SOME ENDOCRINE AND RELATED FACTORS INFLUENCING SPERMATOGENESIS

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In the last two or three decades, important papers have appeared concerning the effects on spermatogenesis of diet including vitamins, of bacterial toxins, of alcohol, of tuberculin, of vasectomy, of testicular transplantations, of radiation of light, of seasonal rhythms, of sexual excess, of venereal disease, and of age, but a complete review of the literature is impossible here. The purpose of this report is to summarize some of the results of recent studies on experimentally induced testicular atrophy and repair and to indicate the possible value of such findings clinically.

HYPOPHYSECTOMY

That gonadal activity is dependent on the anterior lobe of the pituitary gland has been known since Crowe, Cushing and Homans[1] performed their notable experiments on dogs. They demonstrated that removal of the posterior lobe of the pituitary gland did not influence the genital development of dogs but that complete removal of the gland resulted in an atrophic condition of these organs. Some investigators have claimed that the genital atrophy following hypophysectomy is due to brain injury. Although brain injury may influence the genitalia, there now remains no question but that prehypophyseal hormones are necessary for normal gonadal activity.

A great portion of our knowledge concerning the effects of hypophysectomy has been the result of observations on the rat, commencing with the classical work of Smith[2]. If male rats are hypophysectomized before puberty, they never become sexually mature. The accessory sex glands remain infantile in appearance and the testes decrease in size. Spermatogonia and Sertoli cells remain normal in appearance but spermatogenesis does not occur. If the operation is performed after maturity, a similar picture results. The testes become greatly reduced in size and much less firm in consistency. Spermatozoa, spermatids, and spermatocytes rapidly disappear from the seminiferous tubules. Sertoli cells and spermatogonia remain, the latter continuing to undergo mitotic division for months after the operation.

As pointed out by Smith[3], destruction of the seminiferous tubules frequently is accompanied by a relative, though not absolute, increase

in the cells of Leydig. Following hypophysectomy, however, the Leydig cells become atrophic in appearance and the testes no longer produce male sex hormone as evidenced by the fact that the accessory sex glands become markedly atrophic. These degenerative changes following hypophysectomy always occur very rapidly following the operation, being noticeable five days later and almost complete in 18 days. In Table 1 the weights of organs of normal rats and of rats

TABLE 1

Average Weight (Grams) of Rats Hypophysectomized for 18 Days and of Rats Hypophysectomized and Treated with 1.5 mg. Androsterone Daily for 18 Days.

	Normal Control	*Hypophysectomized Control*	*Hypophysectomized Treated*
No. of Animals	8	4	7
Testes	2.41	0.54	1.57
Seminal Vesicles	0.745	0.096	0.431
Ventral Prostate	0.325	0.034	0.239
Dorsal Prostate	0.185	0.042	0.139

hypophysectomized for 18 days are compared. There can be no doubt but that the hypophysectomized rats were lacking in male sex hormone. The importance of this will be discussed later.

Recently, studies have been made of the scrota of hypophysectomized rats. We have shown[4] that the scrotum behaves as do the other accessory sex organs in that it rapidly regresses following hypophysectomy. About one week following hypophysectomy, the scrotum has greatly decreased in size and we believe this to be due, in part at least, to lack of male sex hormone. Hamilton[5] has also adduced evidence indicating that the scrotum is controlled by male sex hormone. Following hypophysectomy, one of the first observable signs of abnormality in the male rat is the disappearance of the testes from the scrotum about ten days after the operation. Our observations have led us to believe that this is not the result of pressure caused by the regressing scrotum but is an actual ascent of the testes into the abdomen.

CRYPTORCHIDISM AND HEAT

It has long been known that in man and most other mammals spermatogenesis does not occur unless the testes occupy a scrotal position. Outstanding among the contributions on this subject are those of Moore and his associates. The subject has recently been reviewed by Moore[6]. Cryptorchidism does not significantly interfere with the

power of the testes to maintain the accessory sex organs for at least a period of months. This is true even when very young animals are made cryptorchid by surgical means—normal sexual development occurs but the spermatogenic elements fail to mature or function. When adult rats or guinea pigs are made cryptorchid, the seminiferous tubules undergo amazingly rapid degeneration so that, at the end of a week or ten days, no spermatozoa and rarely spermatids can be found. Later, even the spermatogonia disappear and only Sertoli cells remain. The testes become very small and flabby. As long as spermatogonia exist, recovery of spermatogenic function will take place if the testis is brought into the scrotum.

Moore has been able to prove quite conclusively that, in those animals in which spermatogenic activity requires the scrotal position of the testes, the scrotum acts as a thermoregulator and that its influence on spermatogenesis is due to this property. The nature of the scrotum is such that its temperature is always several degrees lower than the intra-abdominal temperature of the animal. If the scrotal temperature is artificially increased, the epithelium of the seminiferous tubules is partially or completely destroyed. Insulation around the scrotum sufficient to force the temperature to that of the adjacent tissues will cause testicular destruction. Temperatures slightly above body heat will cause tubular damage in a few minutes.

The reason for the survival of the testes in the abdominal cavities of some mammals is unknown. It may be that a more complete investigation of the changes in body temperature will throw some light on the subject. A recent and interesting investigation by Riley[7] on the diurnal nature of spermatogenesis in the sparrrow indicates that short periods of low temperature may be of considerable importance in spermatogenesis in certain species. In the sparrow, the temperature varies from 110° F. during the day to 103° F. during the night. It is only during the period of lowered temperature that spermatogenesis occurs. If the light and dark of day and night be artificially reversed, the temperature of the bird is reduced during astronomical daytime and spermatogenesis proceeds during the afternoon.

The Injection of Pituitary and Pituitary-Like Hormones and Parabiosis

The question of the number and the nature of gonadokinetic hormones in the anterior lobe of the pituitary gland is one of the most controversial in endocrine literature. Whether or not any of these substances directly influence spermatogenesis remains open to question. There is, however, no doubt concerning the fact that the genital atrophy which occurs following hypophysectomy can be completely repaired by im-

plantation or injection of pituitary substance. This includes recovery of the ability of the testes to produce spermatozoa.

Unfortunately, from the point of view of the work under discussion, more investigation of pituitary-gonadal relationship has been made using female than male animals. Smith, in reviewing the subject of the gonadotropic hormones, is inclined to agree with those workers who believe that there are at least two gonadotropic factors—one the "follicle-stimulating" (FSH) and the other the "luteinizing" (LH) hormone. The names obviously are derived from the action in the female but it is believed by those who adhere to this idea that FSH acts as a gametokinetic hormone in both sexes and that LH stimulates the theca cells in the ovary and the interstitial cells in the testes. The experiments of Greep, Fevold and Hisaw[8] lend strong support to the view of the duality of pituitary gonadotropic function. Proponents of this idea also feel that nature has demonstrated a difference between gonadotropic hormones and that during the menopause and following castration the blood and urine contain largely FSH, probably of pituitary origin. It is also pointed out that the gonadotropic hormone of human pregnancy urine, although possibly not of hypophyseal origin, meets most of the requirements of a true LH.

In this laboratory we have carried out experiments designed to elucidate this problem more completely. Smith and Leonard[9] and Evans and his associates[10] state that the seminiferous epithelium is stimulated by extracts of pregnancy urine. This is denied by Collip[11]. We find that intensive treatment with pregnancy urine of hypophysectomized adult rats in which the testes have regressed will result in a limited degree of stimulation of the germ cell line. The bulk of evidence seems to show that pregnancy urine is not a pure interstitial cell-stimulating hormone (comparable to LH).

The problem of the type of hormone coming from the pituitary of castrated animals was next investigated[12]. The technic employed appeared to us to be as unequivocal as any available. In testing for any endocrine product, the most sensitive test animal is one from which the gland producing that hormone is removed. Therefore, in testing for gonadotropic hormone we used hypophysectomized animals. The problem of the frequency of treatment of the test animals was precluded by uniting them in parabiosis with the experimental animals and thus having a continuous administration of hormone. From the results of these experiments, there could be no doubt but that the pituitary glands of castrated rats produced a greater amount of gonadotropic substance than did those of normal animals. There was, however, no indication of any selective gametokinetic or interstitial cell stimulating activity.

THE INJECTION OF ANDROGENS

Effect of Injection of Androgens in Hypophysectomized Animals

For some time there has been no doubt that adequate doses of male sex hormone will prevent atrophy of accessory sexual organs in hypophysectomized as well as in castrated rats. Freud[13] demonstrated this in castrated hypophysectomized rats using hombreol or testicular extracts. Vatna[14] observed repair of the accessory glands of hypophysectomized rats using bull testis extracts but was unable to detect any repair of the testicular tubules if injections were begun three weeks after the operation. Walsh, Cuyler and McCullagh[15] studied the effect of androtin (fat soluble sex hormone of human male urine) on adult hypophysectomized rats and found that it prevented atrophy not only of the accessory organs but also of the testes. The urinary extracts were unquestionably free of pituitary or pituitary-like hormone. Nelson and Gallagher[16] recently confirmed this work and considerably extended it by demonstrating that urinary androgens would not only maintain the normal appearance of the testes of hypophysectomized rats over a very long period but that these rats were capable of siring normal litters. Nelson[17] has also shown that, in addition to androgens, progesterone possesses the property of maintaining spermatogenesis in the absence of the hypophysis and of hypophyseal hormones. Eight synthetic androgenic sterols* have been quantitatively investigated in this laboratory, the findings on only three of which have been reported[4]. The most important androgen in urine appears to be androsterone. Table 1 shows the effect of the injection of this substance into hypophysectomized rats. Although at this dose level (1.5 mg. daily), the testes of the treated animals were somewhat smaller than those of the normal controls, histological examination revealed normal spermatogenesis in the majority of the tubules. Using the weights of the prostate glands as a criterion of dose, these animals received only 75 per cent of a maintenance dose. Possibly with a larger dose there would be no signs of tubular degeneration. In this case, occasional tubules distributed throughout the whole of the gland showed signs of degeneration quite comparable to untreated hypophysectomized controls. Of the androgens so far investigated by us, the most efficient from the point of view of the maintenance of spermatogenesis in the absence of the hypophysis is dehydroandrosterone acetate. Spermatogenic stimulation under these circumstances is a common property in varying degrees of all androgens tested.

Maintenance of the testis tubules in hypophysectomized rats is not limited to pituitary extracts, pituitary-like hormones from urine, or

*Supplied through the courtesy of Dr. Erwin Schwenk of the Schering Corporation, Bloomfield, N. J.

gonadal hormones. Hisaw, Greep and Fevold[18] report that yeast extracts prevent degeneration of the seminiferous tubules of hypophysectomized rats. All substances which have been demonstrated to have this property also possess the property of maintaining the scrotum. This, of course, is to be expected since spermatogenesis cannot occur without the scrotum.

None of the androgens investigated have, as far as we can determine, prevented the usual atrophy of the interstitial cells of the testes in hypophysectomized rats. In this the androgens differ from the gonadotropic hormones. The indications are at present that there is another important difference between the effect of these two types of hormones. It has been noticed by Nelson[17] as well as in this laboratory that if the testes are permitted to regress following hypophysectomy the injection of androgens will not cause their regeneration.

Effect of Injection of Androgens Into Normal Male Animals Not Producing Spermatozoa

Moore and Price[19] showed that from 3 to 12 bird units of androgenic bull testes extracts led to a reduction in size of as much as 50 per cent in the testes of growing rats as compared to littermate controls. More recently, Moore and Price[20] showed that similar results were obtained when young rats were treated with androsterone. Wells and Moore[21] observed that in the ground squirrel (which is an annual breeding type as opposed to the constant breeding rat) a definite stimulation of spermatogenesis occurred following the administration of androgens from male urine and testes, as well as androsterone. During the first year of life, immature animals were caused to produce spermatozoa four months earlier than normal controls. The injection of androgens caused adult squirrels to produce spermatozoa normal in appearance and activity during seasons when they normally show no signs of testicular activity. One of the effects of the administration of androgens into sexually inactive squirrels was that the testes, which at that time are normally cryptorchid, descended into the scrotum. This may have been all that was necessary to cause spermatogenesis. Evidence is still lacking therefore that the androgens are directly gametokinetic.

Effect of Injection of Androgens Into Normal Adult Male Rats

At the present time, various androgens may be purchased for clinical use and further knowledge concerning possible undesirable reactions resulting from their administration seems to be indicated. This is particularly true when the problem of spermatogenesis is considered. It has been shown that androsterone produces a deleterious effect in young rats. The rapidity with which such animals recover from this

condition has not been reported. Moore and Price[20] showed that prolonged injection of large doses of androsterone into adult male rats did not have a deleterious effect upon the testes.

Androgens are known to depress pituitary activity. McCullagh and Walsh[22], using the parabiotic technic, injected urinary androgens into castrated rats and controlled the hyperactivity of the pituitary glands of the castrated animals. It seemed possible that large doses of androgens might depress the normal pituitary gland in such a fashion that, although no serious damage appeared during the course of the injection, hypogonadism of pituitary origin might result subsequent to the cessation of injections. For this reason, normal adult male rats have been

TABLE 2

Body and Organ Weights (Grams) of Normal Adult Rats Injected with Androstenediol (3 mg. in 0.5 cc. Sesame Oil) Subcutaneously Daily for 18 Days. Rats Were Autopsied at Various Intervals Following the Injection Period.

Final Body Weight	*Pituitary*	*Testes*	*Seminal Vesicles*	*Ventral Prostate*	*Days Autopsied after Final Injection*
274	.007	2.96	.728	.370	1
239	.008	1.98	1.455	.472	1
278	.007	2.82	.692	.272	1
266	.008	2.80	.814	.342	4
216	.006	2.59	1.265	.348	4
220	.008	2.74	.962	.500	6
182	.007	2.17	1.248	.671	6
275	.010	2.60	.762	.395	8
284	.008	2.33	.741	.349	8
243	.007	2.71	.578	.344	11
242	.006	2.69	.739	.406	11
287	.007	3.26	.574	.290	14
285	.008	1.38	.849	.287	14
219	.007	2.72	.468	.157	14
245	.008	2.84	.957	.381	18
262	.007	2.98	.611	.225	18
310	.010	2.71	1.111	.483	20
Uninjected Normal Controls (Average Weights for 8 Animals Sacrificed at Varying Periods During the Experiment).					
277	.008	2.85	1.026	.348	(4-20)

injected with large doses of androgens and subjected to necropsy at various periods after the last injection.

Table 2 shows the effect of the daily injection of 3 mg. of androstenediol for 18 days. By previous experiments, it had been ascertained that the dose of hormone would more than maintain the

accessory sexual glands of hypophysectomized or castrated adult male rats. The animals were sacrificed at periods varying from 1 to 20 days after the last injection. An examination of the weights of the organs indicates neither stimulation nor depression of the sexual glands.

TABLE 3

Body and Organ Weights of Normal Adult Rats Injected with Testosterone Propionate (1.5 mg. in 0.5 cc. Sesame Oil) Daily for 18 Days. Rats Were Autopsied at Various Intervals Following Injection Period.

Final Body Weight	*Pituitary*	*Testes*	*Seminal Vesicles*	*Ventral Prostate*	*Days Autopsied after Final Injection*
280	.007	2.52	2.220	.646	1
237	.007	2.74	1.859	.541	1
238	.007	2.84	1.909	.610	1
299	.008	3.06	2.194	.606	4
223	.006	3.14	1.666	.537	4
270	.007	2.63	1.989	.538	6
241	.007	2.24	1.351	.561	6
298	.007	2.54	.892	.346	8
204	.005	2.12	1.051	.325	8
213	.005	2.24	.882	.462	11
222	.007	2.18	1.112	.389	11
233	.007	2.69	.....	.391	14
233	.006	2.77	.942	.330	14
244	.007	2.51	1.357	.364	14
286	.007	2.60	1.082	.437	18
281	.007	2.89	1.322	.428	18
293	.009	2.41	.992	.467	20
218	.006	2.40	.705	.260	20
Uninjected Normal Controls (Average Weights for 9 Animals Sacrificed at Various Periods During the Experiment).					
241	.007	2.56	.923	.298	(4-20)

The experiment was repeated (Table 3) using the more potent compound testosterone propionate in doses of 1.5 mg. daily for 18 days. The animals were examined as in the preceding group. It had previously been determined that 1.5 mg. of testosterone propionate was not only more than sufficient to maintain the accessory glands of castrated rats but that it also caused marked changes in the pituitary glands. The weights of the organs do not indicate that the testes or pituitary glands were influenced in any way by this treatment.

The seminal vesicles and prostate were distinctly enlarged at the end of the injection period and returned to normal within twenty days. Had there been any marked hypofunction of the pituitary gland, the

curve of decrease in weight of the accessory glands would have been expected to be much sharper.

Discussion

It is well known that androgenic activity on the part of the testes is an essential factor in the care and transportation of spermatozoa from the time of their production to the time of their deposition in the female genital tract. Recent experiments show that androgenic activity is necessary for the production of spermatozoa. Our studies lead us to believe that the maintenance of the scrotum and the control of the factors which cause the testes to retain their scrotal position are dependent on the presence of androgens. As shown by the experiments on cryptorchid animals, these conditions are essential before spermatogenesis can proceed.

It remains to be determined whether or not androgens have a function other than the preparation of the stage for spermatogenesis. New experiments will have to be devised before it will be known whether or not androgens are directly gametokinetic.

The interpretation of the experiments concerning the rôle of the pituitary gland in the process of spermatogenesis is even more difficult. In the male it would seem that the most essential gonadotropic function of the pituitary gland is the stimulation of the interstitial cells of the testes. These cells produce androgens essential for spermatogenesis. The question then arises as to the reasons for postulating a gametokinetic activity on the part of the anterior lobe of the pituitary gland. The most important point experimentally is the fact that, by the use of androgens, it has been impossible to cause repair of posthypophysectomy atrophy of rat testes, whereas pituitary extracts will bring about this restoration. If one wishes to believe that the pituitary has no gametokinetic effect in the male rat, one can argue that the interstitial cell-stimulating hormone causes the testes to produce different and more effective androgens than those which we have injected. The existence of androsterone as well as testosterone indicates definitely the possibility that other intermediate androgens exist normally.

Because spermatogenesis cannot occur unless the testes are in the scrotal position, it is difficult to conceive that a purely gametokinetic effect may be demonstrated by the injection of pituitary preparations into male rats. Until new methods are available, it seems improbable that a final answer can be given to the problems suggested in this paper.

Sterility in the Human Male

Masculine ego has been blamed in part for the amazing lack of investigation into the responsibility of the male for barren marriages.

Although it has been recognized for years that potency did not indicate fertility, it is only within the last decade that serious efforts have been made to devise methods for the diagnosis and treatment of male sterility.

From the history and physical examination of a patient, facts of considerable diagnostic value may be garnered. The most valuable criterion, however, of fertility is to be found in the examination of the semen. This requires considerable experience and skill. The diagnosis of fertility by glancing down a microscope and seeing a few motile spermatozoa in a condom specimen is worse than useless in that it misleads both patient and physician. Hotchkiss[23] outlines the most essential points in the technic of sperm analysis; Moench[24] has investigated the problem of thorough examination of semen; methods are available for making accurate counts of the sperm; and technics for collecting and staining for proper examination of motility and morphology may be found in the literature. Just as proper methods must be used in hematology and other laboratory work, they must also be employed in the examination of semen.

When examination of the semen indicates low grade fertility or sterility, that does not constitute proof of any endocrine disorder nor is it necessarily an indication for endocrine therapy. Various other influences such as those mentioned in the introductory part of this paper should be considered and, so far as possible, corrected or ruled out as contributing factors to the inadequacy of the semen. If, however, there is still reason to suspect that the condition is of endocrine origin, further studies should be made to ascertain the nature of the endocrinopathy. In this regard, biological assays of blood and urine for sex hormones may be of considerable value. In primary testicular deficiency, the decrease in the production of androgens is frequently accompanied by increased gonadotropic activity. If the hypogonadism is of pituitary origin, there is also a decrease in output of androgens but never an indication of increased gonadotropic hormone.

When lack of fertility can be shown to be of endocrine origin, the prognosis is not particularly good. Chute[25] has recently summarized the problem of endocrine factors in human male sterility and correctly feels that many therapeutic failures have been due to indiscriminate and unscientific use of endocrine products. For example, there seems to be little reason for the administration of pituitary hormone to individuals who already excrete an excess of that substance. This type of therapy should be applied only when there is good reason to believe that the pituitary is deficient. Some authors report salutary effects in sterility by the use of pituitary-like hormone from urine and others have been very disappointed in this form of therapy. Possibly, gonado-

tropic preparations from pituitary glands will prove to have a greater gametokinetic effect.

The indiscriminate use of androgens is to be discouraged and numerous warnings have appeared in the literature. In view of recent researches, however, the statement that the gonads cannot be stimulated by androgens must be reconsidered and cautious clinical trials in appropriate cases by experienced endocrinologists would not seem to be contraindicated.

In the treatment of sterility of endocrine origin, the general health of the patient must be considered and the thyroid gland must not be overlooked. It is improbable that the thyroid hormone exerts any specific influence on the gonads; nevertheless, thyroid therapy is indicated when it is well tolerated by the patient.

Summary

Removal of the anterior lobe of the pituitary gland in rats results in cessation of the androgenic and reproductive functions of the testes. In hypophysectomized rats, the androgenic activity of the testes can be maintained or revived only by anterior pituitary (AP) or anterior pituitary-like (APL) hormones. Spermatogenic activity in hypophysectomized rats, however, can be maintained, if treatment is begun shortly following hypophysectomy, by a variety of substances: androgenic products, progesterone, and yeast, as well as by AP or APL hormones. On the other hand, if treatment is postponed for a considerable time following hypophysectomy, it has been found that only AP substance can restore gametogenesis to a completely normal condition. Regardless of the type of stimulation needed to maintain or revive spermatogenesis in the rat, it has been shown that this function can occur only if the testes occupy a scrotum which can exert a beneficial thermoregulatory effect. Recent studies have shown that such scrotal function is at least in part controlled by the male sex hormone. The significance of this observation is discussed with reference to the importance of androgenic activity to spermatogenesis, and to the difficulty encountered in postulating the existence of a pure hypophyseal gametogenic hormone.

Studies in normal adult rats have indicated that rather large doses of androgenic substances administered daily for 18 days failed to have any macroscopically visible deleterious effects on the testes, sexual accessory organs, or pituitary gland during, or as late as 20 days following, the injection period.

Sterility in men is discussed in the light of recent experimental findings. While caution is urged in the employment of hormonal therapy in

sterility, there would seem to be no objection in some instances to a judicious use of AP or APL hormones, as well as of androgenic substances.

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