg/kg/hr. Animals so treated had no significant elevation of their plasma NEFA levels and did not develop hyperlipemia. Dexamethasone also failed to potentiate the effect of ACTH, for simultaneous infusion of the 2 agents at rates of 0.5 mg/kg/hr and 5 mU/kg/hr, respectively, gave results that did not differ from those observed when ACTH alone was injected. Furthermore, intramuscular injection of 150 mg of cortisone acetate into rabbits did not raise plasma NEFA levels appreciably nor produce hyperlipemia within 24 hours. In other experiments it has been found that rabbit adipose tissue when incubated *in vitro* with exceedingly small amounts of corticotropin A_1 releases NEFA into the medium. Taken together, these studies indicate that corticotropin acts directly on rabbit adipose tissue and affects its metabolism independently of the adrenal cortex.

Discussion. The reported effects on the lipid metabolism of rabbits of a single injection of pituitary peptides, reputedly distinct from known hormones, were herein duplicated by repetitive injections or infusion of purified corticotropin A_1. The plasma NEFA response to a single injection of corticotropin was of equivalent magnitude, but somewhat more prompt and of much shorter duration than that elicited by PAP. Hyperlipemia, however, could not be produced even by a very large single injection of corticotropin, whereas a sufficiently large injection of crude pituitary extract or of PAP can induce hyperlipemia within 10 to 24 hours. On the other hand multiple injections repeated at short intervals or continuous intravenous infusion of corticotropin was just as effective as PAP in prompting increased plasma NEFA and hyperlipemia. These findings indicate that corticotropin has comparable adipotropic activity but, in contrast to PAP, a brief survival *in vivo*. This shorter effective half-life is probably due to a faster rate of destruction or inactivation rather than more rapid renal excretion, since the NEFA rise was not significantly prolonged in nephrectomized animals. It is possible that the adrenals, and perhaps other organs which are known to extract ACTH from plasma, may be relatively indifferent toward the pituitary adipotropic peptides, thus permitting the latter longer survival *in vivo* and hence more protracted biological activity.

Sustained elevation of plasma NEFA levels approaching the maximum plasma albumin transport capacity appears to be followed regularly by fatty infiltration of the liver and kidneys and subsequent discharge of triglycerides into the plasma leading to the development of hyperlipemia. This sequence has recently been observed in the dog in response to epinephrine and norepinephrine infusion (16), and in the rabbit in response to PAP (17). A similar mechanism for the pathogenesis of hyperlipemia appears to be operative in rabbits given corticotropin infusions, as reported herein, and in other experiments, in the duck. It seems likely that prolonged elevation of NEFA may be responsible for some of the hyperlipemias observed clinically in disease states in human beings.

The corticotropin-induced hyperlipemia described here differs from that induced by injection of cortisone in several respects. Hyperlipemia usually appeared after a few hours of corticotropin infusion, whereas quite large injections for several days are required for the cortisone hyperlipemia(18). Furthermore, the hyperlipemia associated with corticotropin subsided despite continued injection, whereas that associated with cortisone persists with continued treatment. The findings that corticotropin caused elevation of plasma NEFA levels in bilaterally adrenalectomized rabbits, that infusion of the synthetic steroid

dexamethasone failed to increase NEFA levels and did not potentiate the lipemia produced by ACTH, and that corticotropin caused release of NEFA from adipose tissue *in vitro,* make it seem likely that the action of corticotropin in producing elevated plasma NEFA and total lipid content is an extra-adrenal one, not mediated by way of the adrenal cortex. This is consistent with the recent demonstration by Boright *et al.* of dissociation between the adrenal and extra-adrenal actions of corticotropin by periodate oxidation of the hormone(19).

Summary and conclusion. Repeated subcutaneous injections or intravenous infusion of corticotropin A_1 in rabbits caused prompt elevation of plasma NEFA levels, accumulation of fat in the liver and kidneys, and hyperlipemia. These responses appear to be evoked by extra-adrenal action of corticotropin A_1.

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Bioassay of Essential Acids. Comparison of Effect of Triglycerides, Methyl and Ethyl Esters. (27761)

H. J. THOMASSON AND J. J. GOTTENBOS (Introduced by E. H. Ahrens, Jr.)
Unilever Research Laboratory, Vlaardingen, The Netherlands

In the bioassay of essential fatty acids (EFA) the fatty acids can be administered to animals (rats) in various forms, *e.g.,* as triglycerides, methyl or ethyl esters.

Preliminary observations have indicated that the response of the EFA-deficient rats is poorer if the fatty acids are in the form of methyl esters instead of triglycerides. In the present investigation the influence of the 3 above-mentioned forms of fatty acids was studied.

Several types of fatty acids were used, *e.g.,* EFA-inactive fatty acids (lauric, myristic and oleic acid) and the active linoleic acid, all in the above-mentioned forms.

Experimental. The EFA bioassay of Thomasson(1) was used. During 5 weeks newly weaned male Wistar rats were fed a fat-free diet *ad libitum*; drinking water was restricted to 14 ml/animal/day. At the end of the preliminary period the mean body weight of the animals was 115 g. The ani-