A Lipolytic Action of Epinephrine and Norepinephrine on Rat Adipose Tissue *in vitro*.* (24355)

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Evidence that epinephrine and the sympathetic nervous system play a role in lipid metabolism and transport has been accumulating for years(1). Recent interest in the possible mechanisms involved has been roused by observations of Gordon and Cherkes(2) and of Dole(3) that injection of epinephrine into human subjects leads to prompt elevation of serum nonesterified fatty acids, suggesting that the hormone may act directly on adipose tissue to accelerate rate of hydrolysis of triglycerides and release of fatty acids. This was confirmed by Gordon and Cherkes(4), and independently by ourselves(5), with the demonstration that incubation of epinephrine with rat epididymal adipose tissue *in vitro* results in release of fatty acids into the incubating medium.

The present report presents data indicating that both epinephrine and norepinephrine stimulate production of nonesterified fatty acids from adipose tissue *in vitro* and that the effect is specific for the l-isomer of each hormone.

Materials and methods. Approximately 50 mg portions of epididymal adipose tissue were removed from 250-350 g Wistar rats which had been fasted overnight and anesthetized by intraperitoneal injection of sodium pentobarbital. The tissue was weighed on a Roller-Smith torsion balance, then incubated in 1 ml of pooled rat plasma at 36°C in a Dubnoff shaking incubator. Heparinized plasma was obtained by exsanguinating male rats *via* the abdominal aorta and used immediately or after refrigeration. A few pilot studies done with serum yielded results comparable with those of heparinized plasma. The gas phase was air or nitrogen. The 10 ml Erlenmeyer flasks used for incubation were left unstoppered. Evaporation was retarded by enclosing the flasks in a humidifying hood. Plasma nonesterified fatty acids were determined on 0.4 ml aliquots of plasma by the method of Dole(3) immediately and 15 minutes or 3 hours after addition of the hormone solution, or an equal volume of distilled water for the controls. Tissue nonesterified fatty acids were determined after rinsing the adipose tissue specimen in distilled water, by macerating them with a glass rod in the same extraction mixture used for plasma, then following the same procedure as in the plasma method. Hormones l-epinephrine bitartrate, d-epinephrine bitartrate, l-norepinephrine bitartrate, and d-norepinephrine chloride were weighed in crystalline form, then brought into solution in distilled water with 0.1 ml of 0.1 N HCl per 100 ml to achieve a pH of 5.0. They are stable in solution for several days if refrigerated. Concentrations of the hormones are expressed in terms of their hydrochloride equivalents per ml of the plasma medium.

Results. When adipose tissue is incubated in rat plasma for 3 hours, no change occurs in fatty acid concentration of the medium (Table I), but fatty acid content of the tissue declines significantly‡ ($p<.01$). In the presence of 5 μg of l-epinephrine, striking increases in fatty acid concentration in both medium and tissue are detectable in 3 hours ($p<.01$). Tissue content of fatty acids increased to 4.5 $\pm$ 0.26 μM/g compared to 2.3 $\pm$ 0.27 μM/g in the control ($p<.01$) after only 15 minutes exposure to 10 μg of l-epine-

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‡ The small but significant difference in tissue content of fatty acids before and after incubation is an artifact of the procedure. It represents the amount of hydrolysis of tissue triglycerides which results from allowing tissue specimens to stand for 3 hours in the undiluted H_2SO_4-isopropyl alcohol-heptane mixture for extracting nonesterified fatty acids. When the initial tissue specimens are diluted promptly after extraction their content of fatty acids is the same as that found in control tissues after incubation. This factor does not influence the significance of the other tissue or plasma differences.

TABLE I. Changes in Nonesterified Fatty Acid Concentrations (Mean ± S.E.) in Medium and Adipose Tissue Induced by l-Epinephrine *In Vitro*.

	Medium			Adipose tissue		
	Initial	15 min.	3 hr	Initial	15 min.	3 hr
			Air			
1. Control	(7) .66 ± .031		(7) .67 ± .039	(6) 2.8 ± .44		(7) 1.2 ± .10
2. l-Epi, 5 μg/ml	(5) .60 ± .046		(5) 1.67 ± .223			(5) 5.8 ± .73
3. Control	(4) .47 ± .040	(4) .51 ± .045			(4) 2.3 ± .27	
4. l-Epi, 10 μg/ml	(4) .45 ± .036	(4) .57 ± .043			(4) 4.5 ± .26	
			Nitrogen			
5. Control	(6) .61 ± .046		(6) .85 ± .062	(6) 2.8 ± .44		(6) 1.4 ± .07
6. l-Epi, 10 μg/ml	(4) .67 ± .014		(4) 1.06 ± .034			(4) 2.0 ± .49

Numbers in parentheses indicate No. of experiments. Nonesterified fatty acids in medium are expressed as μM of fatty acid/ml of medium and in the tissue as μM of fatty acid/g of tissue. Initial values are contrasted with those found after incubating adipose tissue aerobically or in nitrogen for 15 min. or for 3 hr, with and without l-epinephrine.

phrine and before the plasma level had changed appreciably. This latter finding strongly suggests that the triglycerides of the adipose tissue rather than those of the plasma are the source of the new fatty acids which appear in the medium. Unrecorded data indicate that plasma incubated without adipose tissue increases very little in fatty acid content, and this slight rise is not influenced by epinephrine. Preliminary assays of the medium for lipase have disclosed no appreciable release of this enzyme from adipose tissue during incubation, due perhaps to the limited contact of heparin with the capillary network of the tissue *in vitro*. It appears that epinephrine acts in this system by speeding hydrolysis of neutral fat within the tissue at least to the stage of lower glycerides and fatty acids. When incubations are carried out anaerobically, in an atmosphere of nitrogen, fatty acids increase in the medium of the controls more than in air and the effect of epinephrine is suppressed in medium and tissue. Lipolytic action of epinephrine must therefore depend on continuing aerobic metabolism of the tissue. Fig. 1 compares l-epinephrine with its d-isomer in respect to potency in releasing fatty acids from adipose tissue, and with l- and d-norepinephrine. As little as 0.1 μg of l-epinephrine brought about a small but significant release of fatty acids ($p<0.02$). Increasing the dose increased the quantity of fatty acids produced to a maximal response at the 5 μg level. Commercial Adrenalin Chloride® was found to have the same order of lipolytic activity in this system as equimolar concentrations of the l-epinephrine bitartrate. D-epinephrine must be present in a concentration about 100 times that of l-epinephrine for equivalent activity, but the slope of dose response curves is similar for both hormones. L-norepinephrine has about the same potency as l-epinephrine, while d-norepinephrine is about 1/200th as potent as the l-isomer. A number of studies in intact animals have shown the l-isomers of epinephrine and norepinephrine to be from 4 to 60 times as potent

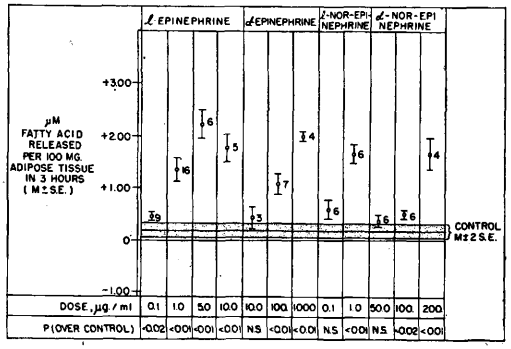

FIG. 1. Influence of epinephrine and norepinephrine on release of nonesterified fatty acids from rat adipose tissue *in vitro*.

as their respective d-isomers(6). The lipolytic process in adipose tissue appears also to be sensitive specifically to the physiological isomers of these hormones.

Discussion. The lipolytic potency of epinephrine found in this study is remarkably similar to that reported earlier by Gordon and Cherkes(4), despite wide differences in conditions of assay. These investigators found that 0.05 μg of epinephrine in 5 ml of Krebs-Ringer phosphate medium containing 5% bovine serum albumin stimulated production of 2.31 μM of fatty acid per gram of adipose tissue per hour, while the control produced 1.31 μM/g/hr. In the present study, 0.07 μg of l-epinephrine in 0.7 ml of rat plasma led to an increase in the medium of 1.50 μM of fatty acid per gram of adipose tissue per hour, while no change occurred in the medium of the control. The studies differ in composition of medium, total amount of adipose tissue per flask, and period of incubation, but the results are quite comparable when allowance is made only for glucose, present in rat plasma but absent from the Krebs-Ringer phosphate medium. Gordon and Cherkes have shown that glucose suppresses production of fatty acids from adipose tissue incubated in Krebs-Ringer phosphate. In this laboratory, the lipolytic effect of epinephrine has been demonstrated in a variety of different media besides plasma, including Krebs-Ringer phosphate, Krebs-Ringer bicarbonate, and saline. Addition of glucose to the medium has been found to have an inhibitory effect on lipolysis in every case (unpublished).

The evidence presented here that norepinephrine is as potent a lipolytic agent *in vitro* as epinephrine and that the lipolytic process in adipose tissue is stimulated preferentially by the physiological isomer of each suggests a role for both hormones in mobilizing fat in the intact animal. The activity of epinephrine in bringing about an increase in serum nonesterified fatty acids and in causing fatty liver *in vivo* is well established(1,2,3). Of special relevance to this study is the recent report of Wadstrom(7) that epinephrine given subcutaneously to rabbits produced within a few minutes an increase in the lower glycerides of adipose tissue, indicating a partial hydrolysis of neutral fat. A few reports are available suggesting comparable activity for norepinephrine. Aujard(8) noted that norepinephrine increased neutral fat content of the liver of rats. Both Havel as well as Estes and Bogdonoff (personal communications) have found that norepinephrine increases plasma levels of nonesterified fatty acids in human subjects and in dogs, though perhaps is less potent in this regard than equivalent doses of epinephrine. Physiologically norepinephrine is in a good position to influence fat metabolism by virtue of its mediation of adrenergic impulses at nerve endplates(6). Beznak and Hasch (9), Hausberger(10), and Clement(11) have shown that interrupting the sympathetic nerve supply to adipose tissue retards locally its loss of fat in rabbits, cats, and rats during fasting and cold exposure. Wertheimer and Shapiro (12) have reported that sympathetic innervation of fat depots must be intact to permit normal depletion of lipid stores which follows certain stresses. These studies, coupled with our demonstration of a lipolytic influence of norepinephrine *in vitro,* support the suggestion that nerve impulses may under certain circumstances act directly to effect mobilization of lipid stores, perhaps by liberating norepinephrine locally at nerve endings within adipose tissue.

Certain other hormones have also been found in this laboratory to induce lipolysis in isolated rat adipose tissue(13). Corticotropin, growth hormone, and thyroid stimulating hormone have this property in common with epinephrine and norepinephrine. The activity of these same hormones in mobilizing fat in the intact rat and mouse(14) strengthens the significance of the *in vitro* observations. Other hormones, such as melanophore stimulating hormone, prolactin, Pitressin®, Pitocin®, serotonin, triiodothyronine, and hydrocortisone, which do not mobilize fat acutely *in vivo,* have been found here not to be lipolytic *in vitro.* A clue to a mechanism for the lipolytic activity of these hormones derives from observation that lipolytic doses of ACTH, epinephrine, and norepinephrine markedly inhibit glucose uptake by isolated adipose tissue, even in the presence of large

doses of insulin (Verner and Engel, unpublished).

Summary. Epinephrine and norepinephrine stimulate production of nonesterified fatty acids from rat adipose tissue when incubated *in vitro* in rat plasma, presumably by stimulating the hydrolysis of neutral fats within the tissue. Incubation under nitrogen markedly inhibits this effect of epinephrine and appears to increase production of fatty acids from control tissues. The d-isomers of epinephrine and norepinephrine are much less active in this system than the physiological l-isomers. indicating a specificity in tissue response. Some implications of this lipolytic activity are discussed.

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On Nonantigenicity of Polysaccharides in Guinea Pigs*† (24356)

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Although antigenicity of polysaccharides has been investigated extensively in rabbits(1) and man (2a, b), there is little in the literature concerning antigenicity of polysaccharides in guinea pigs(3). The availability of good technics for immunization, production of "delayed hypersensitivity," and the detection of antibody prompted a reinvestigation of the problem from several approaches.

Methods. Antigen and antibody. Both pneumococcal polysaccharides SIII and rabbit anti SIII were obtained from Dr. M. Heidelberger. Dextran preparations B512F and 3079 were obtained from Northern Regional Research Laboratory and the Pharmacia Co. respectively. Human antisera against these materials were obtained by immunization of normal, healthy medical students(2b). Five times recrystallized ovalbumin (Ea) was prepared by the method of Kekwick and Cannan (4). Anti Ea sera were obtained from rabbits after a course of intravenous injections of alum precipitated Ea. The antibody content of various sera was determined by employing quantitative precipitin technics developed by Heidelberger and co-workers(5).

Methods. 1. Antigens used for immunization of guinea pigs were prepared several ways: a) Dextran preparation or pneumococcal SIII was mixed in a complete adjuvant mixture containing Arlacel C, Bayol F and dead mycobacteria(6,7). The final concentration of polysaccharide was 20 μg/ml. b) Specific precipitates of either dextran-anti dextran, SIII-anti SIII or Ea-anti Ea were prepared according to procedure employed by Uhr, Salvin and Pappenheimer(8). In this procedure precipitates are formed in antibody

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