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STIMULATION OF THE SEX ACCESSORIES OF
HYPOPHYSECTOMIZED MALE RATS BY NON-GONADOTROPHIC
HORMONES OF THE PITUITARY GLAND

By

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While the ventral prostates of mature rats that have been gonadectomized rapidly assume the characteristics of the castrate state, those of gonadectomized immature animals retain a functional appearance up to the age of puberty (Price, 1936). Since the testes have been removed, an extra-gonadal source must be responsible for the post-operative maintenance of the sex accessories in the latter instance. Two endocrine glands have been implicated in the process, namely, the adrenal and the pituitary. Both glands are known to be the source of a number of secretion products; however, just which hormones are responsible for the observed maintenance of the ventral prostate in the gonadectomized immature rat is unknown, and the mechanism through which the maintenance is accomplished is not understood.

There seems to be little doubt but that the adrenal gland is capable of producing an androgenic hormone or hormones (Parkes, 1945, and Gassner *et al.*, 1951). Davidson & Moon (1936) were among the first to show that crude extracts of ACTH¹ do maintain the secondary sex accessories of the young castrated rat in a condition superior to that of the accessories of the saline-injected control. It was further demonstrated that this maintenance is effected through the adrenals (Davidson, 1937, and Nelson, 1941). Studies with more highly purified preparations have yielded variable results. The negative

1. The following abbreviations are used throughout: ACTH, adrenocorticotrophic hormone; ICSH, interstitial cell-stimulating hormone; FSH, follicle-stimulating hormone; TSH, thyrotrophic hormone; HCG, human chorionic gonadotrophin.

findings reported by a number of investigators seem to imply that crude extracts may contain stimulatory substances that are not present in the purified ACTH preparations (*Moore*, 1953). The possibility has been suggested that ICSH may be involved in the process (*Greep & Jones*, 1950); experimental evidence in support of this thesis is lacking.

It is only fair to note that much of the previous work has been done in non-hypophysectomized castrated males, which usually require high hormonal dosages. Since our own experience with this type of experiment was not satisfactory, in the present study only hypophysectomized animals have been employed. The problem of the extra-gonadal stimulation of the secondary sex accessories has been reinvestigated in the male rat, with five of the six recognized anterior pituitary hormones. Since thyrotrophic hormone is not available in a purified form, thyroxin has been substituted for it. All conclusions are based on gravimetric and histological data obtained with highly purified hormones carefully assayed for any possible contamination.

E X P E R I M E N T A L

Male rats of the Long-Evans strain were castrated at 43 days of age and hypophysectomized at 44 days; twenty-four hours later, a regimen of single daily injections was initiated. The pituitary hormones were dissolved in physiological saline except for the ACTH, which was administered as a suspension in a 5% beeswax-95% peanut oil medium. These were given intraperitoneally. The thyroxin and the steroid preparations were given subcutaneously, the latter in the form of sesame oil suspensions. On the day following the tenth injection, the animals were anesthetized with nembutal; the adrenals, ventral prostates and seminal vesicles were removed and weighed. These tissues were immediately fixed in neutral formalin, and were subsequently sectioned and examined for confirmatory histological evidence. The paraffin method, with the hematoxylin-eosin stain, was employed for the sex accessories; the freezing technique, with the Scharlach R stain, was used for the adrenals.

The hypophysectomized-castrated-adrenalectomized animals were subjected to a similar regimen. When the animals were 43 days of age, the testes and the right adrenal together with all detachable surrounding fatty tissue were removed; hypophysectomy was performed at 44 days, and the left adrenal was removed at 46 days. Although all groups had access to a 1% solution of NaCl, triply-operated rats survived for the brief ten-day experimental period only when a small amount of corticoid was injected or when bread and milk was substituted for the regular wet mash-pellet diet.

The growth hormone, isolated from anterior lobes of bovine pituitary glands, and the active core from a chymotryptic digest of the hormone, were prepared by previously reported procedures (*Li*, 1954, and *Li et al.*, 1956). The other pituitary preparations were derived from whole sheep glands. The ACTH-F fraction was obtained from acid-acetone powder, and possessed an adrenal ascorbic acid-depleting activity of 20-25 I.U. per mg. (*Li et al.*, 1955). The purified lactogenic hormone was essentially free from biologically active contaminants (*Cole & Li*, 1955). The FSH from sheep pituitary glands was obtained by ammonium sulfate precipitation followed by alcohol fractionation, and subsequent zone electrophoresis on starch (*Raacke et al.*, unpublished).

The ICSH² was also derived by the ammonium sulfate-alcohol fractionation procedure (*Li et al.*, 1942).

Other hormones used in this work were obtained from various commercial sources. The HCG, assaying 1000 I. U./mg., came from Leo Pharmaceutical Products, Copenhagen, Denmark. The DL-thyroxin was a Squibb product; the sources of the steroids are listed in Table 6.

RESULTS

Effect of Castration and of Hypophysectomy on the Ventral Prostate. The atrophy of the ventral prostate and seminal vesicles produced by hypophysectomy is more severe than that resulting from castration (Figs. 1 a and 1 b). However, large doses of whole sheep pituitary powder given to hypophysectomized animals tend to restore the weights of the sex accessories to the

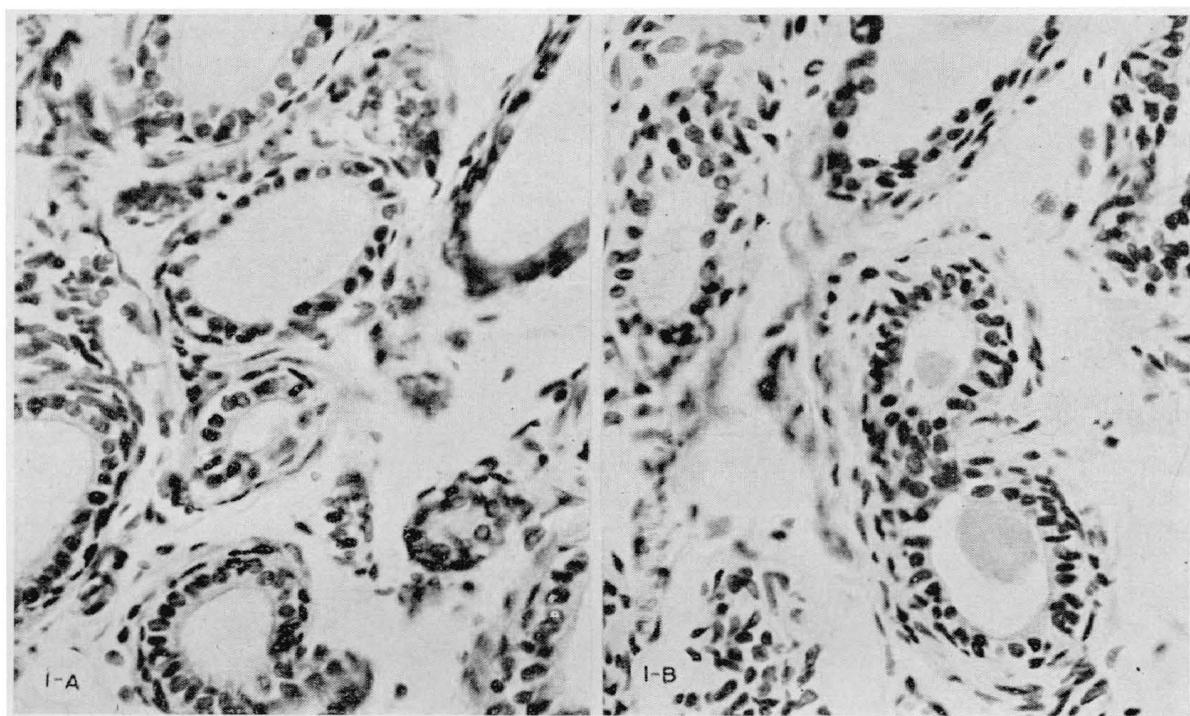


Fig. 1.

Ventral prostate of the Long-Evans rat, operated on at 44 days and sacrificed at 55 days. H and E Stain, $\times 385$.

1 A. Castrated male. Weight of prostate, 18.0 mg.

1 B. Hypophysectomized male. Weight of prostate, 11.4 mg.

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2. From the weights of the adrenals (Table 2), it was apparent that some ACTH was present in the ICSH preparation; frozen sections of the adrenal stained with Scharlach R confirmed this premise inasmuch as the distribution of the sudanophil-staining lipid was typical of that seen with low dosages of ACTH.

castrate level. Approximately 0.005 mg. of testosterone propionate promotes a similar restoration. These data are summarized in Table 1.

Effect of Gonadotrophins and of Thyroxin. None of the gonadotrophin preparations used, namely ICSH, FSH, HCG and lactogenic hormone, were found to stimulate growth of the male sex accessories in the absence of the testes and the pituitary gland. Weight values for the ventral prostate and seminal vesicles are given in Table 2.

Effect of Growth Hormone. Pituitary growth hormone given at dosages of 0.25 mg. and above was found consistently to increase the weight of the ventral prostate of the hypophysectomized-castrated Long-Evans above the prostatic weight of the control animal (Table 3). With such dose levels, the nuclei showed a vesicular appearance, and the connective stroma was stimulated, as Figs. 2 a and 2 b illustrate. Similar but less striking effects were seen in the seminal vesicles. This stimulatory effect exercised by growth hormone on the sex accessories could also be detected in the absence of the adrenal glands if a small amount of an active corticoid (hydrocortisone or desoxycorticosterone) was administered simultaneously.

Effect of ACTH. The hormonal dosage is a critical factor in demonstrating the capacity of the adrenals to produce androgen under ACTH stimulation. A dose insufficient to restore the weight of the glands to that of the glands of a comparable non-hypophysectomized animal induced no detectable androgen secretion (Table 4). At slightly higher levels, growth of the ventral prostate and seminal vesicles was detectable gravimetrically and histologically; Fig. 2 c illustrates the nature of this growth. However, high dosages of ACTH, accompanied by evidence of hypercorticoidism, invariably obliterated the effect. Moreover, this effect is not realized in the adrenalectomized animal in either the absence or presence of a small amount of exogenous adrenal steroid. In this respect, it is evident that the mechanisms whereby ACTH and growth hormone exercise their effects on the sex accessories are separate and distinct from one another.

Effect of Combined Treatment with Growth Hormone and ACTH. Histological evidence that growth hormone and ACTH do act independently of each

Fig. 2.

Ventral prostate of the Long-Evans rat, operated on at 44 days and injected with pituitary preparations for ten days.

- 2 A. Hypophysectomized-castrated male. Weight of prostate, 11.2 mg.
- 2 B. Hypophysectomized-castrated male injected with 0.50 mg. of growth hormone per day. Weight of prostate, 17.4 mg.
- 2 C. Hypophysectomized-castrated male injected with 0.050 mg. of ACTH-F per day. Weight of prostate 15.6 mg.
- 2 D. Hypophysectomized-castrated male injected with 0.50 mg. of growth hormone plus 0.050 mg. of ACTH-F per day. Weight of prostate, 15.0 mg.

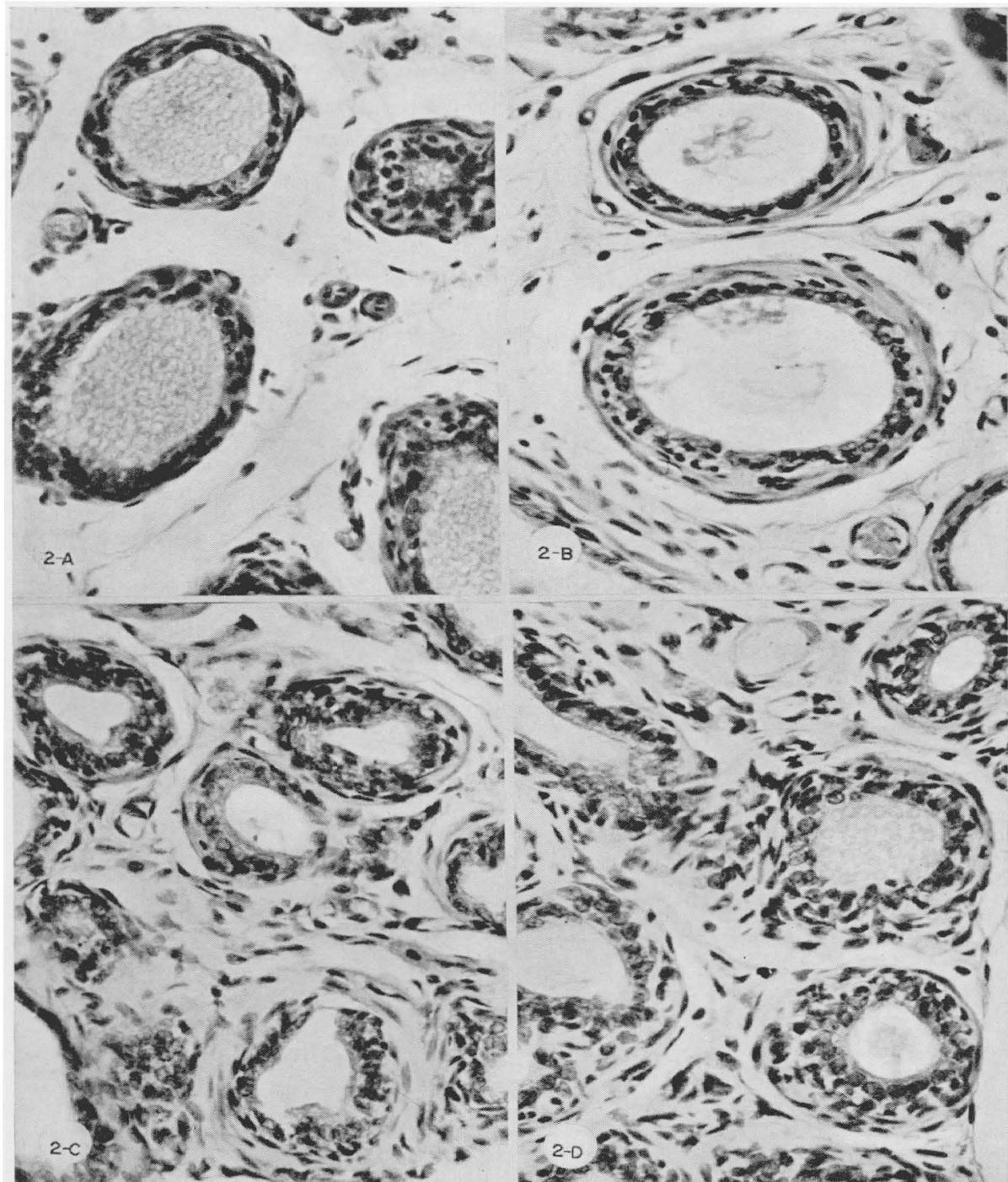


Fig. 2.

Table 1.

Effect of Castration, Hypophysectomy, and Replacement Therapy with Whole Pituitary Extracts or Testosterone Propionate.

Animals*	Treatment	Daily Dose	No. of Rats	Body Weights		Adrenals	Ventral Prostate	Seminal Vesicles
				Onset	Autopsy			
		mg.		gm.	gm.	mg.	mg.	mg.
N	Saline	0	5	141	199	36.0 ± 3.1**	140.0 ± 10	132.5 ± 10
C	»	0	4	129	167	32.0 ± 2.7	18.5 ± 1.1	11.9 ± 0.7
H-C	»	0	4	112	115	9.9 ± 0.5	13.3 ± 0.7	10.6 ± 1.1
						p < 0.01†		
♂	»	Whole sheep pituitary	25	4	111	117	20.9 ± 0.8	14.7 ± 0.2
	»	»	50	5	111	117	28.9 ± 2.1	16.9 ± 0.8
						p = 0.01	14.2 ± 1.1	p < 0.1
	»	Sesame oil	0	3	121	110	10.3 ± 0.4	11.0 ± 0.2
	»	Testosterone††	0.003	4	121	113	11.8 ± 0.9	12.1 ± 0.8
	»	»	0.005	5	109	111	—	19.3 ± 1.7
	»	»	0.007	4	113	115	11.4 ± 0.7	22.7 ± 1.7
								13.7 ± 0.9

* Animals operated at 44 days and sacrificed at 55 days. N = Nonoperated; C = Castrated; H = Hypophysectomized.

** Mean ± standard error.

† The experimental group indicated was compared with the appropriate control. p values were calculated from Fisher's (1944) table of t.

†† Testosterone propionate in sesame oil, Schering Corporation, Bloomfield, New Jersey.

Table 2.

Effect of Gonadotrophins and Thyroxin on the Weights of the Sex Accessories of the Hypophysectomized-Castrated Male Rat.*

Treatment**	Daily Dose	No. of Rats	Body Weights		Adrenals	Ventral Prostate	Seminal Vesicles
			Onset	Autopsy			
	mg.		gm.	gm.	mg.	mg.	mg.
Saline	0	4	120	113	9.8 ± 0.8†	13.7 ± 0.4	13.2 ± 1.1
ICSH	0.50	6	118	120	14.2 ± 0.8	12.3 ± 1.3	13.2 ± 1.8
FSH	0.50	4	111	110	9.7 ± 1.0	11.3 ± 0.9	14.8 ± 2.1
HCG	0.50	4	121	104	10.4 ± 0.4	12.7 ± 1.4	13.9 ± 1.5
Lactogenic hormone	0.50	3	118	118	11.3 ± 1.2	13.6 ± 1.2	14.1 ± 3.4
»	5.00	4	114	119	10.8 ± 0.8	11.1 ± 0.6	13.0 ± 0.8
Thyroxin	0.003	5	126	117	11.2 ± 0.6	11.9 ± 0.5	15.5 ± 2.7

* All animals castrated at 43 days, hypophysectomized at 44 days and sacrificed at 55 days.

** Injections begun on day following hypophysectomy and continued for 10 days.

† Mean ± standard error.

Table 3.

Effect of Growth Hormone on the Weights of the Sex Accessories of the Hypophysectomized-Castrated Male Rat
Before and After Adrenalectomy.

Animals*	Treatment	Daily Dose	No. of Rats	Body Weights		Adrenals	Ventral Prostate	Seminal Vesicles
				Onset	Autopsy			
♂	H-C	Saline	mg.	8	gm.	gm.	mg.	mg.
	"	Growth hormone	0.25	4	116	129	10.0 ± 0.4**	13.9 ± 0.6
	"	"	0.50	8	125	148	12.5 ± 0.7	14.5 ± 1.8
	"	Growth hormone††	0.50	4	113	142	14.7 ± 0.9	16.3 ± 0.5
							p < 0.001†	p < 0.01
							p = 0.001	p < 0.10
	H-C-A	Cpd. F§	0.10	3	104	101	12.9 ± 0.5	15.6 ± 1.0
	"	Growth hormone	0.50	6	111	125	0	16.1 ± 2.3
	"	Growth hormone	0.50				p = 0.001	p = 0.10
	"	+	+	4	117	141	0	10.1 ± 1.2
		Cpd. F	0.10				p = 0.10	12.5 ± 1.3
	"	Growth hormone	0.50					13.1 ± 1.4
		+	+	4	122	128	0	14.3 ± 1.8
		DOCAS§§	0.01				16.3 ± 1.2	13.4 ± 1.1

* All animals castrated at 43 days, hypophysectomized at 44 days and sacrificed at 55 days.

H = Hypophysectomized; C = Castrated; A = Adrenalectomized.

** Mean ± standard error.

† The experimental group indicated was compared with the appropriate control.

†† The active core of a chymotrypsin-digested preparation.

§ Hydrocortisone in saline suspension, Sharp & Dohme, Philadelphia, Pa.

§§ Desoxycorticosterone injected as a sesame oil solution.

Table 4.

Effect of ACTH on the Weights of the Sex Accessories of the Hypophysectomized-Castrated Male Rat
Before and After Adrenalectomy.

Animals*	Treatment	Daily Dose	No. of Rats	Body Weights		Adrenals	Ventral Prostate	Seminal Vesicles
				Onset	Autopsy			
♂	H-C	Saline	0	3	133	122	9.6 ± 1.3**	13.0 ± 0.9
	"	ACTH-F Fraction	0.020	4	117	114	21.8 ± 2.2	13.3 ± 0.7
	"	"	0.050	4	128	110	43.7 ± 3.7	16.9 ± 1.9
							p = 0.10***	p < 0.20
	"	"	0.065	4	114	96	53.0 ± 6.5	14.5 ± 1.8
	"	Saline	0	6	122	120	8.8 ± 0.3	11.6 ± 1.2
	"	α-corticotrophin†	0.020	3	119	102	27.6 ± 4.7	14.8 ± 4.7
	"	"	0.030	4	112	100	58.3 ± 8.6	15.6 ± 2.5
							p < 0.20	
	H-C-A	"	0.030	4	112	101	0	9.2 ± 2.0
	"	DOCA††	0.100	4	111	114	0	12.3 ± 1.6
	"	α-corticotrophin + DOCA	0.030 + 0.100	6	106	113	0	11.0 ± 1.1
								11.9 ± 0.5

* All animals castrated at 43 days, hypophysectomized at 44 days and sacrificed at 55 days.

** Mean ± standard error.

*** The experimental group indicated was compared with the appropriate control.

† The data for α-corticotrophin is taken from Li *et al.*, J. Exper. Med., in press.

†† Desoxycorticosterone injected as a sesame oil solution.

other in stimulating the prostate is available from studies with both hormones, given simultaneously. While a small amount of cytoplasm could be detected within the epithelial cells of the animals treated with growth hormone, approximately twice the amount appeared in the cells of the animals that were simultaneously injected with ACTH in a dosage that was itself effective in producing prostatic enlargement (Fig. 2 d). Weight values, *per se*, do not reflect this improvement.

With respect to the adrenal gland itself, distinct complementary influences on the part of these two hormones are apparent. When growth hormone was administered together with a low dose of ACTH (0.020 mg. ACTH-F), the two effects were simply additive as evidenced by the weights of the adrenal glands. However, when growth hormone was administered together with ACTH, at dose levels of the latter sufficient to produce adrenals larger than those of the intact animals, the effects were no longer merely additive, but a synergism between the two hormones was evident (Table 5). For example, 0.065 mg. of the ACTH preparation increased the adrenal weights to 53.0 mg., whereas the same level of ACTH given concurrently with 0.50 mg. of growth hormone produced glands averaging 79.7 mg. Evidence of this synergism between ACTH and growth hormone is also apparent in the animals injected with 0.050 mg. of ACTH and 0.50 mg. growth hormone.

Histologically detectable changes in lipid distribution accompany the increases in the weights of the adrenal glands. Lipid depletion of the inner zones of the cortex, such as is seen ordinarily with similar doses of ACTH, was obtained with 0.50 mg. of the ACTH-F fraction, but, although only trace amounts of sudanophil-staining material remained in the adrenal cortices of the animals treated with 0.50 mg. of the ACTH-F fraction, the adrenals taken from a corresponding group injected with the same dose of ACTH plus 0.50 mg. of growth hormone contained considerable quantities of sudanophilic material. The lipid was distributed throughout the fasciculata and reticularis, and sinusoidal distension was very marked. Analogous differences were seen between the group treated with 0.065 mg. of ACTH alone and that given a combination of 0.065 mg. ACTH and 0.50 mg. growth hormone.

Effect of Steroid Hormones. All the C₂₁ steroids employed in these studies were administered at a dose level of 0.50 mg. per day. This dose is 100 times the dose of testosterone propionate required for restoring the weight of the ventral prostate to that of the non-hypophysectomized-castrated animal. Corticosterone, cortisone, hydrocortisone and progesterone were without effect on the reproductive accessories. On the other hand, 17- α -OH-progesterone, 11-desoxy-17-OH-corticosterone and 11-dehydro-corticosterone displayed similar androgenic capacities equivalent to approximately 0.004 mg. of testosterone propionate. DOCA showed only a slight positive effect. These results are summarized in Table 6.

Table 5.

Effect of the Combined Administration of Growth Hormone and ACTH on the Weights of the Sex Accessories of the Hypophysectomized-Castrated Male Rat.*

Treatment	Daily Dose	No. of Rats	Body Weights		Adrenals	Ventral Prostate	Seminal Vesicles
			Onset	Autopsy			
	mg.		gm.	gm.	mg.	mg.	mg.
Saline	0	4	115	117	10.2 ± 0.4**	14.6 ± 0.7	11.5 ± 0.7
Growth hormone	0.50	8	125	148	14.7 ± 0.9	16.3 ± 0.5	14.1 ± 1.0
ACTH	0.020	4	117	114	21.8 ± 2.2	13.3 ± 0.7	13.0 ± 1.8
»	0.050	4	128	110	43.7 ± 3.7	16.9 ± 1.9	16.3 ± 1.7
»	0.065	4	114	96	53.0 ± 6.5	14.5 ± 1.8	13.7 ± 0.8
Growth hormone + ACTH	0.50 + 0.020	3	118	136	24.2 ± 0.7	17.6 ± 1.6	16.6 ± 2.5
»	0.50 + 0.050	4	130	117	57.5 ± 1.1	16.0 ± 1.5	18.1 ± 2.1
»	0.50 + 0.065	4	118	104	79.7 ± 7.7	13.8 ± 1.2	16.0 ± 0.7

* All animals castrated at 43 days, hypophysectomized at 44 days and sacrificed at 55 days.

** Mean ± standard error.

† The experimental group indicated was compared with the appropriate control.

Table 6.
Effect of Steroids on the Weights of the Sex Accessories of the Hypophysectomized-Castrated Male Rat.*

Treatment**	Body Weights		Adrenals	Ventral Prostate	Seminal Vesicles
	Onset	Autopsy			
Sesame oil	121	110	10.3 ± 0.4***	11.0 ± 0.2	12.5 ± 1.3
Corticosterone†	107	108	11.7 ± 0.6	11.4 ± 0.9	12.0 ± 0.9
Cortisone†	108	100	10.5 ± 0.02	12.5 ± 1.6	14.3 ± 0.5
Hydrocortisone††	132	113		12.1 ± 1.5	17.5 ± 2.8
11-desoxy-17-OH-corticosterone†††	132	133		14.9 ± 1.3	22.8 ± 2.6
				p < 0.5§	p = 0.02
11-dehydro-corticosterone†††	137	129		15.4 ± 2.8	20.2 ± 2.4
				p = 0.2	p = 0.05
Desoxy-corticosterone	106	115	10.8 ± 0.5	13.4 ± 0.5	12.7 ± 0.6
17-OH-progesterone†††	108	112	10.4 ± 1.1	14.1 ± 1.7	13.1 ± 1.2
				p < 0.2	
Progesterone§§	108	113	11.7 ± 0.7	12.4 ± 0.7	9.8 ± 0.8

* All animals castrated at 43 days, hypophysectomized at 44 days and sacrificed at 55 days.

** Injections begun at 45 days and continued for 10 days; daily dose = 0.50 mg.; 4 animals/group.

*** Mean ± standard error.

† Merck and Co., Inc., Rahway, New Jersey.

†† Sharp and Dohme, Philadelphia, Pa.

††† The Upjohn Co., Kalamazoo, Mich.

§ The experimental group indicated was compared with the appropriate control.

§§ Parke Davis Co., Detroit, Mich.

DISCUSSION

In the present investigation we have found that none of the gonadotrophins (FSH, ICSH, HCG or prolactin) were effective in inducing any detectable improvement in the ventral prostate or seminal vesicles of hypophysectomized-castrated rats at a daily dose level of 0.50 mg. given for a ten-day period. DL-thyroxin at a dose of 0.003 mg. was likewise ineffective. On the other hand, the administration of either the ACTH-F fraction or α -corticotrophin did result in an improvement in the sex accessories that could readily be detected in histological sections, even though the increase in the weights of the glands were not statistically significant. As expected, no stimulation was obtained in the adrenalectomized animal with any ACTH treatment. Furthermore, the efficacy of ACTH in stimulating the production of a detectable amount of androgen was related to the dose level that was injected. While at moderate dosages the hormone was effective in stimulating the ventral prostate and seminal vesicles, with either low or high dosages there was no evidence of stimulation.

The studies with corticosterone and hydrocortisone indicate that the stimulation of the secondary sex accessories of the hypophysectomized-castrated animal probably cannot be attributed to an overproduction of either of these two C₂₁ glucocorticoids. On the other hand, the results obtained with 17- α -OH-progesterone, 11-dehydrocorticosterone and 11-desoxy-17-OH-corticosterone reveal that these steroids do manifest a slight stimulatory capacity at a level of 0.50 mg. Inasmuch as they have been identified as intermediates in the synthesis of the glucocorticoids by the adrenal glands, it would appear that these or similar precursors to corticosterone or hydrocortisone may well be responsible for the prostatic stimulation obtained with moderate dosages of ACTH. In this connection, it is interesting to note that a similar explanation has been proposed for the production of substantial quantities of androgen by the adrenal glands of human patients showing the virilism characteristic of the adrenogenital syndrome (*Jailer*, 1953, and *Dorfman*, 1955).

It is not possible, nonetheless, to explain the observed androgenic effects as a simple enzyme-saturation phenomenon which would lead to the secretion of testoid precursors of corticosterone or hydrocortisone by the adrenal gland. Were these intermediates, or any others, responsible for the androgenicity, prostatic stimulation should be accentuated rather than obliterated with high doses of ACTH; however, it was observed that animals given sufficient ACTH to induce severe hyperplasia and grossly visible hyperaemia of the adrenal glands did show distinct signs of hypercorticoidism. Although the prolonged administration of ACTH to the rabbit favours the release of larger quantities of the more potent hydrocortisone (*Kass et al.*, 1954), there is no published data to indicate that a similar shift in the secretory pattern of the adrenal gland occurs in the rat. Nevertheless, it seems quite likely that the absence of detectable stimulation of the ventral prostate at high levels of

ACTH, as observed in this study and as reported by other investigators (*VanderLaan*, 1953, and *Gaunt & Parkins*, 1933), may well be attributed to the antianabolic or catabolic reactions accompanying the hypercorticoid condition. In the light of our experience with ACTH administration, it seems rather unlikely that the production of substantial amounts of adrenal androgen can be demonstrated in »*in vivo*« studies unless some way can be found to block the synthesis of the highly-active glucocorticoids.

Another important consideration evolving from this study concerns the relationship of growth hormone to other endocrine-mediated reactions. *Ingle* (1952) has suggested for some time that adrenal steroids play a permissive role in physiological processes. Our own results in triply-operated rats treated with growth hormone support this thesis. In the absence of the adrenals, the prostatic improvement regularly seen following the administration of growth hormone was obtained only when an adrenal steroid was also given. Whereas the effect of growth hormone is independent of the adrenal gland, *per se*, it is dependent on the presence of a small amount of an active corticoid.

It is of interest to note that the adrenal gland manifests significant weight increases with a daily dose of growth hormone as low as 0.25 mg. The increase cannot be attributed to a trace contamination of ACTH. In frozen sections of the adrenal stained with Scharlach R, no change in the fat distribution can be detected following treatment with growth hormone. Moreover, a growth hormone preparation that has been treated with chymotrypsin is as effective as an untreated preparation in promoting a weight increase in the adrenal gland. This is important since chymotryptic digestion rapidly destroys the biological activity of ACTH under conditions where full growth hormone potency is retained in the growth hormone digest (*Li et al.*, 1956 a and b). It therefore seems highly probable that if an ACTH contaminant were present in the growth hormone preparation in trace amounts, it would be attacked by the enzyme and its activity destroyed. The fact that the active core of the chymotrypsin-treated growth hormone promotes a significant increase in adrenal weight constitutes further evidence that growth hormone itself is effective in stimulating the adrenal gland. Finally, the marked enlargement of the gland and the lipid engorgement of the individual cortical cells that are obtained with high ACTH doses and growth hormone are atypical of any effect observed with ACTH alone. From their studies on adrenocortical mitotic activity in the hypophysectomized rat, *Cater & Stack-Dunne* (1954) have also drawn the conclusion that there is an effect, not attributable to any contamination by ACTH, exercised by growth hormone preparations on the adrenal gland.

Distinct histological changes accompany the marked increases in the adrenal weights observed with high doses of ACTH and growth hormone. The overall picture leaves one with the impression that while ACTH is moving the sudanophil material out of the adrenal, growth hormone is replacing it. Growth hor-

mone could accomplish this by simply making available to the gland the raw materials which are rapidly being used up in the secretory process. Inasmuch as considerable amounts of stored lipid material is present in the adrenal of the hypophysectomized animal, it is extremely difficult to ascertain what subtle changes may be occurring at lower doses of ACTH. Consequently, these observations, strictly speaking, apply only to a hyperactive gland, and the significance of these findings in relation to physiological processes remains to be demonstrated. Nevertheless, it seems reasonable to assume that growth hormone performs a similar function with all dose levels of ACTH.

S U M M A R Y

The participation of pituitary factors in the maintenance of the sex accessories has been reinvestigated in the adolescent hypophysectomized-castrated male rat. Thyroxin and the gonadotrophins (incl. prolactin) were found to be without effect. Corticotrophin (ACTH), on the other hand, induced histologically detectable androgen production at moderate dose levels. Slight, but statistically insignificant, weight increases in the ventral prostate and seminal vesicles were associated with the observed histological improvement. The effect was obliterated at high dose levels of ACTH which were accompanied by signs of hypercorticoidism.

Growth hormone also was found to be involved in the restoration process. Its effect is independent of the adrenal gland, *per se*, and manifests itself histologically in a general improvement in the secretory epithelium and connective tissue stroma of the accessory structures. Adrenal enlargement invariably resulted from growth hormone administration. Evidence is presented in support of the thesis that the effect of growth hormone on the adrenal is distinct from the effect of ACTH and that growth hormone actually enhances the action of the latter.

A C K N O W L E D G M E N T S

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