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Morphometric Studies of Compensatory Testicular Hypertrophy in the Rat after Hemicastration

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Abstract

The quantitative nature of the compensatory testicular hypertrophy following unilateral castration was examined in groups of immature Wistar-derived male rats hemicastrated at 10, 20, 30, 40 and 50 days of age and completely castrated 20 days later. Hemicastration resulted in compensatory hypertrophy of the remaining testis and it decreased as the animals aged. In 30-day-old rats compensatory testicular hypertrophy was 33% while at 70 days of age hypertrophy was reduced to 2%. The increase in testis weight of hemicastrated rats was correlated with an increase in total seminiferous tubule length and a larger cross-sectional area which was due in part to the greater number of germ cells per testis. Sertoli cell number did not increase significantly in the hemicastrated testis but more germ cells were associated with each Sertoli cell.

Introduction

Removal or damage to one of a paired glandular organ frequently results in compensatory hypertrophy, compensatory hyperplasia, or both, of the cells of the remaining or intact gland. The occurrence of compensatory testicular hypertrophy in the immature rat testis after hemicastration has been reported (Jacobsohn and Norgren 1965; Yaginuma *et al.* 1969; Ojeda and Ramirez 1972; Stewart *et al.* 1973; Cunningham *et al.* 1978). On the other hand, Shellabarger (1963) failed to demonstrate an increase in weight of the testis of rats hemicastrated at 30 days of age and killed 14 days later. The degree of compensatory gonadal hypertrophy in male rats decreased with age (Ojeda and Ramirez 1972), and was absent in mature animals (Setchell and Waites 1972; Gomes and Jain 1975; Howland and Skinner 1975; Lindgren *et al.* 1976).

The increase in weight of the remaining testis after unilateral castration appeared to be due to an increase in Sertoli cell numbers (Cunningham *et al.* 1978), but testicular hypertrophy occurring in hemicastrated young puberal rats was thought by Liang and Liang (1970) to be due in part to an increase in the interstitial cells, and was not true hypertrophy of the tubules. In the ram, it was reported that diameter (Voglmayr and Mattner 1968) and length (Hochereau-de Reviers *et al.* 1976) of the seminiferous tubules of hemicastrated animals were significantly greater than in intact animals.

As the nature of the hypertrophy occurring in the contralateral testis after hemicastration does not appear to have been adequately described, particularly in regard to cell type and number, a study has been conducted to examine the quantitative nature of the hypertrophy of the testis and to ascertain the age at which hypertrophy ceases to occur in rats following hemicastration.

Materials and Methods

Wistar-derived rats were maintained on commercial rat pellets and water *ad libitum* under controlled conditions of temperature and lighting (22°C, light duration from 6.00 a.m. to 6.00 p.m.).

Animals were weighed, lightly anaesthetized with ether and hemicastration performed through a scrotal incision at 10, 20, 30, 40 and 50 days of age; the remaining testis was removed and examined 20 days later for evidence of compensatory hypertrophy; alternate left and right testes were removed, weighed, and immediately fixed for at least 24 h in Susa's fixative. The tissue was embedded in paraffin, sectioned at 5 μm and stained with haematoxylin and eosin (Humason 1972).

Testes collected at hemicastration were considered as control or intact groups while those removed at autopsy 20 days later were the hemicastrated groups. The numbers of individuals for the intact and hemicastrated groups are shown in Table 1.

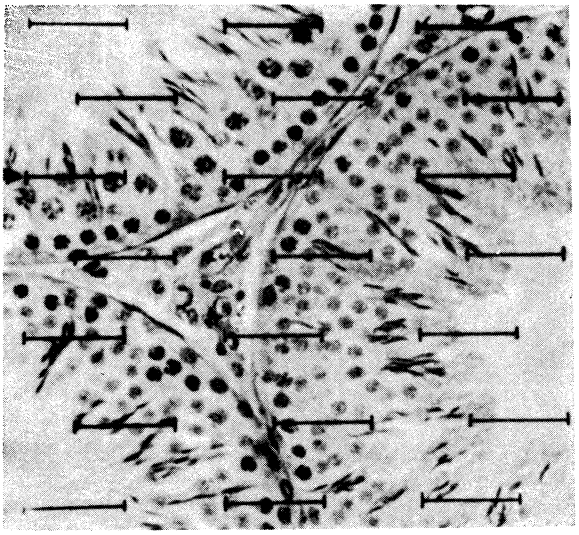

Fig. 1. Weibel multipurpose stereological test system with 21 lines and 42 end-points superimposed on profiles of seminiferous tubules.

Stereological techniques were employed to obtain quantitative morphological information. The 42-point graticule of Weibel *et al.* (1966) which was used has an area containing 21 lines of constant length arranged in seven equidistant and parallel rows (Fig. 1). Calibration of the area of the graticule for each magnification used enabled tubular length and cross-sectional area to be estimated. The slides were assessed for seminiferous tubule length (× 125) (Elias *et al.* 1971), cell number (× 1250) (Aherne 1967; Underwood 1970) and the volume fraction of the testicular components (× 125) (Weibel *et al.* 1966). Cross-sectional area of the seminiferous tubule (Weibel *et al.* 1966) was assessed at magnifications depending on the size of the tubule. The occupancy of Sertoli cells by germ cells was determined by the ratio of germ cell and Sertoli cell numbers.

The results were analysed by two-way analysis of variance with unequal sample size, using the method of unweighted means (Keppel 1973). Means of the observed items for the intact group were compared with those of the hemicastrated animals, and, when the interaction was significant, comparison between the two groups at each day of age was made.

Results

Hemicastration resulted in slightly faster growth of rats and the hemicastrated groups had higher body weights than the intact groups (Table 1). The age at which

hemicastration was performed and its effects on the remaining testis of the rats are shown in Table 1. Compensatory testicular hypertrophy (CTH) occurred and the testicular weights of the hemicastrated rats were significantly greater than those of the intact animals (Table 1). Hemicastrated rats at 40, 50 and 70 days of age had significantly larger testes than intact animals of the same age. However, the mean testicular weight per 100 g of body weight was greater in hemicastrated rats at 30, 40 and 50 days of age than in control animals. The percentage of CTH (based on testis weight per 100 g of body weight) decreased with age, falling from 33% at 30 days of age to 2% at 70 days (Table 1).

Table 1. Compensatory hypertrophy of the rat testis: weight changes

Mean values (±standard error of means) for the remaining testis in rats hemicastrated at different ages are compared with means for the equivalent testis in intact animals. a,b = significantly different from the corresponding intact group : a, $P<0{\cdot}05$; b, $P<0{\cdot}01$. * $P<0{\cdot}05$. ** $P<0{\cdot}01$

No. of animals	Age (days)	Body wt (g)	Testis wt (mg)	Testis wt per 100 g body wt (mg)	Compensatory hypertrophy (%)[A]	
					Testis wt	Testis wt per 100 g body wt
			Intact rats			
11	30	85·66±3·95	332·20±28·16	375·56±19·86		
12	40	110·58±3·35	593·21±41·05	534·37±31·86		
16	50	151·58±4·00	845·94±43·88	551·85±18·74		
14	60	170·90±9·43	1232·14±36·17	739·45±32·33		
16	70	191·84±10·15	1161·93±42·36	615·21±17·91		
			Hemicastrated rats			
12	30	76·57±4·83	382·79±28·85	499·45±17·79[b]	15·23	32·98
16	40	108·83±6·12	732·87±30·69[a]	684·12±22·85[b]	23·54	28·02
10	50	173·05±9·21[a]	1160·10±49·56[b]	643·06±41·40[b]	37·14	16·52
12	60	190·36±4·82[a]	1277·21±50·41	674·85±30·91	3·66	−8·73
14	70	209·42±6·59	1301·57±26·76[a]	626·72±15·87	12·02	1·87
			Overall analysis of variance			
Degrees of freedom		1,122	1,121	1,121		
F		4·74*	30·79**	19·37**		
Error mean squares		618·98	19651	7385·37		

[A]Compensatory hypertrophy (%) $= \dfrac{\text{Mean hemicastrated testis weight—Mean intact testis weight}}{\text{Mean intact testis weight}} \times 100.$

Hemicastration increased significantly the length of the seminiferous tubules per testis (Table 2). In hemicastrated animals 40 and 50 days old the cross-sectional area of the seminiferous tubules was increased (Table 2). The number of cells within the seminiferous epithelium per testis in intact and hemicastrated rats increased considerably as the animals aged (Table 2). There was no significant difference in Sertoli cell number between the intact and hemicastrated groups at any age but the number of germ cells in the testis of the hemicastrated animals was significantly greater than that of intact animals (Table 2). Sertoli cell occupancy increased significantly as the animals of both groups became older, but hemicastration led to an earlier increase in occupancy. Sertoli cell occupancy in hemicastrated rats at 40, 50, 60 and 70 days of age was 30·3, 16·1, 33·9 and 10·5% higher respectively than

Table 2. Compensatory hypertrophy of the rat testis: histological changes

Mean values (± standard error of means) for the remaining testis in rats hemicastrated at different ages are compared with means for the equivalent testis in intact animals. a,b = significantly different from the corresponding value for intact rats: a, $P<0{\cdot}05$; b, $P<0{\cdot}01$. * $P<0{\cdot}05$. ** $P<0{\cdot}01$

No. of animals	Age (days)	Tubule length per testis (m)	$10^3 \times$ Tubular area (mm^2)	$10^{-6} \times$ No. of germ cells per testis	$10^{-6} \times$ No. of Sertoli cells per testis	Sertoli cell occupancy[A]
			Intact rats			
11	30	27·26±1·20	22·52±1·24	226·2±20·8	62·2±5·4	3·68±0·24
12	40	36·95±2·52	31·21±1·24	404·6±38·3	52·6±3·2	7·91±0·07
16	50	40·12±1·50	39·67±2·69	935·7±77·0	57·8±4·2	16·81±1·29
14	60	48·12±1·10	49·74±1·00	1158·9±47·9	74·5±6·2	16·52±1·51
16	70	41·91±2·05	54·59±1·58	1395·9±70·5	83·8±6·2	17·09±0·87
			Hemicastrated rats			
12	30	29·97±0·94	24·48±1·19	270·0±31·6	74·7±5·6	3·56±0·20
16	40	36·94±1·14	39·47±1·16[b]	513·4±35·9	51·3±3·6	10·30±0·84
10	50	44·70±1·47	49·47±1·92[b]	1149·2±66·9[a]	61·1±3·5	19·46±1·69
12	60	48·81±2·12	50·77±1·59	1367·2±100·7[a]	66·1±6·2	22·10±2·44
14	70	45·00±1·03	55·54±1·39	1369·4±75·8	74·6±5·2	18·94±1·53
			Overall analysis of variance			
Degrees of freedom		1,115	1,89	1,87	1,87	1,87
F		4·65*	20·55**	8·13**	0·039	8·18**
Error mean squares		$3{\cdot}31 \times 10^7$	0·226	$3{\cdot}57 \times 10^{16}$	$2{\cdot}71 \times 10^{14}$	18·05

[A] Ratio of germ cells to Sertoli cells.

Table 3. Volume fractions of the seminiferous tubule, interstitial cells, and interstitial space in testes of intact and hemicastrated rats at different ages

b, significantly different ($P<0{\cdot}01$) from the corresponding value for intact rats. ** $P<0{\cdot}01$

No. of animals	Age (days)	Percentage mean (±s.e.m.) volume fraction Seminiferous tubule	Interstitial cells	Interstitial space
		Intact rats		
11	30	92·1±0·5	3·2±0·3	3·8±0·2
12	40	91·1±0·4	4·3±0·4	3·5±0·2
16	50	92·2±0·5	3·5±0·2	3·4±0·3
14	60	92·3±0·2	3·5±0·2	3·4±0·3
16	70	92·1±0·4	3·5±0·3	3·6±0·4
		Hemicastrated rats		
12	30	91·9±0·4	3·0±0·3	4·0±0·3
16	40	91·7±0·5	3·7±0·3	3·8±0·3
10	50	89·8±0·6[b]	3·8±0·2	5·8±0·5[b]
12	60	91·2±0·3	4·2±0·3	3·9±0·3
14	70	91·5±0·3	3·9±0·2	3·8±0·3
		Overall analysis of variance		
Degrees of freedom		1,85	1,85	1,85
F		7·93**	0·46	13·55**
Error mean squares		0·00017	0·00007	0·000005

that in intact animals of the corresponding age. The volume fractions of the seminiferous tubules and interstitial space were significantly greater for the intact animals (Table 3). However, the volume fraction of the interstitial cells was similar in both groups.

Discussion

Compensatory testicular hypertrophy develops when one testis is absent, maldescended, or damaged in any way provided that the contralateral testis is normal. In this study CTH of the contralateral testis diminished as the age of the hemicastrated animals approached 60 days. Testicular weight data (mg/100 g of body weight) showed that the degree of CTH occurring after hemicastration was inversely related to sexual maturation and this is consistent with the reports of Ojeda and Ramirez (1972), and Cunningham *et al.* (1978) who found that hypertrophy of the remaining testis did not occur in rats hemicastrated at 45 days of age or older.

The increase in the weight of the testis after unilateral castration was due in part to an increase in germ cell number. This agrees with the view of Santolaya and Burgos (1978) who concluded that the primary factor for the increase in testicular weight was an increase in the number of cells in the seminiferous tubules, and with the findings of Johnson (1978) that in adult and young puberal bulls hypertrophy could be attributed to an increase in activity of the seminiferous epithelium. Hochereau-de Reviers and Courot (1978) noted that hemicastration of impuberal rats resulted in an increase in Sertoli cell and stem spermatogonia numbers in the remaining testis; however, in prepubertal animals, hemicastration promoting hypertrophy of the contralateral testis led to an increase in stem spermatogonia but not in Sertoli cell numbers. In the toad, Thyagaraja and Sarkar (1970) also found that the increase in testicular weight following hemicastration was due to an increase in the numbers of sperm bundles and cell nests per seminiferous tubule.

Previous work reported an increase in the seminiferous tubule diameter of the contralateral testis after unilateral castration in the rat (Liang and Liang 1970), toad (Thyagaraja and Sarkar 1970), bull (Johnson 1978), and ram (Voglymayr and Mattner 1968; Johnson *et al.* 1971). The present results also found that the area of the seminiferous tubule of hemicastrated rats was significantly larger than that of intact animals. Moreover, the length of the seminiferous tubules per testis was significantly greater for the hemicastrated group (Table 2). This supports the findings of Hochereau-de Reviers *et al.* (1976) that in the ram the hypertrophy of the remaining testis following unilateral orchidectomy was caused by an increase in the total length of the seminiferous tubules with an associated increase in intertubular elements.

The results of volume fraction estimations of the testicular components showed that the hemicastrated testis contained more interstitial space, but there was no difference in the volume fraction of interstitial cells. This does not support the results of Liang and Liang (1970) who concluded that the increase in testicular weight after hemicastration was due in part to an increase in the interstitial cell number.

Normal testicular growth is regulated by the pituitary gonadotrophins (Davies 1981) and hemicastration has been shown to raise FSH levels preferentially (Ojeda and Ramirez 1972; Ramirez and Sawyer 1974; Moger 1977; Cunningham *et al.* 1978; Walton *et al.* 1980). Serum testosterone concentrations have been shown to be significantly reduced by hemicastration (Moger 1977) and the transient decrease in

blood steroid levels after removal of one gonad may reduce the negative feedback effects of the steroids and trigger an increase in pituitary gonadotrophin secretion which causes the remaining testis to increase in size and secretory activity until the gonadotrophin control system reaches a new steady state.

In conclusion, the present results show that hypertrophy occurring after hemicastration is restricted to immature rats and the effects are noted in the unilaterally castrated rats up to 50 days of age. The hypertrophy of the testis is related to an increase in the number of germ cells accommodated by each Sertoli cell. The histological preparations used did not permit changes in the surface area of Sertoli cells to be determined and the increase in occupancy could be related to either increased surface area of the Sertoli cells or a greater density of germ cells associated with the Sertoli cell surface.

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