

# MORPHOLOGICAL CHANGES IN THE TESTIS UNDERGOING HYPERTROPHY AFTER UNILATERAL CASTRATION

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The testis of rats undergoing compensatory hypertrophy after unilateral castration at the age of 2-3 and 10 days is indistinguishable from the testis of control animals undergoing a mock operation with respect to the diameter of the seminiferous tubules, the diameter of the nuclei of cells of the spermatogenic epithelium and Leydig's cells, and also to the relative percentages of interstitial and tubular tissue, but the number of spermatocytes in pachyneme and the number of spermatids are increased. The data showing intensification of spermatogenesis in the testis undergoing compensatory hypertrophy are discussed in connection with the problem of physiological degeneration of the spermatogenic epithelium and the mechanism of development of compensatory hypertrophy of the testis.

Unilateral castration of rats at the age of 1 to 22 days causes an increase in weight of the residual testis on the average up to 65% of the combined weight of two testes of control animals [3]. It has not yet been shown, however, whether changes take place in the structure of the hypertrophied testis [4, 6, 7].

Figarova [4] studied the testis of 15-day rats unilaterally castrated at the age of 5 days and found no increase in the quantity of interstitial tissue or in the diameter of the convoluted tubules in the hypertrophied testis and no increase in the rate of maturation of the spermatogenic epithelium. No investigations to discover whether the number of spermatogenic cells is changed in the testis undergoing hypertrophy after removal of the opposite organ could be found in the literature.

The object of the present investigation was to study the morphological features of the hypertrophied testis in unilaterally castrated rats.

## EXPERIMENTAL METHOD

Experiments were carried out on 110 noninbred young rats. Unilateral castration was performed on the rats of the age of 2-3 or 10 days and also on adult rats. A mock operation was performed on the control animals. The rats undergoing operation at the age of 2-3 days were sacrificed 30 (series I) and 60 (series II) days after the operation, while those castrated at the age of 10 days were sacrificed after 23 days (series III) and the adult rats 70 days after castration (series IV). The animals were killed by decapitation, the testes were fixed in Zenker-formol, and paraffin sections 5  $\mu$  in thickness were stained with PAS-hematoxylin. The ratio between the amounts of tubular and interstitial tissue was determined by Chalckly's method in Pakenas' modification [1], as follows. A glass marked with a grid and with five hairs of different lengths glued to it was inserted into the ocular. The occasions when the ends of these hairs coincided with the tubules and with the interstitial tissue were recorded (1000 measurements for each case). The diameter of the convoluted tubules of the testis was measured by means of a screw-adjusted ocular-micrometer, the mean value being calculated from 100 measurements for each case. The diameter of the nuclei of the spermatogenic cells (spermatocytes in preleptoneme and pachyneme) was measured with the screw-adjusted ocular micrometer under a 90 $\times$  objective, and the diameter of 100 nuclei of Leydig's cells

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TABLE 1. Effect of Unilateral Castration on State of Spermatogenesis and on Diameter of Convoluted Tubules in Testes of Rats of Different Ages ( $M \pm m$ )

Age of animals at time of operation (days)	Time after operation (days)	Group of animals	Wt. of testis (in body wt. at time of sacrifice)	Percent hypertrophy	Diameter of convoluted tubules ( $\mu$ )	No. of cells of spermatogenic epithelium per transverse section through seminiferous tubules		
						spermatocytes in preleptoneme	spermatocytes in pachyneme	spermatids in stage VII of development
2-3	60	Experimental Control P	$8,3 \pm 0,2$ $6,5 \pm 0,1$ 0,01	13,9	$224,9 \pm 1,4$ $223,4 \pm 1,3$ 0,76	$218,0 \pm 9,5$	$270,8 \pm 10,6$	$919,0 \pm 25,0$
						$201,5 \pm 5,5$ 0,18	$240,3 \pm 5,9$ 0,02	$777,4 \pm 21,7$ 0,01
10	23	Experimental Control P	$5,8 \pm 0,4$ $4,1 \pm 0,2$ 0,01	20,7	$141,8 \pm 2,6$ $144,1 \pm 2,9$ 0,17	$227,5 \pm 6,9$	$253,5 \pm 8,9$	$184,8 \pm 19,0$
						$224,6 \pm 5,8$ 0,39	$201,8 \pm 14,6$ 0,01	$132,0 \pm 28,2$ 0,01
Adults	70	Experimental Control P	$7,6 \pm 0,1$ $7,2 \pm 0,1$ 0,05	0	$222,0 \pm 4,6$ $182,9 \pm 17,0$ 0,12	$284,2 \pm 4,6$	$333,7 \pm 9,7$	$1150,6 \pm 40,5$
						$316,2 \pm 12,7$ 0,06	$358,8 \pm 21,4$ 0,38	$1150,2 \pm 45,3$ 1,0

was also measured for each case. Spermatocytes in preleptoneme and pachyneme and spermatids in stage VII of development were counted in 40 transversely divided tubules for each case. The number of Sertoli cells also was counted in the same tubules. The results of the quantitative analysis were expressed as ratios of 100 Sertoli cells. All the calculations - measurement of the diameter of the convoluted tubules and of the nuclei of the spermatogenic cells and Leydig's cells, quantitative analysis of the spermatogenic cells in the testis of the unilaterally castrated and control animals - were carried out strictly at stage VII of the cycle of the spermatogenic epithelium [5]. Statistical analysis of the results was carried out by the Fisher-Student method. Differences were significant for which  $P \leq 0.02$ .

## EXPERIMENTAL RESULTS

The results of the experiments of series I showed that the weight of the residual testis was 70% of the combined weight of the two control testes. Meanwhile the hypertrophied testis of the unilaterally castrated rats was indistinguishable from that of the control animals in the diameter of the nuclei of the spermatogenic cells and Leydig's cells. The relative percentages of interstitial and tubular tissue in the hypertrophied testes were the same as in the intact testes of the control animals.

The results of the experiments of series II, III, and IV are summarized in Table 1. The weight of the residual testis in the rats castrated at the ages of 2-3 and 10 days reached 63.9 and 70.7%, respectively, of the combined weight of the two testes in control animals; in rats unilaterally castrated in the adult state no compensatory increase in size of the residual testis was observed. Measurement of the diameter of the convoluted tubules in the testes of the unilaterally castrated rats in all three series of experiments showed no difference compared with the intact testes of the control rats. The number of spermatocytes in preleptoneme in the hypertrophied testes of animals aged 33 and 62-63 days was indistinguishable from the control (Table 1). Determination of the number of more highly differentiated cells of the spermatogenic epithelium, namely spermatocytes in pachyneme and spermatids in stage VII of development showed a significant increase in the number of cells of this type in the hypertrophied testes of the rats in the experiments of series II and III, but no increase in the number of spermatogenic cells in the late stages of development of the testes of the adult rats, which did not increase in size after removal of the opposite organ.

The results thus show that the testis undergoing compensatory hypertrophy contains more spermatocytes in pachyneme and more spermatids in stage VII of development than in the control.

These results are interesting in connection with the study of the dynamics of development of compensatory hypertrophy of the testis in rats in early ontogeny. It was found that after the completion of muosis (which occurs in rats at the age of 23-24 days) the animals ceased to react by enlargement of the testis and by a change in secretion of follicle-stimulating hormones

(FSH) by the pituitary to unilateral castration. This suggested that compensatory hypertrophy of the testis is linked with the state of spermatogenesis in the organ and the character of SFH secretion by the pituitary. The results of the present investigation, indicating a change in the intensity of spermatogenesis in the testis undergoing compensatory hypertrophy, confirmed this hypothesis.

The fact that the number of spermatocytes in preleptoneme in the hypertrophied testis was not greater than in the control testis while the number of spermatocytes in pachyneme and the number of stage VII spermatids were increased, suggests that during the development of compensatory hypertrophy of the testis degeneration of the cells of the spermatogenic epithelium is less marked.

During physiological degeneration of the spermatogenic epithelial cells about 50% of the theoretically expected number of spermatozoa has been observed to die [2]. However, the question of the factors determining degeneration of the cells of the spermatogenic epithelium has not yet been solved. It can be assumed from observations showing that compensatory hypertrophy of the testis depends on FSH secretion that the increase in FSH secretion observed during the development of compensatory hypertrophy [3] inhibits degeneration of the sex cells. The increase in size of the testis after removal of the opposite organ is not regarded as being attributable entirely to an increase in the number of spermatogenic cells; lengthening of the convoluted tubules evidently took place also. This suggestion, repeatedly found in the literature [4, 6] has not yet been confirmed experimentally because of the lack of an adequate method of investigation.

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