A change in the frequency of the degenerating spermatogonia in the rat

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Summary. In right and left testes of the same animals, the frequencies of degeneration of type A spermatogonia were almost equal. When one testis was removed, there was a negative correlation of the frequency of the degenerating spermatogonia in this testis and the other after 25 days.

Introduction

The degeneration of germinal cells in the seminiferous epithelium is regularly seen in normal spermatogenesis in many mammalian species (for review, see Roosen-Runge, 1973). Roosen-Runge (1973) described this phenomenon as “the degree and the modes of the cell loss are species-specific”. In rats, this type of germinal-cell loss occurs at definite stages of the cycle of the seminiferous epithelium (Roosen-Runge, 1955; Clermont, 1962; Huckins, 1971). Type A spermatogonia degenerate during their mitotic stages (Clermont, 1962; Huckins, 1971), spermatocytes degenerate during their meiotic divisions (Roosen-Runge, 1955; Clermont, 1962), and spermatids do so during stage IX–XI of the cycle (Clermont, 1962). Spermatogonial degeneration is a specific feature in the rat and some quantitative data have been reported (Clermont, 1962; Ohwada & Tamate, 1975; Huckins 1978). At stage XII of the cycle, the average frequencies of the degenerating type A spermatogonia were 8.1% in Sherman rats (Clermont, 1962) and 7.7% in Holtzman rats (Ohwada & Tamate, 1975). In Charles River rats, the frequency of the type A₂ spermatogonia has been reported as 8.9% in whole mount tubules (Huckins, 1978). The value for Holtzman rats showed a rather large variance among individuals. In this paper we report the change in the frequency of the degenerating type A spermatogonia at stage XII of the cycle in Wistar rats.

Materials and Methods

The 24 Wistar rats used were 10 months of age. They were fed *ad libitum* on ground rat chow and tap water and were kept in 12 h light (06:00–18:00 h)/24 h. Pieces of testis were collected between 14:00 and 15:00 h and were fixed in Zenker–formalin fluid for 20–22 h. After embedding in paraffin wax, 5 µm sections were stained with the Periodic acid–Schiff–haematoxylin technique. The stages of the cycle of the seminiferous epithelium were identified according to the classification of Leblond & Clermont (1952) and Perey, Clermont & Leblond (1961). At stage XII of the cycle, the number of type A spermatogonial nuclei, normal and degenerating, were counted and used to calculate the frequencies of the degenerating cells.

In Exp. 1, both testes of 10 rats were removed and the percentages of the degenerating spermatogonia at stage XII of the cycle were calculated for each pair of testes.

In Exp. 2, one testis was removed from each of 14 rats under anaesthesia with pentobarbitone sodium. The remaining testis was removed at slaughter 25 days later.

Results

Experiment 1

As shown in Table 1, there was no difference in the average ($\pm$ s.d.) frequencies between right ($6.8 \pm 1.9\%$) and left ($7.3 \pm 2.1\%$) testes and the correlation coefficient was 0.7747 ($P < 0.01$). The regression equation was y (% degeneration in left testis) = $1.50 + 0.84x$, where x = % degeneration in right testis (Text-fig. 1a).

Table 1. Frequency of degenerating type A spermatogonia at stage XII in right and left testis of rats

Animal	Right testis			Left testis		
	No. of tubular cross-sections	Total no. of spermatogonia	No. of degenerating spermatogonia (%)	No. of tubular cross-sections	Total no. of spermatogonia	No. of degenerating spermatogonia (%)
1	88	351	24 (6.8)	105	472	35 (7.4)
2	118	503	41 (8.2)	144	719	79 (11.0)
3	114	489	13 (2.7)	92	428	16 (3.7)
4	108	390	22 (5.6)	86	366	23 (6.3)
5	99	419	30 (7.2)	126	504	49 (9.7)
6	89	359	38 (10.6)	91	359	33 (9.2)
7	113	555	44 (7.9)	125	590	44 (7.5)
8	102	557	35 (6.3)	87	391	27 (6.9)
9	97	451	28 (6.2)	102	462	25 (5.4)
10	100	501	34 (6.8)	108	454	26 (5.7)

Text-fig. 1. Correlation of the frequency of degenerating spermatogonia at stage XII between (a) right and left testes of the same rats and (b) 'removed' and 'remaining' testes 25 days after hemicastration in the same rats.

Experiment 2

As shown in Table 2, the testis remaining for 25 days after unilateral castration did not show any 'compensatory hypertrophy' with respect to the frequencies of the degenerating spermatogonia (7.3 ± 2.1 (s.d.)% in the 'removed' and $8.3 \pm 1.9\%$ in the 'remaining' testis). The regression equation for the values in the testes on each side was $y = 12.18 - 0.52x$, and the negative correlation (-0.5563) was significant ($P < 0.05$) (Text-fig. 1b).

Discussion

In the rat testis, type A spermatogonia degenerate mostly at stage XII of the cycle (Clermont, 1962; Huckins, 1971) and the frequencies were variable among individuals, ranging from 2 to

Table 2. Frequency of degenerating type A spermatogonia at stage XII in one testis ('removed') and that 'remaining' for 25 days

Animal	'Removed' testis			'Remaining' testis		
	No. of tubular cross-sections	Total no. of spermatogonia	No. of degenerating spermatogonia (%)	No. of tubular cross-sections	Total no. of spermatogonia	No. of degenerating spermatogonia (%)
1	125	463	40 (8.6)	125	603	58 (9.6)
2	109	407	38 (9.3)	92	504	33 (6.5)
3	98	395	22 (5.6)	108	447	56 (12.5)
4	125	532	36 (6.7)	141	592	50 (8.4)
5	109	402	37 (9.2)	100	483	25 (5.2)
6	103	465	42 (9.0)	113	449	31 (6.9)
7	129	551	43 (7.8)	113	535	42 (7.9)
8	122	528	20 (3.8)	113	540	42 (7.8)
9	116	552	38 (6.9)	97	504	40 (7.9)
10	102	416	37 (8.9)	134	602	55 (9.1)
11	106	497	13 (2.6)	109	452	51 (11.3)
12	112	497	35 (7.0)	125	500	46 (9.2)
13	151	563	41 (7.3)	97	601	51 (8.5)
14	120	701	70 (10.0)	96	459	30 (6.5)

14% (Clermont, 1962; Ohwada & Tamate, 1975). The data of Exp. 1 showed that, in the right and left testes of the same animals, the frequencies of the degenerating cells were almost equal. In Exp. 2, although the values varied widely amongst individuals, their range was again between 2.6 and 12.5% and there was no difference in average values between the 'removed' and the 'remaining' testes. However, there was a negative correlation between these two values in the same animals as shown in Text-fig. 1(b).

There was no testicular hypertrophy after unilateral castration in mature rabbits (Edwards, 1940) and rats 45 days of age or older (Vreeburg, van Andel, Kort & Westbroek, 1974; Cunningham, Tindal, Huckins & Means, 1978). In the present study, no hypertrophy was observed by 25 days after surgery and the changes observed in Exp. 2 can be considered not to be an effect of the unilateral castration. The negative correlation observed in Exp. 2 is therefore important and shows that changes are due to some other factor(s) and may be periodic. We suggest that the frequency of the degenerating spermatogonia at stage XII of the cycle in the rat is not a fixed value in each animal, but fluctuates within a certain limit and could be periodic. The apparent individual variance in the frequencies observed may be the result of this periodic change.

We are puzzled to find this unexpected time-dependent change of the frequencies of the degenerating spermatogonia in the rat. Potten, Al-Barwari, Hume & Searle (1977) reported that approximately 10% of normal mitoses are not successful and result in cell death by apoptosis in the ileal crypt cell population in the mouse, and that the peak of apoptosis appears 1-3 h after the peak of mitotic figures in the circadian rhythm. Tamate & Fell (1977) have drawn attention to the significance of the apoptosis in the control of cell kinetics of the ruminal epithelium in the sheep. The only possible explanation for our finding is that the degeneration of type A spermatogonia in the rat is a phenomenon based on a certain time-dependent schedule which also influences a similar schedule of proliferation rate in these cells through some unknown kinetic factors.

To confirm a periodicity in the frequency of degenerating spermatogonia, we are now examining rats killed 8 and 12 weeks after the unilateral castration.

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