Fat Mobilization by Purified Corticotropin in the Mouse.*† (25216)

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Purified corticotropin (ACTH) is known to produce acutely fatty liver and ketonemia in rats and mice(1). These signs of accelerated fat catabolism appear not to result from stimulation of the adrenal cortex but to reflect a direct action of ACTH on intermediary fat metabolism of extra adrenal tissues(2). Evidence is available that the similar activity of growth hormone (STH) is not due to mutual contamination but represents another of many effects common to the two hormones (1,2). The suggestion has been made that both ACTH and STH accelerate fat catabolism by stimulating fat mobilization from peripheral depots(3,4). In support of this viewpoint are their lipolytic effects on rat adipose tissue *in vitro*(5). Growth hormone has considerably less and more variable lipolytic activity *in vitro* relative to ACTH however than would be expected if this direct action of each hormone on adipose tissue were the sole source of their more nearly equal ketogenic potencies in intact animals. Data presented here provide further evidence *in vivo* that ACTH and STH do speed the loss of stored lipid from depots of fasted mice and that this action of ACTH is independent of adrenal stimulation.

Materials and methods. Swiss mice weighing 18-21 g were fasted overnight, anesthetized with ether, and one testis, alternately right or left, removed from each through a scrotal incision. The epididymal fat pad was then carefully dissected away, hormones were given intraperitoneally in a volume of 0.1 ml, and the mouse allowed to recover from anesthesia. It was maintained on tap water for 6 hours, then reanesthetized and the second fat pad and liver removed. Total lipids were determined on fat pads and liver by the method of Handler(6). The difference in total extractable lipids between the two pads was taken as a measure of fat mobilization, expressed in Table I both in mg and as per cent of total lipids in the initial pad. One group of mice was adrenalectomized through dorsal incisions at the time the first fat pad was removed and just prior to treatment with hormones. This group was maintained for the subsequent 6 hours on 0.9% saline. Preliminary studies demonstrated no significant difference in lipid content of the 2 pads of the same mouse when they were removed together, as Stoerck and Porter have found in the rat (7). The hormones used were hydrocortisone as the hemisuccinate, The Upjohn Co.; oxycel adsorbed ACTH, Wilson Laboratories, lot No. 102621, approximately 140 U.S.P. units per mg; growth hormone, Endocrinology Study Section, Nat. Inst. of Health, BGH-1.

Results. No mobilization of lipid was detected in the untreated controls during the 6 hour period of observation, as shown in the upper section of Table I. Growth hormone and ACTH, however, both caused significant losses of depot lipid. A small or large dose of hydrocortisone failed to effect similar lipid depletion, indicating that neither ACTH nor STH was acting through stimulation of the adrenal cortex. The liver fat values of these intact mice were not significantly different. Previous studies(1) have demonstrated that female mice of this same strain develop fatty livers within 4 hours of receiving ACTH or STH intraperitoneally. Average values for liver fat in this study were highest for the groups treated with growth hormone or corticotropin and lowest in the groups given hydrocortisone. Failure to achieve significance in these differences may relate to the use of male animals, which appear to be more resis-

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TABLE I. Lipid Mobilization from Epididymal Fat Pads of Fasting Mice.

Treatment	No. animals	Avg body wt (mg)	Epididymal lipid (mg) Initial	Final	Difference	% difference	mg liver fat per 100 mg liver
			Intact				
None	15	18.2	57.1	56.4	− .8 ± .99	− 2.5 ± 1.94	5.45 ± .321
STH .5 mg	12	17.8	63.8	54.6	*−9.3 ± 1.17*	*−17.3 ± 2.54*	6.13 ± .454
ACTH .1	8	19.4	48.7	40.3	*−8.4 ± 1.28*	*−20.8 ± 4.80*	5.96 ± .542
HC .1	9	20.1	60.3	57.2	−3.0 ± 3.10	− 5.1 ± 5.32	5.01 ± .239
HC 1.0	8	18.2	59.5	57.7	−1.8 ± 1.25	− 4.1 ± 2.34	4.82 ± .121
			Adrenalectomized				
None	22	20.7	82.0	75.8	−6.2 ± 1.69	− 7.9 ± 1.73	5.11 ± .243
p					<.05	<.05	
HC .1 mg	9	21.9	88.6	86.7	−1.9 ± 1.17*	− 2.6 ± 1.47‡	4.34 ± .340*
ACTH .1	23	20.7	95.7	87.9	−8.2 ± 1.94†	− 8.1 ± 1.58§	6.43 ± .316†
HC .1 mg + ACTH .1 mg	21	20.7	93.4	84.8	−8.7 ± 2.13†	− 9.6 ± 2.06§	6.23 ± .291†

Values are means ± stand. errors. Those in *italics* are significantly different ($p < .01$) from control value at top of respective column.

† $p < .01$ over *. § $p < .02$ over ‡. $p < .05$ shown between means compared. STH—growth hormone. ACTH—corticotropin. HC—hydrocortisone as the hemisuccinate.

tant to fatty liver than the female. Under the conditions of this study loss of depot fat in the intact mice appears to be a better indicator of fat mobilization than development of fatty liver.

Further evidence that ACTH stimulates fat mobilization by means other than adrenal stimulation may be seen in the lower section of Table I. Mice adrenalectomized after removal of the first fat pad lose significantly more lipid from the remaining pad in 6 hours than do mice with intact adrenals. The controls necessary to identify this increased fat mobilization with the adrenal deficit rather than with the more extensive surgery were not done. Levy and Ramey(8), however, have shown in the fasted rat that hydrocortisone administration or sham adrenalectomy inhibits mobilization of depot fat, whereas adrenalectomy promotes it. Furthermore, in the present study hydrocortisone inhibited fat loss from depots of adrenalectomized mice. ACTH failed to accelerate fat loss over the effect of adrenalectomy alone (Table I) due perhaps to a pre-existing maximal stimulus to fat mobilization. ACTH given to adrenalectomized mice together with hydrocortisone, however, overcame the inhibition of fat depletion which occurred with this dose of hydrocortisone alone. Further evidence of fat mobilization by ACTH in adrenalectomized mice is the significantly higher values for liver fat in the animals treated with ACTH or ACTH plus hydrocortisone than those treated with hydrocortisone alone.

Discussion. The data presented here serve as a link between earlier studies demonstrating (a) ketosis and fatty liver following ACTH and STH administration *in vivo* and (b) acceleration of lipolysis in isolated adipose tissue exposed directly to the same hormones *in vitro*. Viewed as a whole the evidence strongly favors the interpretation that both hormones may act directly on adipose tissue depots to promote lipid mobilization. The scant but significant lipolytic effect of STH on adipose tissue *in vitro* relative to ACTH leaves open the possibility, however, that growth hormone may deplete fat depots *in vivo* by more than this one means. The relative importance to fat mobilization of endogenously secreted hormones, including epinephrine and norepinephrine, still is uncertain. The results of this study are nevertheless consistent with the possibility that increased ACTH secretion in response to adrenalectomy contributed to the greater fat loss from depots of the adrenalectomized mice than occurred when the adrenals were left intact. Hydrocortisone has not been found to inhibit lipolysis when applied directly to adipose tissue *in vitro*(5). It seems possible that it may inhibit fat mobilization in the adrenalectomized mouse by opposing the secretion

of endogenous ACTH. Alternative explanations have not been excluded, however, such as stimulation by hydrocortisone of insulin secretion from the pancreas. Further suggestive evidence that endogenously secreted pituitary hormones may effect fat mobilization is the well known tendency of hypophysectomized rats to become relatively obese when fed and to resist lipid depletion when fasted despite their proneness to hypoglycemia. Knobil(9) has demonstrated that adipose tissue from hypophysectomized rats releases smaller amounts of fatty acid *in vitro* than that from normal rats, whereas treatment of the hypophysectomized rats with STH restores this defect toward normal. The contributions of increased insulin sensitivity and lowered basal metabolic rate to impaired fat mobilization of such animals are yet to be evaluated.

Summary. Purified corticotropin and growth hormone both stimulate lipid mobilization from the epididymal fat depots of fasting mice. Hydrocortisone failed to induce lipid loss in intact mice and inhibited fat mobilization in adrenalectomized mice, indicating that the lipid mobilizing effect of corticotropin is not mediated by adrenal stimulation. Furthermore, inhibition of fat loss by hydrocortisone in adrenalectomized mice could be overcome by simultaneous administration of corticotropin. The results demonstrate an extra-adrenal action of corticotropin on fat mobilization in the intact animal similar to that induced by growth hormone.

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Mechanism of Inhibition of Gastric Secretion by Fat in the Intestine.* (25217)

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It is well known that gastric secretory activity and gastric motility are inhibited when fat is present in the proximal small bowel(1). This inhibitory effect of fat has been documented by experiments in dogs and humans, and is said to be mediated by an agent circulating in blood(2). The nature of this agent is unknown. Lim *et al.*(3) prepared from mucosa of small and large bowel of dogs a crude extract capable of inhibiting gastric secretion in dogs. They postulated that these extracts and also those prepared later from hog small bowel mucosa contained the hormone "enterogastrone" which under physiologic conditions is released by the small bowel mucosa in the presence of fat. Whereas "enterogastrone" preparations inhibit gastric secretion in dogs and to a lesser degree in humans, they fail to inhibit gastric secretion in the rat(4). Our finding(5) that parenteral administration of bile salts inhibited gastric secretion in rats suggested the possibility of increased resorption of bile salts incident to fat digestion and absorption being the mechanism responsible for gastric inhibition by fat. Our purpose was to determine if rat gastric secretion was inhibited when fat was present in the small intestine and, if so, to what extent this inhibition was affected by presence

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