males, the degree of success in prediction would be quite small.

Summary. When whole saliva samples were collected at 9:00 a.m. from 323 males and 159 females under stimulation of 3 large rubber bands and from 503 males and 179 females utilizing no exogenous stimulants, the following conclusions could be drawn: 1. No significant differences in volume attributable to age were noted. Rate of flow for males was significantly higher than that for females. Rate of flow for both males and females was significantly higher when exogenous stimulation was utilized. 2. No significant increase or decrease in chloride values attributable to age was noted. 3. For females, volume and chloride were negatively correlated while, for males, a positive correlation was found with exogenous stimulation and a negative correlation when no such stimulation was used. For both males and females, chloride levels were significantly higher when exogenous stimulants were used.

The author expresses his appreciation to Miss Muriel Stahnke for statistical analysis and to J. F. McAnear and R. R. Hogue for technical assistance.

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Received October 1, 1957. P.S.E.B.M., 1958, v97.

Synergistic Effect of Somatotropin with α-Corticotropin on the Adrenal of Hypophysectomized Rats.* (23892)

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Although an antagonism is often operative between adrenocorticotropic hormone (ACTH) and growth hormone (somatotropin) with respect to certain anabolic effects of the latter(1), it is becoming apparent that the 2 hormones may also act in synergism. Cater and Stack-Dunne(2) have studied the combined effect of somatotropin and ACTH in the hypophysectomized rat and demonstrated that the resulting increase in adrenal weight is greater than the sum of increases obtained with each alone. More re-

* Supported in part by grants from Am. Cancer Soc. and Nat. Inst. H., U.S.P.H.S. (RG-2907).

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cently, we have demonstrated a striking synergism between partially-purified ACTH and somatotropin, reflected in increases in weight of adrenal glands, and have described the lipid changes that accompany administration of these 2 hormone preparations(3). These studies have now been repeated with α-corticotropin (α-ACTH), and the combined effects of somatotropin and this pure peptide are reported here.

Methods. Male rats of the Sprague-Dawley strain were hypophysectomized at 50 days of age; daily injections were begun on fifth post-operative day and continued for 4 days. The somatotropin solution was injected intraperitoneally; thyroxin and α-ACTH were injected subcutaneously, the latter as suspension in a delaying vehicle that contained 5% beeswax and 95% peanut oil. At autopsy, the adrenal glands were removed and immediately fixed in 10% neutral formalin; subsequently, they were dissected free from surrounding fatty tissue and weighed on Roller-Smith torsion balance. For lipid studies, frozen sections of the adrenal, 10 μ thick, were prepared and stained with either sudan black B or with oil red-O. In all instances, sections in the region of adrenal vein were selected for study. The growth hormone was prepared from bovine pituitaries according to procedure previously described(4). It was estimated to contain 0.04 i.u. of thyroid-stimulating hormone (TSH)/mg,‡ but was free from any detectable follicle-stimulating hormone, interstitial cell stimulating hormone or ACTH when assayed at dose level of 10 mg in hypophysectomized rats. The α-ACTH was isolated from sheep pituitary glands(5), and the DL-thyroxin was a product of E. R. Squibb Co.

Results. The adrenal gland of the rat atrophies markedly following removal of the anterior pituitary; nine days after hypophysectomy, changes in weight of gland (Table I) as well as its histology had occurred. In frozen sections, it was evident that the *zonae fasciculata* and *reticularis* were shrunken, and cortical cells of these zones displayed signs of atrophy characteristic of the hypophysectomized animal; a distinct sudanophobe zone was also present. Although the cells of the *zona glomerulosa* retained their normal size, the lipid distribution within them was clearly abnormal. Coarse droplets of sudan-staining material were distributed intermittently in the glomerular cells adjacent to the sudanophobe zone, whereas the cells lying just beneath the outer capsule were completely devoid of lipid.

Treatment with α-ACTH. As expected, α-ACTH promoted substantial weight increases in the adrenal gland of hypophysectomized Sprague-Dawley rats. Administration of a daily dose of 0.025 mg promoted weight increase of approximately 100% over the average adrenal weight of the control group and restored the weight of glands to that of intact control. This dose caused marked hypertrophy of fascicular cells, a considerable diminution in their lipid content, and a redistribution of lipid throughout the *zona glomerulosa.* With a dose of 0.10 mg, hypertrophy of the fascicular cells was accentuated and lipid depletion was nearly complete. Whatever lipid could be detected was present in the *zona reticularis* and the *zona glomerulosa,* although even in the latter it was somewhat depleted. Sinusoidal distention accompanied cellular hypertrophy and no sudanophobe zone was apparent in adrenals of the injected animals. These histological changes were similar to those obtained in hypophysectomized rats of the Long-Evans strain(6).

Treatment with α-ACTH and somatotropin. Somatotropin administered at daily dose levels of 0.050 mg and 0.250 mg increased the weights of adrenal glands from the control value of 16.3 mg to 21.3 mg and 20.3 mg, respectively, the 2 dose levels being equally effective in increasing size of the gland. Despite these changes in weight, no changes in lipid distribution were observed. Furthermore, the dose of α-ACTH and not that of the somatotropin determined the degree of synergism between the 2 hormones. From the Table it is clear that somatotropin given in conjunction with 0.10 mg of α-ACTH was considerably more effective in enhancing adrenal weight than when given with 0.025

‡ We wish to thank Dr. R. W. Bates of the Nat. Inst. of Arthritis and Metabolic Diseases for assay of TSH contamination in growth hormone preparation.

TABLE I. The Effect of α-Corticotropin, Growth Hormone, Thyroxin* and Combinations Thereof on Adrenal Weight of Hypophysectomized Sprague-Dawley Rat.†

Treatment	Daily dose, mg	Body wt, g: Pre-hyp'ed	Body wt, g: Day of 1st inj.	Body wt, g: At autopsy	Adrenal, mg	Thymus, mg
Normal controls	0	139	151	160	28.7 ± 1.3‡	436 ± 34
Saline	0	145	129	131	16.3 ± .5	338 ± 35
α-corticotropin	.025	139	129	125	28.5 ± .5	0
	.100	143	129	118	43.8 ± 2.2	0
Growth hormone	.050	139	131	141	21.3 ± .9	357 ± 45
	.250	145	131	136	20.3 ± 1.7	374 ± 38
Thyroxin	.005	146	132	132	16.7 ± .5	308 ± 35
α-corticotropin + growth hormone	.025 + .050	149	128	130	32.4 ± 3.0	98 ± 39
Idem	.025 + .250	151	131	134	33.5 ± 2.9	138 ± 36
"	.100 + .050	152	133	134	53.7 ± 2.5	0
"	.100 + .250	136	129	129	54.9 ± 5.3	0
α-corticotropin + thyroxin	.025 + .005	146	132	124	27.6 ± 1.2	133 ± 38

* DL-thyroxin, E. R. Squibb & Co.

† Male rats hypophysectomized at 50 days of age, inj. subcut. at 54 days once daily for 4 days, 4 rats/group.

‡ Mean ± stand. error.

mg of α-ACTH. Histological examination of the adrenal glands from animals treated with given dose of α-ACTH or with the same dose of α-ACTH with somatotropin did not reveal any clear-cut difference in general distribution of lipid or in amount that could be detected within the cortical cells.

Treatment with α-ACTH and thyroxin. As already noted, the somatotropin preparation used in this study did exhibit a limited degree of TSH activity. To establish that the effects observed with somatotropin would not be duplicated by small amounts of thyroxin, one group of animals was injected with 0.005 mg of DL-thyroxin and another group was injected with 0.005 mg of DL-thyroxin and 0.025 mg of α-ACTH. This treatment effected no change in adrenal weight or in lipid distribution.

Discussion. The changes that occur in the adrenal cortex of the rat following hypophysectomy have already been thoroughly described(7), the atrophy being more severe the longer the post-operative interval. Comparisons between results obtained with Sprague-Dawley and the Long-Evans strains reveal that the two are comparable with respect to both adrenal atrophy(8) and to sensitivity to exogenous α-ACTH(6).

An alteration in lipid distribution in the *zonae glomerulosa, fasciculata* and *reticularis* was observed following hypophysectomy. Furthermore, administration of α-ACTH caused redistribution of sudanophil material in the cortical cells of all 3 zones of the adrenal cortex. These observations confirm those reported by Wexler, *et al.*(9) and suggest that the *zona glomerulosa* may not be independent of the control of the anterior pituitary. On the other hand, Deane and Greep and their collaborators have demonstrated that either stimulation or atrophy of the *zona glomerulosa* can be induced with appropriate alterations in the sodium-potassium ratio(10-12); similar effects have been reported for the rhesus monkey(13). Nonetheless, observations of other workers that changes in lipid distribution do occur in the *zona glomerulosa* following administration of ACTH to rat(9,14) and mouse(15,16) suggest some pituitary control. Recently, Lever, in a study of the fine structure of cortical cells with the electron microscope, has observed that although atrophy of glomerular cells after hypophysectomy is

much slower than that of other zones, these cells eventually do show a loss of cytological detail, and that ACTH restores them toward a normal condition(17). This indicates again that the *zona glomerulosa* is influenced by the pituitary and suggests that ACTH is the pituitary factor that is responsible for these changes.

Evidence is also available that secretion by the adrenal gland of both gluco- and mineralocorticoids is subject to pituitary influence. Singer and Stack-Dunne demonstrated that output of mineralocorticoid from adrenal cortex falls markedly after hypophysectomy and that maintenance of the hypophysectomized rat with corticotropin, permits the animal to respond to acute stimulation with ACTH in an essentially normal fashion(18). In addition, *in vitro* studies have shown that when ACTH is added to the incubation medium, some aldosterone-like material, along with a considerable amount of corticosterone, is released by the isolated adrenal(19,20). On the basis of our studies and those cited above, it may be concluded that the *zona glomerulosa* in both rat and mouse is not independent of pituitary control and that the pituitary principle ACTH does act on this zone.

Marked hypertrophy of the adrenal gland can be attained with α-ACTH alone. However, we have observed consistently that the combination of ACTH and somatotropin is more effective in increasing the weight of adrenal gland than is ACTH alone(3). Although there is no suggestion from these results that the capacity of somatotropin preparations to enhance effectiveness of α-ACTH can be attributed to contamination with any other hormone, dosages of growth hormone were selected which provided a maximal degree of synergism with minimal amount of hormone. Dosages of 0.050 mg and 0.250 mg were equally effective under our conditions, and the synergism observed between α-ACTH and growth hormone was related to dosage of α-ACTH. Cater and Stack-Dunne, in investigations on mitotic activity in the adrenal of the hypophysectomized rat, have likewise concluded that growth hormone preparations have an effect on the adrenal cortex of the hypophysectomized rat not attributable to any contamination by ACTH(2). Enlargement of adrenal gland of intact but not of the hypophysectomized rat has also been reported following administration of somatotropin(21). Thus, in these instances where a synergism between growth hormone and ACTH has been demonstrated, the effect obtained appears conditioned by level of either endogenous or exogenous ACTH. More direct studies measuring liberation of corticoids from the adrenal gland, however, have to date provided no support for this contention(18).

Summary. The adrenal gland of hypophysectomized Sprague-Dawley rat showed substantial atrophy 9 days after operation. Histologically-detectable changes in lipid distribution were observed in the *zonae glomerulosa, fasciculata* and *reticularis* following hypophysectomy, and a redistribution of lipid in all 3 zones was effected with α-ACTH. In addition, a synergism between α-ACTH and pituitary growth hormone (somatotropin) was demonstrated, the magnitude of the effect being conditioned by level of exogenous α-ACTH. These results indicate that somatotropin operates synergistically with ACTH when the 2 hormones are administered in combination to the hypophysectomized rat.

The author wishes to acknowledge the able technical assistance of Charles W. Jordan, Jr. and Peggy Woodward Randolph.

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Received January 8, 1958. P.S.E.B.M., 1958, v97.

Electrophoretic Studies on Serum Proteins of Young Germfree, Conventional and Antibiotic Treated Conventional Chickens.*† (23893)

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One of the characteristics of the germfree animal is a low level of antibody-containing globulin fractions. Thorbecke *et al.* found that in the germfree chicken a low level of gamma globulin persisted throughout the first 3 months of life. In the conventional‡ bird, during the same time period, this antibody containing fraction steadily increased(1). Meanwhile, we showed that conventional chickens treated orally from birth with 50 mg procaine penicillin G/kg diet develop certain organ characteristics which are qualitatively similar to those seen in germfree animals(2, 3). In essence, these consist of lower weight and less lymphoid tissue content of such organs which are normally in close association with an abundant microbial flora. Comparable changes have been reported by others(4, 5,6,7). Present evidence points to an influence of the antibiotic on composition and or metabolism of the intestinal flora as the most likely explanation of this effect on the host. It was therefore considered of interest to know whether oral administration of procaine penicillin G to young growing chickens, which was able to produce such definite "germfree-like" morphological features, would have an influence on the globulin fractions of the serum.

Methods. White Leghorn chickens of mixed sexes were used at 35, 75 and 125 days of age. The germfree birds were hatched into the Reyniers germfree system(8,9). After hatch the experimental animals were maintained in commercial brooders and in germfree units respectively. Percentage composition of diet (L-289F) was as follows: yellow corn 50, soybean meal 23, wheat midlings 9, casein 7, alfalfa 2, fish meal 2, meat scraps 2, minerals 4, vitamins and carriers 1. Throughout the experiment steam-sterilized diets were used (121°C, 25 min). Diet and tap water were offered *ad lib.* The present regimen allowed normal growth of both conventional and germfree animals. Procaine penicillin G was administered at 50 mg/kg diet. For further details, see Gordon *et al.*(3). All animal material indicated as germfree tested so up to moment of sacrifice, with the exception of the 125-day-old group. These animals became contaminated accidentally with a pure culture of *Micrococcus sp.*, 1 to 6 days before termination of experiment. Specific name of the contaminant was not determined. As previous experience has shown that in all probability no major changes in serum pattern can be ex-

* These studies were aided by contract between Office of Naval Research and University of Notre Dame.

† The authors are indebted to Charles Pfizer and Co. for supplying practical diets, to Eli Lilly and Co. for procaine penicillin and testing its potency after sterilization, to Dr. John G. Trump of the Mass. Inst. of Technology for radiation-sterilization of the antibiotic and to Mrs. Marge H. Beaver for performing electrophoretic analysis.

‡ Indicating animals of normal stock.