trations, it is pertinent that similar, but more evanescent, changes in plasma FFA are induced by doses as low as 0.1 unit per kilo body weight. Even if smaller quantities of oxytocin were effective in reducing plasma FFA, the physiological significance of this systemic effect would remain obscure in view of the rapidity with which this hormone is destroyed *in vivo*(16). It is quite possible, however, that the effect on the circulating FFA reflects an action at or near the site of secretion of oxytocin in the nuclei of the hypothalamus.

Irrespective of the physiological significance of the action of oxytocin on circulating FFA, the present findings provide a tool for dissociating the effects of insulin which are dependent upon utilization of glucose by the muscles from those which are dependent upon utilization of glucose by adipose tissue.

Summary. Intravenous injection of oxytocin results in a decrease in concentration of free fatty acids in the plasma of non-diabetic and alloxan-diabetic dogs.

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Interaction of Growth Hormone with ACTH, TSH, and FSH in the Hypophysectomized Rat.* (27417)

CHRISTOPHER LONGCOPE† AND JOSEPH W. JAILER‡
(Introduced by Nicholas P. Christy)
Departments of Medicine and Obstetrics and Gynecology, College of Physicians and Surgeons, Columbia University, New York City

Whereas purified ACTH is extremely effective in depleting adrenal ascorbic acid and in stimulating adrenal steroidogenesis, it is not so effective as the less pure substance in increasing adrenal weight in the hypophysectomized animal(1). This dichotomy of effect has been demonstrated by Li(2), Cater and Stacke-Dunne(3), and Dixon, Young *et al.* (4). The latter investigators prepared pituitary fractions relatively rich in adrenal weight-maintaining activity and others which were noted to be more active in depleting adrenal ascorbic acid. The nature of the adrenal weight-maintaining substance is not clear.

Jailer, Longson and Christy(5) have demonstrated the presence of a factor in the

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† Fellow of the Dazian Foundation for Medical Research, 1959-1960. Present address: Dept. of Medicine, Univ. of Washington, School of Med., Seattle.

‡ Deceased.

plasma of patients with Cushing's syndrome due to adrenal cortical hyperplasia which is capable of partially preventing the adrenal atrophy normally occurring after hypophysectomy in rats. This factor has been shown by Christy and Drucker(6) to disappear after pituitary irradiation. That this might possibly be related to growth hormone was suggested by the fact that some patients with acromegaly also possessed an adrenal weight-maintaining factor in their plasma(7).

Lostroh(8) has shown that growth hormone administered simultaneously with ACTH to hypophysectomized rats potentiates the effect of the latter hormone on the weight of the adrenal. No evidence relative to its effect on plasma corticosteroid levels was presented. In human subjects, Liddle *et al.*(9) were able to demonstrate an increased response to ACTH by pretreatment with an equine pituitary extract; a similar response could not be demonstrated with ACTH pretreatment.

The purpose of the present work was to ascertain whether growth hormone was capable of potentiating the steroidogenic effect of ACTH in the rat and also to determine whether the biologic activity of the thyrotropic and gonadotropic hormones could be potentiated by growth hormone.

Materials and methods. Sprague-Dawley hypophysectomized rats weighing 70-80 g at time of hypophysectomy were weighed and divided into groups. For the adrenal weight maintenance experiments(10) injections were begun 24-48 hours after hypophysectomy and continued for 10 days. For the adrenal repair(10) and for all other experiments, injections were started 9-10 days after hypophysectomy and continued for 3 days according to the dosage schedules noted in the tables. In all experiments, the rats were sacrificed on the day after the last injections. The glands were removed and dissected free of fat, and wet weights were obtained. Dry weight of the adrenals was obtained after heating in an oven at 135°C for 48 hours.

Corticosterone levels in blood. At time of sacrifice, blood was collected in heparinized tubes, and the serum was prepared and frozen until used. Corticosterone levels were measured by a modification of the Guillemin technic(11). For quantification of the response to ACTH§ and growth hormone (GH)¶ or both together, the animals received the hormones intraperitoneally 45 minutes prior to sacrifice.

I^{131} Uptake. Rats were given 0.5 μc of I^{131} intraperitoneally on the third day of injections of TSH‖ and GH. Twenty-four hours later they were sacrificed and the thyroids were dissected out, cleaned of fat, weighed, and placed in tubes with 1.0 ml of formalin and counted in a well-type scintillation counter.

Solutions. Solutions of hormones were made up in saline before each experiment and were kept at 4°C.

Results. When ACTH was injected alone in either the adrenal maintenance or repair experiments, there was no significant change in body weight, although adrenal weights were increased (Tables I and II). GH, when administered alone, caused an increase in body weight, but only when given for 10 days did it significantly increase adrenal weight. However, the potentiation by GH of the effect of ACTH on adrenal weight was marked in both experiments and in both sexes.

Pretreatment with ACTH and/or GH did not alter basal corticosterone levels significantly in comparison with controls (Table III). When rats were given ACTH 45 minutes before sacrifice, the groups pretreated with ACTH and GH were significantly different from their controls but not from each other. When the adrenal response was tested with both ACTH and GH, levels of corticosterone produced were again similar to those produced by ACTH alone.

As noted (Tables IV and V) there was no good correlation between thyroid weight and hormone administration. The increase in weight with GH could be accounted for by the amount of TSH known to be present in the GH preparation. There was, however, no potentiation of thyroid weight increase when

§ Corticotropin, Upjohn Co., Kalamazoo, Mich.

¶ Kindly supplied by Endocrinology Study Section, Nat. Inst. Health.

‖ TSH (Armour) kindly supplied by Dr. K. S. Sterling.

TABLE I. Adrenal Repair Experiment (3 days).

Injection	Rats No.	Sex	Initial body wt (g)	Final body wt (g)	Adrenal wt* (mg) (±S.E.) Wet	Dry
Control						
.2 ml saline bid	5	♀	79.0	83.8	12.1 (± .43)	3.12 (±.26)
Idem	5	♂	76.4	74.6	8.5 (± .48)	
Growth Hormone						
.5 mg bid ip	5	♀	80.0	101.8	12.9 (± .43)	3.37 (±.17)
1.0 " " "	8	♂	88.0	96.0	11.6 (± .53)	
ACTH						
0.5 unit tid sc	5	♀	79.8	83.8	18.4 (± .77)	4.80 (±.26)
Idem	8	♂	83.0	75.0	17.1 (± .82)	
ACTH and GH						
.5 unit tid sc	5	♀	79.6	94.2	22.8 (± .62)	5.99 (±.15)
.5 mg bid ip	8	♂	83.0	92.0	25.5 (±1.51)	5.99 (±.15)

* Significance of difference of means(12) between groups is as follows:
$P < .01$ for Control vs. ACTH, both wet and dry weights.
for ACTH vs. ACTH and GH, both wet and dry weights.

TABLE II. Adrenal Weight Maintenance Experiment (10 days).

Injection	Rats No.	Sex	Initial body wt (g)	Final body wt (g)	Adrenal wt (mg) (±S.E.) Wet
Control					
Saline	5	♂	76.4	74.6	8.5 (± .48)
Growth Hormone					
1.0 mg bid ip	7	♂	77.0	119.4	14.0 (±1.05)
ACTH					
.5 unit tid sc	9	♂	73.0	70.0	26.4 (±1.20)
ACTH and GH					
.5 unit tid sc					
1.0 mg bid ip	9	♂	79.0	123.0	52.1 (±2.53)

TABLE III. Plasma Corticosterone Levels.

Rats injected for 3 days with:	Plasma corticosterone level* (μg/100 ml, ±S.E.) No test injection before sacrifice	.5 unit ACTH ip 45 min before sacrifice
Control		
.2 ml saline bid	9.4 (±1.22)	21.0 (±2.03)
Growth Hormone		
.5 mg bid	9.4 (±1.31)	18.3 (± .88)
ACTH		
.5 unit tid	11.2 (±2.36)	33.0 (±2.05)
ACTH and GH		
.5 unit tid	14.3 (±2.34)	35.3 (±1.19)
.5 mg bid	.5 unit ACTH ip	.5 unit ACTH, .5 mg GH,
ACTH and GH		
.5 unit tid	before sacrifice	ip 45 min before sacrifice
.5 mg bid	35.5 (±1.19)	36.9 (±2.02)

* Significance of difference of means(12) between groups is as follows:
$P < .01$ for response to ACTH of ACTH and ACTH plus GH vs. Control and GH groups.

TABLE IV. Effects of TSH and GH on Thyroid Weight and I^{131} Uptake.

Rats injected for 3 days with:	No. of rats	Initial body wt (g)	Final body wt (g)	Thyroid wt (g)	% uptake (±S.E.)
Control .2 ml saline bid	7	68.5	72.1	1.64	.28 (±.02)
GH .5 mg bid	6	68.5	86.1	3.38	1.05 (±.14)
TSH .5 unit qd	7	68.9	72.4	3.13	3.48 (±.28)
TSH .5 unit qd GH .5 mg bid	6	68.1	80.0	2.77	5.57 (±.44)

TABLE V. Effects of TSH and GH on Thyroid Weight and I^{131} Uptake.

Rats injected for 3 days with:	No. of rats	Initial body wt (g)	Final body wt (g)	Thyroid wt (g)	% uptake (±S.E.)
Control .2 ml saline bid	5	80.8	84.0	3.5	.37 (± .11)
GH .5 mg bid TSH .5 unit qd	5	82.0	90.4	3.8	9.18 (±1.11)
TSH 10 milliunit* qd TSH .5 unit qd	5	76.8	77.0	4.16	9.01 (±1.33)

* Calculated to be the TSH in 1.0 mg GH.

TABLE VI. Effects of GH and PMS on Ovarian Weights.

Rats injected for 3 days with:	No. of rats	Initial body wt (g)	Final body wt (g)	Ovarian wt (mg) (±S.E.)*
Control .2 ml saline bid	5	71.8	74.4	7.9 (± .71)
GH .5 mg bid	5	71.4	92.4	10.3 (± .45)
PMS 10 units qd	5	73.2	75.7	34.2 (±1.72)
GH .5 mg bid PMS 10 units qd	3	80.0	96.2	32.4 (±2.03)

* Significance of difference of means(12) between groups as follows: $P < .01$ for Control vs. PMS.

GH was given simultaneously with TSH. Similarly, although TSH caused the expected significant increase in I^{131} uptake, the addition of GH did not potentiate this over and above the increase expected from the TSH present in the GH.

GH also did not potentiate the effect of the gonadotropin preparation (pregnant mares' serum—PMS)** on ovarian weights (Table VI).

Comment. GH and ACTH are synergistic in both the adrenal weight repair and maintenance tests. In contrast to the findings of Lanman(13) who used pregnancy plasma, the present data indicate that the synergistic action is not mediated through the testis, since the effect was similar in females as well as males. That this increase in weight is not due to increased water content is brought out by the fact that there is a significant difference in the dry weight of the adrenals of the ACTH group as contrasted to the ACTH plus GH group. Under the conditions of these experiments, however, the enlarged adrenals produced by the combination of GH and ACTH did not elicit a greater increase in blood levels of corticosterone in response to ACTH

** PMS kindly supplied by Leo Pharmaceutical Products.

than did the smaller adrenals of the ACTH-treated rats. Our data do not explain why GH potentiates only the adrenotropic (not the steroidogenic) action of ACTH.

Growth hormone did not potentiate the effect of TSH or PMS on their respective target organs nor did it affect the iodide-trapping mechanism of the thyroid in association with TSH stimulation.

Summary. 1. The effect of administration to hypophysectomized rats of GH simultaneously with other pituitary hormones was investigated. 2. Growth hormone potentiates the effect of ACTH on adrenal weight but does not increase the steroidogenic response of the adrenal to ACTH. 3. GH did not enhance the effect of TSH on thyroidal weight or iodide uptake, nor did it augment the effect of PMS upon ovarian weight.

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A Comparison of Erythropoietin* Bioassays. (27418)

RICHARD L. DEGOWIN, DIANA HOFSTRA AND CLIFFORD W. GURNEY
(Introduced by L. O. Jacobson)
Department of Medicine and the Argonne Cancer Research Hospital,† University of Chicago, Chicago, Ill.

Attempts to explain the mechanism by which the body reconstitutes its red blood cell mass after hemorrhage led investigators to the discovery of a humoral stimulant of erythropoiesis(1). This hormone, present in the plasma of anemic animals, has not yet been isolated, but characteristics of highly purified preparations suggest it is a glycoprotein(2). Jacobson and Goldwasser(3) have suggested that short periods of hypoxia initiate erythropoietin secretion which in turn stimulates erythropoiesis, and with frequent minute adjustments the equilibrium of the normal red cell mass is thus maintained within normal limits.

While Gurney *et al.* have demonstrated erythropoietic activity in 10-fold concentrated Borsook extract of normal human plasma by using the hypophysectomized rat assay(4), erythropoietin has not been found in raw plasma from humans. Critics of the theory that erythropoietin normally regulates red cell production argue that the hormone is absent from plasma of non-anemic animals and that it is elicited as a "panic mechanism" (5) to compensate for blood loss. Proponents of the theory contend that, because only slight steady stimulation is required to sus-

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