QUANTITATIVE HISTOLOGICAL STUDIES ON THE INJURY OF SPERMATOGENESIS INDUCED BY ENDOTOXIN IN RATS

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Summary. A characteristic, reversible injury to spermatogenesis was induced by the intraperitoneal injection of *S. marcescens* endotoxin into rats. Quantitative histological studies of the testes revealed an inhibition of the maturation divisions of spermatocytes. The reversible character of the injury was due to the high degree of resistance of type A spermatogonia.

The significance of the clinical consequences of the presence of endotoxin is becoming increasingly apparent. The present paper gives an account of studies on the effect of endotoxin on testes and spermatogenesis.

Fifty-six, mature, male Wistar-strain rats were used. The average body weight of the animals was 150 g. Sublethal doses of *S. marcescens* endotoxin (0.1 ml/100 g body weight) were injected intraperitoneally (i.p.). Control animals were injected i.p. with the same volumes of physiological salt solution. Rectal temperatures were taken every 30 min during the first 12 hr following treatment. The animals were weighed and then killed by decapitation on the 1st, 4th, 9th, 16th, 25th, 36th, 49th and 64th day after treatment. Testes were weighed separately, fixed in 4% formol solution for 48 hr, embedded in paraffin and 6- μ sections were stained with haematoxylin and eosin.

Changes in spermatogenesis were detected by the method of quantitative analysis described by Roosen-Runge & Giesel (1950). Nuclear volumes of spermatogonia and of spermatocytes were determined by karyometry in each phase of spermatogenesis using a magnification of $\times 3000$ (Palkovits, Czeizel & Palkovich, 1966). In the majority of cases, the spermatocytes were identical to primary spermatocytes. Two diameters were measured for 150 nuclei, the longest and that which bisected it at right angles. Nuclear volumes were calculated in μ^3 with the help of the parameters described by Inke & Fischer (1961).

The number of spermatids was also determined. Only completely regular tubules with circular cross-sections removed from animals killed on the 4th day were studied in these experiments. Mean values were calculated from the measurements of ten tubules.

Seven rats died during the first 24 hr after the injection of endotoxin. No

further loss occurred later. A definite decrease in the average rectal temperature occurred after endotoxin treatment (Text-fig. 1). The average temperature reached its minimum at the 2nd hour with extremes of 35.2 and 36.8° C which may be compared to the values for the control animals of 37.2 and 39° C.

No significant differences in the body weight were found but a significant 21.5% decrease of testis weight occurred as early as the 1st day following treatment (Table 1). The lowest testicular weight was found on the 4th day, when a decrease of approximately 30% occurred.

TEXT-FIG. 1. Changes of body temperature in control (○) and endotoxin (●) treated animals.

TABLE 1

CHANGES OF TESTIS WEIGHT IN CONTROL AND TREATED ANIMALS DURING THE EXPERIMENTS

Days after treatment	Control		Treated with endotoxin		Changes in average testis weight (treated/ % control)
	Weight of left testis (mg) $\pm$ S.D.	Weight of left testis/100 g body weight	Weight of left testis (mg) $\pm$ S.D.	Weight of left testis/100 g body weight	
1	874 $\pm$ 12.5	559	558 $\pm$ 137.5	450	78.5
4	980 $\pm$ 16.2	605	521 $\pm$ 28.3	428	70.7
9	912 $\pm$ 62.0	533	737 $\pm$ 42.5	461	86.5
16	1162 $\pm$ 56.5	567	883 $\pm$ 197	488	86.2
25	1261 $\pm$ 117.0	521	1146 $\pm$ 132	516	99.0
36	1262 $\pm$ 84.8	595	1371 $\pm$ 56.4	571	96.1
49	1294 $\pm$ 90.5	539	1246 $\pm$ 63.2	504	93.3
64	1426 $\pm$ 31.1	520	1415 $\pm$ 93.3	502	96.5

Histological preparations showed no indication of the presence of any actual qualitative injury.

Mean values obtained by using the method of Roosen-Runge & Giesel (1950) are shown in Table 2. An increase in phases 4 and 5 of spermatogenesis and a decrease in phases 6 and 8 were characteristic of the 1st day after treatment. An essentially similar pattern was found on the 4th day. A significant increase in phase 5 was obvious, but to a lesser extent, on the 9th, 16th and also on the 25th day. Deviations in the patterns of treated and control animals were not found on the 36th day, or later.

Although karyometry showed no significant alterations in the spermatogonia, it did reveal characteristic changes in the spermatocytes (Table 2). The increase in the nuclear volume and the succeeding division of the spermatocytes of the control animals took place in phase 3. The divisions of the spermatocytes were displaced by phase 5 on the 1st, 4th and 9th day after the injection of endotoxin and the preceding increase in the nuclear volume of the spermatocytes was even more significant. Divisions took place in phase 3 again on the 16th, 25th and 36th day, though the increase in the nuclear volume of the spermatocytes was still greater in the treated than in the control group. Karyometric values found on the 49th day did not differ from those of the control animals.

The most obvious change in the number of spermatids was found in phase 6.

TABLE 2

CHANGES OF THE INCIDENCE OF ROOSEN-RUNGE PHASES AND OF THE NUCLEAR VOLUME OF PRIMARY SPERMATOCYTES DURING THE EXPERIMENTS

R-G stage	Control group	Treated groups							
		I	II	III	IV	V	VI	VII	VIII
I %	4	1.6	7.6	4	6	7	6.5	6.5	5
μ^3	63.7	45.1	51.3	80.5	103.0	14.0	40.0	61.3	62.4
II %	3.9	6	8	2.5	3.3	4	4	6	6
μ^3	84.0	30.2	78.0	100	119	123	126	87.4	86.2
III %	12.9	10.3	8.6	6	5	5.6	6.5	16.5	18.5
μ^3	103.4	64.4	118.0	56.3	134	148	167	124.3	108.3
IV %	9.9	17.3	12.3	5.5	3.3	5.6	8	7	8
μ^3	37.3	77.3	135	105	99.1	119	82.6	45.7	40.1
V %	12	17	15.3	21	22.6	19.3	12.5	8.5	10.5
μ^3	41.3	115.5	140	124	87.5	131	45.0	49.4	43.6
VI %	26.2	16.3	19.6	25.5	25.3	28	25.5	16.5	23
μ^3	45.7	24.7	102.8	108	28.3	32.5	77.7	47.6	46.1
VII %	12.5	13.6	14.3	13.5	6.3	9.3	10.5	12.5	10
μ^3	46.8	60.0	48.9	121	123.5	28.8	56.9	47.9	47.4
VIII %	21.0	8.6	12.3	22	28.6	19.3	26.5	26	19
μ^3	57.2	76.1	107.5	60.5	76.9	73.6	95.1	50.4	51.3

% = incidence of Roosen-Runge phases; μ^3 = nuclear volume of spermatocytes.

While control animals had an average of 111 spermatids/tubule in this phase, animals injected with endotoxin had only seventy-six spermatids/tubule, almost a 30% decrease.

The general effect of the injection of *S. marcescens* endotoxin was characterized by a 14% mortality caused by endotoxin shock and by hypothermia developing after treatment in rats.

The presence of endotoxin resulted in a quantitatively distinct and characteristic injury to the testes, reflected in the decrease in the weight of the testis of the treated animals. Quantitative histological studies produced a more exact definition of the rate of injury and contributed to a determination of the primary site of injury. Studies on the phases of spermatogenesis showed a definite increase in phase 5 following the less significant initial differences. Karyometry

showed a corresponding inhibition in the divisions of primary spermatocytes which was represented partly by an obvious increase in nuclear volume and partly by a chronological shift in divisions. The inhibition of maturation divisions resulted in a decrease in the number of spermatids. A decrease in the number of more mature spermatogenic cells occurring as a result of the inhibition of divisions can thus account for the loss of testis weight.

Studies of the parameters involved showed that the changes induced by the endotoxin were of a reversible character since each parameter reached a normal level sooner or later, depending on the sensitivity of the test. Thus, testis weight incidence of the different phases of spermatogenesis and changes in nuclear volume needed 25, 36 and 49 days, respectively, to reach normal levels. Moreover, type A spermatogonia appeared to possess a high level of resistance to the effects of the endotoxin.

The pattern of the injury to spermatogenesis following the injection of endotoxin parallels that resulting from the presence of some other noxious agents. The maturation division of the primary spermatocyte can be considered as the primary site of injury by endotoxin, as in those injuries induced by X-ray irradiation (Oakberg, 1955), heat (Chowdhury & Steinberger, 1964) and the inhalation of cigarette smoke (Viczián, 1968).

REFERENCES

- CHOWDHURY, A. K. & STEINBERGER, E. (1964) A quantitative study of the effect of heat on germinal epithelium of rat testis. *Am. J. Anat.* **115**, 509.
- INKE, G. & FISCHER, J. (1961) Über methodische Fragen der Kernvariationsstatistik. *Z. mikrosk.-anat. Forsch.* **67**, 104.
- OAKBERG, E. F. (1955) Sensitivity and time of degeneration of spermatogenic cells irradiated in various stages of maturation in the mouse. *Radiat. Res.* **2**, 369.
- PALKOVITS, M., CZEIZEL, E. & PALKOVICH, I. (1966) A spermatogenesis karyometriai vizsgálat. (Karyometric analysis of spermatogenesis). *Kisérlet. Orvostud.* **18**, 586.
- ROOSEN-RUNGE, E. C. & GIESEL, I. O. (1950) Quantitative studies on spermatogenesis in the albino rat. *Am. J. Anat.* **87**, 1.
- VICZIÁN, M. (1968) The effect of cigarette smoke inhalation on spermatogenesis in rats. *Experientia*, **24**, 511.