of 50 to 100 cm of water pressure were observed. These pressures are of the same magnitude as those obtained with control animals.

Discussion. Although total destruction of lymphoid elements of the appendix was not observed in these studies, suppression of these elements *per se* did not appear to modify appendical secretion significantly. Rather, decrease in secretion appeared to be related more to damage of epithelial elements than of lymphoid follicles.

The goblet cell apparently is not responsible for the large volume of secretion occurring in rabbit appendix. Stimulation of goblet cells was produced in several experiments with pilocarpine and mustard oil as evidenced in mucicarmine stained sections. In none of these animals was there an increased volume of secretion. Irradiation led to increase in numbers of goblet containing cells of surface epithelium and crypt walls, but to decreased rather than increased secretion.

Obliteration of papillae with conversion of crypts into globular cyst-like spaces following closure of crypt orifices suggests that secretion occurs at least in part in these crypts. Since cells of crypt walls appear to be predominately mucous cells, and since these do not appear to contribute significantly to the secretory process, epithelial cells overlying the papillae must be responsible. The role of surface epithelium and subepithelial elements was not clarified in these experiments. However, as it was possible to destroy surface epithelium by silver nitrate and have secretion continue, the epithelial elements of the papillae must contribute significantly to the process.

Summary. 1. Large volumes of fluid produced by vermiform appendix of the rabbit, result from a cellular process requiring oxygen utilization. 2. Suppression of lymphoid elements of the appendical wall produces no significant decrease in secretory volume or pressure. 3. Stimulation of mucous cells is not associated with augmented secretion. 4. Destruction of surface epithelium reduces but does not abolish secretion. It would appear that secretion stems primarily from epithelial elements overlying papillae in crypts.

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ACTH *in vitro* on Release of Nonesterified Fatty Acids from Adipose Tissues of Adrenalectomized Rats. (24866)

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The role of hormones in regulation of fat mobilization is not clear. During fasting plasma nonesterified fatty acids (NEFA) are released from fat depots and are transported to other tissues(1). It has been shown that factors affecting concentration of NEFA in plasma similarly affect their release from adipose tissue incubated *in vitro*(2,3). Addition of ACTH to incubating medium enhances release of NEFA from adipose tissue(4). Injection of this hormone into animals causes an increase in hepatic lipids which is prevented by adrenalectomy(5,6,7,8). Presumably, increased hepatic lipid is due to higher rates of mobilization of fat from adipose tissue(9). Thus, it is possible that the augmented level of liver lipids found in ACTH treated animals is due partially to increased release of NEFA from adipose tissue and transport of these fatty acids to the liver where they are esterified(10). The purpose of this investigation was to determine whether in rats, previous adrenalectomy had an effect on release of NEFA by epididymal adipose tissue, on incu-

TABLE I. Effect of Adrenalectomy on NEFA Release from Epididymal Rat Adipose Tissue.

Animal treatment	No. of animals	μM NEFA released/g adipose tissue/3 hr
None	4	2.86 ± .69*
Adrenalectomy + water	6	.55 ± .20
" + saline	6	.66 ± .06

* Mean ± stand. error of mean.

bation *in vitro*, and the response of this tissue to added ACTH.

Material and methods. Sprague-Dawley, male, albino rats, weighing 150-250 g were adrenalectomized in one stage under ether anesthesia, given 1% sodium chloride or tap water and fed Purina Fox Chow. They were weighed daily. Each group contained 4 to 6 animals. On fifth postoperative day, and after 24 hour fast, the animals were given sodium pentobarbital and decapitated. Epididymal fat bodies were removed, weighed on Roller Smith Torsion balance and incubated at 36°C for 3 hours with shaking in 4 ml of Krebs Ringer phosphate buffer containing 5% bovine albumin(2). Each epididymal fat body served as control for the contralateral tissue to which ACTH was added. ACTH (ACTHAR, 10 units/ml, lot # 10808, Armour Labs) was diluted with Krebs Ringer phosphate buffer and added at concentrations of 25 and 2.5 milliunits/ml of incubation medium. NEFA was measured at beginning and end of incubation period. Analysis was based on modification of extraction procedure described by Dole(11), using isooctane rather than heptane and washing the isooctane with dilute sulfuric acid as described by Gordon(1). Two ml of incubation medium were placed in conical glass stoppered centrifuge tubes containing 8 ml of a mixture of isopropyl alcohol and 3 N sulfuric acid (40:1); 4 ml of water and 10 ml of isooctane were added. Tubes were shaken for one minute, allowed to stand for approximately 15 minutes, then centrifuged at 300 x g for 5 minutes. The top layer was transferred to another glass stoppered centrifuge tube and 9 ml of approximately 0.002 N sulfuric acid was added; tubes were shaken for 30 seconds, centrifuged for 5 minutes at 300 x g and the bottom layer removed. The washing procedure was repeated once with 0.002 N sulfuric acid and twice with distilled water. After final wash, the tubes were centrifuged and 5 ml aliquots were removed from the isooctane fraction. Titration was carried out in 12 ml conical centrifuge tubes as described by Gordon(1). The results are expressed as μM of NEFA released/g adipose tissue (wet weight) during 3 hour incubation. Experiments in which palmitic acid was added to plasma in concentration of 2.65 meq/liter indicated 96-100% recovery. Lactic acid when added to plasma to a concentration of 1 N did not appear in the washed isooctane extract.

Results. Incubation of epididymal adipose tissue from normal rats causes a significant release of NEFA into the incubation medium (Table I). The amount of NEFA released/g of adipose tissue is markedly decreased by adrenalectomy; no significant difference was found between animals maintained on tap water or 1% sodium chloride (Table I). This finding was confirmed in 3 experiments.

Addition of ACTH at concentrations of 25 and 2.5 milliunits/ml of incubation medium caused a highly significant increase in NEFA released from adipose tissue of normal animals. Three times as much NEFA was released into the medium/g of tissue with 25 milliunits as with 2.5 milliunits of ACTH (Table II). Following adrenalectomy, addition of 25 milliunits ACTH elicited about one-half the NEFA released by adipose tissue of normal animals whereas 2.5 milliunits had no significant effect. There was no difference between adrenalectomized animals receiving tap water or saline (Table II) in the release of NEFA at either dose of ACTH.

TABLE II. Effect of ACTH on Release of NEFA from Adipose Tissue of Normal and Adrenalectomized Animals.

Animal treatment	Milliunits ACTH/ml	No. of animals	μM NEFA released/g tissue/3 hr*
None	25	6	13.14 ± .81†
Adrex + water	"	5	5.49 ± .93
" + saline	"	5	6.08 ±1.32
None	2.5	6	3.75 ± .76
Adrex + water	"	6	.41 ± .25
" + saline	"	6	.16 ± .01

* Differences between NEFA release of treated tissues and contralateral controls.

† Mean ± stand. error of mean.

Discussion. It has been suggested that circulating NEFA, in part, is the means by which fatty acids are transported from depots to active tissues in the fasting state(12). Dole's results on NEFA composition(13) and Gordon's studies on arteriovenous differences indicate that the source of circulating NEFA is adipose tissue(1). That starvation, carbohydrate feeding, epinephrine, and insulin influence release of NEFA from adipose tissue *in vitro*(2) in a manner compatible with the effects observed *in vivo*(1,11) support the theory that plasma NEFA, during fasting, originates from adipose tissue. The view that fatty acids released on incubation of adipose tissue result from hydrolysis of tissue esterified fatty acids is based on the finding that the amount released exceeds the initial concentration of NEFA in the tissue. The observation that mono- and diglycerides, hydrolysis products of triglycerides, are increased in adipose tissues following injection of epinephrine (which increases NEFA mobilization) supports the idea that plasma NEFA originates in adipose tissue and are derived from the triglycerides(14).

Our results indicate that hydrolysis of esterified fatty acids present in adipose tissue, occurs to a smaller extent in adrenalectomized animals than in normal animals. During fasting less fat is found in livers of adrenalectomized animals than normal, indicating less mobilization(5). This is what would be expected from the *in vitro* results.

Adrenalectomy in our experiments diminished markedly or abolished the increased release of NEFA from adipose tissue resulting from varying amounts of ACTH. These results are compatible with the finding that adrenalectomy prevented the increase in hepatic lipids in animals given ACTH(5,6,7,8).

White and Engel(4) reported that *in vitro,* minimum concentration of ACTH necessary to demonstrate a lipolytic effect in adipose tissue from normal animals was greater than that required to stimulate adrenal gland *in vitro* or *in vivo.* They suggested that these results would be expected if ACTH specifically stimulated the adrenal gland. On the other hand, our results show a significant lipolytic effect demonstrable with 1/50th the concentration reported by White and Engel. This concentration of ACTH is well within the range reported for *in vitro* effects of ACTH on adrenal tissue(15). Since ACTH causes release of NEFA from adipose tissue in absence of adrenals, in which the hormone is active *in vitro,* it appears that ACTH has an independent lipolytic effect on adipose tissue.

Thus, it is possible that ACTH has a physiological role in regulation of fatty acid mobilization.

Summary. 1. Adrenalectomy decreases release of nonesterified fatty acids (NEFA) from rat epididymal adipose tissue. 2. Addition of small amounts of ACTH *in vitro* to adipose tissue increases release of NEFA. This effect is markedly diminished in adrenalectomized animals. 3. It is suggested that ACTH may have a physiological role in fatty acid mobilization not mediated by adrenal cortical tissue.

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