EFFECT OF HORMONES ON SPERMATOGENESIS IN THE RAT

by

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Department of Anatomy,
McGill University,
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"Why sometimes I've believed as many as
six impossible things before breakfast."

L.C.

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INTRODUCTION

PURPOSE

It is ironic that the organ which was instrumental in the birth of the science of endocrinology (Berthold, 1849) should itself remain the subject of continual controversy as to the precise role hormones play in its functioning. The testis unlike the other endocrine organs, does not lend itself readily to the classical procedure of extirpation, for with ablation of the endocrine function one has of necessity also removed the primary target in the form of the seminiferous tubules. Consequently, indirect methods of negating the secretion of hormones by the testis, such as removal of trophic hormones of the pituitary by hypophysectomy, must be adopted with the resultant lack of precision in the interpretation of the observable results of such a procedure.

Despite these difficulties, certain facts have emerged concerning the effects of hormones on the process of spermatogenesis. Testosterone, produced by the interstitial cells of the testis, appears to be the primary hormone involved in the maintenance of the seminiferous epithelium. The interstitial cell stimulating hormone (ICSH) of the anterior pituitary, in turn, is responsible for stimulation of the production of testosterone. The role of the other pituitary hormones, the gonadotropins, follicle stimulating hormone (FSH) and prolactin (LTH) and non-gonadotropins such as growth hormone (GH), is not so clear. The contribution, if any, of the other endocrine glands, such as the thyroid and the adrenal

cortex, to the normal functioning of the testis is even less understood.

Among the enormous amount of literature on the subject of endocrine control of the testis, one finds few attempts made to assess the effects of hormones on the number of the various types of germ cells in the testis. The exceptions are the investigations on the hypophysectomized rat by Mess (1952) and Clermont and Morgentaler (1955) and on the ram during the non-breeding season by Ortavant (1958). Ortavant's study was comparable to those on the hypophysectomized rats from the point of view that it constituted an evaluation of the effect of a lack of pituitary hormones on germ cell number.

The work of Boccabella and Materazzi (1964) is unique in that it is the only investigation in which the effects of administration of various hormones on spermatogenesis were assessed by careful counting of the germ cells of the epithelium. Specifically,these authors examined the effect of thyroxin and growth hormone, administered in conjunction with testosterone propionate, on the number of germ cells in the testis of the hypophysectomized rat. In these animals the seminiferous epithelium was allowed to regress and then spermatogenesis reinitiated by hormone therapy.

The present investigation was undertaken by study of the effectiveness of various gonadotropic and non-gonadotropic hormones with or without testosterone propionate in maintaining the normal number of germ cells in the adult rat. In all experiments reported, hormone

therapy was instituted shortly after pituitary ablation before post-operative regression had occurred. Thus, an attempt has been made to evaluate the role of hormones in the maintenance of spermatogenesis rather than in the restoration of the process.

SUMMARY OF PREVIOUS LITERATURE

Histology of the Seminiferous Epithelium of the Normal Rat

Spermatogenesis is defined as the process by which undifferentiated cells, the spermatogonia, present in the seminiferous epithelium of the adult male animal produce the male gametes, the spermatozoa.

Figure 1 represents schematically the morphologic and topographic characteristics of the various cell types encountered in histolotical preparations of the rat testis. The most primitive spermatogonia or Type A, presenting a finely granulated chromatin pattern and at least one nucleolus, are observed along the basement membrane of the seminiferous tubules. These cells undergo several mitotic divisions (described below) and produce the intermediate spermatogonia, (Figure 1, second row, left) simultaneously propagating themselves. The Type A spermatogonia, therefore, constitute the stem cells of the seminiferous epithelium. The intermediate spermatogonia, having a slightly more granular chromatin pattern than the Type A, divide by mitosis and produce the Type B spermatogonia. The latter cells in turn, undergo mitosis to produce the resting or pre-leptotene, primary spermatocytes.

The appearance of the resting primary spermatocytes initiates the onset of the long meiotic prophase. During this so-called resting phase the DNA of the spermatocytes is duplicated in preparation for meiosis. Gradually increasing in nuclear volume the spermatocytes pass through leptotene (L), zygotene (Z), pachytene (P) and diakenesis (Di) phases of the meiotic prophase. Completion of the first maturation division produces the transient secondary spermatocytes (II, Figure 1, third row, right).

The secondary spermatocytes immediately undergo the second maturation division of meiosis and produce the spermatids seen in the fourth and fifth rows of Figure 1. These spermatids gradually evolve, shedding their excess cytoplasm and transforming into spermatozoa which are released into the lumen of the seminiferous tubule. This process of evolution is referred to as spermiogenesis.

The process of spermiogenesis, best visualized in PA-Schiff-hematoxylin stained preparations, can be divided into four consecutive phases; the Golgi, the cap, the acrosome and the maturation phase, Leblond and Clermont (1952) have further divided these phases into 19 steps.

The Golgi phase persists for three steps (step 1 - step 3). Step 1, the Golgi zone of the young spermatid becomes weakly PA-Schiff positive. Step 2, one to four tiny intensely stained granules called the pro-acrosomic granules appear in the Golgi zone. Step 3, these granules fuse into a single, large acrosomic granule. During the Golgi phase, iron hematoxylin staining has, in addition, revealed two

centrioles and the formation of a fine filament which will become the flagellum of the future spermatozoon.

During the cap phase the acrosomic granule flattens against the nuclear membrane and the head cap grows out over the nucleus. In this phase, Leblond and Clermont identified four steps (step 4 - step 7). Step 4 is characterized by a flattening of the acrosomic granule against the nuclear membrane. During step 5 the membrane of the head cap is seen first to protrude beyond the acrosomic granule covering one quarter of the nuclear surface. Step 6, the head cap expands until it covers one third of the nuclear surface, reaching its maximum development in step 7 when it covers one half. Iron hematoxylin staining of the cap phase reveals the two centrioles and flagellum moving toward the nucleus. One centriole comes into contact with the nuclear membrane at the pole opposite the acrosome; the other centriole, to which is attached the developing flagellum, becomes orientated perpendicular to the first.

The acrosome phase consists of seven steps (step 8 - step 14). Step 8, the spermatid nucleus rotates so that the head cap becomes orientated toward the basement membrane. Step 9, the nucleus begins to elongate and to flatten. Step 10, the elongated spermatid nucleus develops a sharp angle (the ventral angle) at its flagellar end. Step 11, a second angle (the dorsal angle) appears opposite the ventral one. Step 12, the acrosomic granule moves along the nuclear membrane and reaches the tip of the spermatid nucleus. At this point the spermatid nucleus has the appearance of a blunt ended rod.

Step 13, the nucleus shortens and displays increased basophillia. The tip of the nucleus pulls free of the acrosome and head cap, creating a space between itself and the head cap. Step 14, the spermatid begins to resemble the spermatozoon. The tip of the nucleus is now free of the head cap and the acrosomic granule forms a crest along one edge.

The last phase, the maturation phase, consists of 5 steps, (step 15 - step 19). Steps 15, 16 and 17 are characterized by displacement and rearrangement of the acrosome and head cap in relation to the nucleus. During step 18 the excess cytoplasm moves along the flagellum and accumulates in the concavity of the cresentric nucleus. This excess cytoplasm is shed as the residual body in step 19. The resulting spermatozoon is released from the epithelium into the lumen of the seminiferous tubule. Thus, the process of spermatogenesis, including spermiogenesis is completed.

Early histologists when examining the seminiferous epithelium of the mammalian testis were struck by the high degree of organization of this complex tissue. The various germ cells encountered in cross sections of seminiferous tubules were always arranged into distinct groups (von Ebner, 1871; 1888; Brown, 1885; Regaud, 1901). Spermatids of a given phase were always found associated with spermatocytes and spermatogonia of a given stage of development. Secondly, they realized that the members of these cell groups evolved in synchrony with each other. Consequently, with time the cells of one association would have evolved into another association. In a given area of seminiferous epithelium the cells would pass through successive

associations until the original one would reappear. Thus this area of epithelium would have passed through one cycle during which time a new crop of stem cells, the spermatogonia would be produced along the basement membrane of the seminiferous tubule and a crop of mature spermatozoa shed into the lumen. In fact Brown (1885) not only realized the cyclic nature of the epithelium but quite accurately stated that for a spermatogonium to evolve into a spermatozoon required the area of epithelium which contained it to pass through four to five cycles.

These groupings or cell associations which appear successively during the cycle of the seminiferous epithelium could be considered as stages of the cycle and could, thus, be used to break down the process of spermatogenesis into various phases. Over the years several classifications of cell associations have been proposed (Brown, 1885; Benda, 1887; von Ebner 1888; Lehossek, 1898; Regaud, 1901; Schoenfeld, 1902; Waldeyer, 1906; Hoof, 1912; and Roosen-Runge and Giesel, 1950).

Although all these classifications, even the earlier ones, are quite valid, they present problems in practical application. In many cases the stages are either poorly defined or their correct identification is dependent on subtle morphological features not always easily discernable to the uninitiated worker. The classification proposed by Leblond and Clermont (1952) overcomes these difficulties. As criteria of identification of the various stages they used only the appearance of the spermatids in PA-Schiff-hematoxylin stained material. The striking changes of nucleus, acrosome and head cap on

the spermatids as they transform into spermatozoa are clearly discernable and thus definition of the stage is facilitated. It is this classification (explained in detail below) that was employed in all the investigations reported in this work.

Figure 1 is divided into columns indicated by Roman numerals I to XIV. These columns depict the cell types found in association with each other in different segments of seminiferous tubule, or in the same segment if viewed at different times.

If, for example, a segment of tubule which contained the cell types depicted in column I could be studied over a period of time, one would find that the cells would transform until the cell types seen in column II would be present. The cell association of II would transform into that of III and so on up to XIV. The cell types of XIV would then transform into the association of I. The segment of tubule under consideration would thus have undergone a cyclic series of changes referred to as the cycle of the seminiferous epithelium. Each cell association is referred to as a stage of the cycle, there being fourteen in all.

In PA-Schiff-hematoxylin stained sections the apperance of the spermatids present in each tubule section can be used as the sole criterion for the identification of the stage of the cycle. Hence the first fourteen steps of spermiogenesis are the "name tags", so to speak, of the fourteen stages of the cycle.

If a segment of tubule is at stage VII of the cycle, along the bottom (Figure 1, column VII, bottom) that is, along the basement

membrane of the tubule are found the Type A spermatogonia. Above the Type A spermatogonia is a generation of resting primary spermatocytes (R), above these are the pachytene primary spermatocytes (P), above the spermatocytes are the step 8 spermatids and above these are the step 19 spermatids which are being released into the lumen of the tubule. One cycle later, a new crop of Type A spermatogonia appears. The Type A spermatogonia, originally present will have become the resting primary spermatocytes, the originally present resting primary spermatocytes will have become the pachytene primary spermatocytes, the originally present pachytene primary spermatocytes will have undergone meiosis and will now have become the step 8 spermatids, the originally present step 8 spermatids will have become the step 19 spermatids which leave the epithelium. Hence, each cycle moves the cell up one row in Figure 1.

The first mitotic division of the Type A spermatogonia occurs in stage IX and the release of the spermatozoa occurs in stage VIII. Therefore it is easily seen that the process of spermatogenesis, that is from stem cell to spermatozoon, requires four complete cycles.

Figure 2 is a photomicrograph of a cross section of seminiferous tubule containing the cells of stage VII. Figure 3 shows the same in longitudinal section. Along the basement membrane are the few Type A spermatogonia and with them are the round dark pre-leptotene or resting primary spermatocytes. Above the resting primary spermatocytes are the large dark pachytene primary spermatocytes. Next are the step 7 spermatids and at the lumen the step 19 spermatids about to

be released from the epithelium. In addition to these cells of the germ line there are the non-germinal Sertoli cells. These cells, not shown in Figure 1, are present in all areas of the seminiferous epithelium.

As early as 1901 Regaud had recognized the various types of cells constituting the germinal epithelium and had realized their proper chronological relations to each other. But not until the work of Roosen-Runge (1951) was a careful study made of the number of divisions the germ cells underwent. Using colchicine, he found that in the normal adult rat the Type A spermatogonia divided in stages IX, XIV and I, the intermediate type spermatogonia in stage IV and the Type B spermatogonia in stage VI. This last division produced the primary spermatocytes. The resultant of these five successive divisions is an approximate 32 fold increase in the number of cells from stem cell to primary spermatocyte.

Clermont and Leblond (1952) also found five peaks of mitosis in the spermatogonia. They reported a division of Type A spermatogonia, not noted by Roosen-Runge, in stage XII. On the other hand, they found only one division in stage XIV and I combined.

Huckins (1965) re-examined spermatogonial mitosis and found that both previous reports were partially correct. She found divisions of the Type A spermatogonia in stages IX' XII and XIV, of a pre-intermediate cell in stage I, of the intermediates in stage IV and of the Type B spermatogonia in stage VI. In addition, in the very young prepuberal rat she also found an extra division of Type A spermatogonia. This mitosis disappeared as the animal matured.

Recently, Bustos (M.Sc. Thesis, 1966; personal communication) in an investigation of whole mount preparations of seminiferous tubules discovered a class of Type A spermatogonia which does not appear to take part in any of the mitotic divisions. These, he speculated, may constitute a reserve group of stem cells which can function in repair of depopulated areas of the seminiferous epithelium. Thus the work of Huckins and of Bustos appears to invalidate some aspects of the renewal scheme of spermatogonia proposed by Clermont (1962). Nevertheless, it appears certain that the spermatogonia undergo 6 mitotic divisions to become spermatocytes. Possibly the spermatogonia have two means of increasing their number; either from a reserve stock of cells (Bustos) or by reverting back to prepuberal type divisions in stage VI (Huckins).

All the above workers realized despite the numerous divisions of the germ cells the number of spermatozoa produced per stem cell was always less than what should have been theoretically possible. Many cells degenerate before they complete their development. There is a loss of spermatogonia, mainly Type A spermatogonia, at stage XII, (10% Clermont, 1962), and a loss of spermatocytes, (2% Roosen-Runge, 1955; 27.4% Clermont, 1962). The latter number of Clermont is most likely correct. Comparison of counts of spermatids versus spermatocytes invariably gives a 3 to 1 ratio rather than the 4 to 1 meiotic ratio expected. There is an approximate loss of 10% of the spermatids as they evolve into spermatozoa (Roosen-Runge, 1955); the most affected cells appearing to be the step 9 to 11 and 17 to 19 spermatid.

When all degenerations are considered it is found that in the normal adult rat only 52% of the theoretical yield of spermatozoa is realized (Clermont, 1962). That is to say, only about half as many spermatozoa are produced per Type A spermatogonium as is theoretically possible.

Effects of Hormones on the Testis

The profound influence of the testis on the rest of the body has been recognized since antiquity. In 1849 Berthold first formulated the concept of the testis secreting a substance into the blood as the cause of these observed effects. However, it was not until the twentieth century that any progress was made in understanding the effect of the "testis on the testis". No method of destroying the interstitial cells or ablating their function without also destroying the seminiferous epithelium was available.

Houssay (1916) succeeded in removing the pituitary of the dog without damage to the adjacent hypothalamus and observed that the genital system, including the seminiferous epithelium atrophied. Moreover, he demonstrated that removal of the anterior lobe only of the gland was responsible for this atrophy. Thus, he introduced a method of ablation of the endocrine function of the testis.

Smith and Engle (1927) perfected this technique of hypophysectomy for small animals and demonstrated that these animals, with minimal special care could be kept alive for extended periods of time. In the same year, McGee succeeded in extracting a lipid soluble androgen from the testis. Butenandt (1930) demonstrated that the esterfication of steriods with organic acids increased the time of their biological

activity. Thus, research into the endocrine control of the testis was greatly facilitated.

Testicular Hormones Early investigations on the effects of androgens on the testis produced ambiguous results. Injection of male sex hormones into the intact animal frequently casued an inhibition of spermatogenesis (Moore and Price, 1932, 1937, 1938; Biddulph, 1939; Selye, 1941; Selye and Friedman, 1941; Meyer, 1948). Small amounts appeared to be more damaging than large. In fact, amounts of testosterone greater than 1 mg appeared to have little effect, if any (Selye and Friedman, 1941). In contrast, Rubenstein and Kurland (1940), Greene and Burrill (1940) and Freeman and Small (1941) reported that androgens appeared to stimulate the seminiferous epithelium.

On the other hand, injection of androgens into the hypophysectomized animal has produced consistent results. This hormone therapy invariably prevented post-operative regression of the seminiferous epithelium (Walsh et al, 1934; Nelson and Gallagher, 1936; Nelson, 1937, 1940; Nelson and Merkel, 1938; Cutuly et al, 1937; Cutuly, 1941, 1952; Wells, 1942; Dvoskin, 1943; Gaarenstroom and de Jongh, 1946). Moreover, Nelson (1940) was able to maintain the fertility of the animals for 169 days after hypophysectomy.

These incongruous effects of androgens in the intact and in the hypophysectomized animal led Ludwig (1950) to re-examine carefully this problem. Her detailed investigation demonstrated that in the intact rat small amounts of testosterone had a damaging effect on the seminiferous

epithelium whereas large amounts maintained the epithelium. However, the weight of the testes of these experimental animals was below that of the intact controls. Testosterone also maintained spermatogenesis in the hypophysectomized animal. Pellets containing this hormone, directly implanted into the testis, supported spermatogenesis throughout the entire organ if the concentration of testosterone in the pellet was high. If the pellet contained a low concentration of hormone spermatogenesis was maintained only in the seminiferous tubules in the area of implantation. This local maintenance caused by hormone pellet implants had been previously noted by Dvoskin (1944). On these observations Ludwig formulated a hypothesis on the effect of androgens both in intact and hypophysectomized animals. Briefly, in the intact animal small amounts of androgens suppresses the gonadotropic hormone production by the pituitary. This lack of gonadotropins damages the seminiferous tubules. Administration of large amounts of testosterone to the intact animal surpasses a threshold whereby the general serum level becomes high enough to stimulate directly the seminiferous epithelium. In the hypophysectomized animal the androgen effects the epithelium directly. Concerning this last point, it is noteworthy that Ludwig used an amount of testosterone (1 mg) in hypophysectomized animals which is sufficent to maintain spermatogenesis in the intact animal.

Direct evidence indicates under physiological conditions that the local level of testosterone in the testis is indeed very high (Linder, 1963). He showed that the level of testosterone in the lymph

of the testis is 2 to 8 times the level in the systemic arterial blood. The level in the spermatic vein is also much higher than the systemic arterial level. However, the lymph is possibly a better reflection of conditions of the intercellular fluid of the testis than is venous blood.

Thus, it is relatively certain that the maintenance of the seminiferous epithelium by exogenous androgen necessitates the administration of hormone in excess amounts. Consequently the general serum level increases until it approaches the level of the intercellular fluid of the testis of the intact animal. Further, this excess amount must be sufficient to overcome the de-activation processes of the liver and kidneys. These organs have been shown to destroy certain amounts of injected steroid hormones (Biskind and Mark, 1939; Samuels, 1949).

Although androgens, particularly testosterone, maintain spermatogenesis both in the hypophysectomized and the intact rat, all workers have invariably reported that the weight of the testis is below that of the normal. It has been postulated that this decrease in weight is due to the atrophy of the interstitial cells in these animals. Also it has been noted that the seminiferous tubules appear to be smaller than those of normal animals (Ludwig, 1950; and others), and this decrease in tubular size might account for the loss of weight.

Concerning the ability of androgens to re-initiate spermatogenesis in the hypophysectomized animal, the results are not so clear. Nelson (1941) and Smith (1944) found that testosterone propionate

restored spermatogenesis after a post hypophysectomy regression had occurred. Other reports stated that re-initiation was possible only if hormone therapy was commenced with 10 days of pituitary ablation (Hamilton and Leonard, 1938; Nelson and Gallagher, 1936; Cutuly et al, 1957; Dvoskin, 1949). Recent workers (Randolph et al, 1959; Woods and Simpson, 1961) have also reported testosterone to be relatively impotent in restoring the seminiferous epithelium of the hypophysectomized rat. However, one could explain the failures to restore spermatogenesis as being due to shortness of hormone therapy. It is possible that these workers did not allow sufficient time for the epithelium to be restored. However, Boccabella (1963) reported an investigation in which testosterone propionate was injected for as long as 110 days. Rats, hypophysectomized for 67 to 70 days, were injected daily with hormone for 35 to 110 days. He found that with this prolonged treatment testosterone propionate restored spermatogenesis in one third of the animals. The response in the other two thirds ranged from nil to erratic. The erratic response was characterized by testes in which seminiferous tubules contained an increase in spermatogonia and spermatocytes, as well as tubules in which there was no change from the untreated condition.

Cell counts in the testes of animals in which the testosterone propionate therapy had succeeded in restoring spermatogenesis (Boccabella and Materazzi, 1964) revealed the number of Type A and Type B spermatogonia and pachytene primary spermatocytes was 20-30% lower than those of the normal rat. Thus, it would seem that testosterone propionate

can restore spermatogenesis in only a very limited manner. The seminiferous epithelium may either not respond at all to hormone therapy or when there is a response the number of germ cells is much less than normal.

In addition to testosterone, the testes also produce androstenedione (Dorfman, 1954; Brinck-Johnson, 1957; Savard, 1961). Both hormones are in equilibrium with one another (see review of Dorfman, 1954); the ratio in spermatic blood being 7:1 testosterone:androstenedione. Thus the injection of one will cause a proportional increase in the other.

The injection of testosterone propionate has long been known to cause an increase in urinary oestrogens (Steinach et al, 1936; Steinach and Kein, 1937; Nathanson, 1939; Haskins et al, 1939). This finding, however, is likely due to a peripheral metabolism of testosterone to oestrogen in that it occurs in the castrate. However it has been demonstrated that oestrogens can be extracted from the testis (Beall, 1940; Haines et al, 1948; Goldzieher, 1952) or from sperm (Schaffenburg, 1954). Thus, the testis appears to produce a hormone that can damage the seminiferous epithelium (Morrel and Hart, 1941; Mark and Biskind, 1941; Meyer, 1948; Selye and Friedman, 1941; Gaarenstroom and de Jongh, 1946). Meyer (1948) showed the damage occurs only when the ratio of oestrogen to testosterone exceeds a certain limit. However, the level of oestrogen in the normal testis is exceedingly low.

There is abundant evidence that the Leydig cells produce the androgen but what of the oestrogens? Maddock and Nelson (1952) and

and Leach et al (1956), both in clinical studies, have shown that the injection of chorionic gonadotrophin increases the output of urinary oestrogen in men, but only when functional Leydig cells are present. At the same time the elements of the seminiferous epithelium, both germ and Sertoli, remain atrophic. They have also shown the urinary oestrogens is a better test for testicular endocrine function than urinary 17 ketosteroids. Over 80% of the urinary oestrogens come from the testes, but less than half of the 17 ketosteroids do.

Possibly the most direct evidence that the Leydig cells are the only source of both testicular androgen and oestrogen is the fact that they have been shown to be the only cells of the testis which contain 3 β-dehydrogenase: an enzyme fundamental to all steroid synthesis (Jirasek and Rabock, 1963).

Discussion of testicular hormones would not be complete without mention of "Inhibitin". This is a hypothetical hormone secreted by the Sertoli cells which is supposed to inhibit the release of FSH from the pituitary. Its existence has been postulated mainly on clinical studies (del Casletto et al, 1947; McCullagh et al, 1948; Teilum, 1950; Howard et al, 1950). Evidence for its existence in the rat has been put forward (Taira and Tarkhan, 1962). They showed that cryptorchidectomy resulted in an increase in FSH release equal to that observed after castration. However the intra-abdominal testis caused little change in ICSH release compared to the increase caused by castration. Their explanation was that the atrophy of all elements

of the germinal epithelium in the crytorchid testis including the Sertoli cells resulted in no inhibitin being produced. However, they have assumed that the Leydig cells of these testes are normal, for there was no increase in ICSH output. The work of Kormono et al (1964) on cryptorchidectomized rats suggests thatthis is not so. They report that secretory activity of the Leydig cells in the intra-abdominal testes is greatly reduced.

Until "Inhibitin" is isolated, purified and identified little progress can be made in its study. Suffice to say the production of oestrogens by the Leydig cells would appear to make the existence of inhibitin superfluous.

Pituitary Hormones Uncertainty as to the purity of pituitary hormone preparations has plagued past investigators examining the function of the pituitary gland in control of the male reproductive system. Being proteins, the pituitary hormones cannot be isolated and identified with the specificity accorded the steroid hormones. Over the years hormone extraction procedures have improved and certain facts regarding the trophic substances of the hypophysis effect on the testis established.

A principal procedure to investigate the role of the pituitary gland in spermatogenesis has been the study of the seminiferous epithelium of the hypophysectomized rat. Smith (1930) described the effects on the testis of the removal of the pituitary gland. Following hypophysectomy, the seminiferous epithelium was observed to atrophy rapidly. Spermatids disappeared from the epithelium within a few days of pituitary ablation; but the spermatocytes and spermato-

gonia, although reduced in number, persisted for months. Despite morphological changes, the Sertoli cells displayed no signs of degeneration. These observations of Smith were confirmed by Cutuly and Cutuly (1940), who postulated that the pituitary hormones were required for spermatogenesis during meiosis; but that the process of spermatogenesis proceeded to this point without benefit of the hypophysis.

In a quantitative study of the effect of hypophysectomy in the adult rat, Clermont and Morgentaler (1955) reported that advanced spermatids (step 17-19) began to degenerate within 3 days post-operative being followed by the step 9-10 spermatids at 6 days. At this latter time there was also degeneration of the spermatocytes. The spermatogonia, however, appeared normal. At subsequent time intervals more and more germ cells degenerated; this degenerative process affecting the evolved germinal element to a greater extent than the lesser evolved. Cell counts in animals which were hypophysectomized for 60 days revealed that the population of the Type A spermatogonia was reduced to about 50%, of the Type A spermatogonia in the intact rat, resting primary spermatocytes to about 20%, the pachytene primary spermatocytes to about 4% and the spermatids to less than 1%. Although morphologically changed the Sertoli cells were never seen to degenerate. The few spermatids which continued to be produced never evolved beyond step 7 of spermiogenesis.

Though greatly reduced in number the germ cells in the hypophysectomized rat have been observed to undergo mitosis and to differentiate

at the same rate as those in the normal animal (Desclin and Ortavant, 1963; Clermont and Harvey, 1965).

Ortavant (1958) investigated the cell populations of the testis of the ram, an animal with a breeding cycle. In the testis of animals during the non-breeding season he found a loss of cells similar to the loss in the hypophysectomized rats. Specifically, the reduction in cell populations is more manifest the more evolved is the cell, although not as great as in the hypophysectomized animal. He found that the rate at which the remaining cells in the non-breeding season ram proliferated and differentiated is the same as in the ram during the breeding season. Thus, it appears that the rate at which germ cells undergo spermatogenesis is independent of hormones.

Recent work of Lostroh (1963) would indicate that care should be taken in assessing the precise effects of hypophysectomy on germ cell number. He found in a large group of rats, considerable individual variation in the state of the seminiferous epithelium. Meiotic divisions with spermatid formation were found in one quarter of the animals whereas in the other three quarters only spermatogonia and a few spermatocytes were found. Yet by all other criteria, lack of weight gain, state of the adrenal cortex, etc., both groups of animals were totally hypophysectomized. Thus, what Clermont and Morgentaler called the "residual spermatogenesis" in the hypophysectomized rat varies considerably from one animal to the next.

The pituitary gonadotropic hormones commonly associated with the male reproductive system are interstitial cell stimulating hormone

(ICSH) and follicle stimulating hormone (FSH). Despite the dogmatic view stated in some textbooks of physiology, that is, ICSH stimulates the Leydig cells to produce androgen and FSH stimulates the seminiferous epithelium (Best and Taylor, 1961) the action of the individual hormones in the male is the subject of considerable discussion. The dilemma arises from the fact that one hormone preparation is frequently contaminated by the other.

Greep and Fevold (1937) found that either ICSH or FSH could maintain spermatogenesis in the hypophysectomized adult rat. ICSH caused a stimulation of the Leydig cells which resulted in maintenance of the accessory organs as well as the seminiferous epithelium. Although spermatogenesis continued the Leydig cells began to atrophy after 35 to 40 days of treatment. FSH maintained spermatogenesis but did not prevent the atrophy of the accessory organs. This finding might indicate that FSH stimulated the seminiferous epithelium directly. On the other hand, it might only indicate that the androgen secretion of the Leydig cells in the FSH treated rats was not sufficient to stimulate the accessory organs but was sufficient to support spermatogenesis in the seminiferous tubule.

Simpson et al (1942) investigating the effects of ICSH obtained similar results to Greep and Fevold. They found that this hormone maintained both spermatogenesis in mature hypophysectomized animals and the development of spermatogenesis in immature hypophysectomized rats. However, the results in the immature rats indicated that the maintenance was variable. If post-operative regression of the seminiferous epithelium was allowed to occur and then hormone therapy instituted

ICSH was found to be ineffective. Restoration of spermatogenesis did not occur yet the prostatic development indicated a stimulation of androgen production by the Leydig cells.

The maintenance of spermatogenesis in the hypophysectomized rat by ICSH has been effected with dosages of ICSH small enough to produce no detectable stimulation of the Leydig cells (Simpson et al, 1944). Several multiples of the dose which maintained spermatogenesis was required to produce detectable amounts of androgen production by the Leydig cells. However, the accessory organ weights were below those of the normal.

Simpson et al (1951) examined the effect of FSH on the testis of the hypophysectomized rat. As observed by Greep and Fevold (1937), they found thatthis hormone could maintain spermatogenesis. Five to 10 times the dosage which stimulated the seminiferous epithelium was required to cause a detectable stimulation of the Leydig cells, and thus the accessory organs. Human chorionic gonadotropin (HCGT) affected both the seminiferous epithelium and the Leydig cells at the same dose. This hormone having similar effects to ICSH, is immunologically related to ICSH but not identical (Goss and Lewis, 1964). FSH and HCGT when injected together were effective at dosages well below the effective dosage of either alone. That is, they are synergistic.

Randolph et al (1959) found that in the mouse ICSH could maintain spermatogenesis in the hypophysectomized animal and it could restore the process if post-operative regression had taken place. In the latter case larger amounts of ICSH were needed than in the former.

Woods and Simpson (1961) re-examined the problem of pituitary control of the testis by studying the effects both of gonadotropins and non-gonadotropins. ICSH was found to be the principal hormone involved in the maintenance of spermatogenesis in the hypophysectomized rat. An extremely small amount of this hormone would maintain the seminiferous epithelium. Larger amounts supported not only spermatogenesis but the accessory organs as well. The weights both of the testes and accessory organs never equalled those of the intact animal. FSH effected slight stimulation of the seminiferous epithelium if used at levels which did not show ICSH contamination. However, ICSH and FSH, if administered together, greatly augmented the effects of each other.

If a post-operative regression of the seminiferous epithelium was allowed to occur, then FSH or ICSH caused but slight restoration of spermatogenesis. The seminiferous epithelium was restored to a functional level only when the two hormones were injected together. Weights of the testes of these animals, however, were less than those of the normal. Woods and Simpson concluded that ICSH was the principal hormone concerned with spermatogenesis in that no other hormone was effective in its absence. Although ineffective alone FSH appeared to be necessary for the restoration of spermatogenesis.

Lostroh (1963) studied the role of ICSH and FSH in the restoration of spermatogenesis in Long-Evans rats which had been hypophysectomized for a prolonged period of time. ICSH alone was found to be ineffective in restoration of the seminiferous epithelium. Spermatogonia and spermatocytes were increased in number but meiosis was not completed.

When FSH and ICSH were administered together meiosis was completed, and spermatids were produced. In no instance was spermatogenesis completely restored, but treatment lasted for only 15 days. Interestingly, FSH did not enhance the effect of ICSH on androgen secretions in the Long-Evans rat. On the other hand in a similar study on the Sprague-Dawley rat Lostroh et al (1963) found that FSH would augment the effects of ICSH on androgen secretion.

Anti-sera to ovine ICSH has been shown to cross react with rat ICSH (Hayashida, 1963), Lostroh et al employed this technique to de-activate any ICSH contaminant of their FSH preparation. When FSH and anti-ICSH were administered mitotic divisions of spermatogonia increased and the Sertoli cells appeared to be stimulated. However, no meiotic divisions of the spermatocytes were observed. ICSH alone produced an increase in mitotic divisions of the spermatogonia but the Sertoli cells and spermatocytes were unaffected. When FSH without anti-ICSH or when FSH and ICSH were injected the spermatocytes completed meiosis. However, the response was constant only in animals in which FSH and ICSH were injected. In the Sprague-Dawley rat, unlike in the Long-Evans rat FSH augmented the effects of ICSH on androgen secretion.

When anti-ICSH alone was injected into the hypophysectomized Sprague-Dawley rat further damage to the seminiferous epithelium was noted. This suggested that there was residual ICSH production in these animals which were supposedly completely hypophysectomized. Lostroh (1963) had also noted in the hypophysectomized Long-Evans rat considerable variation in the state of atrophy of the seminiferous

epithelium.

Lostroh and co-workers concluded that ICSH and FSH are both required for the restoration of spermatogenesis in the hypophysectomized rat. ICSH alone stimulates androgen secretion and mitotic division of spermatogonia. FSH alone stimulates mitotic division of spermatogonia and the Sertoli cells. Both hormones must be present for meiosis and thus, spermatid formation.

Other workers have postulated the role of FSH in supporting mitotic division of spermatogonia in the rat (Gaarenstroom and de Jongh; 1946) and in the frog (Burgos and Fadman, 1957; Lofts, 1961; van Oordt and Lofts, 1964). Comparison of effects of FSH in the amphibian with those in the rat should however, be made with reservations. The testes of these two animals are dissimilar both in morphology and endocrine response. For example, treatment with testosterone will cause complete cessation of spermatogonial division in the frog (van Oordt and Basu, 1960), whereas this response is not observed in the rat.

Murphy (1965) made intertesticular injections of FSH into hypophysectomized rats and observed an increase in the number of Sertoli cells per tubular cross section. This was interpreted as evidence supporting the action of FSH on Sertoli cells. Yet other factors such as change in tubular size may cause this apparent increase. He himself admitted that no Sertoli cell divisions were seen. Thymidine labelling would be expected if these cells divided. This however is not observed in the adult rat (Clermont et al, 1959; Nebel and Murphy,

1960; Clermont and Harvey, 1965). In fact these cells undergo their last division before the animal is 20 days old (Clermont and Perey, 1957; Huckins, 1965). The experiments of Murphy are dubious for no stimulation of germ cells or Leydig cells was observed.

Papkoff (1965) assessed the action of ICSH and FSH in the Sprague-Dawley rat. His procedure to purify each hormone was: 1) ICSH was removed from the FSH preparation by incubation with urea, a procedure which inactivates ICSH but does not affect FSH (Ellis, 1961); 2) FSH was removed from the ICSH preparations by treatment with neuraminidase which inactivates FSH but does not affect ICSH (Ellis, 1961). When these purified preparations were tested each was found to stimulate androgen secretion in the Sprague-Dawley rat.

It is noteworthy however, that germ cells in tissue culture appear to require neither ICSH nor FSH (Steinberger et al, 1964). Neither hormone was effective in causing embryonic germ cells to differentiate into spermatogonia and spermatocytes. However, FSH caused differentiation of the primitive supporting elements into Sertoli cells.

In summary, in the male rat it appears that ICSH alone will maintain spermatogenesis. FSH will augment the effects of ICSH. FSH alone has a potential effect on the seminiferous epithelium, possibly on the Sertoli cells. In the Sprague-Dawley rat, unlike the Long-Evans rat, RSH and ICSH both stimulate androgen secretion. Both hormones appear to be necessary to bring about restoration of spermatogenesis if atrophy of the seminiferous epithelium has occurred.

From the literature the relationship between testosterone and ICSH or FSH appears to be as follows. Testosterone inhibits ICSH synthesis and ICSH release by the pituitary gland (Purves and Griesbach, 1954; Gans, 1959; Paesi and van Rees, 1960 a; van Rees and Paesi, 1960; Davidson et al, 1960). Concerning FSH, testosterone appears to stimulate FSH synthesis and to inhibit FSH release (Greep and Jones, 1950; Purves and Griesbach, 1954; Paesi and van Rees, 1960 b; Shipley, 1962; Bogdanove, 1964). In contrast, the work of Davidson et al (1960) suggests that testosterone may inhibit not only FSH release but its synthesis as well. Concerning the feed-back mechanism of testosterone on the two gonadotropins, the work of Paesi and van Rees (1960 a, b) indicates that it is an indirect feed-back mediated by the hypothalamus.

The third gonadotropic hormone of the pituitary gland is lactogenic hormone (LTH). In 1946 Huggins and Russell noted that hypophysectomy caused a greater atrophy of the prostate gland than did castration. This observation indicated that some factor, present in the pituitary, affected the prostate. Greyhack et al (1955) also reported a greater atrophy of the prostate in the hypophysectomized animal as compared to the castrated animal. In addition they noted that the prostate of these animals was less responsive to testosterone propionate treatment than those of the castrated animals. However, if LTH was injected with testosterone propionate in the hypophysectomized animal the prostate responded like that of a castrated animal treated with testosterone propionate alone. LTH, when administered alone, had no effect. The synergism of LTH with ICSH has been reported by other investigators

(Segaloff et al, 1956; Lostroh et al, 1958; Woods and Simpson, 1961). These authors failed to establish any dose-response relationship for LTH. The action of LTH appeared to be all-or-none without a negative feed-back mechanism. Whereas castration has no effect on the amount of LTH present in the male pituitary, large amounts of testosterone propionate will increase its secretion (Woltheus, 1963).

Lostroh et al (1958) demonstrated that LTH was effective only in the Sprague-Dawley rat, but ineffective in the Long-Evans rat. Woods and Simpson (1961) noted that LTH augmented the effect of ICSH not only on the prostate but on the testis as well. It is noteworthy that LTH administered to birds has a detrimental effect on the testis, inhibiting spermatogenesis (Lofts and Marshall, 1956).

Adrenal Hormones It has long been known that certain steroids other than testosterone have a beneficial effect on spermatogenesis (Nelson, 1937; Selye and Friedman, 1941; Masson, 1945, 1946). The major source of steroids in the body, other than the testis, is the adrenal cortex. In the rat it appears that the testis has a regulatory influence on the adrenal and not vice versa. Injection of testosterone propionate will partially reverse the changes in the adrenal cortex caused by hypophysectomy (Culuty et al, 1938; Kalant and Sellers, 1957; Roy and Mahesh, 1964). The mitotic activity of the epidermis appears to be controlled by a balance of testicular and adrenal hormones (Bullough, 1952 a, b). The spermatogonia of the testis, like the basal cells of the epidermis, constitute a renewal system. However the mitotic activity of the spermatogonia appears to be independent of

hormones (Ortavant, 1958; Desclin and Ortavant, 1963; Clermont and Harvey, 1965).

Woods and Simpson (1961) found that adrenocorticotropic hormone (ACTH) had no effect on the testis of the hypophysectomized rat. It was ineffective alone, or when injected with FSH or ICSH. Thus, it appears that the adrenal gland does not contribute to the process of spermatogenesis.

Thyroid Hormone The thyroid gland has been indirectly implicated in the regulation of the testis. In 1939, Smelser reported that thyroidectomy caused a reduction in the testis and accessory organ weights. Thyroidectomy is also known to reduce the gonadotropic content of the pituitary gland (Evans and Simpson, 1930; Stein and Lisle, 1942; Contapoulus et al, 1958). Contapoulus et al also noted a decrease in the weights of the testis and accessory organs. On the other hand, Jones et al (1946) found the thiouricil-produced hypothyroidism caused a decrease in fertility in the adult rat. In the mouse, Bruce and Slaviter (1957) reported that thyroidectomy caused no decrease in fertility.

Woods and Simpson (1961) found that thyrotropic hormone injected into hypophysectomized rats produced no increase in weight either in the testis or the accessory organs. This was observed whether the TSH was injected alone or with FSH and ICSH.

Thyroxin, with or without GH has been found to produce a stimulation of the testis (Randolph et al, 1959; Boccabella, 1963). Boccabella has shown that if thyroxin is injected in conjunction with testosterone propionate and GH, the response of the atrophied seminif-

erous epithelium of a hypophysectomized rat is greater than when testosterone propionate and GH are injected alone.

Growth Hormone Growth hormone (GH) has also been implicated in the hormonal control of the male reproductive system. Like LTH it enhances the effect of testosterone propionate or ICSH on the prostate of the hypophysectomized rat (Huggins et al, 1955; Lostroh et al, 1958; Lostroh and Li, 1957; Randolph et al, 1959; Woods and Simpson, 1961). Again like LTH, no dose-response relationship can be established for GH. Lostroh et al (1958) have shown that GH is synergistic to testosterone propionate in the Sprague-Dawley but not in the Long-Evans rat. GH has been shown to augment the effect of both ICSH (Woods and Simpson, 1961) and testosterone propionate (Boccabella, 1963) not only on the prostate but on the testis as well.

The effectiveness of GH administered with testosterone propionate on the testis was assessed by Boccabella and Materazzi (1964) by cell counts. As described above they hypophysectomized rats, allowed 67 to 70 days of post-operative regression of the epithelium and then commenced hormone therapy. Testosterone propionate alone restored spermatogenesis in one third of the animals. But testosterone propionate administered with GH restored the process in two thirds of the rats. Cell counts revealed that the number of cells in these latter animals was equal to that of the normal, whereas the number was subnormal in animals treated with testosterone propionate alone. Thus, it would seem that GH, although ineffective alone, is necessary to restore spermatogenesis in the hypophysectomized rat to its normal level.

From this brief discussion of hormonal control of spermatogenesis, it is clear that one must distinguish between the maintenance and the restoration of this process. The maintenance of spermatogenesis appears dependent on either ICSH or testosterone, although FSH will augment the effect of both. In the restoration of spermatogenesis either ICSH or testosterone appears relatively impotent. FSH must be also present to effect a constant restoration. Moreover, GH, LTH and thyroxin, each ineffective alone, will improve the restoration caused by both ICSH and FSH. Finally, the responsiveness to hormones may vary between one strain of rats and another.

MATERIALS AND METHODS

GENERAL

For all studies reported in this dissertation, adult male Sprague-Dawley rats, obtained from Charles River Laboratories, Boston, Mass., were used. The animals were maintained on a diet of Purina Lab Chow and tap water; a 5% solution of sucrose and 0.9% solution of sodium chloride being substituted for the water in the case of the hypophysectomized and adrenalectomized groups respectively. Regular weighings were made and the weights recorded.

Hormone Preparations

All hormones were purchased from Nutritional Biochemicals, Cleveland, Ohio. These were testosterone propionate, DL-thyroxin, ovine follicle stimulating hormone, ovine interstitial cell stimulating hormone, porcine growth hormone, Raben type, and ovine lactogenic hormone. With the exception of testosterone propionate all hormone solutions were made fresh every three days. The concentration of all solutions was adjusted so that the total volume injected daily was 0.2cc. Storage was at $5^{o}C$.

Surgical Procedures

Hypophysectomy The pituitary gland was removed by the supplier using the parapharyngeal approach perfected by Smith (1927). The animals arrived on the third post-operative day and experimentation commenced on the second day after arrival.

Completeness of pituitary ablation was judged, where treatment permitted, on the basis of a complete lack of any gain in body weight during the course of the experiment, by gross inspection of the sella turcica for pituitary fragments at the time of sacrifice, and histological examination of the thyroid and adrenal. In some cases, such as treatment with growth hormone, certain of these criteria could not be used.

Adrenalectomy The removal of the adrenals was performed under ether anaesthesia using aseptic technique. An incision was made in the skin and muscular wall of the back just below the rib cage thus exposing the dorsal aspect of the kidney. The adrenal gland was gently isolated from the renal fat pad by blunt dissection with fine curved forceps. The procedure was repeated on the opposite side and the skin wound closed with metal clamps. If the capsule of the gland was accidentally broken during removal the animal was discarded.

Adrenalectomy was judged as incomplete if the animals displayed any marked increase in weight during the course of the experiment. At sacrifice the abdominal cavity was examined for accessory adrenal glands. The kidney with fat pad was carefully removed, washed and particularly scrutinized for remaining adrenal tissue.

Thyroidectomy Removal of the thyroid was also performed under ether anaesthesia. A midline incision was made in the neck exposing the trachea and thyroid. Using blunt forceps the gland together with its fascial capsule was gently grasped and slowly stripped from

the trachea.

Completeness of thyroidectomy was based on a histological examination of sections of trachea for remaining fragments of the gland.

SPECIFIC EXPERIMENTS

Normal Rats

Before ascertaining the effects of various endocrine conditions on the testes it was first necessary to determine the number of germ cells in the normal adult rat. Consequently 6 adult 280 gram rats were sacrificed and the cell number determined.

Effect of Age

The effect of age on germ cell number in adult rats was determined by obtaining a group of 30 normal animals. Five were sacrificed when the average body weight of the group was 200 grams, 5 when it was 250 grams, 5 when it was 300 grams and 5 when they obtained a weight of 400 grams.

Testosterone Propionate

Effect in Normal Rats Five normal 220 gram rats were injected daily for 45 days with 3 mgms of testosterone propionate in 0.2cc olive oil. Because of the critical importance of the results obtained, this experiment was later repeated.

Effect in Hypophysectomized Rats Twenty hypophysectomized 200 gram rats were injected daily with 3 mgms of testosterone propionate

in 0.2cc of olive oil. Hormone therapy commenced on the 5th post-operative day and continued up to the time of sacrifice of the animals in groups 35, 45, 55 and 60 days after pituitary ablation.

Effects of Adrenalectomy

Five 380 gram adrenalectomized rats along with 5 sham-operated, pair-fed controls were sacrificed 45 days post-operative.

Pituitary Gonadotropins

Effects of Interstitial Cell Stimulating Hormone (ICSH) in Hypophysectomized Rats Three groups of 5 hypophysectomized rats were injected respectively with 10 μg of ICSH, 15 μg of ICSH or 20 μg of ICSH in 0.2cc of saline. Hormone therapy commenced on the 5th post-operative day. Sacrifice was on the 35th post-operative day. Three additional groups were similarly treated and sacrificed 45 days post-operative.

Effects of Follicle Stimulating Hormone (FSH) in the Hypophysectomized Rats Three groups of 5 hypophysectomized 200 gram rats were injected respectively with 100 μg of FSH, 150 μg of FSH or 200 μg of FSH in 0.2cc of saline. Hormone therapy commenced on the 5th post-operative day. Sacrifice was on the 35th post-operative day. An additional three groups were similarly treated and sacrificed on the 45th post-operative day.

Combination of Testosterone Propionate with the Gonadotropins

Testosterone Propionate and ICSH in Normals Five normal 220 gram rats were injected with 3 mg of testosterone propionate in 0.2cc of olive oil and 15 μg of ICSH in 0.2cc of saline. Sacrifice

was on the 45th day of treatment.

Testosterone propionate and FSH in Normals Five normal 220 gram rats were injected daily with both 3 mg of testosterone propionate in 0.2cc of olive oil and 150 µg of FSH in 0.2cc of saline. Sacrifice was on the 45th day of treatment.

Testosterone Propionate in Normals A third group of 5 normal rats was injected with 3 mg of testosterone propionate in 0.2cc of olive oil. These animals acted as controls for the above two groups and repeated a critical previous experiment.

Effects of Testosterone Propionate in Hypophysectomized Rats on a Short Term Basis

To test the initial effect of hormone treatment, a group of 20 hypophysectomized adult rats were treated with testosterone propionate in the usual manner. The animals were sacrificed in groups 3, 6, 9, 12 and 26 days post-operatively. Counts of Type A spermatogonia and Sertoli cells only were made.

Effect of Lactogenic Hormone (LTH)

Two groups of 5 hypophysectomized 220 gram rats were injected with 3 mg of testosterone propionate in 0.2cc of olive oil and 3 mgm of testosterone propionate in oil plus 1 IU of lactogenic hormone in 0.2cc of saline respectively. Sacrifice was 30 days post-operative.

Effects of the Non-Gametogenic Hormones

Effect of Thyroidectomy Five 240 gram thyroidectomized rats along with 5 sham operated controls were sacrificed 45 days post-

operatively.

Effect of Thyroxin and Growth Hormone in the Hypophysectomized Rat Hypophysectomized rats were divided into four groups, all were injected with 3 mg of testosterone propionate in oil: in addition, group 2 received 2 µg of thyroxin in alkaline saline (pH 8), group 3 received .5 USP of growth hormone (Raben type) in alkaline saline (pH 10), and group 4, 2 µg of thyroxin and .5 USP of growth hormone. A group of normal rats were maintained for growth rate estimation. Sacrifice was on the 30th post-operative day.

Effect of Hypophysectomy and Growth Hormone To check for possible gonadotropin contamination of the growth hormone two groups of hypophysectomized rats were used, one was left untreated and the other injected daily with .5 USP of growth hormone in 0.2cc saline. Sacrifice was on the 30th post-operative day.

HISTOLOGICAL PROCEDURES

Fixation

The testes, epidiymedes, prostate and seminal vesicles were removed and fixed in Zenker's formol. After 30 minutes fixation the testes were cleared of excessive fat and carefully dissected free. Small cuts were made in the tunica albuginea at each pole of the organ to allow the fixative to penetrate more rapidly. The tissue was then fixed for 24 hours.

After fixation the tissue was washed in running tap water for 24 hours and then stored in 70% alcohol.

During storage the testes and male accessory organs were trimmed completely free of connective tissue and fat, blotted dry and quickly weighed to .001 gram accuracy.

Sectioning and Staining

The tissues consisting of two cross sections of each testes and representative samples of the prostate, seminal vesicles and vas deferens were dehydrated in ethanol, paraffin embedded and sectioned at 5 $\mu \pm 1 \mu$. Staining was with PA-Schiff and Harris hematoxylin. This staining is desirable in that the Schiff reagent stains the head caps and acrosomes of the developing spermatids making identification of the different stages of spermatogenesis possible.

HISTOMETRIC PROCEDURES

Cells Counted

In each animal the cells were enumerated in 40 perfect cross sections of seminiferous tubules showing the cellular association of Stage VII of Leblond and Clermont (1952; see Figures 1, 2, 3)). This stage gives a good,general overall picture of the process of spermatogenesis, the cells present being the dormant stem cells, that is the Type A spermatogonia, resting or preleptotene spermatocytes, primary spermatocytes in the mid pachytene phase, and step 7 spermatids which is just prior to the process of elongation of the nucleus of the cell as it transforms into a spermatozoon. Also counted were the supporting nurse cells, that is, the cells of Sertoli. The Sertoli

cells have large irregular nuclei hence only nuclei with the prominent single nucleolus in the plane of section were counted. Selection of the tubules was completely at random.

The counts were averaged for each group and expressed as the number of cells per tubular cross section.

Correction Factors

In that the various germinal cells have different nuclear diameters, the larger cells will by chance appear more frequently in cross section than those of small diameter. Thus comparison between number of cells in cross section is inaccurate when based on the crude counts. Correction for this variation was achieved by applying Abercrombie's Formula (Abercrombie, 1946).

$$TC = CC \times \frac{S}{S + d}$$

where TC = true counts, CC = crude counts, S = section thickness and d = nuclear diameter.

These measurements were obtained by using a Filar micrometer; section thickness being determined by measuring the thickness of the occasional folds in the tissue (Swift and Rasch, 1956).

Sertoli Cell Correction Factor

Before comparison of the cell counts of one group with another certain facts had to be considered. One was that due to treatment and/or histological processing there could be shrinkage of the seminiferous tubules which could necessarily be different from one group to the next. The reduction in diameter is easily determined in histological

section but the dimunition of length is much more difficult to ascertain. The shortening is serious in that proportionately more cells would appear in 5 μ cross section of the tubules. However in that the Sertoli cells are not seen to either divide or degenerate in the adult animal any change in their number per cross section would be indicative of a change in length of seminiferous tubule. Hence, the ratio of number of Sertoli cells counted in each experimental group to the number per tubular cross section in the normal animal could be used as an index of reduction in length of the seminiferous tubules in the experimental animals.

This use of the Sertoli cell number (Clermont and Morgentaler, 1955; Boccabella and Materazzi, 1964) is referred to as the "Sertoli Cell Correction Factor". (See Appendix for discussion of this feature.)

RESULTS

Germ Cell Number in the Normal Intact Sprague-Dawley Rat

The average weights of the body, testes and accessory organs are given in Table 1. Figure 4 shows a typical histological section of seminiferous tubules of the normal intact rat.

Table 2 shows the number of germ and Sertoli cells per tubular cross section, corrected and uncorrected by Abercrombie's Formula. Sertoli cell nuclei are irregular and cannot be accurately measured. Thus, only cells which contained a single prominent nucleolus were counted. As nuclear diameters of germ cells in the various experimental groups were identical to those of Table 2, only the corrected counts are given in the subsequent tables. All the following experimental groups and their controls are compared to this group of normal intact rats.

Effects of Age on Germ Cell Number in the Normal Adult Rat

Table 3 shows the average weights of the testes and accessory organs of normal rats of various body weights. The age difference between 206 and 255, 255 and 301, 301 and 400 grams is 7, 9 and 24 days respectively. Thus, the difference in age between the youngest and oldest group is 40 days. This interval of time is during the most active period of body growth in the sexually mature Sprague-Dawley rat.

An increase, corresponding to the increase in body weight, occurs in the accessory organs. Each increment in body weight is mirrored by a concomitant increase in weight in the epididymes and seminal vesicles and prostate. However, the increase in testis weight is very small

being only significant between 250 and 300 grams ($.01 > p > .001$).

The average number of germ and Sertoli cells per tubular cross section in each of the four groups of normal rats is shown in Table 4. The tissues of these groups were shrunken during histological processing, rendering the counting of cells difficult. This shrinkage resulted in the number of cells, including the Sertoli cells, being increased in number per tubular cross section as compared with those of the previous normal intact group (Table 2).

Between 206 and 400 grams there is no significant change in the number of Type A spermatogonia ($.3 > p > .2$), in the number of resting primary spermatocytes ($.4 > p > .3$) or in the number of pachytene primary spermatocytes ($.9 > p > .8$). In the case of the step 7 spermatids there is an increase in the number of cells per tubular cross section between 206 and 400 grams ($p < .001$).

Effects of Testosterone Propionate on Germ Cell Number in Normal and Hypophysectomized Rats

Table 5 shows the average weights of the body, testes and male accessory organs of hypophysectomized and normal rats treated with testosterone propionate as well as oil-injected, sugar-fed, normal controls. The body weight of both the control rats and the normal rats injected with testosterone propionate are greater than those of the hypophysectomized animals. The testes weights of both normal rats treated with testosterone propionate or oil are slightly higher than those of the previous intact normal rats (Table 1). The testes of the hypophysectomized testosterone propionate treated groups are considerably smaller than those of the normals. In all hormone treated groups the

seminal vesicles and prostate are large as compared to the oil-injected groups. The epididymes, however, are not greatly changed.

Histological examination of the testes of hypophysectomized and normal rats receiving testosterone propionate reveal (Figures 6, 7) a picture quite similar to the intact untreated normal (Figure 4). However, in both the normal and hypophysectomized animals receiving the hormone, degenerating cells, usually advanced spermatids, were often encountered. These degenerating cells were seen lying near the basement membrane, presumably phagocytosed by the Sertoli cells. This phenomenon is also observed in intact animals but appeared more common in these experimental hormone treated groups. The seminiferous tubules although basically normal appeared to be of a smaller diameter than those of the intact normal rat (Figure 4). The Leydig cells were very atrophied with small dark nuclei.

Table 6 gives the average number of germ and Sertoli cells per tubular cross section corrected by Abercrombie's Formula. The germinal cells in the hypophysectomized rats or normal rats receiving testosterone propionate are reduced in number as compared to the intact normal rats (Table 2). All germ cell types in hormone treated animals are less than in the intact controls (Table 2; p values in all cases were less than .05). The Type A spermatogonia in the oil-injected, sugar-fed controls appears to be higher than those of the intact normal rat of Table 2. This difference was not, however, significant ($.2 > p > .1$). The variations in the number of cells in hypophysectomized animals 35 to 60 days post-operative were not greatly different, thus the entire lot was averaged (Table 7).

Effects of Adrenalectomy on Germ Cell Number

Due to the death of one animal in each group during the course of the experiment all values presented below represent the average of four animals.

Table 8 shows the average weights of the body, testes and accessory organs in adrenalectomized and sham-operated, pair-fed controls, 45 days post-operative. The restriction of food intake in the control animals has resulted in a lack of gain in body weight identical to that caused by adrenalectomy. The testes and accessory organs of both groups are, however, not different from those of the normal (Table 1). The histology of the testis of adrenalectomized animals (Figure 5) was not noticeably different from that of the normal intact rat (Figure 4).

Table 9 shows the average number of germ and Sertoli cells per tubular cross section in adrenalectomized and sham-operated, pair-fed controls. In all cases the counts compare favourably to those of the normal intact rat (Table 2).

Effect of ICSH and FSH on Germ Cell Number in the Hypophysectomized Rat

Table 10 presents the average weights of the body, testes and accessory organs in hypophysectomized rats treated with either FSH or ICSH and sacrificed 35 days post-operative.

Considering first the weights of the prostate and seminal vesicles it is seen that these organs are very small as compared to those of the normal intact rat (Table 1). Histologically, however, they did not resemble exactly those from an untreated hypophysectomized rat.

For example, the epithelium of the prostate displayed tall columnar cells indicating a certain degree of stimulation. On the other hand there was very little secretory product in the lumen of either the prostate or seminal vesicles. This stimulated condition of the accessory organs was more apparent in the FSH treated than in the ICSH treated hypophysectomized rats.

The weights of the testes were in all cases quite low as compared to the normal intact (Table 1). The dosage of 150 µg of FSH appeared to give the best maintenance on the basis of testis size. The dosage of 15 µg of ICSH appeared to give the best maintenance on the basis of testis size. In the groups of ICSH treated hypophysectomized rats receiving 10 µg or 20 µg of the hormone the seminiferous epithelium of some of the animals had undergone marked atrophy (Figure 13).

Histologically the testes of FSH treated animals (Figure 8) were superficially normal. The same was true for the group receiving 15 µg of ICSH (Figure 9). However, in both FSH and ICSH treated hypophysectomized rats seminiferous tubules were on occasion observed to contain a mass of degenerating cells (Figure 12).

The Leydig cells in both FSH treated and in ICSH treated animals displayed signs of active stimulation. The nuclei were large and pale, the cytoplasm copious and vacuolated (Figures 14 and 15). This stimulated condition of the Leydig cells could be correlated with maintenance of the seminiferous epithelium. In animals where there was a marked atrophy of the seminiferous tubule (10 µg and 20 µg of ICSH) the Leydig cells were often observed to be small with dark nuclei

thin strands of cytoplasm.

In animals treated with FSH or ICSH for 45 days the occurrence of atrophic seminiferous tubules increased. So much so that in each group only one or two animals had testes in which spermatozoa were being produced. The atrophy made cell counts so variable as to be useless.

Cell counts were made only on the 150 µg FSH treated and 15 µg ICSH treated hypophysectomized rats, 35 days post-operative. The averaged corrected counts of germ and Sertoli cells are shown in Table 11. Compared to the normal intact animals (Table 2) it can be seen that a reduction in the number of germ cells has occurred ($p < .01$ to $< .001$). However, the number of Sertoli cells has increased as compared to the normal intact rat.

Effects of a Combination of Testosterone Propionate with FSH or ICSH on Germ Cell Number in the Normal Rat

The weights of the body, testes and accessory organs of normal rats treated with testosterone propionate alone, and treated with testosterone propionate and FSH or ICSH, are given in Table 12. The weights are similar to those of the normal intact rat (Table 1), except those of the seminal vesicle and prostate which due to the testosterone propionate are very large. The weights of the testes and accessory organs in the two groups receiving a combination of hormones are larger than those receiving testosterone propionate alone. The weight of the seminal vesicle and prostate in the testosterone propionate FSH treated group are significantly larger ($.05 > p > .025$).

However, it must be remembered that these weights are of fixed tissue and the variation in the amount of secretory material in the seminal vesicles and prostate may result in significant differences.

Histologically the testes (Figures 10 and 11) appear similar to those of the normal intact animal. The Leydig cells of animals which received either FSH or ICSH with testosterone propionate were stimulated whereas the Leydig cells in those treated with testosterone alone were atrophied.

Table 13 shows the average number of germ and Sertoli cells per tubular cross section in normal rats treated with testosterone propionate or testosterone propionate with FSH or ICSH. The cell counts in the testosterone propionate treated group are similar to those of the previous experiment on the effects of this hormone in the normal rat (Table 6). However, in the case of the Type A spermatogonia, the number of cells in this experiment (Table 13) are less than in the previous one (Table 6; $.01 > p > .001$). The resting primary spermatocytes in the testosterone propionate-ICSH treated normals are not significantly different ($.4 > p > .3$) from those of the normal intact rat (Table 2). The step 7 spermatids are in the testosterone propionate-FSH treated rats are also not significantly different ($.2 > p > .1$) from those of the normal intact rat (Table 2). All other germ cells in these three groups of hormone treated normal rats are significantly different from those of the normal intact rat (p values $< .02 - < .001$). The Sertoli cells in these normal rats treated with testosterone propionate and testosterone propionate with

FSH or ICSH are increased in number per tubular cross section as compared to normal intact rats.

Effects of Testosterone Propionate in Hypophysectomized Rats on a Short Term Basis

Counts of Type A spermatogonia and Sertoli cells only were made in these hypophysectomized rats treated with testosterone propionate, and sacrificed 3, 6, 9, 13 and 26 days post-operative. The counts of Type A spermatogonia were corrected by the Sertoli cell correction factor and the results depicted graphically (Figure 23). At 3, 6 and 9 days post-operative the number of Type A spermatogonia is not significantly different from that of the normal intact rat (Table 2) the p values being $.8 > p > .7$, $p > .9$ and $.9 > p > .8$ respectively. At 13 and 26 days post-operative the number of cells is significantly less than that of the intact rat ($.001 > p$).

Effects of Lactogenic Hormone in Hypophysectomized Rats

Table 14 presents the average weights of the body, testes and accessory organs in hypophysectomized rats treated with testosterone propionate alone or with testosterone propionate and LTH. As in other hypophysectomized, hormone treated rats, the testicular weights are lower than those of the normal intact rat (Table 1). Due to the testosterone the seminal vesicles and prostate are quite large. Histologically the seminiferous epithelium of the rats receiving testosterone propionate alone or testosterone propionate and LTH (Figure 16) appeared similar to that of the normal intact rat.

Cell counts (Table 15) revealed that the number of germ cells in both hormone treated groups is less than that of the normal intact rat (Table 2; p values all less than .05). The Sertoli cells were, on the other hand, increased in number as compared with the normal intact rat. There is little difference between the number of germ cells in the testosterone propionate treated rats as compared with the testosterone propionate-LTH treated rats. The step 7 spermatids display the greatest difference but this was not significant ($.1 > p > .05$).

Effects of the Non-Gametogenic Hormones

Effects of thyroidectomy Table 16 presents the average weights of the body, testes and accessory organs of thyroidectomized and sham-operated control rats sacrificed 45 days post-operative. The weights of the testes and accessory organs are similar to those of the normal intact rat (Table 1), in fact they are slightly greater. It should be noted that the sham-operated group appeared to have suffered from some type of low grade infection as they did not grow throughout the duration of the experiment.

Histologically the testes of the thyroidectomized rats (Figure 17) appeared to be normal.

Counts (Table 17) of germ and Sertoli cells in the thyroidectomized and sham-operated controls revealed that there is a slight reduction in the number of the evolved germ cells in the thyroidectomized rats. The Type A spermatogonia are present in number equal to that of the normal intact rat (Table 2). However, the resting primary spermato-

cytes and pachytene primary spermatocytes are significantly less ($.01 > p > .001$ and $.02 > p > .01$, respectively). The reduction in spermatids is however, not ($.2 > p > .1$).

Effects of Thyroxin and Growth Hormone in Hypophysectomized Rats

Table 18 gives the average weights of the body, testes and accessory organs of hypophysectomized rats treated with testosterone propionate, testosterone propionate and thyroxin, testosterone propionate and GH and testosterone propionate, thyroxin and GH, sacrificed 30 days post-operative. The weights of the testes, in all groups including those receiving GH are below those of the normal intact rat (Table 1). The seminal vesicles and prostate, due to the testosterone are much larger.

Completeness of hypophysectomy in the two groups receiving GH was judged on the basis of histological examination of the adrenal and thyroid glands and gross examination of the sella turcica at the time of sacrifice.

Histologically the testes of the various groups appeared to be similar to that of the normal intact rat; treatment with testosterone propionate and thyroxin (Figure 18), with testosterone propionate and GH (Figure 19) and with testosterone propionate, thyroxin and GH (Figure 20) did not cause any apparent change in the histology of the seminiferous epithelium.

Counts of germ and Sertoli cells (Table 19) revealed that in all four experimental groups the number of germ cells was less than that of the normal intact rat (Table 2). P values range from $< .01$ to

$<.001$. The Sertoli cells are increased in number as compared with the normal rat.

Effects of Hypophysectomy and Growth Hormone This experiment was done primarily to assay the GH preparation for gonadotropin contaminants. Table 20 shows the average weights of the body, testes and accessory organs in untreated hypophysectomized rats and hypophysectomized rats treated with GH sacrificed 30 days post-operative. The GH treatment caused an increase in body weight but resulted in no change in the testes or accessory organs as compared with the untreated hypophysectomized rat.

Figure 32 depicts the growth curves of untreated hypophysectomized rats, GH treated hypophysectomized rats treated with GH and a group of normal intact rats which were maintained as controls. At the termination of the experiment the intact rats weighed considerably more than did the GH treated hypophysectomized rats. However the hypophysectomized rats did not respond to the GH therapy until about the tenth post-operative day (6 day of hormone therapy), consequently they weighed less than the control animals.

Histologically the testes of the GH treated rats (Figure 22) were not different from the untreated hypophysectomized rats (Figure 21). In both groups many seminiferous tubules were observed to be devoid of spermatids, other tubules contained a few very young spermatids.

The Leydig cells in the GH treated rats were atrophied indicating no gonadotropin contamination of the GH preparation.

Cell counts (Table 21) revealed a great loss of germ cells in

both the untreated and GH treated hypophysectomized groups. The more evolved the cell type the greater the loss. There was no significant difference between the number of germ cells in the untreated hypophysectomied rats as compared with the GH treated hypophysectomized rats. The Sertoli cells of both groups were greatly increased in number per tubular cross section as compared with the normal intact rat (Table 2).

The correction of the germ cell counts by the Sertoli cell correction factor involves calculation of the percentage increase in the number of Sertoli cells in the experimental groups. The average number of Sertoli cells per tubular cross section in each group is presented in Table 22. The percentage of cells in the experimental groups as compared to the normal intact rat is given in the second column of the table.

The corrected values of germ cell number in each of the experimental groups are presented in Table 23. This data was used in the construction of the histiograms used in the Discussion. The experimental groups represented in the various histiograms are denoted by the corresponding figure number of each of the histiograms.

DISCUSSION

THE EFFECTS OF HORMONES ON SPERMATOGENESIS

The effects of hormones on spermatogenesis are commonly assessed by two procedures: 1) by testing the effectiveness of various hormones in support or maintenance of spermatogenesis in hypophysectomized animals, in which hormone therapy is commenced immediately after pituitary ablation; 2) by testing the effectiveness of various hormones in re-initiation or restoration of spermatogenesis in animals which are hypophysectomized, regression of the seminiferous epithelium is allowed to occur and then hormone therapy is commenced. The second procedure is generally considered a more rigorous test of hormonal requirements of spermatogenesis. Hormones, for example LTH and GH, which do not appear to be essential in maintenance of spermatogenesis, on the other hand appear to be beneficial in restoration of the process (Woods and Simpson, 1961). However, the interpretation of data from restoration experiments is complicated by extreme variability of response between individual animals. Specific hormone therapy has been observed to restore spermatogenesis in some animals while having little effect in other animals of the same experimental group, (Boccabella, 1963). The work of this author suggests that long-term hypophysectomy may cause irreparable damage to the seminiferous epithelium of some animals. Consequently hormone therapy will be ineffective.

Hormonal maintenance of spermatogenesis likely constitutes a condition closer to the physiological one than does hormonal restoration

of spermatogenesis. Long-term hypophysectomy possibly results in damage such as loss of enzymatic systems which must be re-activated before spermatogenesis can be re-established. Hormones, not normally required for spermatogenesis may be necessary to re-activate these enzyme systems and thus restore spermatogenesis. For example the extreme atrophy of the Sertoli cells may have to be repaired before the germ cells can be stimulated. Thus, restoration experiments may not be determining just the hormones which physiologically are required by spermatogenesis. Furthermore, hormones which have been shown to be necessary for restoration of spermatogenesis may have subtle effect on the maintenance. These subtle effects should, however, be detectable by counting germ cells, a procedure which has been used in this investigation.

In discussion of the effect of hormones on the seminiferous epithelium three aspects of the process of spermatogenesis must be clearly distinguished and defined. 1) The rate of spermatogenesis is the time taken by the germ cells to evolve through the various phases of spermatogenesis whereby Type A spermatogonia evolve into spermatozoa. The word rate in this case denotes only duration, and does not imply quantity per unit time. Thus, rate of spermatogenesis is the time taken for a spermatogonium to evolve into a spermatozoon, but does not imply the number of spermatozoa produced per unit time. 2) The yield of spermatogenesis is the number of evolved germ cells produced per lesser evolved cell. Thus, one may speak of the yield of spermatocytes per spermatogonium, of spermatids per spermatocyte

and of spermatozoa per young spermatid. The total yield of spermatogenesis is therefore, the yield of spermatozoa per Type A spermatogonium. In the present investigation the most evolved germ cell counted was the step 7 spermatid. Thus in the discussion, the yield of spermatogenesis will refer to the number of step 7 spermatids produced per Type A spermatogonium. 3) The stem cell population or Type A spermatogonia, are the cells which give rise to the differentiating spermatocytes and renew their own number. Discussion of stem cells includes their absolute number as well as their renewal.

Effect of Hormone on the Rate of Spermatogenesis

Histiograms (Figures 24, 26, 27, 28 and 30) reveals that therapy with various hormones produces a reduction in the number of germ cells per tubular cross section in comparison with the normal, intact rat. A 20-30% reduction in all germ cells occurred in normal or hypophysectomized rats in which spermatogenesis had been maintained by exogenous hormones. Thus, the testes of these animals appear to be producing fewer spermatocytes, spermatids and spermatozoa than the testes of normal intact rats. However, if hormone therapy has resulted in an acceleration of the rate of spermatogenesis the testes of these hormone treated rats may still be producing the same number of spermatogonia, spermatocytes and spermatids per unit time as is the normal. The observation of fewer germ cells in histological section may be but an indication of this accelerated rate. Thus, the possibility of hormones accelerating the rate of spermatogenesis must be examined.

The work of Ortavant (1958) in the ram, Desclin and Ortavant (1963)

in the rat, Clermont and Harvey (1965) in the rat and Heller and Clermont (1964) in man suggest that hormones have no effect on the rate of spermatogenesis. Study of the effects of a lack of hormones, stimulating hormones and depressant hormones on spermatogenesis revealed no change in the rate of spermatogenesis. These findings have led the above investigators to postulate that the rate of spermatogenesis is a hormone independent, species-specific, biological constant.

However, Huckins (1965) reported an exception to the constancy of the rate of spermatogenesis. She observed that the rate in a very young animal was at first more rapid than in the adult. The rate, however, became equal to that of the adult before sexual maturation.

Boccabella and Materazzi (1964) reported that testosterone propionate treatment in hypophysectomized rats resulted in the alternation of the rate at which the germ cells evolved. However, their determination of the rate was merely an estimate. Desclin and Ortavant (1963) and Clermont and Harvey (1965) found that testosterone propionate caused no change in the rate of spermatogenesis in the hypophysectomized rats. In both these latter studies the exact phase of evolution of labelled germ cells was ascertained and consequently the rates of evolution precisely determined. However, the work of Boccabella and Materazzi was a study in restoration, whereas those of Desclin and Ortavant and Clermont and Harvey were studies on maintenance. This difference in experimental procedures may possibly account for the discrepency in results.

Desclin and Ortavant (1963) and Clermont and Harvey (1965) found

that complete hypophysectomy (i.e. lack of all the pituitary hormones) resulted in no change in the rate of spermatogenesis. Consequently, it has been assumed that none of the hormones employed in the present investigation cause changes in the rate of spermatogenesis. Hence, a decrease in number of germ cells per tubular cross section indicates an actual loss of germ cells, rather than an acceleration in the rate.

Effects of Hormones on the Yield of Spermatogenesis

The number of spermatocytes, spermatids and spermatozoa produced per spermatogoium, or yield of spermatogenesis is the second aspect of spermatogenesis possibly affected by hormones. Germ cell degeneration during division and differentiation resulting in a 10.6% loss of the spermatogonia during mitosis, a 27.4% loss of spermatocytes during meiosis (Clermont, 1962) and a 10% loss of spermatids during spermiogenesis (Roosen-Runge, 1955) has been reported in the normal intact rat. These germ cell degenerations in the normal intact rat result in the yield of spermatozoa being 52% only of the theoretical yield (Clermont, 1962). Thus, the administration of exogenous hormones into the normal rat could possibly improve the yield of spermatogenesis.

Concerning the effect of hypophysectomy on the yield of spermatogenesis, Clermont and Morgentaler (1955) reported that within 10 days after hypophysectomy all advanced spermatids disappeared from the seminiferous epithelium (i.e. the yield of spermatozoa became nil). The less evolved spermatocytes and young spermatids, although greatly reduced in number, persisted as long as 60 days post-operative. Thus, hormones should affect the yield of spermatogenesis in the hypophy-

sectomized rat.

In the present investigation the most evolved germ cell enumerated was the step 7 spermatids. Thus, yield of spermatogenesis refers in the discussion below to the number of step 7 spermatids produced per Type A spermatogonia.

Steroid Hormones Injection of testosterone propionate into hypophysectomized rats (Figure 24) greatly increases the number of spermatocytes and spermatids compared to the number of these cells in the untreated hypophysectomized rat. However, compared to the normal intact rat the number of spermatogonia, spermatocytes and spermatids is reduced in the testosterone propionate treated hypophysectomized rat. Calculation of the ratio of step 7 spermatids to Type A spermatogonia (i.e. the yield) from the data of Table 23 reveals that the ratio in the normal intact rat is 113:1 and in the hypophysectomized testosterone propionate treated rat it is 103:1. Thus, although the absolute number of germ cells in hypophysectomized testosterone propionate treated rats is below that of the normal, the yield of spermatids per Type A spermatogonia remains similar. This similarity to the normal of the yield in hypophysectomized testosterone propionate treated rats is reflected in Figure 24 by the similarity in the degree of reduction of each of the cell types.

Injection of testosterone propionate into normal rats causes the germ cells to be reduced in number in a manner similar to the reduction in number in hypophysectomized rats treated with this hormone (Figure 24). From the data of Table 23 the ratio of step 7 spermatids to Type A

spermatogonia in normal testosterone propionate treated rats is 106:1. Thus, although the number of cells in these normal rats is reduced the yield of spermatogenesis remains the same. Hence it would appear that testosterone is the main steroid hormone involved in the maintenance of the normal yield of spermatogenesis. However it does not cause the normal yield to be exceeded.

Other than the testis, the main source of steroid hormones in the body, some of them androgenic, is the adrenal cortex. Figure 25 shows that the removal of the adrenal gland has little effect either on absolute number of germ cells or on the yield. The number of the various germ cells is the same as in the normal intact animal. The actual yield calculated from Table 23 is 117 spermatids per Type A spermatogonia compared to 113 in the normal intact rat. Thus, testosterone appears to be not only the main steroid hormone involved in the maintenance of the yield of spermatogenesis, but also to be the sole steroid hormone involved.

Pituitary Gonadotropins Figure 26 represents the number of germ cells found in hypophysectomized rats treated with FSH or ICSH. Again as in testosterone propionate treated rats the number of cells is below that of the normal intact rat. When the yield of spermatids per Type A spermatogonia is calculated from Table 23 it is found to be 108:1 in ICSH treated rats and 132:1 in FSH treated rats. Thus, ICSH maintains the yield in a manner similar to testosterone propionate. FSH appears to greatly improve the yield. However, the reader should be cautioned that the number of Type A spermatogonia in the FSH treated animals

is lower than the ICSH treated groups. On the other hand the number of step 7 spermatids is higher. Calculation of ratios results in a summation of these two differences and causes a higher value for the yield. Furthermore, a Chi squared test for difference in proportion revealed that all germ Type A spermatogonia, resting spermatocytes, pachytene spermatocytes and step 7 spermatids in the FSH treated group were reduced by the same proportion, thus the apparent improvement in yield by FSH is likely only due to variation in the data. FSH most likely has no additional effects to that of ICSH.

Figure 27 depicts the relative number of germ cells per tubular cross section in normal rats treated with testosterone propionate, testosterone propionate and FSH, or testosterone propionate and ICSH. As was noted in the Results, the number of resting primary spermatocytes in the testosterone propionate ICSH treated group and the number of step 7 spermatids in the testosterone propionate FSH treated was not significantly different from that of the normal intact rat. However, after correction of the counts with the Sertoli cell correction factor (Table 23) these values are now significantly below those of the normal.

Calculation of the ratio of step 7 spermatids to Type A spermatogonia (i.e. the yield) in the normal rats treated with testosterone propionate alone, or with testosterone propionate and FSH and testosterone propionate and ICSH, produces ratios of 143:1, 154:1 and 151:1 respectively. These are considerably in excess of the yield found in normal intact rats (113:1). Examination of Figure 27 reveals that the point in spermato-

geneses at which the yield is improved is between the Type A spermatogonia and the resting primary spermatocytes. In proportion to the number in the normal intact rat the resting primary spermatocytes are present in greater number than are the Type A spermatogonia. The improvement in the yield occurs in the number of resting primary spermatocytes per Type A spermatogonia. This improved yield is then carried through the pachytene primary spermatocytes and step 7 spermatids. FSH or ICSH do not appear to improve further the effects of testosterone propionate alone in normal rats.

Before drawing conclusions from the results of this experiment (Figure 27) the results of the previous experiment (Figure 24) in which testosterone propionate was injected into normal rats must be reviewed. In this experiment no improvement in yield of spermatids was found. Thus, doubt is cast on the validity of one or other experiment. Examination of the protocols of both experiments revealed no valid reason for discarding either. It would thus appear that the response of normal rats to injection of testosterone propionate can vary considerably. Comparing Figure 24 and 27 it is seen that the variation is in the number of Type A spermatogonia. Thus, the variation in yield is not due to an increase in the number of spermatids found but to a decrease in the number of Type A spermatogonia.

However, if testosterone propionate injected into normal rats can in certain instances improve the yield of spermatids per spermatogonia, then it must be emphasized that the improvement in yield is accompanied by a loss in absolute number of all germ cells. Secondly

FSH or ICSH do not appear to improve further the yield resulting from testosterone propionate therapy alone.

A noteworthy difference between the normal rats receiving FSH or ICSH for 45 days and the hypophysectomized animals injected with the same hormones and sacrificed 45 days post-operative, was that the Leydig cells in the normal animals were highly stimulated. In the hypophysectomized rats the Leydig cells were atrophied. Because of these results in normal animals it was likely not antibody formation to the hormones which caused the failure in the hypophysectomized rats. It would seem that the Leydig cells of the hypophysectomized rats could not withstand the prolonged stimulation whereas the Leydig cells of the normal rats, due to better nutritional and metabolic conditions, were more resistant.

The role of ICSH, accepted by most modern workers, is that it stimulates the Leydig cells to produce androgen (Woods and Simpson, 1961; Lostroh et al, 1963; Papkoff, 1965). The similarity of cell counts and yield of spermatids per spermatogonia in testosterone propionate treated hypophysectomized rats and ICSH treated hypophysectomized rats supports this finding. Although FSH produced an improvement in the yield, the significance of the results are doubtful. It most likely has no effects additional to those of ICSH. Also in the normal rats FSH produced no effects additional to those produced by testosterone propionate alone or testosterone propionate and ICSH. The similarity of effects of FSH to those of ICSH or testosterone propionate plus the absence of additional effects would suggest that

either FSH acts in a manner identical with ICSH, or that the FSH preparation was contaminated with ICSH. Consequently the ICSH would be the active principal. The difficulty of removal of ICSH from FSH preparations has been previously reported (Woods and Simpson, 1961). On the other hand pure FSH acts like ICSH in respect to androgen secretion in the Sprague-Dawley rat (Papkoff, 1965).

It has been suggested that both FSH or ICSH are required at meiosis (Lostroh et al, 1963). However, in the present experiment either hormone or testosterone propionate alone would support meiosis in the hypophysectomized rat. However the above mentioned investigators studied the effects of hormones on restoration of spermatogenesis in long-term hypophysectomized rats, whereas the present investigation studied the effects of hormones on maintenance of spermatogenesis.

The injection of LTH has been shown to have a beneficial effect on the male reproductive system when injected with ICSH (Segaloff et al, 1956; Lostroh et al, 1956; Woods and Simpson, 1961). It has also been shown to augment the effects of testosterone propionate on the prostate (Greyhack et al, 1955). Thus, presumably the effects of LTH with ICSH is through the androgen produced by ICSH injection.

Examination of Figure 28 indicates that LTH administered with testosterone propionate produced no additional effects to the administration of testosterone propionate alone. The yield of step 7 spermatids per Type A spermatogonia in the testosterone treated group in this case is 124:1 and in the LTH treated group it is 105:1 (calculated from data of Table 23). In fact LTH appears to have a detrimental effect. However, due to the relatively large standard errors of the counts (see

Table 15), the difference between testosterone propionate and testosterone propionate and LTH treatment is not significant. Hence, LTH does not increase the yield of cells above the yield produced by testosterone propionate alone, nor does it make the yield less.

Non-gametogenic Hormones Figure 29 depicts the relative number of germ cells in the thyroidectomized rats. The Type A spermatogonia are present in the same number as in the normal intact rat. The number of resting and pachytene primary spermatocytes is slightly decreased. The yield of step 7 spermatids per Type A spermatogonia is 103:1, (calculated from data of Table 23). This is not greatly reduced from the 113:1 ratio found in the normal intact rat. Thus, the contribution of the thyroid in maintenance of the yield of spermatogenesis is rather minor.

Another hormone not normally considered as being gametogenic is growth hormone (GH). However, it has been shown to augment the effects of other hormones on the testis (Huggins et al, 1955; Lostroh et al, 1956; Lostroh and Li, 1957; Randolph et al, 1959; Woods and Simpson, 1961; Boccabella, 1964).

Figure 30 depicts the relative number of cells in hypophysectomized rats treated with testosterone propionate alone or with thyroxin, or with GH or with thyroxin and GH. All germ cells, Type A spermatogonia, resting primary spermatocytes, pachytene primary spermatocytes and step 7 spermatids are reduced in number as compared to the intact normal rat. The yield of step 7 spermatids per Type A spermatogonia in testosterone propionate treated, testosterone propionate thyroxin treated,

testosterone propionate GH treated and testosterone propionate thyroxin GH treated is 100:1, 107:1, 109:1 and 111:1 respectively, as compared to 113:1 in the intact rat (calculated from data of Table 23). Thus, treatment with thyroxin or GH does not improve the yield above that resulting from testosterone propionate treatment alone.

Figure 30 shows the GH had absolutely no effect on germ cell number in the hypophysectomized rat. Hypophysectomized rats treated with GH alone present the same loss of cells as do the untreated hypophysectomized animals, but in the GH treated animals somatic growth continued (Figure 32). Therefore, GH does not have any subtle effects on the testis which are masked by the injection of large amounts of testosterone propionate.

One instance where a slight improvement in the yield of spermatogenesis was observed in the present investigation was in the intact animals with increasing age (Table 4). The yield of step 7 spermatids per Type A spermatogonia improved from 97:1 in 206 gram rats to 128:1 in 400 gram rats. Thus, increasing age appears to slightly increase the yield of spermatogenesis.

Before concluding this discussion on the effects of hormone on yield of spermatogenesis one point is worthy of note. Change in testicular weight has frequently been used to assess the effects of hormones on spermatogenesis. In the present investigation hormone therapy was found to cause a loss of germ cells. A decrease in testicular weight was also consistently observed. However, this loss of weight was found in some instances to vary from one hormone group to the next when the loss of germ cells did not. For example, in Table 5

the testicular weight in normal rats treated with testosterone propionate is greater than in hypophysectomized rats treated with the same hormone. But cell counts reveal no difference in loss of germ cells in these two groups (Figure 24). Thus, it would appear that testicular weight is a poor criterion of germ cell content.

In conclusion, the effects of hormones on the yield of spermatogenesis appears to be as follows: 1) Hypophysectomy produces a rapid decrease in the yield. 2) Injection of testosterone propionate into a hypophysectomized rat will maintain the yield at a normal level but will not cause the yield to exceed the normal level. The absolute number of stem cells or Type A spermatogonia reduced, the number of spermatocytes and spermatids being reduced proportionally. 3) ICSH or FSH will produce similar results to those of testosterone propionate. The effects of ICSH or FSH appear to be due to stimulation of androgen production. 4) The injections of thyroxin, LTH or GH with testosterone propionate into the hypophysectomized rat do not result in any augmentation of the effects of testosterone on the yield of spermatogenesis. 5) Removal of the thyroid or adrenal glands have little or no effect on either yield of spermatogenesis or on absolute number of germ cells. 6) Injection of testosterone propionate into normal rats results in a loss of germ cells. The yield of spermatids per spermatogonia in these animals may vary from a yield similar to the normal to a greatly increased yield. However, when the yield is observed to be increased the absolute number of stem cells is found to be greatly decreased. FSH or ICSH injected with testosterone propionate into the intact rat

produce no additional effects to those produced by testosterone propionate alone. Increasing age appears to be the only condition where the number of stem cells remains constant and the yield of spermatids increases.

Thus it appears that testosterone is the principal hormone concerned with maintenance of the normal yield of spermatogenesis. The only other effective hormones being ICSH or FSH which stimulate production of testosterone.

Effect of Hormones on the Stem Cell Population

The third aspect of the effect of hormones on spermatogenesis is their influence on the stem cell population. Pituitary ablation in the rat results in a decrease in the number of stem cells, or Type A spermatogonia (Clermont and Morgentaler, 1955). This decrease is characterized by a marked loss of cells during 10-15 days post-operative, followed by a gradual and continuous loss of cells to 60 days post-operative.

The number of spermatogonia in testosterone propionate treated hypophysectomized rats remains nearly constant during 1-9 days post-operative (Figure 23). Between 9-13 days there is a sharp decrease in number of Type A spermatogonia. Then, the number of these cells remains constant to 26 days post-operative. In the first group of experiments it was observed that the number of Type A spermatogonia remained unchanged in hypophysectomized rats treated with testosterone propionate 35-60 days post-operative (see Table 6. Note: the values of this table have not been corrected by the Sertoli cell correction factor and consequently are higher than those of Figure 23). Thus,

testosterone propionate would appear to prevent the continuous loss of Type A spermatogonia observed in the untreated hypophysectomized rat (Clermont and Morgentaler, 1955). On the other hand testosterone propionate treatment does not prevent the sharp initial fall in stem cells, occurring before 15 days post-operative.

It may be argued that failure of testosterone propionate therapy to maintain the spermatogonia is due to the fact that the hormone therapy was not begun until the 5th post-operative day. However, this fails to explain an identical loss of Type A spermatogonia in normal animals treated with this hormone. Furthermore, in a pilot experiment hypophysectomized rats were treated daily with testosterone propionate beginning 3 hours after pituitary ablation. This treatment likewise failed to maintain the Type A spermatogonia. A group of hypophysectomized rats were also injected every 12 hours, rather than daily. Again, hormone therapy failed to maintain the normal number of spermatogonia. Hence, testosterone appears to have only minor effects on the maintenance of the stem cell population of the testis.

The fact that testosterone propionate therapy in normal rats results in the same dimunition of Type A spermatogonia as testosterone propionate therapy in hypophysectomized rats suggests that a pituitary factor under the control of testosterone, is involved in the maintenance of the stem cell population. In the hypophysectomized rat this factor is absent, and in the testosterone propionate treated normal rats secretion of this factor is suppressed. However, the administration of FSH or ICSH does not improve the effects on stem cell number of testosterone propionate therapy alone (Figure 27), nor does LTH (Figure 28) nor does GH (Figure 30).

Boccabella and Materazzi (1964) reported that injection of both testosterone propionate and GH restored the normal number of Type A spermatogonia in the hypophysectomized animal, whereas injection of testosterone propionate alone did not. This finding was not confirmed in the present investigation. However, the above authors investigated the effects of hormones on the restoration of spermatogenesis in the long-term hypophysectomized rat whereas the present investigation studied the effects of hormones in maintenance of spermatogenesis in the hypophysectomized rat. Because of this difference of procedure the variation in result may be attributed to the following observations.

Bustos (personal communication, 1966) in a study of whole-mounts of seminiferous tubules of the normal rat found two classes of Type A spermatogonia: A_1 which divide, renew themselves and give rise to differentiating intermediate spermatogonia and are thus the stem cells of spermatogenesis; and A_0 which do not appear to undergo mitosis. Bustos speculated that the A_0 constitute a reserve of stem cells, which under certain conditions, could divide to repopulate denuded areas of seminiferous epithelium.

Recently Dym (personal communication, 1966) studying the effects of ionizing radiation on the seminiferous epithelium, observed that after irradiation of the testis, the A_1 spermatogonia disappeared from the seminiferous epithelium, while the A_0 remained. Thereafter the normal number of A_1 spermatogonia reappeared, suggesting that the A_0 spermatogonia repopulate the damaged seminiferous epithelium. In fact, the number of A_1 spermatogonia may exceed the original number.

This "rebound" phenomenon has been reported in man (Heller et al, 1950; Heckel et al, 1951; Heller at al, 1959). Suppression of spermatogenesis in man by androgen or oestrogen treatment is followed by a recovery period in which sperm counts exceed the original counts.

The observations of Dym would suggest that the mechanism which initiates division of the A_0 spermatogonia is serious damage to the seminiferous epithelium. It is possible that the findings of Boccabella and Materazzi (1964) indicate the damage of the seminiferous epithelium produced by long-term hypophysectomy will also initiate the A_0 spermatogonia to divide and repopulate the seminiferous epithelium, but they require GH to do this.

The present investigation was a study of the effects of hormones on the maintenance of the seminiferous epithelium. The presence of numerous spermatocytes and spermatids possibly suppressed the divisions of the reserve stem cell, the A_0 spermatogonia. Thus, these cells did not divide and replace the 20-30% loss of Type A spermatogonia.

To explain the reduction in the number of Type A spermatogonia in hormone treated rats, one is invariably forced to postulate a hormonal lack as being the cause. But what is the hormone involved? Hypophysectomy causes a loss in number of Type A spermatogonia. Treatment with testosterone propionate does not appear to curtail this loss. Results of testosterone propionate treatment in normal rats reveal a similar loss, suggesting that a pituitary hormone which is under the control of testosterone is involved. However, neither ICSH nor FSH produce an increase in the number of Type A spermatogonia when injected alone or with testosterone. The secretion of LTH may also be under

the control of testosterone (Woltheus, 1963). However, injection of LTH does not improve the number of Type A spermatogonia. It has been suggested that GH secretion can be suppressed by very large amounts of testosterone propionate (Meyer, 1948). GH also does not increase the number of Type A spermatogonia. The adrenal or thyroid gland do not appear to have any effect on the number of stem cells or Type A spermatogonia.

Thus, if the maintenance of normal numbers of spermatogonia is indeed under hormonal control, then it is either due to an unknown factor possibly of pituitary origin, or it is due to a delicate balance of hormones both testicular and pituitary. This possibility of an unknown factor is doubtful because of the findings of Woods and Simpson (1961). These authors found that injection of fresh pituitary homoginates would not maintain testicular weights in the hypophysectomized animal better than injections of FSH or ICSH. Therefore the failure of the number of Type A spermatogonia to be maintained in hormone treated rats would appear to be due to hormonal imbalances caused by the injection of exogenous hormone.

The Mode of Germ Cell Loss in Hormone Treated Rats

In the experiment on the effects of short term testosterone propionate therapy in the hypophysectomized rat (Figure 23), it was noted that the number of Type A spermatogonia remains constant until 9 days post-operative. A loss of the spermatogonia occurs between 9 and 13 days. At 13 days the seminiferous tubules were occasionally found to contain masses of degenerating spermatocytes and spermatids. Preliminary counts of the resting and pachytene spermatocytes of these

rats revealed that these cells were reduced by about 20%. This finding would suggest that at about 13 days post-operative in testosterone propionate treated hypophysectomized rats the seminiferous epithelium non-selectively sloughs a certain percentage of all germ cell types, spermatogonia, spermatocytes and spermatids. Thus, the reduction in resting and pachytene primary spermatocytes and step 7 spermatids is not a reflection of an initial loss of Type A spermatogonia as was previously reported (Harvey, 1963, 1965).

The above conclusion is also supported by indirect evidence. The duration of the cycle of the seminiferous epithelium in the Sprague-Dawley rat is 12.9 days (Clermont and Harvey, 1965). The reduction in Type A spermatogonia occurs at 13 days post-operative. It will require one cycle before this reduction is manifest by the resting primary spermatocytes, two cycles before it is manifest by the pachytene primary spermatocytes and three cycles before it is manifest by the step 7 spermatids. Consequently an initial reduction in stem cells or type A spermatogonia would not be detected as a similar reduction in the step 7 spermatids until 51.7 days after pituitary ablation (i.e. the initial 13 days plus the duration of the 3 cycles). But after 30 days post-operative (Figure 30) there is a proportional loss in the step 7 spermatids and the Type A spermatogonia. Thus, the reduction in number of germ cell in hormone treated rats appears to affect all cell types at approximately the same time.

The observation that at approximately 13 days post-operative the seminiferous epithelium non-selectively sloughs a certain percentage

of the germ cells suggests that the seminiferous tubules in these hormone treated rats can no longer contain the large number of cells found in the normal intact animal. Moreover, the percentage of reduction of each germ cell tupe is constant suggesting that the target structure of the various hormones may not be the germ cells themselves, but some other element of the seminiferous tubules. The other elements are the myo epithelial-like cells of the limiting membrane and the Sertoli cells.

In discussion of the effects of hormones on the seminiferous epithelium, one has a tendency to concentrate attention on the germ cells and disregard the Sertoli cells. Granted, the Sertoli cells compared to the germ cells constitute, in some respects, a static cell population. In the young rat they are not observed to undergo mitosis after 20 days of age (Clermont and Perey, 1957; Huckins, 1965). In the hypophysectomized rat they are not observed to degenerate as do the germ cells (Clermont and Morgentaler, 1955). Although the Sertoli cells are not observed to degenerate in the hypophysectomized animal they are observed to undergo morphological changes (Clermont and Morgentaler, 1955). These cells become greatly reduced in both cytoplasmic and nuclear volume. The cytoplasm becomes vacuolated and loses its numerous processes. Injection of certain hormones, specifically FSH, has been reported to result in a marked response in the Sertoli cells but little response in the germ cells (Lostroh et al, 1963; Murphy, 1965). Indeed the Sertoli cells are the only element of the seminiferous epithelium which has been observed to respond to hormones in tissue

culture (Steinberger et al, 1964). Since the germinal elements are intimately associated with the numerous cytoplasmic processes of the Sertoli cells, any change in the shape or size of these supporting cells could result in a loss of germ cells due to insufficient support.

Thus, the effects of hormones on spermatogenesis may not be on the germ cells themselves but rather on the Sertoli cells which support these germ cells.

SUMMARY AND CONCLUSIONS

The effects of hormones on spermatogenesis has been, over the years, the subject of numerous investigations. Despite the amount of work published on this topic, very few studies have attempted to assess quantitatively, by cell counting, the effects of hormones on spermatogenesis. Consequently a study was undertaken to evaluate the effectiveness of various hormones in maintaining germ cell number in the rat. Hormone therapy was commenced shortly after pituitary ablation. Thus the investigation examined the effects of hormones on the maintenance of spermatogenesis rather than on the restoration or re-initiation of spermatogenesis in the hypophysectomized rat.

In the first part of the investigation hypophysectomized rats were treated with testosterone propionate commencing on the 5th post-operative day and continuing up to the day of sacrifice 35 to 60 days post-operative. Histological examination of the testes of these animals revealed that spermatogenesis was maintained by the hormone therapy. Cell counts revealed no change in the number of germ cells between 35 and 60 days post-operative. However, comparison of the counts with those of the normal intact rat revealed that all germ cells in the hypophysectomized testosterone propionate treated rats were reduced by 20-30%. This reduction in number affected all germ cell types equally. Thus, the yield of spermatids per spermatogonia remained the same as in the normal intact rat.

Testosterone propionate injection into normal rats caused a reduction in number of cells similar to that found in the hypophysectomized testosterone propionate treated animals.

Injection of FSH or ICSH into hypophysectomized rats was also found to maintain spermatogenesis. However, this maintenance failed if therapy was continued beyond 35 days post-operative. Cell counts in hypophysectomized animals in which spermatogenesis was maintained revealed no difference in the maintenance resulting from FSH or ICSH. The germ cells of either group, on the other hand, were reduced as compared to the number in the normal intact rat. As in the testosterone propionate treated hypophysectomized rats, the reduction in number in FSH or ICSH treated rats affected all cell types equally. Thus, the yield of spermatids to spermatogonia remained unchanged. The similarity of results in ICSH treated hypophysectomized rats with the results in testosterone propionate treated hypophysectomized rats suggests that the ICSH maintained spermatogenesis by the stimulating of the Leydig cells to produce androgen. The similarity of the results of FSH therapy with the results of either ICSH therapy, or testosterone propionate therapy suggests that the action of FSH is due to one of two reasons. Either FSH has no effect on the maintenance of spermatogenesis, but ICSH contamination of the preparation is the active principle, or the action of FSH in the Sprague-Dawley rat is identical to that of ICSH. That is, FSH stimulates androgen secretion which results in maintenance of spermatogenesis.

One qualitative difference between the effects of FSH and ICSH was noted. All dosages of FSH used resulted in consistent maintenance of spermatogenesis in hypophysectomized rats up to 35 days post-operative. On the other hand, only one dosage of ICSH caused consistent maintenance.

This suggests that for practical purposes FSH is the pituitary hormone of choice in maintaining spermatogenesis. On the other hand it may indicate a necessity of FSH for spermatogenesis which is not readily apparent.

Cell counts of normal rats treated with testosterone propionate and FSH or testosterone propionate and ICSH revealed that the number of germ cells was reduced as compared to the number in the normal intact animal. However, the number of Type A spermatogonia was reduced proportionally more than was the number of spermatocytes and spermatids. Thus calculation of the yield of spermatids per spermatogonia resulted in an increased value compared to that of the normal intact rat, suggesting that ICSH or FSH injected into the normal rat will increase the yield of spermatogenesis. However, the control group of testosterone propionate treated normal rats in this experiment displayed the same increased yield, unlike the previous experiment in which the yield in testosterone propionate treated rats had remained similar to the yield in normal intact animals. This would suggest that normal rats may vary considerably in their response to exogenous hormones. However, there was no marked difference in the increase in yield of spermatids per spermatogonia between the normal rats treated with testosterone propionate and the normal rats treated with testosterone propionate and FSH or ICSH. Thus, neither FSH nor ICSH will augment the effects produced in the normal rat by testosterone propionate alone.

GH or LTH, hormones which have been shown to augment the effects of testosterone propionate or ICSH in the restoration of spermatogenesis

were found to have no augmentive effects to testosterone propionate in the maintenance of spermatogenesis in the hypophysectomized rat.

The removal of the adrenal or the thyroid was not found to result in any change in germ cell number in the rat. Thus, these two glands do not appear to be essential for the maintenance of spermatogenesis.

Hence it is concluded that:

1) Testosterone appears to be the main hormone responsible for the maintenance of spermatogenesis in the normal or hypophysectomized rat.
2) ICSH or FSH will also maintain spermatogenesis. Their effect does not appear to be on the seminiferous epithelium directly but rather through the stimulation of androgen production by the Leydig cells.
3) LTH or GH do not appear to have any role in the maintenance of spermatogenesis.
4) The thyroid and adrenal hormones likewise have no role in maintenance of spermatogenesis.
5) Exogenous hormones, testosterone, ICSH or FSH, which maintain spermatogenesis, cannot maintain the normal number of germ cells found in intact rats. In all groups hormone treated the number of cells is reduced.
6) The hormones which are found to maintain spermatogenesis are found to maintain the normal yield of advanced germ cells per stem cell, but do not cause the yield to exceed the normal one. The one

instance where the normal yield was found to be exceeded was in hormone treated normal rats. However, in this instance the yield appeared to be improved only because the number of stem cells had been greatly reduced. The yield of spermatids per spermatogonia appears, however, to improve with age without a concomittant loss of stem cells.

7) Failure to maintain the normal number of germ cells with hormones appears to be due to an upset of balance of the various hormones rather than due to a lack of a specific hormone.

8) The reduction of germ cell number in hormone treated rats was observed to be due to a non-selective sloughing of a percentage of all germ cells, spermatogonia, spermatocytes and spermatids. This suggests that the seminiferous tubules of hormone treated rats can no longer contain the large number of cells found in the normal rat. Thus, the target structure of the hormone may not be the germ cells themselves, but some other element of the seminiferous tubules such as the Sertoli cells.

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TABLES

TABLE 1

AVERAGE WEIGHTS IN GRAMS OF BODY AND ORGANS

OF NORMAL INTACT RATS

Body weight	294
Testes	2.843
Epididymes	0.884
Seminal Vesicles and Prostate	1.999

TABLE 2

NUMBER OF GERM CELLS PER TUBULAR CROSS SECTION OF THE INTACT NORMAL RAT

	Type A Spermatogonia	Resting Spermatocytes	Pachytene Spermatocytes	Step 7 Spermatids	Sertoli Cells
Crude Counts	2.32 ± .07	73.3 ± 1.57	83.9 ± 1.06	259.2 ± 4.5	18.1 ± .3
Nuclear Diameter in u	7.80	5.72	8.08	6.56	(section thickness) 5.92
True Counts	1.09 ± .03	37.0 ± .7	36.0 ± .5	123.0 ± 2.1	

TABLE 3

AVERAGE WEIGHTS IN GRAMS OF BODY AND ORGANS

OF NORMAL RATS OF VARIOUS AGES

Body Weight	Testes	Epididymes	Seminal Vesicles and Prostate
206	2.161	0.306	0.690
255	2.373	0.451	1.121
301	2.742	0.583	1.662
400	2.758	0.946	2.457

TABLE 4

NUMBER OF GERM CELLS PER TUBULAR CROSS SECTION OF NORMAL RATS OF VARIOUS AGES

Body Weight	Type A Spermatogonia	Resting Spermatocytes	Pachytene Spermatocytes	Step 7 Spermatids	Sertoli Cells
206	1.15 ± .05	42 ± 1.8	40 ± 1.6	112 ± 3.9	19.8 ± .4
255	1.20 ± .06	41 ± .8	40 ± .6	121 ± 3.2	19.5 ± .4
301	1.19 ± .07	38 ± .6	37 ± 1.5	125 ± 4.0	19.4 ± .3
400	1.10 ± .07	40 ± .6	39 ± .9	132 ± 2.5	20.4 ± .6
Mean	1.15	40	39	123	19.8

TABLE 5

AVERAGE WEIGHTS IN GRAMS OF BODY AND ORGANS OF RATS

TREATED WITH TESTOSTERONE PROPIONATE

Treatment	Body Weight	Testes	Epididymes	Seminal Vesicles and Prostate
Normal oil injected	483	3.643	1.323	3.394
Hypophysectomized Testosterone 35d.	191	2.233	0.945	4.458
Hypophysectomized Testosterone 45d.	209	2.368	1.053	4.596
Hypophysectomized Testosterone 55d.	207	2.172	0.978	4.994
Hypophysectomized Testosterone 60d.	199	2.017	0.845	4.378
Normal plus Testosterone 45d.	384	3.007	1.407	6.055

TABLE 6

NUMBER OF GERM CELLS PER TUBULAR CROSS SECTION OF HYPOPHYSECTOMIZED RATS TREATED WITH TESTOSTERONE PROPIONATE

Treatment	Type A Spermatogonia	Resting Spermatocytes	Pachytene Spermatocytes	Step 7 Spermatids	Sertoli Cells
Normal oil injected	1.20 ± .05	34 ± .3	36 ± .9	124 ± 2.6	17.1 ± .2
Hypophysectomized Testosterone 35d.	.96 ± .09	28 ± .6	28 ± .9	109 ± 2.7	19.8 ± .3
Hypophysectomized Testosterone 45d.	.94 ± .05	27 ± .5	29 ± .5	92 ± 5.9	19.6 ± .2
Hypophysectomized Testosterone 55d.	1.00 ± .05	29 ± .4	29 ± .8	89 ± 2.4	20.1 ± .1
Hypophysectomized Testosterone 60d.	.94 ± .05	31 ± .8	31 ± 1.4	109 ± 2.5	19.8 ± .2
Normal plus Testosterone 45d.	.92 ± .01	26 ± .6	29 ± .8	93 ± 6.0	19.1 ± .3

TABLE 7

AVERAGE NUMBER OF GERM CELLS PER TUBULAR CROSS SECTION OF RATS TREATED WITH TESTOSTERONE PROPIONATE

Treatment	Type A Spermatogonia	Resting Spermatocytes	Pachytene Spermatocytes	Step 7 Spermatids
Normal oil injected	1.20 ± .05	34 ± .3	36 ± .9	124 ± 2.6
Hypophysectomized plus Testosterone (35 to 60 days)	.97 ± .04	29 ± 1.2	29 ±2.4	100 ± 7.3
Normal plus Testosterone	.92 ± .01	26 ± .6	29 ± .8	93 ± 6.0

TABLE 8

AVERAGE WEIGHTS IN GRAMS OF BODY AND ORGANS OF ADRENALECTOMIZED RATS

Treatment	Body Weight		Testes	Epididymes	Seminal Vesicles and Prostate
	At Operation	At Sacrifice			
Normal (sham operated, pair fed)	390	391	2.864	1.237	2.378
Adrenalectomized	379	381	2.928	1.135	2.463

TABLE 9

NUMBER OF GERM CELLS PER TUBULAR CROSS SECTION OF ADRENALECTOMIZED RATS

Treatment	Type A Spermatogonia	Resting Spermatocytes	Pachytene Spermatocytes	Step 7 Spermatids	Sertoli Cells
Sham Operated	1.10 ± .05	32.7 ± .6	32.9 ± .6	115.9 ± 1.8	18.4 ± .3
Adrenalectomized	1.05 ± .05	35.8 ± .7	35.8 ± .6	122.8 ± 1.9	17.9 ± .3

TABLE 10

AVERAGE WEIGHTS IN GRAMS OF BODY AND ORGANS OF HYPOPHYSECTOMIZED RATS TREATED WITH FSH OR ICSH

Treatment	Body Weight	Testes	Epididymes	Seminal Vesicles and Prostate
FSH 200 µg daily	215.6	1.942	.579	.525
FSH 150 µg daily	219.6	2.206	.653	.543
FSH 100 µg daily	217.6	1.718	.510	.349
ICSH 20 µg daily	211.4	.998	.305	.311
ICSH 15 µg daily	212.0	1.652	.409	.329
ICSH 10 µg daily	205.4	.949	.332	.461

TABLE 11

NUMBER OF GERM CELLS PER TUBULAR CROSS SECTION OF HYPOPHYSECTOMIZED RATS TREATED WITH FSH OR ICSH

Treatment	Type A Spermatogonia	Resting Spermatocytes	Pachytene Spermatocytes	Step 7 Spermatids	Sertoli Cells
FSH 150 µg	.74 ± .03	32 ± 0.4	29 ± 0.5	98 ± 1.7	21 ± 0.3
ICSH 15 µg	.80 ± .03	31 ± 0.4	29 ± 0.5	87 ± 1.5	21.5 ± 0.3

TABLE 12

AVERAGE WEIGHT IN GRAMS OF BODY AND ORGANS OF NORMAL RATS TREATED WITH TESTOSTERONE PROPIONATE AND FSH OR ICSH

Treatment	Body Weight	Testes	Epididymes	Seminal Vesicles and Prostate
Testosterone	365	2.649	1.091	4.910
Testosterone and FSH (150 µg)	320	2.810	1.209	5.761
Testosterone and ICSH (15 µg)	350	2.918	1.111	5.524

TABLE 13

NUMBER OF GERM CELLS PER TUBULAR CROSS SECTION OF NORMAL RATS TREATED WITH TESTOSTERONE PROPIONATE AND FSH OR ICSH

Treatment	Type A Spermatogonia	Resting Spermatocytes	Pachytene Spermatocytes	Step 7 Spermatids	Sertoli Cells
Testosterone	0.72 ± .04	32.3 ± .4	30.1 ± .3	102.3 ± .7	20.5 ± .3
Testosterone plus FSH	0.77 ± .04	33.7 ± .4	32.0 ± .3	119.8 ± .8	20.7 ± .8
Testosterone plus ICSH	0.75 ± .04	38.4 ± .7	33.7 ± .4	113.2 ± 1.6	20.5 ± .2

TABLE 14

AVERAGE WEIGHT IN GRAMS OF BODY AND ORGANS OF HYPOPHYSECTOMIZED RATS TREATED WITH TESTOSTERONE PROPIONATE AND LACTOGENIC HORMONE

Treatment	Body Weight	Testes	Epididymes	Seminal Vesicles and Prostate
Hypophysectomized Testosterone	213	2.355	.769	3.760
Hypophysectomized Testosterone plus LTH	204	1.837	.689	3.681

TABLE 15

NUMBER OF GERM CELLS PER TUBULAR CROSS SECTION OF HYPOPHYSECTOMIZED RATS TREATED WITH TESTOSTERONE PROPIONATE AND LACTOGENIC HORMONE

Treatment	Type A Spermatogonia	Resting Spermatocytes	Pachytene Spermatocytes	Step 7 Spermatids	Sertoli Cells
Hypophysectomized Testosterone	.86 ± .03	28.5 ± .8	27.9 ± .4	106.1 ± 5.8	21.2 ± .5
Hypophysectomized Testosterone plus LTH	.80 ± .03	29.7 ± 1.4	25.6 ± 1.1	83.8 ± 9.1	21.5 ± .8

TABLE 16

AVERAGE WEIGHT IN GRAMS OF BODY AND ORGANS OF THYROIDECTOMIZED RATS

Treatment	Body Weight	Testes	Epididymes	Seminal Vesicles and Prostate
Thyroidectomized 45 days	276	3.301	1.156	2.795
Sham operated	217	3.145	.980	2.270

TABLE 17

AVERAGE NUMBER OF GERM CELLS PER TUBULAR CROSS SECTION OF THYROIDECTOMIZED RATS

Treatment	Type A Spermatogonia	Resting Spermatocytes	Pachytene Spermatocytes	Step 7 Spermatids	Sertoli Cells
Thyroidectomized	1.10 ± .05	32.5 ± 1.1	32.8 ± 1.1	113.9 ± 5.8	18.3 ± .2
Sham operated	1.01 ± .04	36.0 ± 1.3	34.9 ± 1.7	123.2 ± 5.3	18.8 ± .4

TABLE 18

AVERAGE WEIGHT IN GRAMS OF BODY AND ORGANS OF HYPOPHYSECTOMIZED RATS TREATED WITH TESTOSTERONE PROPIONATE, THYROXIN AND GROWTH HORMONE

Treatment	Body Weight	Testes	Epididymes	Seminal Vesicles and Prostate
Hypophysectomized Testosterone	203	1.728	.625	3.567
Hypophysectomized Testosterone plus Thyroxin	208	1.794	.674	3.373
Hypophysectomized Testosterone plus Growth Hormone	280	2.155	.822	3.773
Hypophysectomized Testosterone plus Thyroxin and Growth Hormone	273	1.826	.717	4.036

TABLE 19

NUMBER OF GERM CELLS PER TUBULAR CROSS SECTION OF HYPOPHYSECTOMIZED RATS TREATED WITH TESTOSTERONE PROPIONATE, THYROXIN AND GROWTH HORMONE

Treatment	Type A Spermatogonia	Resting Spermatocytes	Pachytene Spermatocytes	Step 7 Spermatids	Sertoli Cells
Hypophysectomized Testosterone	.85 ± .03	25.9 ± 1.6	28.5 ± 3.3	84.6 ± 5.5	19.2 ± .4
Hypophysectomized Testosterone plus Thyroxin	.81 ± .04	30.1 ± 1.4	26.9 ± .6	85.6 ± 5.9	20.4 ± 1.0
Hypophysectomized Testosterone plus Growth Hormone	.83 ± .04	26.9 ± .9	27.8 ± .4	100.7 ± 5.2	19.7 ± .8
Hypophysectomized Testosterone, Thyroxin and Growth Hormone	.78 ± .02	27.9 ± .4	26.9 ± .8	87.0 ± 12.4	18.8 ± .2

TABLE 20

AVERAGE WEIGHTS IN GRAMS OF BODY AND ORGANS OF HYPOPHYSECTOMIZED RATS TREATED WITH GROWTH HORMONE

Treatment	Body Weight	Testes	Epididymes	Seminal Vesicles and Prostate
Hypophysectomized 30 days	196	.551	.148	.243
Hypophysectomized plus Growth Hormone	261	.504	.156	.277

TABLE 21

NUMBER OF GERM CELLS PER TUBULAR CROSS SECTION OF HYPOPHYSECTOMIZED RATS TREATED WITH GROWTH HORMONE

Treatment	Type A Spermatogonia	Resting Spermatocytes	Pachytene Spermatocytes	Step 7 Spermatids	Sertoli Cells
Hypophysectomized 30 days	.92 ± .06	21.9 ± .6	9.3 ± 2.2	7.3 ± 2.7	28.8 ± 1.8
Hypophysectomized plus Growth Hormone	.87 ± .08	20.5 ± .5	7.0 ± .7	4.8 ± 1.0	29.2 ± .8

TABLE 22

NUMBER OF SERTOLI CELLS PER TUBULAR CROSS SECTION AND THEIR PERCENTAGE OF THE NORMAL VALUE

Treatment	Number of Sertoli Cells	Percentage of Normal
Untreated Normal	18.1	100
Normal plus Oil	17.1	95
Hypophysectomized plus Testosterone	19.8	110
Normal plus Testosterone	19.1	106
Sham Adrenalectomized pair fed	18.4	102
Adrenalectomized	17.9	99
Hypophysectomized plus FSH	21.0	116
Hypophysectomized plus ICSH	21.5	119
Normal plus Testosterone (2nd experiment)	20.5	114
Normal plus Testosterone plus FSH	20.7	114
Normal plus Testosterone plus ICSH	20.5	113
Hypophysectomized Testosterone	21.2	117
Hypophysectomized Testosterone, Lactogenic Hormone	21.5	119
Thyroidectomized	18.3	100
Hypophysectomized Testosterone	19.2	106
Hypophysectomized Testosterone plus Thyroxin	20.4	113
Hypophysectomized Testosterone plus Growth Hormone	19.7	109
Hypophysectomized Testosterone Thyroxin and Growth Hormone	18.8	104
Hypophysectomized plus Growth Hormone	29.2	161
Hypophysectomized 30 days	28.8	159

TABLE 23

NUMBER OF GERMINAL CELLS PER TUBULAR CROSS SECTION OF THE VARIOUS EXPERIMENTAL GROUPS CORRECTED BY THE SERTOLI FACTOR

	Treatment	Type A Spermatogonia	Resting Spermatocytes	Pachytene Spermatocytes	Step 7 Spermatids
	Untreated Normal	1.09	37	36	123
	Normal plus Oil	1.27	36	37	130
24	Hypophysectomized plus Testosterone	.88	26	26	91
	Normal plus Testosterone	.85	25	27	89
25	Sham Adrenalectomized pair fed	1.10	33	33	116
	Adrenalectomized	1.05	36	36	123
26	Hypophysectomized plus FSH	.64	28	25	85
	Hypophysectomized plus ICSH	.67	26	24	73
27	Normal plus Testosterone (2nd experiment)	.63	28	26	90
	Normal plus Testosterone plus FSH	.68	30	28	105
	Normal plus Testosterone plus ICSH	.66	33	30	100
29	Thyroidectomized	1.10	33	33	114
28	Hypophysectomized Testosterone	.83	28	27	103
	Hypophysectomized Testosterone Lactogenic Hormone	.76	29	25	80
	Hypophysectomized Testosterone	.80	24	27	80
30	Hypophysectomized Testosterone plus Thyroxin	.78	29	26	83
	Hypophysectomized Testosterone plus Growth Hormone	.80	26	27	97
	Hypophysectomized Testosterone, Thyroxin and Growth Hormone	.78	28	27	87
31	Hypophysectomized plus Growth Hormone	.84	20	7	5
	Hypophysectomized 30 days	.88	21	9	7

FIGURES

Figure 1 The 14 Cell Associations of the Seminiferous Epithelium of the Rat

Each column number I to XIV schematically represents the various cell associations found in the seminiferous epithelium of the rat. The cells of any one column synchronously develop into those of the adjacent column to the right and thusly up to XIV, which evolves into I. When a given area of seminiferous epithelium has passed through all fourteen and returns to its starting point a cycle has occurred. Each cell association is referred to as a stage of the cycle of the seminiferous epithelium.

Cell Types:

A = Type A spermatogonium
In = Intermediate spermatogonium
B = Type B spermatogonium
R = Resting primary spermatocyte
L = Leptotene primary spermatocyte
Z = Zygotene primary spermatocyte
P = Pachytene primary spermatocyte
Di = Primary spermatocyte in diakinesis
II = Secondary spermatocyte

1 to 19 Spermatids of the nineteen steps of spermiogenesis. The appearance of the first fourteen steps in PA-Schiff-hematoxylin stained sections can be used as the sole criterion for identification of the stages of the cycle of the seminiferous epithelium.

Subscript $_m$ indicates mitosis

(Taken from Clermont et al, 1959)

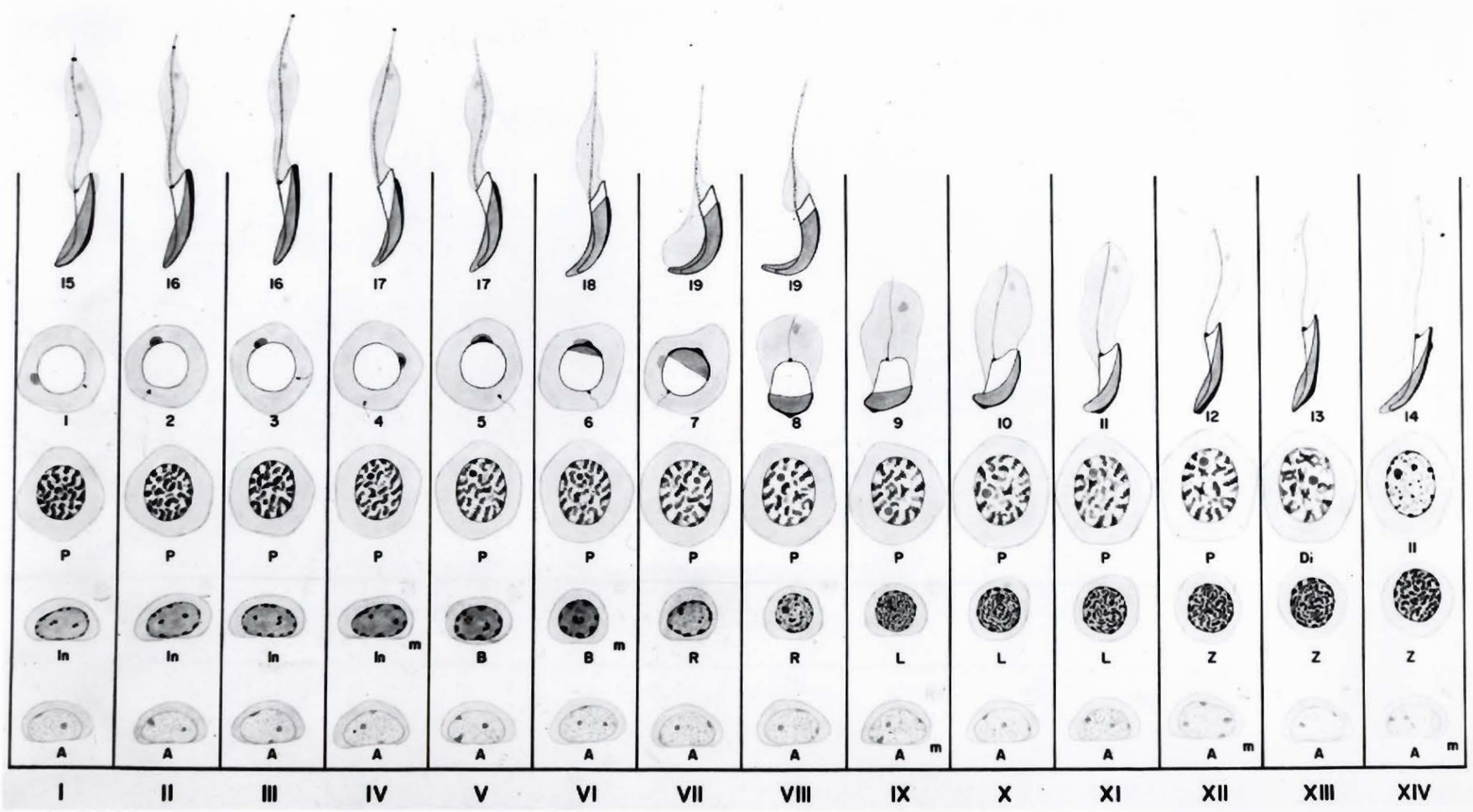
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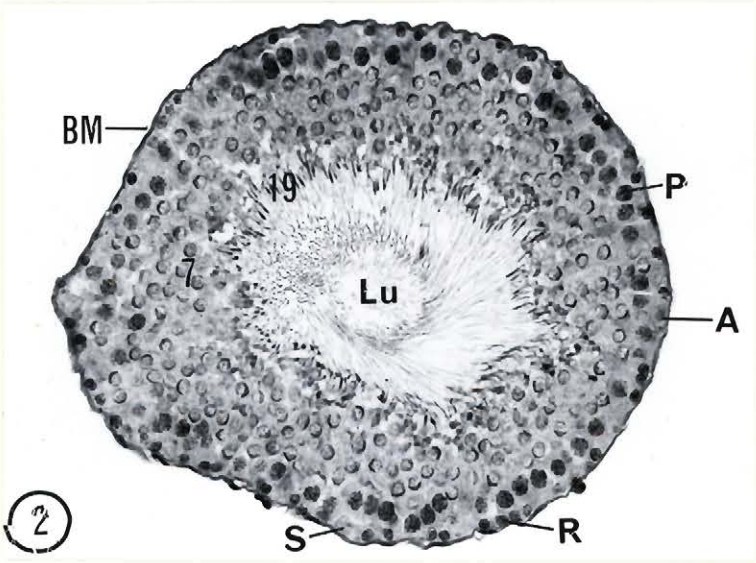
Figure 2 Photomicrograph of a cross section of seminiferous tubule stained with PA-Schiff-hematoxylin. This section depicts the cell association of stage VII of the cycle of the seminiferous epithelium. All cell counts were made on cross sections of similar tubules. (Magnification X 500)

A = Type A spermatogonium
R = Resting primary spermatocyte
P = Pachytene primary spermatocyte
7 = step 7 spermatid
19 = step 19 spermatid
S = Sertoli cell
BM = Basement membrane
Lu = Lumen of tubule

Figure 3 Photomicrograph of a longitudinal section of seminiferous tubule stained with PA-Schiff-hematoxylin, showing the cell association of stage VII. It is noteworthy that the epithelium appears essentially the same as in cross section of Figure 2. (Magnification X 800)

See legend, Figure 2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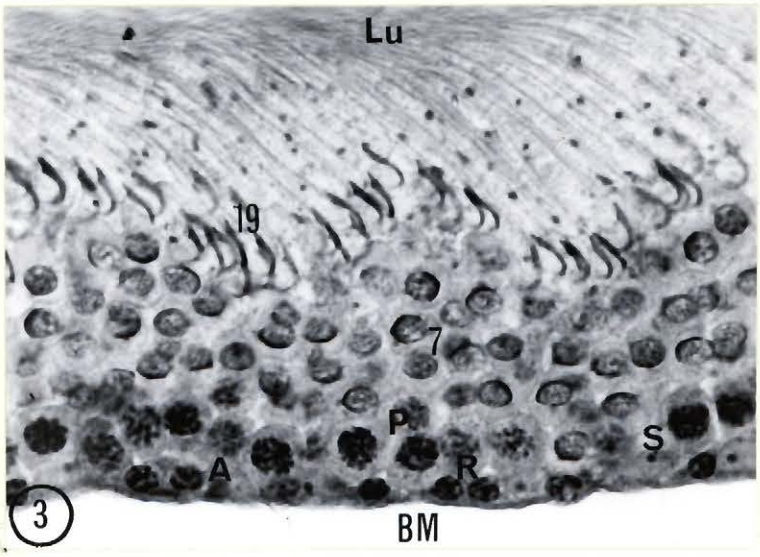
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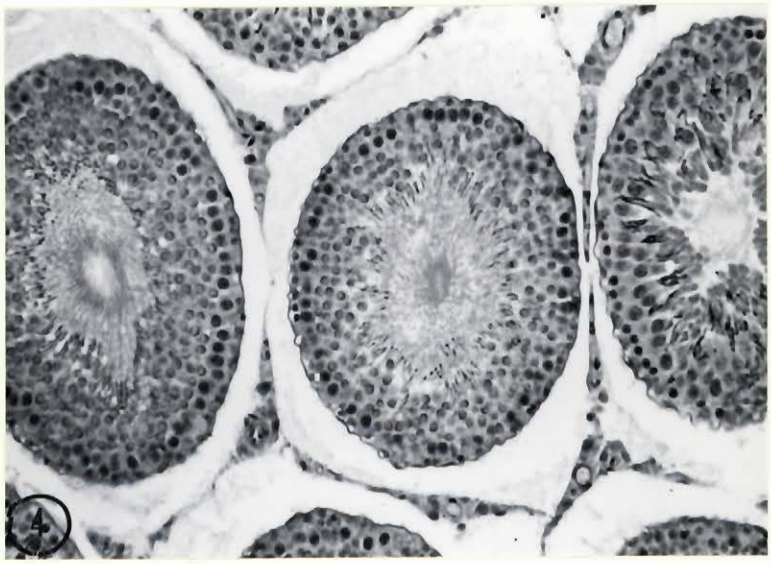
Figure 4 Photomicrograph of testis of normal adult rat. PA-Schiff-hematoxylin. (Magnification X 240)

Figure 5 Photomicrograph of testis of adrenalectomized rat, 45 days. Note that the testis appears normal as in Figure 4. PA-Schiff-hematoxylin. (Magnification X 240)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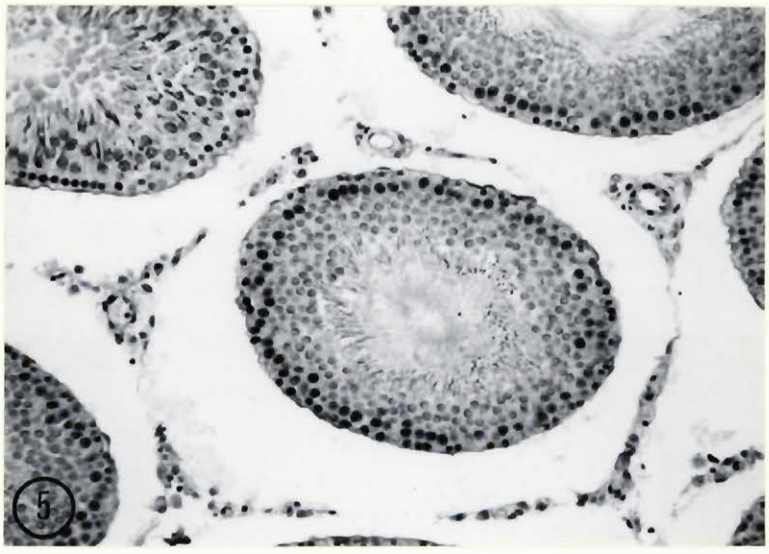
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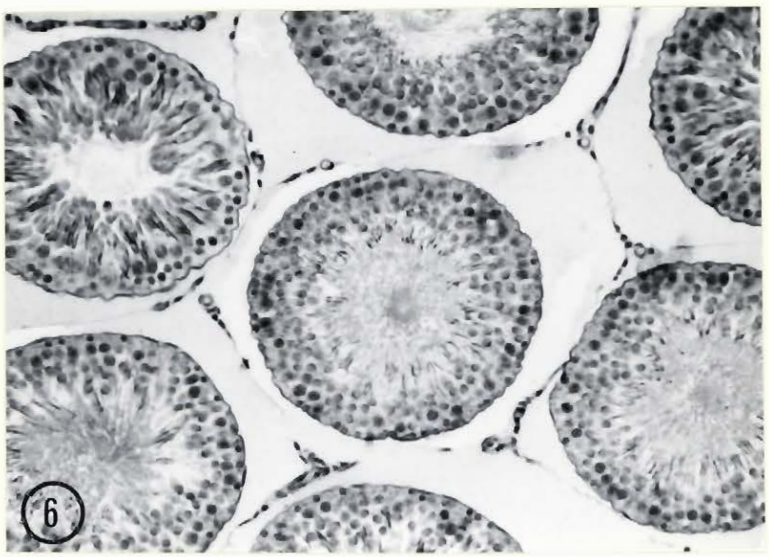
Figure 6 Photomicrograph of section of testis of a hypophysectomized rat treated daily with 3 mgm of testosterone propionate, 45 days post-operative. Note that, although similar to normal rat of Figure 4, the seminiferous tubules are somewhat reduced in diameter. PA-Schiff-hematoxylin. (Magnification X 240)

Figure 7 Photomicrograph of section of testis of a normal rat treated daily with 3 mgm of testosterone propionate for 45 days. The seminiferous tubules, like those of the hypophysectomized rat treated with testosterone propionate, are smaller than those of the normal rat (Figure 4). PA-Schiff-hematoxylin. (Magnification X 240)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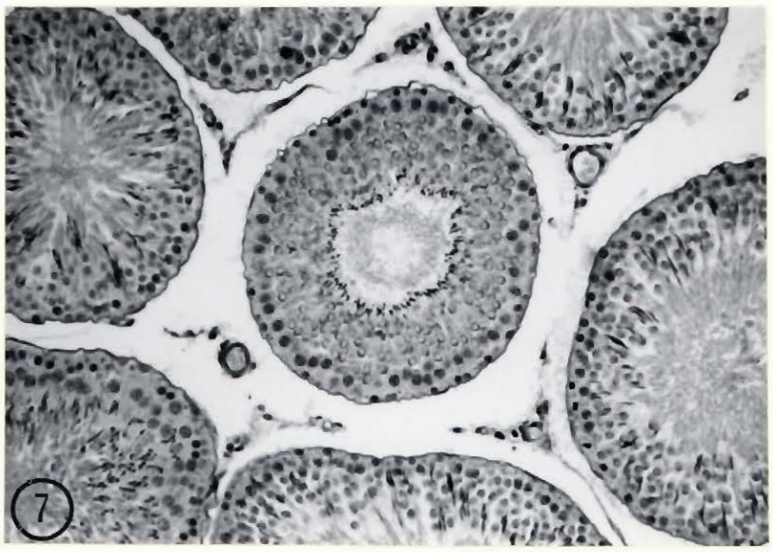
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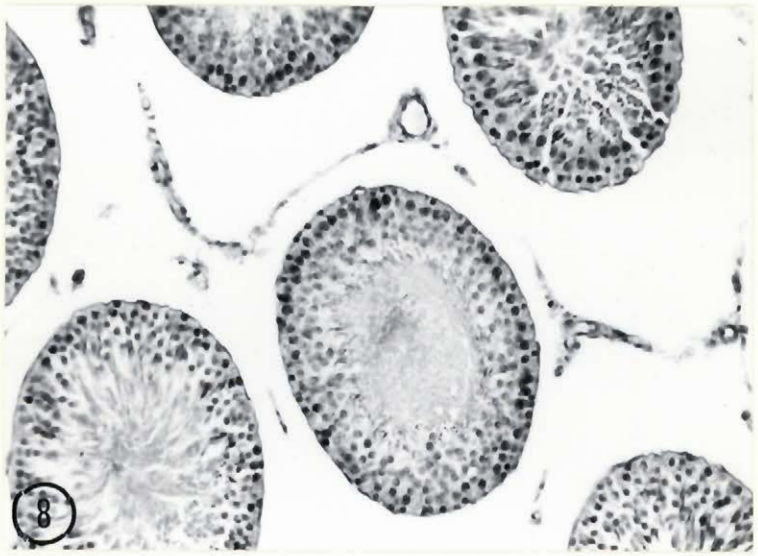
Figure 8 Photomicrograph of testis of hypophysectomized rat treated daily with 150 µg of FSH, 35 days post-operative. Spermatogenesis is well maintained. The tubules, however, are smaller than those of the normal animal. Although not apparent in this area and at this magnification, the interstitial cells display signs of activity. See Figure 14.
PA-Schiff-hematoxylin. (Magnification X 240)

Figure 9 Photomicrograph of testis of a hypophysectomized rat treated daily with 15 µg of ICSH, 35 days post-operative. Spermatogenesis is well maintained, but again the seminiferous tubules are smaller than those of the normal animal. See Figure 15 for high magnification of the interstitial cells.
PA-Schiff-hematoxylin. (Magnification X 240)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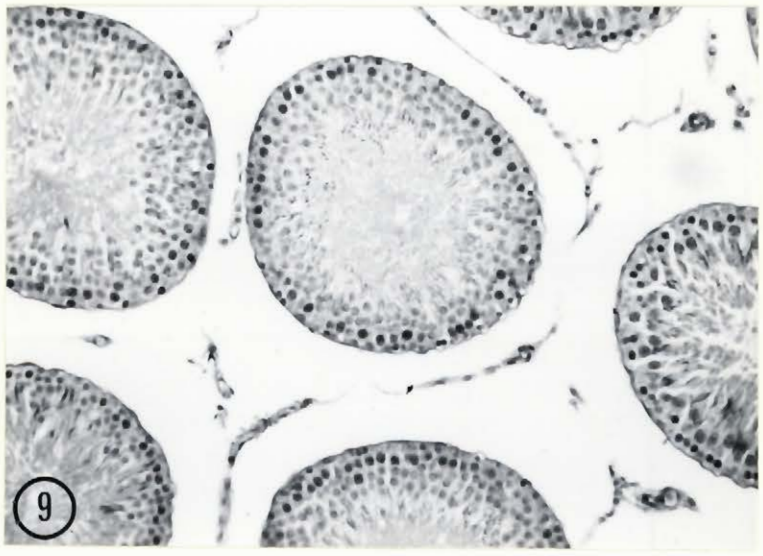
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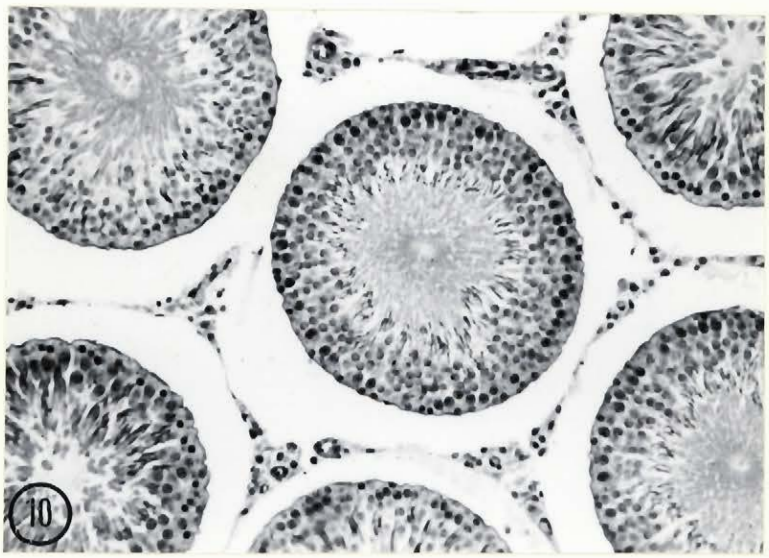
Figure 10 Photomicrograph of testis of normal rat treated daily with 3 mgm of testosterone propionate and 150 µg of FSH for 45 days. Although apparently normal, the seminiferous tubules, like those of the normal rat treated with testosterone propionate alone (Figure 7), are smaller than those of the normal animal (Figure 4). Note that the interstitial cells at this magnification appear stimulated.
PA-Schiff-hematoxylin. (Magnification X 240)

Figure 11 Photomicrograph of testis of a normal rat treated daily with 3 mgm of testosterone propionate and 15 µg of ICSH. Spermatogenesis is well maintained but the seminiferous tubules may be slightly smaller than those of the normal rat.
PA-Schiff-hematoxylin. (Magnification X 240)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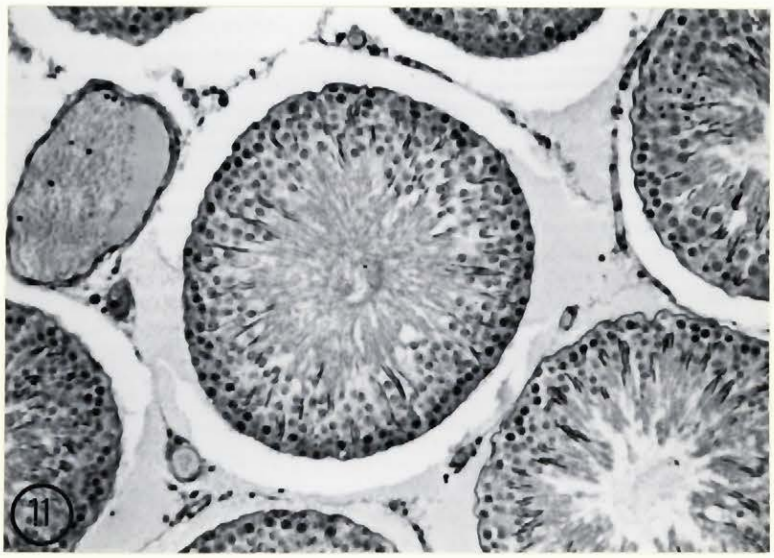
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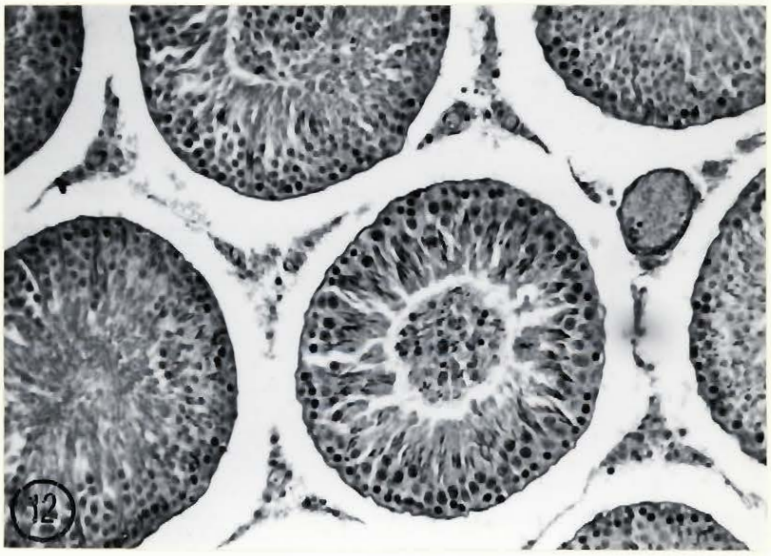
Figure 12 Photomicrograph of testis of a hormone treated rat (hypophysectomized-FSH) showing degenerating cell mass in the lumen of a seminiferous tubule. These degenerating cells were occasionally observed in all hormone treated groups, normal as well as hypophysectomized.
PA-Schiff-hematoxylin. (Magnification X 240)

Figure 13 Photomicrograph of testis of a hypophysectomized rat treated daily with 15 µg of ICSH, 45 days post-operative, revealing the appearance of the testis when hormone therapy had failed to maintain spermatogenesis. Comparison with the testis of untreated hypophysectomized rat (Figure 21) indicates that the impairment of the process is not so severe, however, in these hormone treated animals. Testes of animals treated with FSH, 45 days post-operative, revealed a comparable condition, the advanced spermatids being no longer present and the seminiferous tubule being greatly reduced in diameter.
PA-Schiff-hematoxylin. (Magnification X 240)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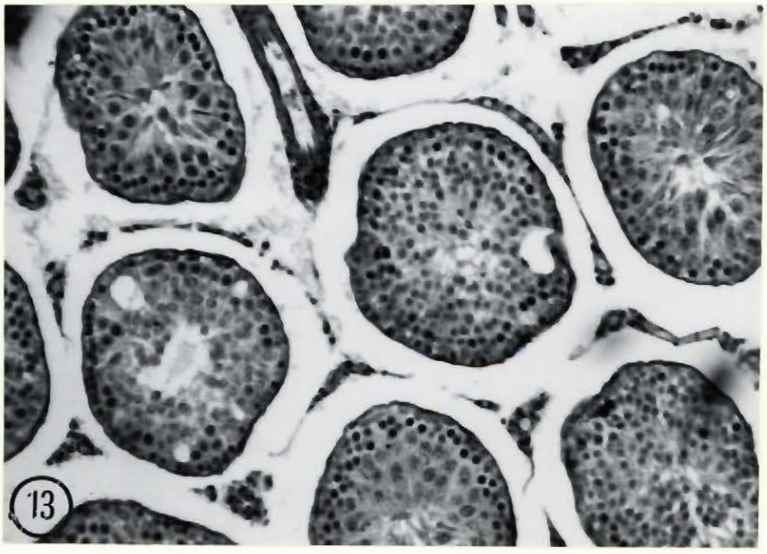
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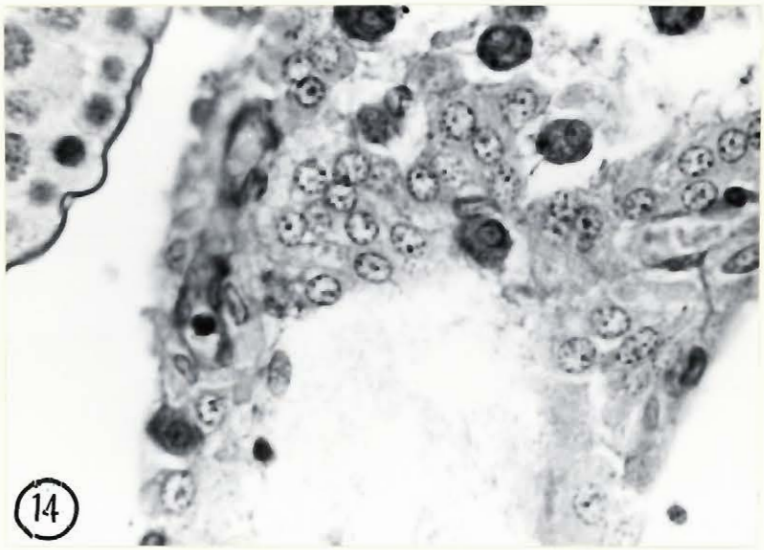
Figure 14 Photomicrograph of interstitial cells of hypophysectomized rat treated daily with 150 µg of FSH, 35 days post-operative. The Leydig cells are large and pale with prominent nucleoli, presumably indicating a high degree of activity. The dark, intensely stained cells are macrophages.
PA-Schiff-hematoxylin. (Magnification X 940)

Figure 15 Photomicrograph of interstitial cells of hypophysectomized rat treated daily with 15 µg of ICSH, 35 days post-operative. The Leydig cells, like those of FSH treated animals (Figure 14), show signs of marked activity.
PA-Schiff-hematoxylin. (Magnification X 940)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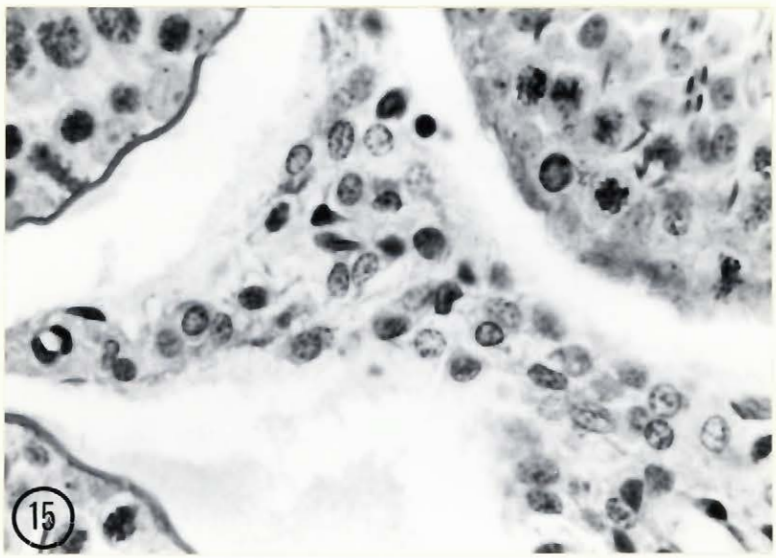
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Figure 16 Photomicrograph of section of testis of hypophysectomized rat treated daily with 3 mgm of testosterone propionate and 1 IU of lactogenic hormone, 30 days post-operative. Spermatogenesis is evidently well maintained as spermatozoa are being released from the epithelium. PA-Schiff-hematoxylin. (Magnification X 250)

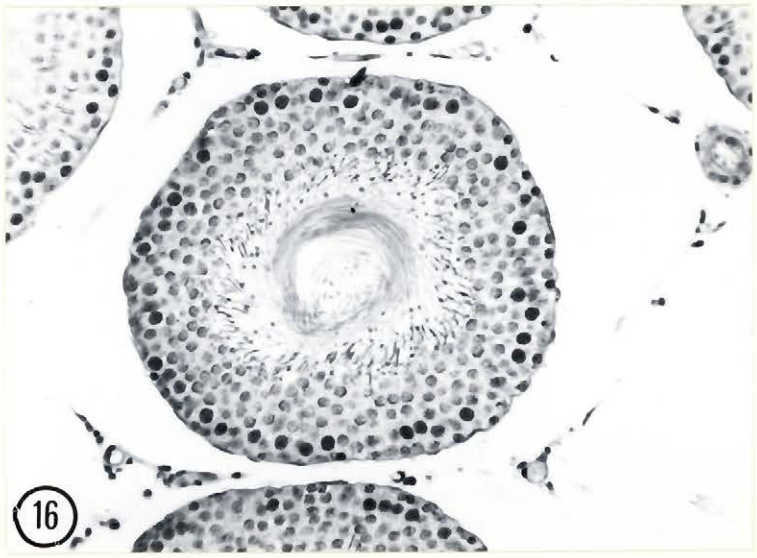
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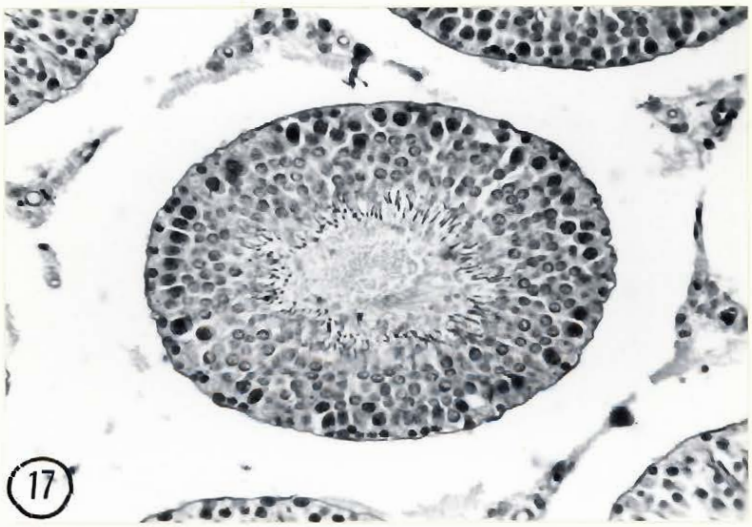
Figure 17 Photomicrograph of section of testes of adrenalectomized rat, 45 days post-operative. The seminiferous tubules appear to be completely normal.
PA-Schiff-hematoxylin. (Magnification X 250)

Figure 18 Photomicrograph of section of testes of a hypophysectomized rat treated daily with 3 mgm of testosterone propionate and 2 µg of DL-thyroxin, 30 days post-operative. The seminiferous tubules display no great difference from those of the normal.
PA-Schiff-hematoxylin. (Magnification X 250)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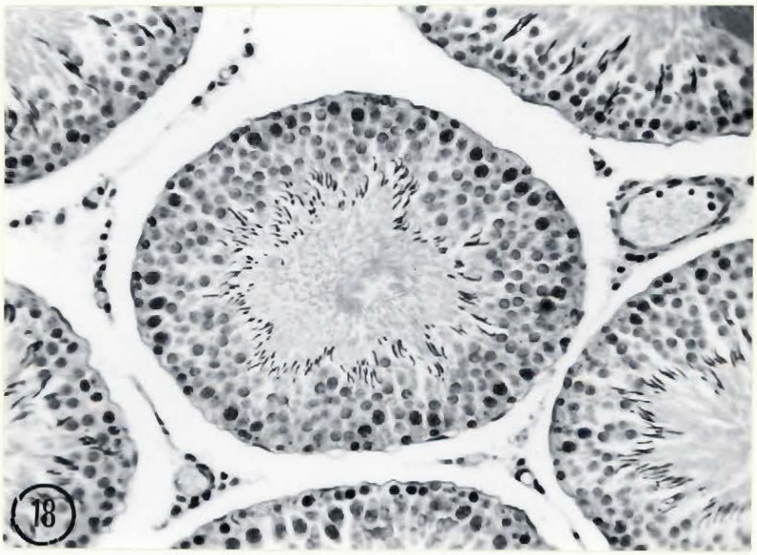
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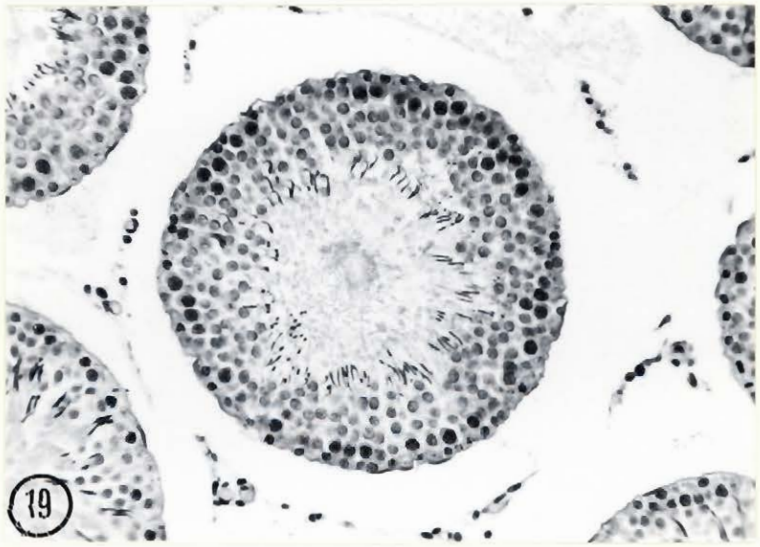
Figure 19 Photomicrograph of section of testis of hypophysectomized rat treated daily with 3 mgm of testosterone propionate and 0.5 USP of growth hormone, 30 days post-operative. The seminiferous tubules are not strikingly different from those of the normal rat.
PA-Schiff-hematoxylin. (Magnification X 250)

Figure 20 Photomicrograph of section of testis of hypophysectomized rat treated daily with 3 mgm of testosterone propionate, 2 µg of DL-thyroxin and 0.5 USP of growth hormone, 30 days post-operative. Again the seminiferous tubules appear similar to those of the normal rat.
PA-Schiff-hematoxylin. (Magnification X 250)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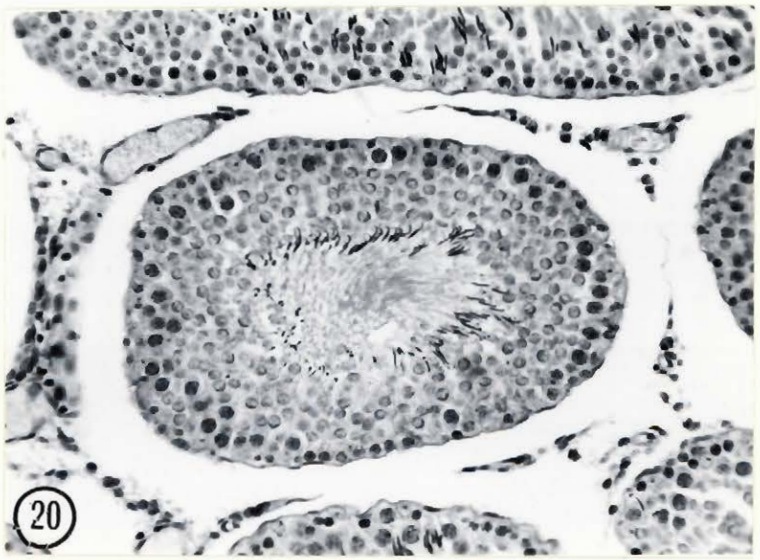
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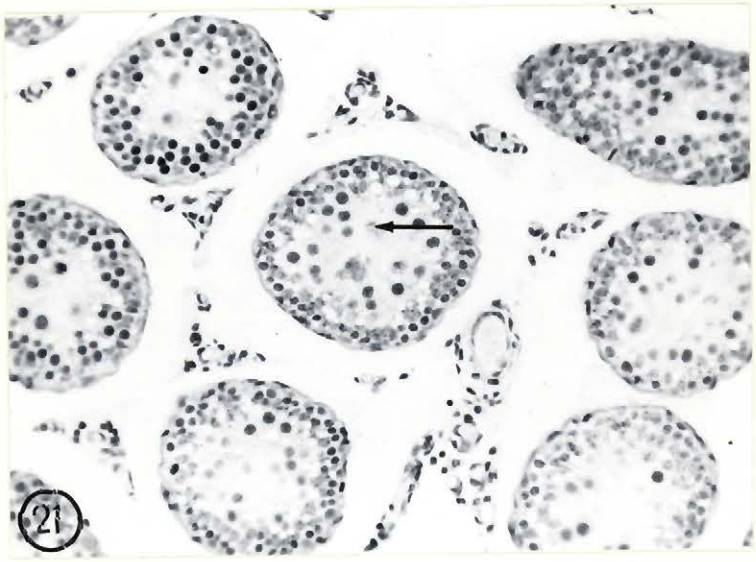
Figure 21 Photomicrograph of testis of an untreated hypophysectomized rat, 30 days post-operative. The seminiferous tubules are greatly reduced in size with a concurrent loss of many of the germ cells. Only the occasional spermatid remains (arrow).
PA-Schiff-hematoxylin. (Magnification X 250)

Figure 22 Photomicrograph of testis of a hypophysectomized rat, treated with 0.5 USP of growth hormone, 30 days post-operative. The picture is basically the same as that of the untreated hypophysectomized animal (Figure 21). Again a few young spermatids are still present (arrow).
PA-Schiff-hematoxylin. (Magnification X 250)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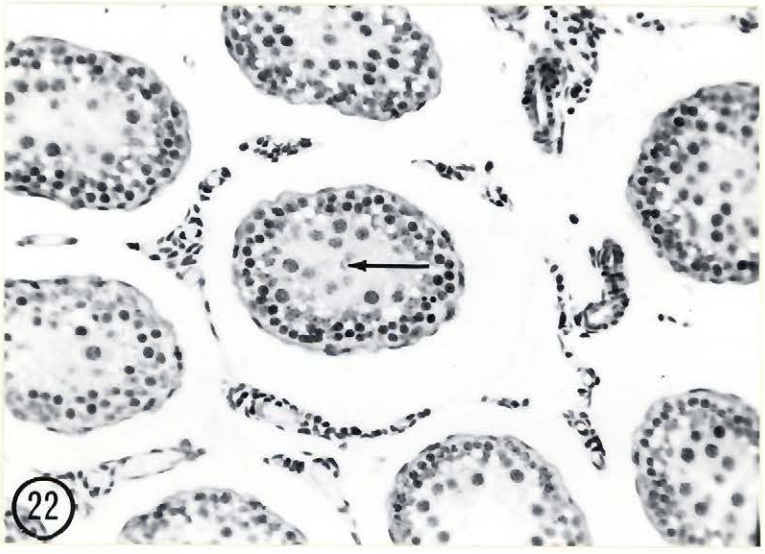
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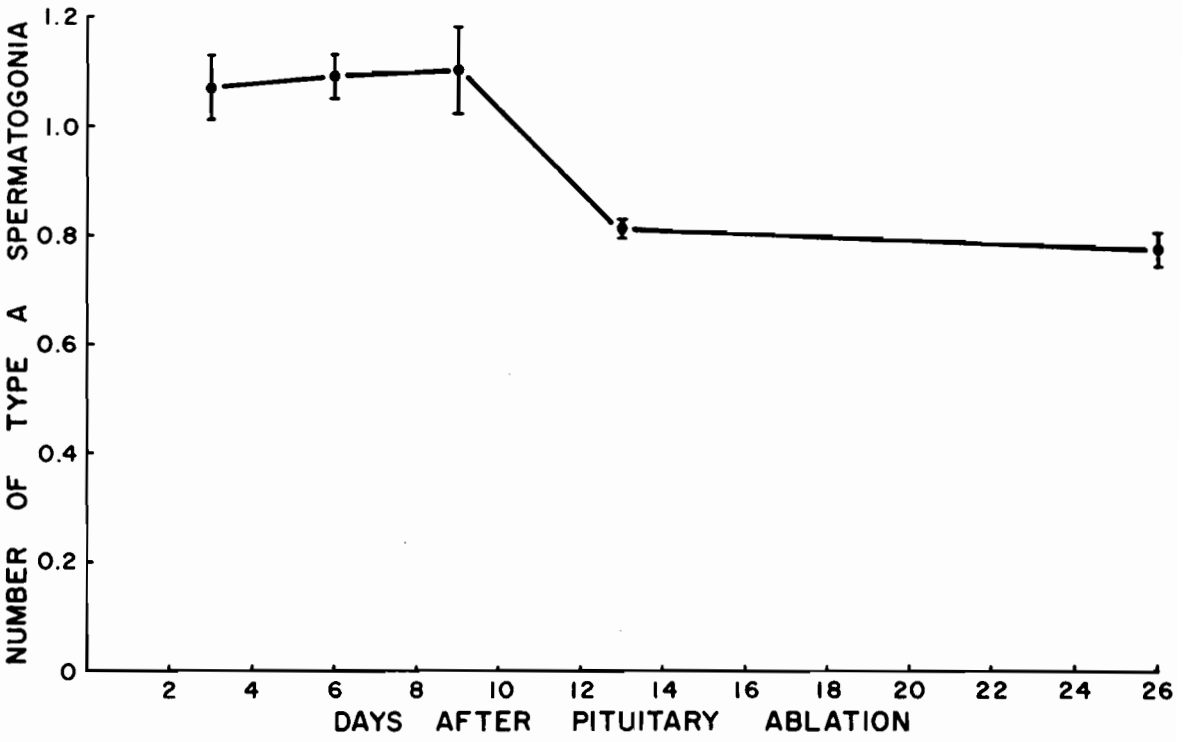
Figure 23 This figure graphically represents the number of Type A spermatogonia per tubular cross section in hypophysectomized testosterone propionate treated rats, 3, 6, 9, 12 and 26 days post-operative. Animals were treated with 3 mgm of testosterone propionate commencing on the 5th post-operative day. Cell counts are corrected by Abercrombie's formula and the Sertoli cell correction factor. Cell number 3, 6, and 9 days post-operative are not significantly different from the number in the intact rat. Cell number 12 and 26 days post-operative are not significantly different from each other but are significantly different from the cell number in the normal animal ($.01 \quad p \quad .001$). Furthermore, the number of Type A spermatogonia per tubular cross section at 12 days post-operative is not significantly different from the number found in testosterone propionate treated hypophysectomized rats 35 to 60 days post-operative.

REDUCTION IN TYPE A CELLS IN HYPOPHYSECTOMIZED RATS TREATED WITH TESTOSTERONE PROPIONATE



Figures 24 to 31 inclusive are histiograms constructed from the data of Table 23. All counts have been previously corrected by Abercrombie's formula and the Sertoli cell correction factor. The number of each cell type in the intact adult rats has been taken as 100% and the number found in the various experimental groups is represented by the appropriate percentage of the normal.

Figure 24 Relative number of germ cells per tubular cross section in rats treated with testosterone propionate.

N = intact adult rats
H+TP = hypophysectomized rats treated daily with 3 mgm of testosterone propionate, pooled values of animals 35 to 60 days post-operative
N+TP = adult rats treated daily with 3 mgm of testosterone propionate for 45 days
H = untreated hypophysectomized rats 44 days post-operative. (Calculated from the data of Clermont and Morgentaler, 1955.)

RELATIVE NUMBER OF GERM CELLS PER TUBULAR CROSS SECTION CORRECTED BY SERTOLI FACTOR: IN TESTOSTERONE TREATED RATS

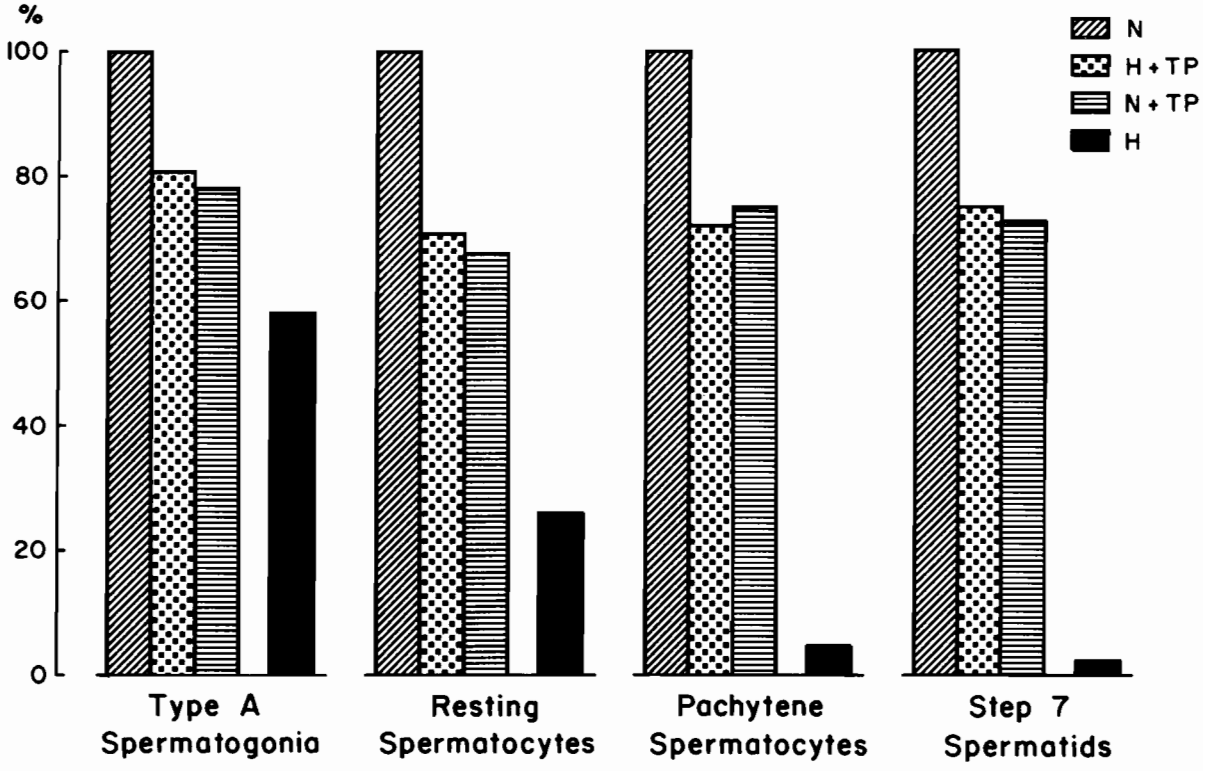


Figure 25 Relative number of germ cells per tubular cross section in adrenalectomized rats.

N = intact adult rats
C = control rats, sham operated, pair-fed
Ad_x = adult rats adrenalectomized for 45 days

RELATIVE NUMBER OF GERM CELLS PER TUBULAR CROSS SECTION: IN ADRENALECTOMIZED RATS

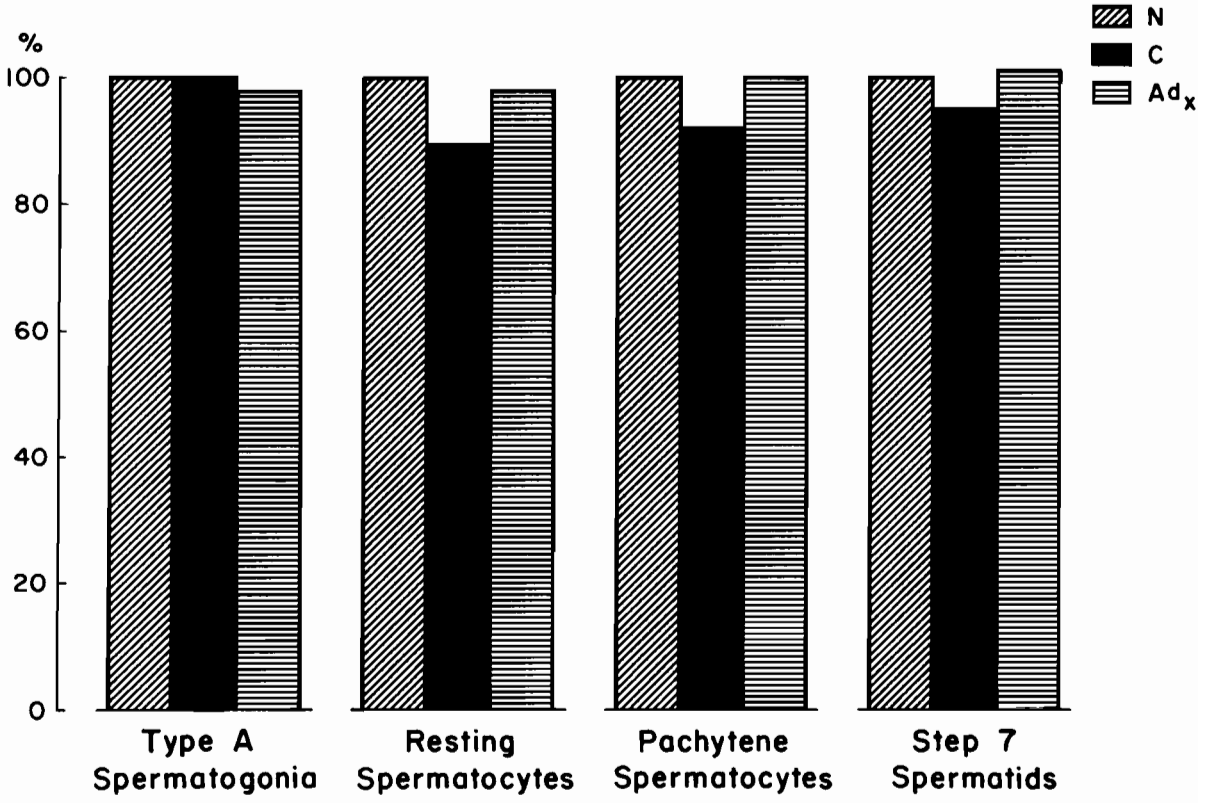


Figure 26 Relative number of germ cells per tubular cross section in hypophysectomized rats treated with FSH or ICSH.

N = intact adult rats
FSH = hypophysectomized rats treated daily with 150 µg of FSH, 35 days post-operative
ICSH = hypophysectomized rats treated daily with 15 ug of ICSH, 35 days post-operative
H = untreated hypophysectomized rat 44 days post-operative. (Calculated from the data of Clermont and Morgentaler, 1955)

RELATIVE NUMBER OF GERM CELLS PER TUBULAR CROSS SECTION CORRECTED BY SERTOLI FACTOR: IN FSH OR ICSH TREATED RATS

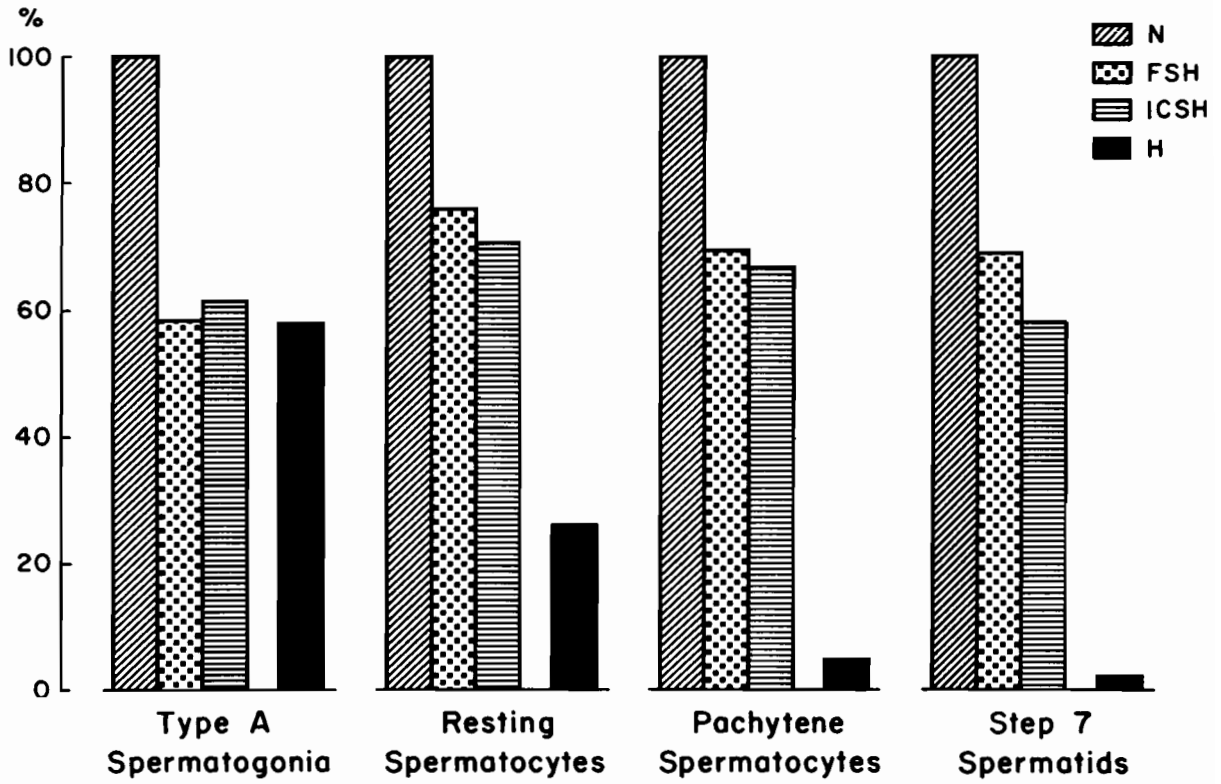


Figure 27 Relative number of germ cells per tubular cross section in adult rats treated with testosterone propionate and FSH or ICSH.

N = intact adult rats
TP = adult rats treated daily with 3 mgm of testosterone propionate for 45 days
TP+FSH = adult rats treated daily with 3 mgm of testosterone propionate and 150 µg of FSH for 45 days
TP+ICSH = adult rats treated daily with 3 mgm of testosterone propionate and 15 µg of ICSH for 45 days

RELATIVE NUMBER OF GERM CELLS PER TUBULAR CROSS SECTION CORRECTED BY SERTOLI FACTOR: IN TESTOSTERONE AND FSH OR ICSH TREATED RATS

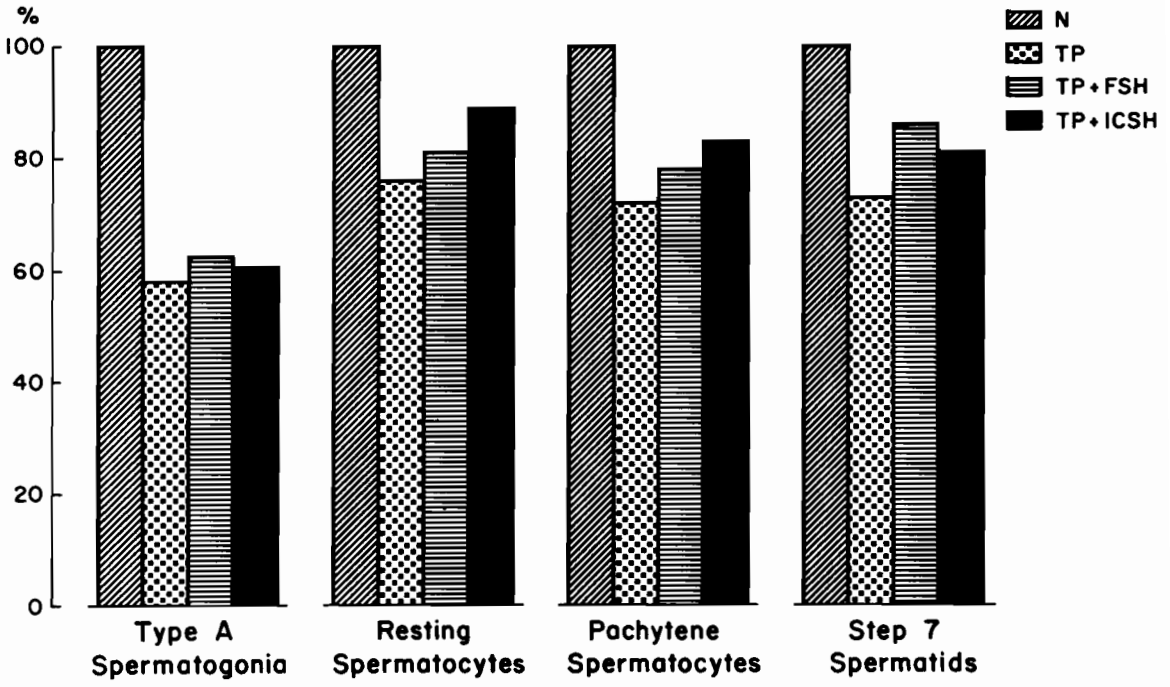


Figure 28 Relative number of germ cells per tubular cross section in hypophysectomized rats treated with testosterone propionate and lactogenic hormone.

N = intact adult rats
TP = hypophysectomized rats treated daily with 3 mgm of testosterone propionate, 30 days post-operative
TP+LTH = hypophysectomized rats treated daily with 3 mgm of testosterone propionate and 1 IU of lactogenic hormone, 30 days post-operative.

RELATIVE NUMBER OF GERM CELLS PER TUBULAR CROSS SECTION
CORRECTED BY SERTOLI FACTOR:
IN TESTOSTERONE OR LACTOGENIC HORMONE TREATED RATS

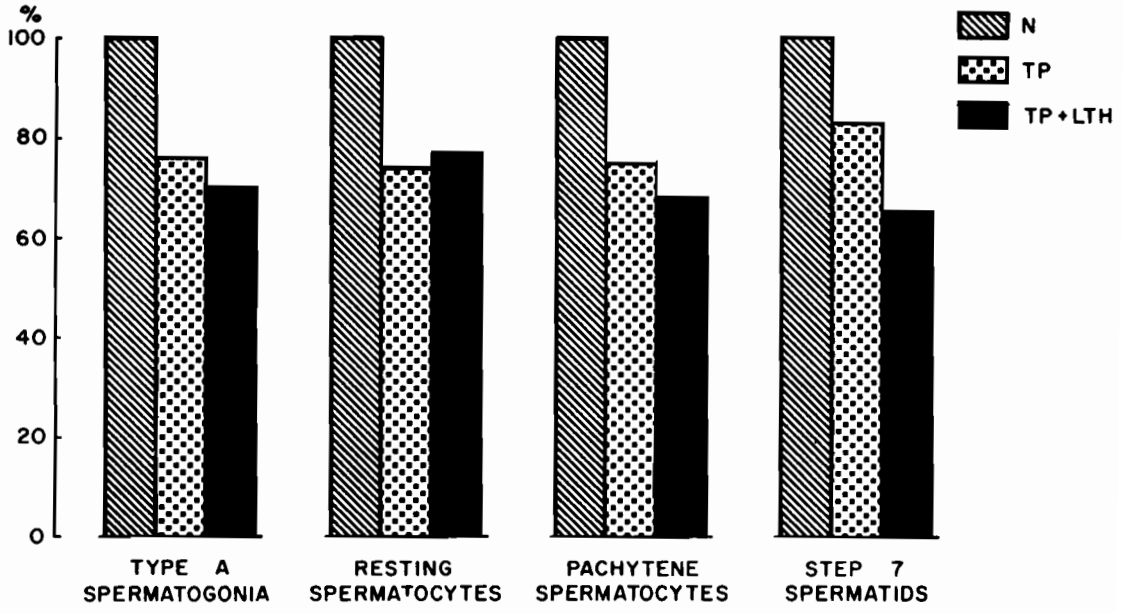


Figure 29 Relative number of germ cells per tubular cross section in normal and thyroidectomized rats.

N = intact adult rats
T_{4_x} = adult rats thyroidectomized for 45 days

RELATIVE NUMBER OF GERM CELLS
PER TUBULAR CROSS SECTION
IN NORMAL AND THYROIDECTOMIZED RATS

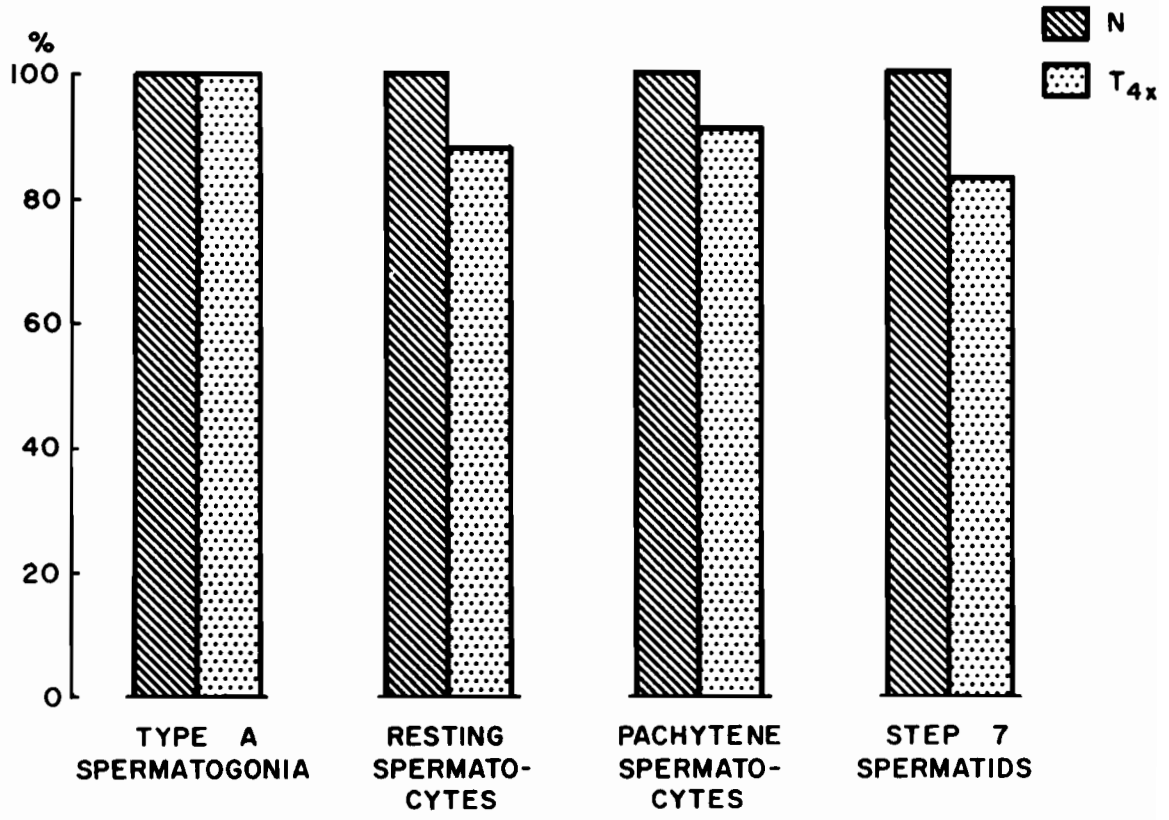


Figure 30 Relative number of germ cells per tubular cross section in hypophysectomized rats treated daily with testosterone propionate, thyroxin and growth hormone.

N = intact adult rat
TP = hypophysectomized rats treated daily with 3 mgm of testosterone propionate, 30 days post-operative
TP:T_4 = hypophysectomized rats treated daily with 3 mgm of testosterone propionate and 2 µg of DL-thyroxin, 30 days post-operative
TP:GH = hypophysectomized rats treated daily with 3 mgm of testosterone propionate and .5 USP of growth hormone, 30 days post-operative
TP:T_4:GH = hypophysectomized rats treated daily with 3 mgm of testosterone propionate, 2 µg of DL-thyroxin and .5 USP of growth hormone, 30 days post-operative

RELATIVE NUMBER OF GERM CELLS PER TUBULAR CROSS SECTION
CORRECTED BY SERTOLI FACTOR:
IN TESTOSTERONE, THYROXIN AND GROWTH HORMONE TREATED RATS

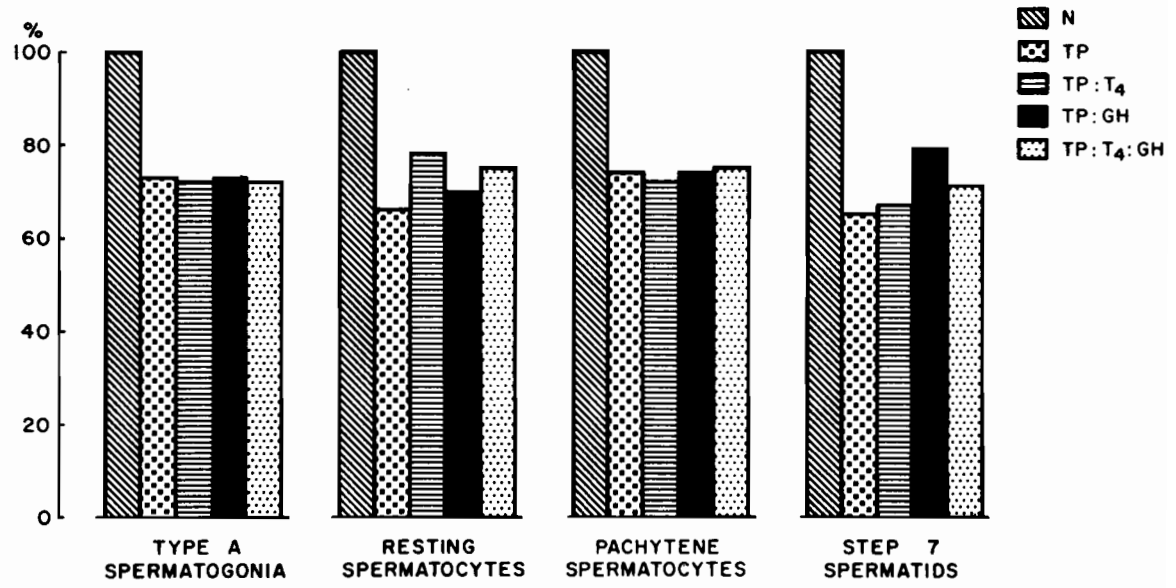


Figure 31 Relative number of germ cells per tubular cross section in untreated hypophysectomized rats and hypophysectomized rats treated daily with growth hormone.

N = intact adult rats
H = untreated hypophysectomized rats, 30 days post-operative
GH = hypophysectomized rats treated daily with .5 USP of growth hormone, 30 days post-operative

RELATIVE NUMBER OF GERM CELLS PER TUBULAR CROSS SECTION
CORRECTED BY SERTOLI FACTOR:
IN HYPOPHYSECTOMIZED AND GROWTH HORMONE TREATED RATS

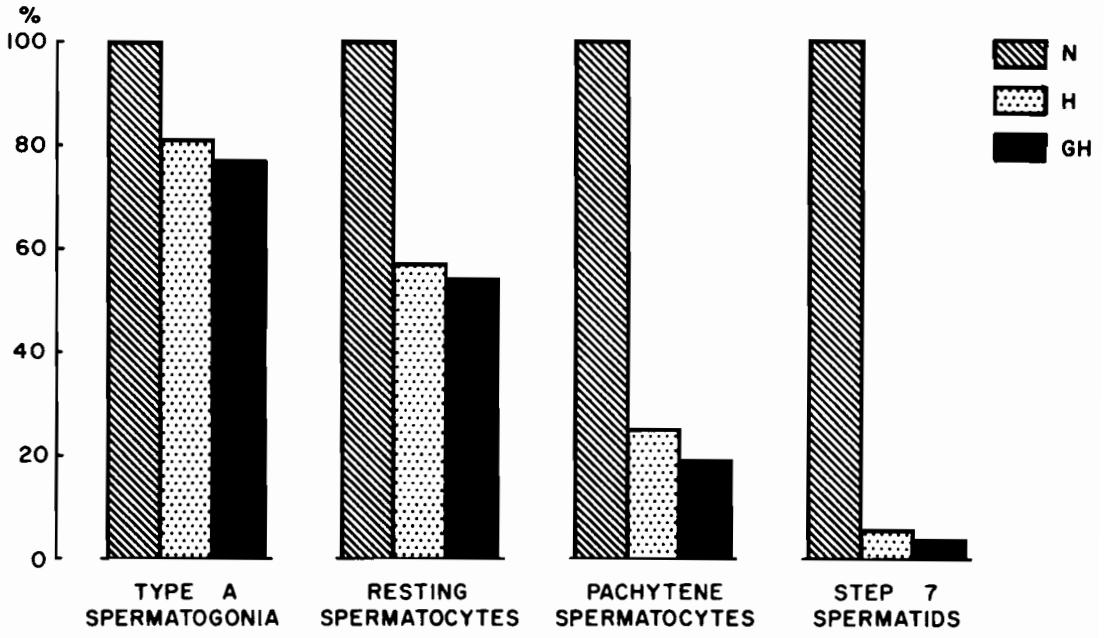
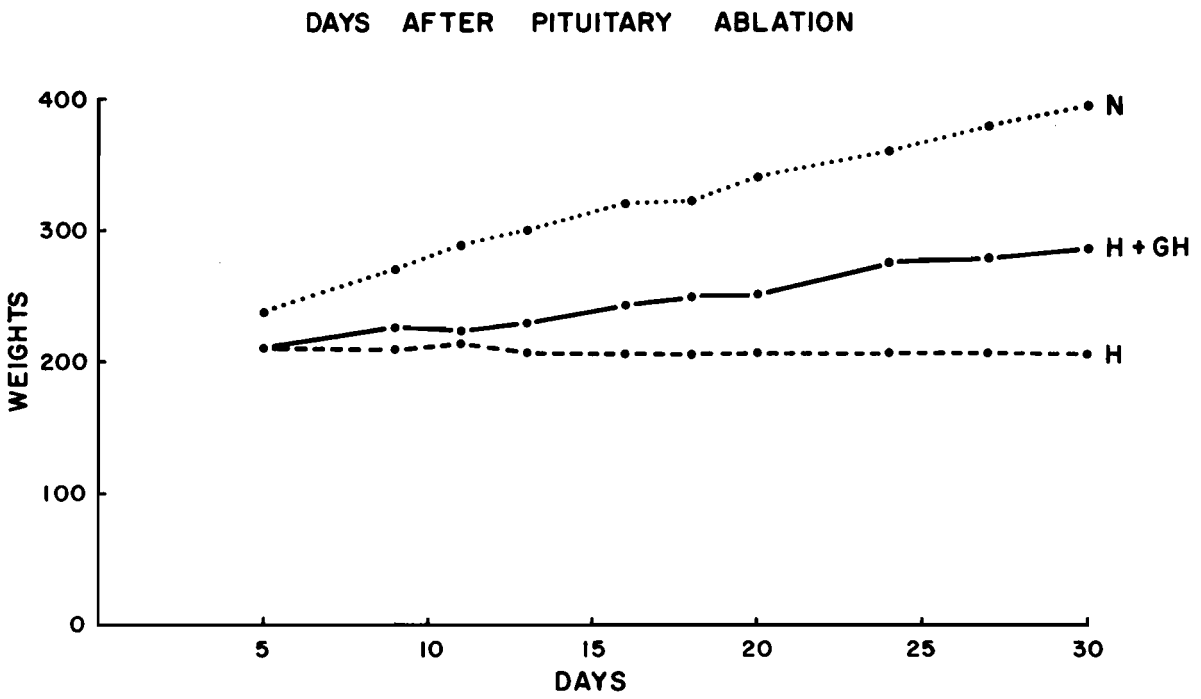


Figure 32 Growth curves of intact adult rats (N), untreated hypophysectomized rats (H) and hypophysectomized rats treated daily with .5 USP of growth hormone (H+GH).


DAYS AFTER PITUITARY ABLATION
400
300
200
100
0
WEIGHTS
5
10
15
20
25
30
DAYS
N
H + GH
H

APPENDIX

SERTOLI CELL CORRECTION FACTOR

Clermont and Morgentaler (1955) proposed the use of the Sertoli cell correction factor to adjust germ cell counts for shrinkage of the seminiferous tubule. In their study of germ cell number in the hypophysectomized rat, it was found that the seminiferous tubules became progressively smaller following hypophysectomy. The decrease in diameter was readily ascertained from measurements in histological sections. However, the reduction in length of the tubules was not. The above authors observed that the number of Sertoli cells increased per tubular cross-section following hypophysectomy. Tubular shrinkage would also result in a larger number of the remaining germ cells to appear in cross section. Consequently, the percentage increase of Sertoli cells could be used as an index of tubular shrinkage, and the counts of germ cell adjusted. These corrected counts of germ cells were felt to present a better indication of germ cell content of the testes of hypophysectomized rats.

In the present investigation the Sertoli cell correction factor was also employed. Treatment with hormones in all instances resulted in a decrease in germ cell number, but in an increase in Sertoli number per tubular cross section. The increase in Sertoli cell number was interpreted as indicating shrinkage of the seminiferous tubules. Following this shrinkage, the number of germ cells would be increased. Although the number of germ cells were found to be less than in the normal, it was felt that the number was not an accurate index of the

degree of cell loss in these hormone treated rats. Consequently, the Sertoli cell number was used to adjust the germ cell counts

The use of the Sertoli cell correction factor depends on the following assumptions; 1) There are no mitosis of the Sertoli cells; 2) Counts of nucleoli are an accurate index of Sertoli cell number; 3) Increase in number of Sertoli cells is an accurate index of shrinkage of the seminiferous tubule.

Firstly, considering mitosis: the Sertoli cells in the young intact rat undergo their last mitotic division before 20 days of age (Clermont and Perey, 1957; Huckins, 1965). Radioautography with thymidine-H^3 may be used as a specific method of detecting dividing cells (Amano et al, 1959). Sertoli cells in the adult rats have not been observed to be labelled with thymidine-H^3 (Clermont et al, 1959; Nebel and Murphy, 1960). Furthermore, Sertoli cells in hormone treated rats are not labelled (Desclin and Ortavant, 1963; Clermont and Harvey, 1965). Thus, it appears safe to assume that increase in the number of Sertoli cells per tubular cross section is not due to mitosis of these cells.

Secondly, considering the reliability of counts of nucleoli as being an index of Sertoli cell number: as stated in Materials and Methods, only Sertoli cells in which the large, prominent nucleolus was present in the plane of section were enumerated. The nuclei of Sertoli cells being large and very irregularly shaped are difficult to measure. Therefore, application of Abercrombie's Formula is impossible. The nucleolus is a relatively small, regularly shaped structure. Counting

of only Sertoli cells which contain this structure should give an accurate index to cell number. This accuracy is conditional on the Sertoli cells containing only one nucleolus. Bustos (1966; personal communication) reported that 95% of Sertoli cells contain one nucleolus. The remaining 5% contain two, one large and one very small. In his investigations he employed DNAse which removes all the chromatin from the nucleus thus making the nucleoli clearly visible. In the present investigation cell counts were made on ordinary histological preparations. Thus, chromatin would mask the small nucleolus in cells containing two of these structures and the cells would appear to have only one nucleolus.

One may argue that hormone therapy may change nucleolar number. However, no reports of this are reported. Furthermore, a survey of several hundred Sertoli cells in the hormone treated rats failed to reveal any increase in the incidence of two nucleoli. Hence, it is concluded that enumeration of Sertoli cell nucleoli is an accurate index of Sertoli cell number.

Thirdly, considering the reliability of Sertoli cell number, increase in cross section as an index of shrinkage of the seminiferous tubules: in the intact rat, the Sertoli cells are known to change considerably in shape during the cycle of the seminiferous epithelium (Leblond and Clermont, 1952). However, the number of cells per area of basement membrane remains constant regardless of the cycle (Bustos, 1966; personal communication). This constancy per area of basement membrane has been interpreted to support the premise that changes in

number of these cells per cross section is an indication of changes in length of the seminiferous tubule.

Table I illustrates the application of the Sertoli cell correction factor. The number of germ cells of two groups of normal, intact rats. In one group the counts were obtained from standard histological sections. In the other group the counts were obtained from sections of testes which had been severely shrunken during histological processing. In this latter group the number of germ cells and Sertoli cells are increased as compared to the former control group. Correction of the counts in the shrunken group brings the number of germ cells into line with those of the control group.

Finally, the frequency distribution of Sertoli cells per cross section of seminiferous tubule was examined (Figure I). The distribution in normal intact animals is slightly skewed. Also shown is the distribution in normal animals in which shrinkage due to histological fixation occurred. The experimental group depicted is FSH treated hypophysectomized rats. This group is purposely shown in that it has been suggested that FSH affects Sertoli cells (Lostroh et al, 1963; Murphy, 1965). (Testosterone propionate treated and ICSH treated rats were also tested and found to give the same distribution as FSH treated.) In Figure I one sees that the shift in distribution of Sertoli cells in the hormone treated group is the same as the shift in the distribution of these cells in tissue which is known to be merely shrunken. This suggests that the increase in number of Sertoli cells in hormone treated rats is most likely a result of simple tubular shrinkage rather than a change in the Sertoli cells themselves.

Figure I This figure is a plot of the number of cross sections of seminiferous tubules containing various number of Sertoli cells. Based on 5 animals per group.

Normal = intact adult rats
Normal shrunken = intact adult rats in which tissue shrinkage of the testis had occurred during histological processing
FSH = hypophysectomized rats treated daily with 150 µg of FSH, 35 days post-operative

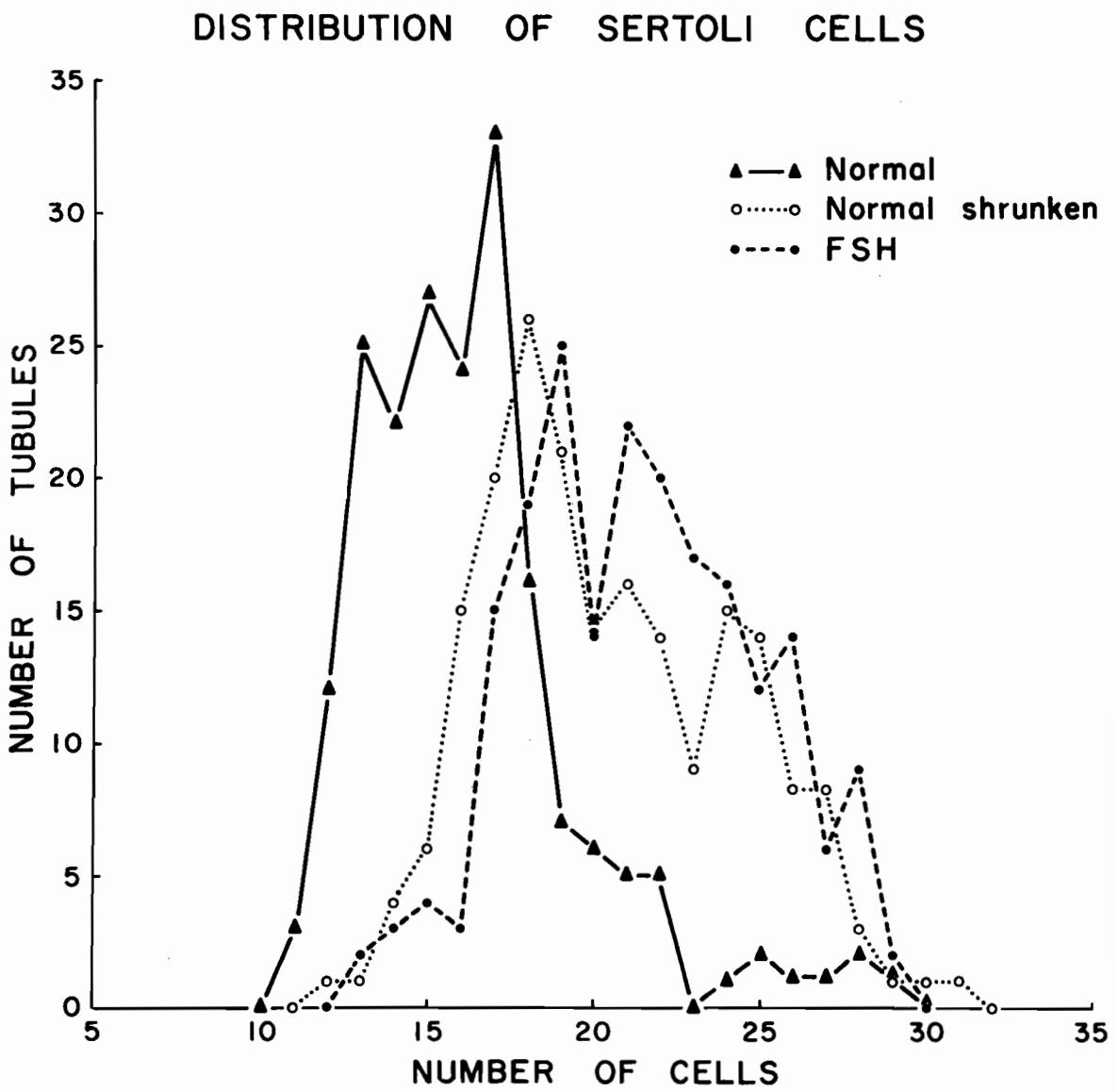
TABLE I

NUMBER OF GERM CELLS PER TUBULAR CROSS SECTION IN TWO GROUPS OF NORMAL RATS:

DEMONSTRATION OF SERTOLI CELL CORRECTION FACTOR

Treatment	Type A Spermatogonia	Resting Spermatocytes	Pachytene Spermatocytes	Step 7 Spermatids	Sertoli Cells
Normal	1.09	37	36	123	18.1
Normal (poorly fixed)	1.15	41	40	125	19.8
Poorly fixed Corrected	1.05	37	36	114	

Sertoli Factor $\frac{19.8}{18.1} \times 100 = 109\%$


DISTRIBUTION OF SERTOLI CELLS
NUMBER OF TUBULES
NUMBER OF CELLS
Normal
Normal shrunken
FSH
0
5
10
15
20
25
30
35

(I)