

THE INFLUENCE OF THE SUPRARENAL CORTEX ON THE GONADS OF RABBITS.

II. THE EFFECTS OF SUPRARENAL INJURY (BY REMOVAL OR FREEZING) ON THE TUBULES AND INTERSTITIAL CELLS (LEYDIG) OF THE TESTIS.

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In a previous paper (1) it was shown that, of seventeen female rabbits which survived double suprarenalectomy for a month or more, striking enlargement of the ovaries was observed in thirteen, or 76 per cent, while of the thirteen rabbits with partial suprarenalectomy, only one showed enlargement of the ovaries. This enlargement consists essentially of an hypertrophy of the interstitial cells.

In view of the fact that enlargement of the suprarenal cortex usually occurs in rabbits and guinea pigs following gonadectomy and also in these animals during pregnancy, and in birds during ovulation, there seems to be some reciprocal relation between the ovary, particularly its interstitial cells, and the suprarenal cortex. It was suggested that the hypertrophy of the interstitial cells following suprarenalectomy may be of the nature of a compensatory hypertrophy.

The observations regarding the ovarian changes prompted us to study the testes of rabbits which survived double or partial suprarenalectomy with particular reference to the cells of Leydig.

LITERATURE.

Little has been published concerning the effects of suprarenalectomy on the gonads. Novak (2) studied the effects of suprarenalectomy on the genital tracts of male and female white rats. He removed the second suprarenals 7 to 14 days after the first. The period of survival after suprarenalectomy varied from several days to months. In some animals there were no effects, particularly in adult

males, and he ascribed this to the well known fact that accessories are found in a large percentage of white rats. He concluded that suprarenal extirpation in white rats induces a hypoplasia or atrophy of the genital tracts that is more pronounced the younger the animal is at the time of operation. Partial supra-renalectomy was without effect. He believed that the main cause of the genital atrophy was the removal of the internal secretion of the suprarenals. Novak, however, did not study particularly the interstitial cells either of the testis or of the ovary.

Elliott (3) stated that he did not observe any significant changes in the ovary or testis of cats following partial or double supra-renalectomy.

The work of Cesa-Bianchi (4) and Pende (5) dealt only with the effects of supra-renalectomy on the ovary.

Hewer (6) fed white rats with the dried cortex of ox suprarenals and injected both lipid and non-lipoid extracts. Her work confirms the observations of others that lipid extracts of the suprarenal cortex cause rats to become extremely fat. In addition, she reported changes in the testes, but they were not of a specific nature.

Recently Leupold (7) attempted to establish a relation between the suprarenal and the testis, mainly on morphological and microchemical grounds. He states that in the male, during sexual activity, the doubly refractive substances and cholesterol of the suprarenals increase. During the periods of sexual quiescence there is a diminution in the cholesterol content. In atrophic testes, cholesterol esters appear in large amounts in the Leydig cells; Leupold believes that these cells resorb the liberated lipoids from the disintegrated tubular epithelium.

In another paper (8) Leupold discusses cholesterol metabolism in relation to spermatogenesis based on his morphological and chemical studies of human autopsy material. He points out that a diminution in lipid content of the suprarenal cortex is associated with a similar diminution of the lipoids in the interstitial cells. The reverse was also found, large amounts of lipid in the suprarenal being associated with large amounts in the interstitial cells.

Leupold attempted to reinforce his morphological studies on the close lipoidal relation between the cells of Leydig and those of the suprarenal cortex by animal experimentation. He carried out unilateral supra-renalectomy on three cats and double supra-renalectomy on one and examined the testes within 8 days after operation. Extensive destruction of the spermatogenic cells was found. From this he concluded that the cholesterol esters of the suprarenal cortex have a protective influence on the sex glands in that they bind toxins which would otherwise destroy these cells, and secondly, that they have a regulatory function on the amount of these esters in the Leydig cells. He supports the old conception that Leydig cells supply the lipoids as foodstuffs to the sperm cells.

However, Leupold's conclusions are based primarily on human autopsy findings and his elaborate hypotheses have not been supported by adequate experimental work.

With regard to the effects of gonadectomy on the suprarenal cortex there are several observations showing that this procedure causes an enlargement of the cortex in rabbits, guinea pigs, and dogs (Theodossieff (9), Schenk (10), and Kolde (11)).

Embryology.

Embryologically, the testis, ovary, and cortex of the suprarenal arise from a common anlage, the urogenital fold, or Wolffian ridge. The vertebrate embryo forms at first an indifferent reproductive gland. The newly formed "testes" or "ovaries" are morphologically alike up to a certain period of development, but later by emphasis of certain characters the sexually differentiated organ is formed. It has been shown for all classes of vertebrates, with the exception as yet of mammals, that the first genital cells have a special origin, probably being derived directly from the segmentation cell. Felix (12), therefore, terms these cells "primary genital cells" in contradistinction to those that are differentiated from the epithelial covering of the reproductive gland, which he terms "secondary genital cells."

Concerning the origin of the cells of Leydig, there are two principal views. According to one they arise from the connective tissue, and according to the other they are epithelial cells derived from the germinal epithelium. Allen (13) and Whitehead (14) have made the most recent studies on the embryology of the cells of Leydig and support the view that they are of connective tissue origin.

Presentation of Data.

This study of the effects of suprarenalectomy on the testis is based on the examination of the gross and microscopic findings in suprarenalectomized rabbits.¹ The tissues were fixed in 10 per cent formalin, embedded in celloidin, and stained with hematoxylin and eosin.

The testes of 48 rabbits subjected to double suprarenalectomy and 45 subjected to partial suprarenalectomy were studied.

Double Suprarenalectomy.—The periods of survival of the 48 rabbits in which bilateral suprarenalectomy was carried out, are as follows:

Rabbits surviving from 1 to 15 days.....	12
Rabbits surviving from 16 to 29 days.....	9
Rabbits surviving from 30 to 60 days.....	9
Rabbits surviving from 61 to 90 days.....	6
Rabbits surviving longer than 90 days.....	12

¹ All operations were performed under ether anesthesia.

Of the twenty-seven rabbits surviving double suprarenalectomy for more than 30 days,² twenty-two showed normal testes microscopically. Of these, three were reported atrophic in the gross, but this apparent atrophy could not be confirmed microscopically.

In this connection it is of interest to recall the conclusions of workers who attempted to correlate the gross and microscopic appearances of testes of men dying of general diseases. Berberich and Jaffé (15) recently studied the testes of 100 human cases in which death was due to general diseases and they came to the conclusion that, with the exception of marked changes, gross findings do not permit of a final interpretation as to the condition of the organ. Simmonds (16) was of the same opinion. Our own experience in a series of 224 patients dying of chronic diseases is in harmony with the expressed view.

The twenty-two rabbits with normal testes microscopically showed well preserved tubules, with normal active spermatogenic elements. The intertubular connective tissue was not increased and there were very few Leydig cells, which were mainly disposed about small blood vessels. The tunica and rete showed no recognizable deviation from the normal. The veins were frequently congested, this being occasionally quite pronounced in animals dying of acute suprarenal insufficiency, with marked cardiovascular asthenia. Two of twenty-seven rabbits, one surviving 41 and the other 115 days after double suprarenalectomy, showed a slight increase in the number of Leydig cells, without any detectable variations in the other elements of the testis. This slight increase may be relative, but if absolute it could be attributed, we believe, to physiological variations rather than to the effects of extirpation of the suprarenal glands. Both rabbits were adults when the second suprarenals were removed.

Three of the twenty-seven rabbits showed atrophic changes in the tubules without definite changes in the Leydig cells. In one animal

² As pointed out many times, accessory cortical tissue is probably present in all rabbits and can be readily demonstrated in about 70 per cent. A larger number of males survive double suprarenalectomy than of females. This is due, we believe, to the more frequent occurrence of accessory cortex which for the most part is found along the spermatic cord. However, skilled surgical technique and careful postoperative care are important factors in survival.

which lived 51 days after removal of the second suprarenal, no apparent cause for the atrophy could be discovered. It was an adult when the second suprarenal was removed. Two other animals, one surviving 35, the other 65 days following removal of the second suprarenals, also showed atrophy of the testes with no changes in the Leydig cells. A possible cause for the atrophy was complete thyroidectomy performed in each case 23 days prior to the removal of the second suprarenal.

Twenty-one rabbits survived suprarenalectomy for less than 30 days. Of these, twelve had normal tubules and Leydig cells, five had atrophic tubules with normal Leydig cells, and one had atrophic tubules with a slight relative increase in the interstitial cells. Of the six rabbits with atrophic tubules, in one no apparent cause for the atrophy could be determined; two were very mangy and weak throughout the period of experimentation; one had paralysis of the hind legs and incontinence during the last month of life; and two had been subjected to thyroidectomy in addition to suprarenalectomy.

Three showed a slight relative or absolute increase in the interstitial cells with normal tubules.

The condition of the interstitial cells and tubules in the 48 bilaterally suprarenalectomized rabbits is summarized as follows:

Condition.	Rabbits surviving over 30 days.	Rabbits surviving less than 30 days.	Per cent.
Normal tubules and Leydig cells.....	21	12	71
Atrophic " " normal Leydig cells.....	4	5	17
" " " increased " "		1	2
Normal " " " " "	2	3	10

Thus the tubules were found to be normal in 81 per cent, while the Leydig cells were unchanged in 88 per cent.

Partial Suprarenalectomy.—45 animals in which the suprarenals were injured by partial removal or by freezing were studied. Seven were eliminated because the testes were not completely developed. Partial suprarenalectomy, however, apparently does not prevent or delay the normal development of the testes as many animals in this group were partially suprarenalectomized before sexual maturity.

Seven other rabbits were eliminated because unilateral or bilateral vasoligature complicated partial suprarenalectomy, although in several instances no effects were observed as a result of this procedure. In one rabbit, vasoligature was performed when the animal was 1 month old; when the testes were examined at 7 months they were fully differentiated. Of the remaining thirty-one rabbits, twenty-six, or 83 per cent, showed normal tubules and interstitial cells; two, or 7 per cent, showed normal tubules with a slight relative or absolute increase in the cells of Leydig; three, or 10 per cent, showed atrophic tubules and in one of these it was associated with a relative increase in the interstitial cells.

DISCUSSION.

Data have been presented concerning 48 rabbits that were subjected to double suprarenalectomy, and 45 in which the suprarenals were injured by partial removal or freezing. In the bilaterally suprarenalectomized rabbits 81 per cent presented microscopically normal seminiferous tubules, and 88 per cent had normal Leydig cells. Partial suprarenalectomy also was without specific effect on the testes. Of those analyzed, 90 per cent showed normal tubules, and 86 per cent showed no change from the normal in the cells of Leydig.

In the few instances in which atrophy of the testes was noted, it was believed that long standing illness, progressive cachexia, or some additional operative procedure, as for instance thyroidectomy, offered a more likely explanation than injury of the suprarenals.

That long standing illness in man has an unfavorable effect upon the testes has been known for some time. Cordes (17) found in chronic diseases an increase in connective tissue, and he noted that this increase was most pronounced in cachectic states. In addition, he reported shrinking of the tubules, hyaline thickening of their walls, and impairment of spermatogenesis. The cells of Leydig showed no constant change, sometimes showing an increase and at other times remaining unchanged. Hansemann (18), Thaler (19), and Berberich and Jaffé (15) have come to similar conclusions.

In an unpublished study of the testes of 224 patients dying of chronic diseases at Montefiore Hospital we have found that fre-

quently these organs showed pronounced injury. The tubules were shrunken, the walls showed hyaline thickening, and spermatogenesis was often impaired, the sperm cells frequently disappearing entirely and leaving only a row of Sertoli cells lining the lumen. The intertubular connective tissue was generally increased. The Leydig cells showed no constant changes. They were relatively increased in about 25 per cent of the cases, but much more frequently they showed no change.

We believe that such factors influenced Novak's results. Double suprarenalectomy in rats frequently induces no serious insufficiency. The hypoplasia or atrophy of the genital tracts reported by Novak was probably brought about by complicating factors.

We were particularly interested in the interstitial cells of Leydig and were struck by the great difference in behavior to double suprarenalectomy between these cells and the interstitial cells of the ovary. Even prior to their discovery in 1850, Berthold (20) was speculating on the dual function of the testis. But since then, in spite of the fact that a large amount of work has been done in attempting to discover and evaluate the internal secretory function of the various constituents of the testis, the complexity of the organ has made it impossible to assign with certainty definite secretory activities to its different components. Transplantation, traumatism, vasoligature, cord ligation, induced cryptorchidism, partial removal, exposure to x-rays and radium, and freezing have all been unsuccessful in dissociating the spermatogenic, Sertoli, and Leydig cells, and for this reason workers have been unable to obtain unequivocal evidence of specific internal secretory activity of any of the elements of the testis, or to extend the observations of Brown-Séquard and his followers on the effects of extracts. That the organ has an important function in addition to the production of the sperm cells there is no doubt. The effects of castration before puberty clearly demonstrate the profound physiological disturbance in the mental and physical development induced by this procedure.

Many have considered that the cells of Leydig and the interstitial cells of the ovary are homologous. The evidence in favor of this view is based on morphological and microchemical studies. More detailed studies have shown that there are sufficient histological

differences between the two to warrant considerable hesitation in accepting this view. The fact that the interstitial cells of the ovary readily undergo hypertrophy after double suprarenalectomy in rabbits while the cells of Leydig show no such response in this animal we have interpreted as physiological and biological evidence against their functional homodynamy.

CONCLUSIONS.

1. Double or partial suprarenalectomy in rabbits produces no specific changes either in the tubules or in the interstitial cells of the testes.
2. This is in sharp contrast with the marked hypertrophy of the interstitial cells of the ovary which usually occurs in rabbits surviving double suprarenalectomy 30 days or more.
3. These facts indicate that the interstitial cells of the testis and ovary are not functionally homologous.

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