10028

Spermatogenesis in Immature Hypophysectomized Rats Injected with Androgens.

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Naturally occurring or synthetic androgenic substances injected into adult rats shortly after hypophysectomy will cause varying degrees of tubular maintenance in the testes,[1-5] but the interstitial cells are not prevented from undergoing atrophy.[2-5] After tubular atrophy in hypophysectomized rats has occurred, it has not been possible to restore spermatogenic activity by means of androgens.[2-5] Maintenance of spermatogenesis in hypophysectomized rats by androgens has not as yet been satisfactorily explained. The possibility that androgens have only an indirect effect on the germinal epithelium, scrotal maintenance being of prime importance,[3, 5] has not been accepted by Nelson and Merckel.[4] Incontestable evidence that male hormones have a direct or only an indirect effect on spermatogenesis is still lacking.

It has seemed of importance in view of our present lack of understanding with regard to the rôle played by androgens in spermatogenesis to study the effects of male hormones in immature hypophysectomized rats. This has seemed especially important since it has been shown that bull testis extracts[6] and androsterone[7] cause marked injury to the testicular tubules of young rats. The purpose of this investigation was to discover whether spermatogenesis would occur in immature hypophysectomized rats injected with androgenic substances. As we have never observed sperm heads, as described by Moore,[8] earlier than the 34th day of life, we selected animals much younger than this to rule out the possibility that sperm

1 Walsh, E. L., Cuyler, W. K., and McCullagh, D. R., *Am. J. Physiol.*, 1934, **107**, 508.

2 Nelson, W. O., and Gallagher, T. F., *Science*, 1936, **84**, 230.

3 Cutuly, E., McCullagh, D. R., and Cutuly, E. C., *Am. J. Physiol.*, 1937, **119**, 121.

4 Nelson, W. O., and Merckel, C. G., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 825.

5 Cutuly, E., McCullagh, D. R., and Cutuly, E., *Am. J. Physiol.*, 1938, **121**, 786.

6 Moore, C. R., and Price, D., *Am. J. Anat.*, 1932, **50**, 13.

7 Moore, C. R., and Price, D., *Endocrinology*, 1937, **21**, 313.

8 Moore, C. R., *Am. J. Anat.*, 1936, **59**, 63.

TABLE I.

Litter	Rat No.	Age at start of expt (days)	Total No. days hypophysectomized	Daily injections: Substance	Amt (gamma)	Oil (cc)	Total No.	Autopsy data: Age (days)	Wt. (g): Testes	Seminal vesicles	Adrenals	Histology of testis tubules†
1	39	27	7	TP‡	50	1/20	6	34	.20	.016	.011	Atrophic
	40	27	7	Hypophysectomized control				34	.19	.006	.012	"
	41	27	—	Normal control				34	.80	.011	.027	IV
2	42	27	12	TP	50	1/20	11	39	.14	.047	.009	Atrophic
	43	27	21	TP	50*	1/20	20	48	.13	.427	.007	"
	45	27	21	TP	50*	1/20	20	48	.11	.655	.008	"
	44	27	12	Hypophysectomized control				39	.11	.005	.008	"
	46	27	—	Normal control				39	1.21	.022	.029	V
3	47	28	16	TP	100	1/20	15	44	.16	.305	.009	Atrophic
	48	28	16	TP	200	1/20	15	44	.23	.541	.016	"
	49	28	16	TP	300	1/20	15	44	.29	.643	.008	"
	51	28	—	Normal control				44	1.47	.040	.031	V
4	71	29	—	Reference control				29	.46	—	.025	II
	66	29	8	TP	2000	1/5	7	37	.54	.231	.018	IV
	67	29	8	TP	2000	1/5	7	37	.59	.196	.015	V
	68	29	8	DA‡	2000	1/5	7	37	.42	.033	.012	II
	69	29	8	Hypophysectomized control				37	.21	.008	.010	Atrophic
	71	29	—	Normal control				37	.83	.020	.022	IV
5	61	30	14	DA	2000	1/5	14	44	.63	.099	.010	V
	60	30	14	TP	2000	1/5	14	44	.59	.592	.014	V
	64	30	14	TP	2000	1/5	14	44	.62	.646	.016	V
	62	30	Normal	TP	2000	1/5	14	44	1.22	.785	.038	V
	59	30	14	Hypophysectomized control				44	.22	.013	.009	I
	65	30	—	Normal control				44	1.35	.053	.025	V

†I to V denote most advanced stage of spermatogenesis seen in tubules. I—primary spermatocytes; II—secondary spermatocytes; IV—sperm heads; V—spermatozoa.

‡TP—testosterone propionate and DA—dehydroandrosterone acetate.

*The last 9 injections were increased to 300 gamma daily.

heads or spermatozoa were present in the testes prior to the beginning of the experiment.

As will be seen in Table I five litters of rats were used. Their ages ranged from 27 to 30 days. In each litter one animal was set aside as a normal control; in 4 of the litters there was also a hypophysectomized control. Litter 4 contained a reference control which was killed at the start of the experiment. Hypophysectomized rats (and one normal rat in litter 5) were injected subcutaneously with testosterone propionate or dehydroandrosterone acetate.* Autopsies were performed 24 hours after the final injection, except in litter 4, where they were performed 48 hours after the last injection. The daily doses ranged from 50γ to 2 mg. Data such as the amount of oil (sesame) vehicle, length of experiment, and autopsy findings will be found in the table. It is realized that the series of animals is small. However, the appearance of sperm heads or spermatozoa in testicular tubules previously lacking such structures is a readily demonstrable and unequivocal criterion. The completeness of all the hypophysectomies was checked by a critical study of serial sections of the pituitary capsules which had been carefully dissected away from the sella. In addition the pieces of gland aspirated into the suction tube at the time of operation were always recovered to see if the fragments (usually 3) could be pieced together to form the whole gland. Finally, no hypophysectomized animal was included in the table unless its autopsy body weight was less than its initial weight, or unless the increase in autopsy body weight was far less than that in normal controls.

The data on litters 1, 2, and 3, in Table I show that testosterone propionate in daily doses as high as 300γ failed to have any effect upon the testes, even though the larger doses produced marked stimulation of the seminal vesicles. Testosterone propionate at the 50γ level caused scarcely any noticeable scrotal stimulation; and even at the 300γ level the scrotal response was weak. This was unexpected, as other observations[9, 10] have indicated that the scrotum is very sensitive to stimulation by male hormone produced by the testes. Since the scrotal turgescence induced by 300γ of testosterone propionate was slight, and despite the fact that the accessories were tremendously enlarged; it was decided to inject male hormone at much higher levels. This was done in litters 4 and 5.

* We are indebted to Dr. Erwin Schwenk of Schering Corporation, Bloomfield, N. J., for the androgenic substances.

9 Cutuly, E., and Cutuly, E. C., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**. 477.

10 Cutuly, E., and Cutuly, E. C., *Endocrinology*, 1938, **22**, 568.

The following results were obtained in litter 4. Two milligrams of testosterone propionate were injected daily for 7 days into rats hypophysectomized when 29 days of age. This amount of hormone caused an increase in testis weight beyond that in the reference control, but not equal to that in the normal control. However, the testis tubules of the injected animals, as well as those of the normal control, contained sperm heads or spermatozoa, while those of the reference control contained only secondary spermatocytes. Dehydroandrosterone acetate at a 2 mg level was unable to advance the condition of the testes beyond that in the reference control; but it did prevent the testes from undergoing the profound atrophy seen in the hypophysectomized control. In litter 5 both dehydroandrosterone acetate and testosterone propionate when given in daily doses of 2 mg for 14 days after hypophysectomy induced the formation of mature spermatozoa in the seminiferous tubules, although the testis weight was only about half that of the normal control. It is interesting to note that the normal animal in litter 5 which received 2 mg of testosterone propionate daily had testes which weighed just slightly less than those of the normal control. Histologically the testes of the injected normal animal showed perfectly normal tubules; but the interstitial cells of the testes were noticeably not as well developed as those in the normal control.

In all the hypophysectomized animals, treated or untreated, the interstitial cells were markedly atrophic, and this would account for some of the weight differences between the testes of these animals and those of the controls. Weight variations, however, were chiefly due to differences in the size of the tubules. This was indicated by measurements on tubules made with a micrometer eyepiece. For example, in litter 4 an average of 20 tubules for each testis measured showed that the diameter of the tubules of the normal control was 189.4μ, while that of rat numbers 66, 67, 68, and 69 was 167.9, 159, 161.8, and 119.5, respectively.

Of special interest was the finding that some of the hypophysectomized animals injected with testosterone propionate (rats Nos. 48, 66, 67, 60, 64) had adrenal glands which were not as small as might have been expected. Examination of histological sections of these glands showed them to be readily distinguishable from sections of normal adrenals, since they possessed typically shrunken cortices. The unexpectedly high weight of these glands was probably mostly due to variable quantities of lipoidal material in the cortex, since it has been demonstrated that the medulla does not change greatly in weight following hypophysectomy.[11] Although the ad-

[11] Cutuly, E., *Anat. Rec.*, 1936, **66**, 119.

renal cortex of rat 66 was characteristically shrunken, mitotic figures were seen in or near the glomerular zone. These observations tend to suggest (a) a possible direct or indirect action of male hormone on the adrenal cortex and (b) a possible connection between the adrenal cortex, male hormone, and spermatogenesis.

It has been very difficult to reconcile the data showing that male hormones maintain spermatogenesis in adult hypophysectomized rats,[1-5] initiate spermatogenesis in ground squirrels,[12] fail to maintain spermatogenesis in hypophysectomized monkeys[13] and hypophysectomized guinea pigs,[14] and cause marked injury to the testis tubules in normal young rats.[6, 7] The present study shows that spermatogenesis can be induced in hypophysectomized rats with previously aspermatic testes. The relatively large dose necessary for this suggests that dosage is possibly responsible for the apparent discrepancies which have been mentioned. Size of accessory organs, as shown by this investigation, is certainly no criterion of the adequacy of the dose of androgen necessary to induce spermatogenesis.

It has already been pointed out that there is little correlation between the ability of an androgen to maintain testis tubules and its ability to stimulate the accessory sexual organs.[4, 5] The present findings not only confirm these reports, but they emphasize the fact that relatively enormous doses of the most potent androgen now available (testosterone propionate) are needed to cause a tubular response. It is difficult to understand why this should be so, especially when it is remembered that in the normal immature rat the testis tubules may develop sperm heads before the seminal vesicles show any appreciable secretory activity.[8] Because of this contrast between normal development and that brought about by a male hormone such as testosterone propionate, the following point seems worthy of mention. If the testis hormone (or hormones) produced by the interstitial cells normally has a stimulating effect on the tubules of the testis, then testosterone propionate is decidedly inferior to testis hormone in this respect. This inferiority on the part of an intensely strong androgenic substance may indicate (1) a real dissimilarity between it and interstitial cell hormone; (2) the inability of such a substance to express itself fully in a hypophysectomized animal, or the inability of organs to respond fully to it; (3) that it acts only indirectly on the spermatogenic elements.

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12 Wells, L. J., and Moore, C. R., *Anat. Rec.*, 1936, **66**, 181.

13 Smith, P. E., and Engle, E. T., mentioned by Scowen, E. F.

14 Scowen, E. F., *Anat. Rec.*, 1938, **70**, 71 (suppl.).

Summary. Testosterone propionate and dehydroandrosterone acetate when administered daily at a 2 mg level to immature hypophysectomized rats induced sperm head or spermatozoon formation in the seminiferous tubules. Smaller doses were ineffective in this respect, even though they caused marked stimulation of the accessory organs. Testosterone propionate seemed in a few instances partially to prevent the adrenal cortex shrinkage which follows hypophysectomy; it is not known whether this effect was the result of a direct or indirect action by male hormone. An explanation which helps to reconcile the apparently discordant reports on the effects of androgens on spermatogenesis is suggested.

10029

Vitamin B_1 in Bacterial Metabolism.*

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Since the work of Peters[1] showing that the oxidation of pyruvic acid in pigeon-brain requires the presence of vitamin B_1, attention has been called to the function of this vitamin in the pyruvate-metabolism of yeast and bacteria. Lohmann and Schuster[2] have demonstrated that the co-enzyme of carboxylase, co-carboxylase, is diphosphorylated vitamin B_1. Lipmann[3] has reported that the phosphorylated vitamin is essential for the dismutation of pyruvate by an acetone preparation of *Bacillus acidificans longissimus.* In the same paper he states that the non-phosphorylated vitamin is without stimulating effect. Krebs[4] reports that no stimulation in the metabolism of pyruvates by *Staphylococcus aureus* could be obtained on the addition of vitamin B_1. However, Hills[5] has reported a marked stimulation in the pyruvate-metabolism of *Staph. aureus* grown in vitamin B_1-deficient media by the simple addition of crystalline vitamin B_1.

The purpose of the present investigation was to determine the

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1 Peters, R. A., *Biochem. J.*, 1936, **30**, 2206.

2 Lohmann, K., and Schuster, Ph., *Angew. Chem.*, 1937, **50**, 221.

3 Lipmann, F., *Enzymologia*, 1937, **4**, 65.

4 Krebs, H. A., *Biochem. J.*, 1937, **31**, 661.

5 Hills, G. M., *Biochem. J.*, 1938, **32**, 383.