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Direct Evidence for Fatty Acid Mobilization in Response to Growth Hormone Administration in Rat.* (24914)

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It has long been known that anterior pituitary gland extracts and purified growth hormone preparations, when administered to experimental animals, increase the concentration of lipids in plasma and the liver while decreasing amount of total body fat (see 1 and 2 for review). More recently, it has been demonstrated that small doses of growth hormone cause a rapid increase in the plasma nonesterified fatty acid (NEFA) concentration in man(3), rhesus monkey(4) and rat (5). These effects of growth hormone on levels of circulating lipid have been interpreted in terms of an increase in mobilization of lipids from adipose tissue but direct evidence for such an interpretation has been lacking. The following experiments were performed to establish the effect of prior growth hormone administration on release of NEFA from isolated adipose tissue *in vitro*.

Material and methods. Male rats of the Charles River strain weighing between 100 and 150 g were used. Hypophysectomies were performed 4 weeks prior to experiment. All animals were fasted 24 hours before autopsy. In the "acute" experiments a single intraperitoneal injection of bovine growth hormone† (3 mg) was administered three hours before sacrifice. In the "chronic" experiments bovine growth hormone (3 mg per rat per day) was given intraperitoneally for 3 days and the rats sacrificed on the day following last injection. Control animals were injected with equal volumes of normal saline. All animals were anesthetized with 30 mg sodium pentobarbital given intraperitoneally. The epididymal fat bodies were removed and incubated individually (2 for each animal) in a system previously described(6). NEFA released into the incubating medium was determined by the method of Dole(7) and expressed as μeq. NEFA released per g wet weight of tissue per hour. Blood glucose concentrations were determined by the method of Nelson(8) and plasma NEFA concentrations by the method of Dole(7).

Results. Hypophysectomy reduced release of NEFA from adipose tissue when compared with normal control animals fasted for the same length of time (Table I). Administration of a single 3 mg dose of growth hormone to hypophysectomized animals 3 hours before sacrifice significantly ($P<.01$) increased NEFA release from adipose tissue *in vitro* (Table I). No difference in blood glucose

TABLE I. Effect of Hypophysectomy and Growth Hormone Treatment on NEFA Release by Adipose Tissue. Ten animals/series; 2 observations/animal.

Rats	NEFA release, μeq/g/hr
Normal control	5.79 ± .44‡
Hypox. + saline	.90 ± .16
" + G.H. (acute)*	2.30 ± .37
" + " (chronic)†	3.40 ± .66

* 3 mg/rat 3 hr before sacrifice.
† 3 mg/rat/day for 3 days.
‡ Mean ± S.E.

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concentration between these animals and their saline-injected controls was observed.

Chronic growth hormone administration (3 mg/rat/day for 3 days) to hypophysectomized rats also increased rate of NEFA release ($P<.01$) from their epididymal fat bodies (Table I). Under these circumstances plasma NEFA and blood glucose concentrations were unchanged by the growth hormone treatment.

Discussion. The results of this study clearly indicate that hypophysectomy inhibits and growth hormone stimulates release of NEFA from adipose tissue and validate the conclusion that the increase in plasma NEFA concentration observed following growth hormone administration signifies increased NEFA mobilization from fat stores. The mode of action of growth hormone in this regard, however, is not clear. That growth hormone does not have a direct lipolytic action on adipose tissue has been suggested by the experiments of White and Engel(9) in which growth hormone was added to adipose tissue *in vitro*.

It has been suggested(10) that the regulation of NEFA mobilization is mediated by availability of glucose for cellular oxidation. If the action of growth hormone is explicable on this basis, *i.e.* by inhibiting glucose oxidation, it was not reflected by alterations in blood glucose concentrations as measured in the present experiments.

Summary. The release of nonesterified fatty acids (NEFA) from epididymal fat bodies removed from normal, hypophysectomized and hypophysectomized growth hormone treated rats has been investigated. Hypophysectomy inhibits and growth hormone administration stimulates NEFA release from adipose tissue *in vitro*. These findings are taken as direct evidence for the concept that growth hormone treatment causes NEFA mobilization from fat stores.

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Quantitative Determination of Infectious Units of Measles Virus by Counts of Immunofluorescent Foci.* (24915)

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Enumeration of infectious animal virus units depends either upon determination of dilution endpoint or upon counting foci of virus growth(1). The latter approach, modeled after bacterial colony count technic, has been utilized to count proliferative and necrotic pocks on animal skin and cornea, and on chorioallantois of embryonic chick. It is now widely employed for counting plaques in cultures of animal cells(2). Many viruses that multiply in animal cells do not form plaques. Demonstration and enumeration of

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