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Effect of Adrenergic Blocking Agents on Fatty Acid Mobilization during Fasting.* (25293)

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Recent studies by Dole and by Gordon and their collaborators(1,2) indicate that a major portion of lipide mobilized from peripheral depots is transported in the form of non-esterified fatty acids (NEFA). It has been demonstrated that concentration of plasma NEFA increases during fasting and that venous blood draining areas rich in adipose tissue contains higher concentrations of NEFA than the blood supplying these areas(2). The additional observation that adipose tissue from fasting rats releases more NEFA *in vitro* than identically treated tissue from fed rats (3) leaves little doubt that the increase in plasma NEFA seen during fasting reflects lipide mobilization. A large body of evidence has been adduced to support the view that fat mobilization from adipose tissue during fasting is a consequence of increased sympathetic discharge(4). Although epinephrine and norepinephrine have been shown to be potent mobilizers of NEFA(2,5), we have shown (to be published) that the adrenal medulla does not play a major role in NEFA mobilization of fasting since adrenalectomized rats maintained with adrenal cortical replacement therapy respond to fasting in the normal fashion with an increase in plasma NEFA. The present paper reports experiments designed to assess the effects of sympathetic blockade at the ganglionic and postganglionic levels on NEFA mobilization during fasting.

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Materials and methods. Female rats (Holtzman) weighing approximately 200 g were used in this study. *Blockade of epinephrine induced NEFA mobilization*: Epinephrine was administered subcutaneously as an oil suspension (1 mg in 0.5 ml peanut oil) to 2 groups of rats. One group received in addition 0.5 mg of ergotamine tartrate in a separate subcutaneous injection. A third group received 0.5 ml of the peanut oil vehicle subcutaneously. The animals were deprived of food at time of injection and were killed 3 hours later. *Blockade of NEFA mobilization during fasting*: Ergotamine tartrate (1.5 mg/rat, total dose) and hexamethonium bromide (15 mg of ion per rat, total dose) were administered in 3 equal subcutaneous injections 0, 4, and 8 hours following removal of food. Dibenzyline was injected under light ether anesthesia as a single dose of 0.5 mg into the tail vein of rats at the beginning of the fasting period. In all cases, control animals were injected with an equal volume of saline by the same route and at the same times. The animals were sacrificed 12 hours after initiation of the fast. At the end of the fasting interval, the animals were anesthetized with ether and blood for NEFA analysis was drawn into a heparinized syringe from the abdominal aorta. NEFA analyses were performed in duplicate by the method of Dole(6) on one ml aliquots of plasma.

Results. The NEFA mobilizing action of epinephrine is completely blocked by 0.5 mg of ergotamine (Table I). None of the au-

TABLE I. Effect of Ergotamine on NEFA Mobilizing Action of Epinephrine. Six animals/series.

Treatment	Plasma NEFA, μEq/ml	P
Control	.529 ± .055*	
Epinephrine	.724 ± .047	<.05
" + ergotamine	.572 ± .016	<.45

* Mean ± S.E.

tonomic blocking agents used had any significant blocking effect on NEFA mobilization during fasting (Table II).

Discussion. These experiments suggest that sympathetic discharge does not initiate NEFA mobilization in response to fasting, since pharmacological blockade at either the effector site (ergotamine and Dibenzyline) or the ganglionic level (hexamethonium) failed to prevent NEFA mobilization. The fact that a single injection of ergotamine completely blocks the NEFA mobilizing action of a large dose of epinephrine indicates that adrenergic mobilization of NEFA is susceptible to pharmacological blockade. Wool *et al.*(7) were able to block the accumulation of liver lipide in the ethionine treated rat with 0.5 mg of ergotamine or adrenal demedullation suggesting that the fat mobilizing action of endogenous epinephrine or norepinephrine is also subject to pharmacological blockade. From these results it would appear that ergotamine was used in adequate dosage to effect at least partial blockade of epinephrine and norepinephrine. Judging the adequacy of the dosage of the Dibenzyline is more complex. Harvey *et al.*(8) reported that 15 mg/kg of Dibenzyline brought about a 70% suppression of the hyperglycemic response to an intravenous dose of 1 μg/kg of epinephrine in rabbits. Nickerson *et al.*(9) found that 3 mg/kg of Dibenzyline could block the effects of 5 μg/kg of epinephrine or norepinephrine on the blood pressure of the cat. There is thus a wide disparity in dose of Dibenzyline necessary to block the different parameters of adrenergic activity and perhaps species differences as well. This is further complicated by our findings (unpublished) that large doses of Dibenzyline (15-25 mg/kg) cause an increase rather than a decrease in NEFA concentration in rats during fasting. The dose of hexamethonium used in these experiments is in excess of the dose (2-5 mg/kg) found to exaggerate the hypoglycemic effects of insulin and reported to produce "effective pharmacological sympathectomy" in the dog(10). Nevertheless, any demonstration that depends solely upon pharmacological blockade is subject to criticism on the grounds that effective blockade may not have been achieved.

TABLE II. Effects of Autonomic Blockade on Plasma NEFA at End of a 12-Hour Fast.

Treatment	No. of animals	Plasma NEFA, μEq/ml	P
Non-fasted	27	.286 ± .027*	
Saline†	6	.654 ± .056	
Ergotamine†	6	.604 ± .045	<.6
Hexamethonium†	6	.585 ± .045	<.3
Saline	5	.508 ± .041	
Dibenzyline‡	6	.664 ± .058	<.1
Saline	5	.632 ± .029	
Ergotamine‡	6	.564 ± .052	<.3

* Mean ± S.E.

† Administered in 3 doses at 0, 4, and 8 hr following removal of food.

‡ Administered as single dose of 0.5 mg at time of food removal.

Our findings that the NEFA mobilization of fasting cannot be suppressed by autonomic blockade agree with the recent report by Levy and Ramey(11) that they could not prevent loss of fat from the epididymal fat body of the fasting rat using autonomic blocking drugs. However, the apparent absence of effect of the sympathetic nervous system on NEFA mobilization during fasting is in direct conflict with reports(4,12) that denervated adipose tissue contained more fat than normally innervated contralateral tissue following a fast. From these studies it was inferred that sympathetic nervous stimulation induces fat mobilization. However, Clement and Shaeffer(13) have shown that denervation interferes with the mobilization of lipide induced by injection of a large dose of epinephrine. But, if sympathetic discharge and hence secretion of an epinephrine-like mediator is necessary for mobilization of lipide from adipose tissue, one would expect the response to exogenous epinephrine to be unaffected or perhaps even enhanced by denervation(14). It should be noted that epinephrine and norepinephrine have been shown to mobilize NEFA effectively when added directly to adipose tissue incubated *in vitro* and hence devoid of

nervous connections(3,15). Further experimentation will be necessary to resolve these paradoxical findings.

Summary. Repeated doses of ergotamine and hexamethonium had no effect on increase in plasma non-esterified fatty acid (NEFA) concentration which occurs during fasting. Dibenzyline was similarly without effect on this response. These findings suggest that the stimulus to increased NEFA mobilization during fasting may not be mediated by the adrenergic nervous system.

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Effect of Injection of Rabbit Leucocytes into Neonatal Rabbits on Subsequent Lymph Node Cell Transfer.* (25294)

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If rabbit lymph node cells are incubated with filtrate of trypsin-treated *Shigella paradysenteriae* and transferred to fresh rabbits, agglutinins to *Shigella* appear in sera of recipients in a few days. It has also been shown (1) that if blood leucocytes obtained from prospective donor rabbits are injected into recipients a week before cell transfer, such agglutinins do not appear. Failure of agglutinins to appear indicates that injection of leucocytes before cell transfer has altered the host environment with respect to transferred cells, with the result that these cells become ineffective in production of agglutinins. This suppressive effect on transferred lymph node cells was considered the result of a homograft reaction, as originally described by Medawar (2) in the case of skin grafts. The present study arose from attempts to produce in the lymph node cell transfer system another, essentially opposite, effect of tissue transplantation, that of acquired tolerance. This state was first experimentally induced by Billingham, Brent and Medawar(3) by injection of fetal mice of one inbred strain with tissue cells obtained from another strain of mice; after birth, injected mice showed tolerance to tissues of donor strain by accepting skin homografts from this strain. Similar results were reported by these authors with rabbits injected during fetal life with rabbit spleen cells(4). Later Billingham and Brent(5) established acquired tolerance even in mice injected soon after birth with spleen cells of donor strain. In rabbits these authors reported that intravenous injection of the newborn with homologous thymocytes or spleen cells resulted in induction of weak tolerance, in only a small

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