EFFECT OF SCROTAL HEATING ON TESTICULAR ENZYMES AND SPERMATOGENESIS IN THE RAT

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Abstract

Immersion of the scrotal testis of the rat in water at 42°C for 30 min caused rapid damage to pachytene primary spermatocytes in stages 1, 2, 3, and occasionally 8 of the seminiferous epithelium. There was cytoplasmic chromophilia with eosin or Alcian blue and binding of the fluorescent dye 2-*p*-toluidinylnapthalene-6-sulphonate to the cell membranes. Rapid dissolution of damaged cells occurred and most had disappeared by 28 hr after heating. Early round spermatids also showed nuclear changes with vacuolation and the development of "signet ring" nuclei.

Quantitative estimates of germ cell damage showed that the pre-meiotic stages were most susceptible to heat and that the later stages showed mainly maturation depletion changes.

Acid phosphatase and leucine aminopeptidase showed early histochemical increases about the damaged pachytene primary spermatocytes and these persisted until the damaged cells had been removed. Changes in the distribution of lactate and succinate dehydrogenases were also seen after heating.

I. INTRODUCTION

The harmful effects of a rise in temperature of the scrotal testis on spermatogenesis were first shown by Moore (1922, 1924*a*, 1924*b*) and Fukui (1923) for several species. The direct application of heat to rat testes (Fukui 1923) also produced testicular degeneration and infertility.

Determination of the relative sensitivity of different cell types to heat has shown species variability. When ram testes were heated to 40°C for about $2\frac{1}{2}$ hr, the number of B-type spermatogonia in the prophase stage of mitosis increased within 12 hr, and a four- to fivefold accumulation occurred within 24–48 hr (Waites and Ortavant 1968). However, spermatogonia of the rat testis were not affected by exposure to 43°C for 15 min (Steinberger and Dixon 1959; Chowdhury and Steinberger 1964, 1970; Collins and Lacy 1969).

The pachytene primary spermatocyte of the ram (Waites and Ortavant 1968; Samisoni and Blackshaw 1971; Samisoni 1972), the pig (Mazzari *et al.* 1968), and the rat (Blackshaw and Hamilton 1970; Chowdhury and Steinberger 1970) have been shown to be damaged within 1–12 hr after heating the testis.

The spermatid has also been shown to be heat-sensitive (VanDemark and Free 1970) and Chowdhury and Steinberger (1970) have demonstrated the early formation of nuclear vacuoles in very young spermatids with eccentric location of the chromatin.

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Changes in enzyme distribution and activity after heat exposure have received little study. Turpeinen *et al.* (1962) observed late changes in succinate dehydrogenase, acid phosphatase, and alkaline phosphatase activity in rat testes heated to 44°C for 20 min, and Blackshaw and Hamilton (1970) have shown very early changes (2 hr post-heating) in the activity and distribution of acid phosphatase in the heated rat testis (42°C for 30 min). Using quantitative methods, Ewing and Schanbacher (1970) found that an early response to the rise in temperature of the cryptorchid rat testis was a decrease in phosphofructokinase activity.

This paper confirms and extends the previous observations of Blackshaw and Hamilton (1970) and quantifies some of the spermatogenic changes produced by heat exposure of the scrotal testis.

II. Materials and Methods

The animals in this study were adult male Wistar rats (180–300 g) with free access to commercial pellets and water. Animal room temperature was regulated to 22±2°C but without positive humidity control.

Rats were anaesthetized by intraperitoneal pentobarbital sodium (40 mg/kg) and placed in a small restraining cage. The hindquarters were immersed in a stirred water-bath sufficient to completely cover the scrotal area. The bath temperature was maintained at 33±0·2°C for the control animals and in a preliminary series of tests at 36, 39, and 42±0·2°C for experimental animals. A temperature of 42°C was selected for the main experiments and in each case the period of immersion was 30 min. All animals were killed by a blow on the head at 1, 2, or 4 hr (initial tests), or 4, 16, 28, 40, 52, or 64 hr (subsequent tests) after scrotal heating.

Testes were either fixed in Susa's fixative (Humason 1967) or rapidly frozen to microtome chucks with a hexane–dry ice mixture. The fixed tissues were embedded in paraffin, sectioned, (7 μm), and stained with haematoxylin and eosin or Feulgen's reagent and Alcian blue (Humason 1967). Frozen sections (10 μm) were stained for acid phosphatase, leucine aminopeptidase, lactate dehydrogenase, and succinate dehydrogenase (Pearse 1960) and mounted in Kaiser's glycerol jelly.

The fluorescent probe 2-*p*-toluidinylnaphthalene-6-sulphonate (TNS) was prepared as a 1·0 mM solution in phosphate-buffered saline (pH 7·4). Frozen sections (10 μm) were fixed in absolute alcohol (15 min), rinsed, and stained in TNS for 1 hr. Sections were either mounted in buffered saline or Kaiser's medium and examined for fluorescence. An HBO 200 lamp was used with exciting filters BG 12 and BG 38, and barrier filters No. 53 and 47, using transmitted illumination with a Zeiss Photomicroscope II.

Sections stained with haematoxylin and eosin were used for quantitative studies on histology. The relative frequencies of the eight stages of spermatogenesis (Roosen-Runge and Giesel 1950) were estimated by random counts of cross-sections of the seminiferous tubules. In those cases where damage to spermatogenesis prevented an immediate and positive identification of the stage type the tubular profile was assigned to an unclassified group. The results were expressed in two ways, either as the total percentage of profiles for each stage or as the percentage of normal profiles for each stage.

Evaluation of changes in the cellular populations of the seminiferous tubules was made by estimation of the volumetric proportions of the nuclei of the cellular elements within the tubules (Chalkley 1943), using the regular 42-point graticule described by Weibel *et al.* (1966); the theory and application of this method to quantitative histology has been discussed by Elias *et al.* (1971).

All quantitative results were examined by analysis of variance; proportions were transformed to angles [arcsin(percentage)$^{\frac{1}{2}}$] before analysis and the mean values reported are derived from the transformed variables (Snedecor and Cochran 1967) rather than the raw data. The frequency of stage 4 was low and it has not been included in tables of results. Proportions for stage frequencies were based on 100 profiles for each testis, but estimates of nuclear volumetric proportions, within the seminiferous tubules, were based on a total of 500 point counts per testis.

III. Results

(*a*) *Histochemistry*

(i) *Acid Phosphatase*

Acid phosphatase showed two distinct patterns of distribution in the normal rat testis (Fig. 1). In the pre-meiotic tubular profiles (stages 1–3) acid phosphatase was extensively distributed in the more peripheral parts of the tubules and tended to form a fine network of granules about the primary spermatocytes, particularly those in the pachytene stage of development. The cytoplasm of the Sertoli cells showed a more even distribution of finer granules.

In the post-meiotic tubules (stages 5–8) acid phosphatase was distributed in the central portions of the tubule, being associated particularly with the long spermatids embedded in the Sertoli cells. Peripheral enzyme activity was relatively slight in these stages.

The earliest changes in acid phosphatase after acute testicular heating were seen, within 2 hr, about the pachytene primary spermatocytes. The usually fine network of granules surrounding these cells was distorted and replaced by a coarse irregular granular net. Increased background staining over these cells was also evident. The intensity of the reaction was greatest at 4 hr and was maintained until at least 16 hr post-heating (Fig. 2). The dissolution of the damaged cells was essentially complete by 28–40 hr after heating and their acid phosphatase reaction became more dispersed at this time (Fig. 3).

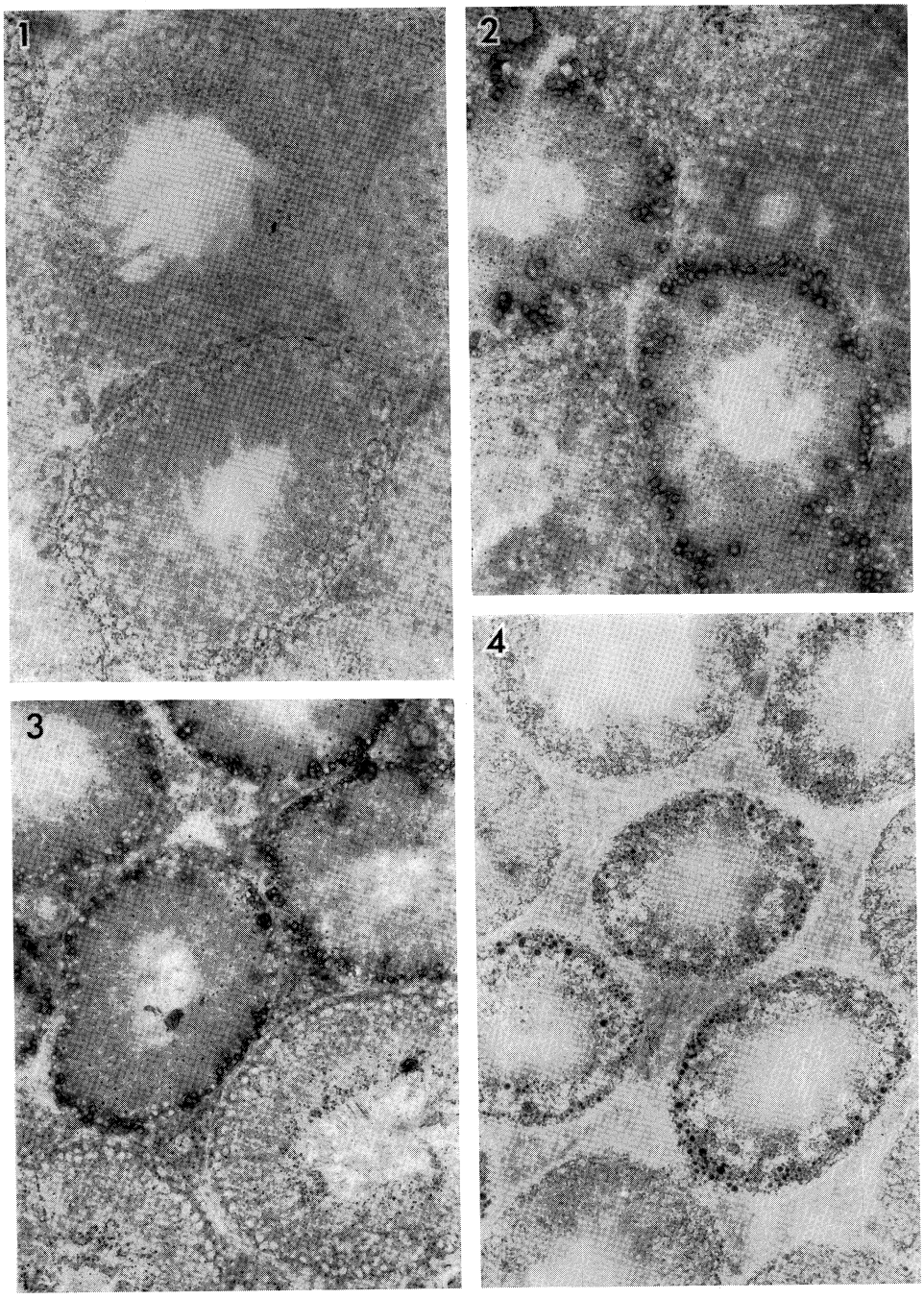
Variation between testes was pronounced and in some cases by 40 hr a strong, finely granular acid phosphatase reaction product spread over much of the profile of damaged tubules. This was maintained until at least 52 hr but had dispersed by 64 hr post-heating (Fig. 4). No significant changes in distribution or intensity of acid phosphatase staining occurred in post-meiotic tubules. Leucine aminopeptidase gave a similar distribution to acid phosphatase in both normal and heated tubules.

(ii) *Lactate Dehydrogenase*

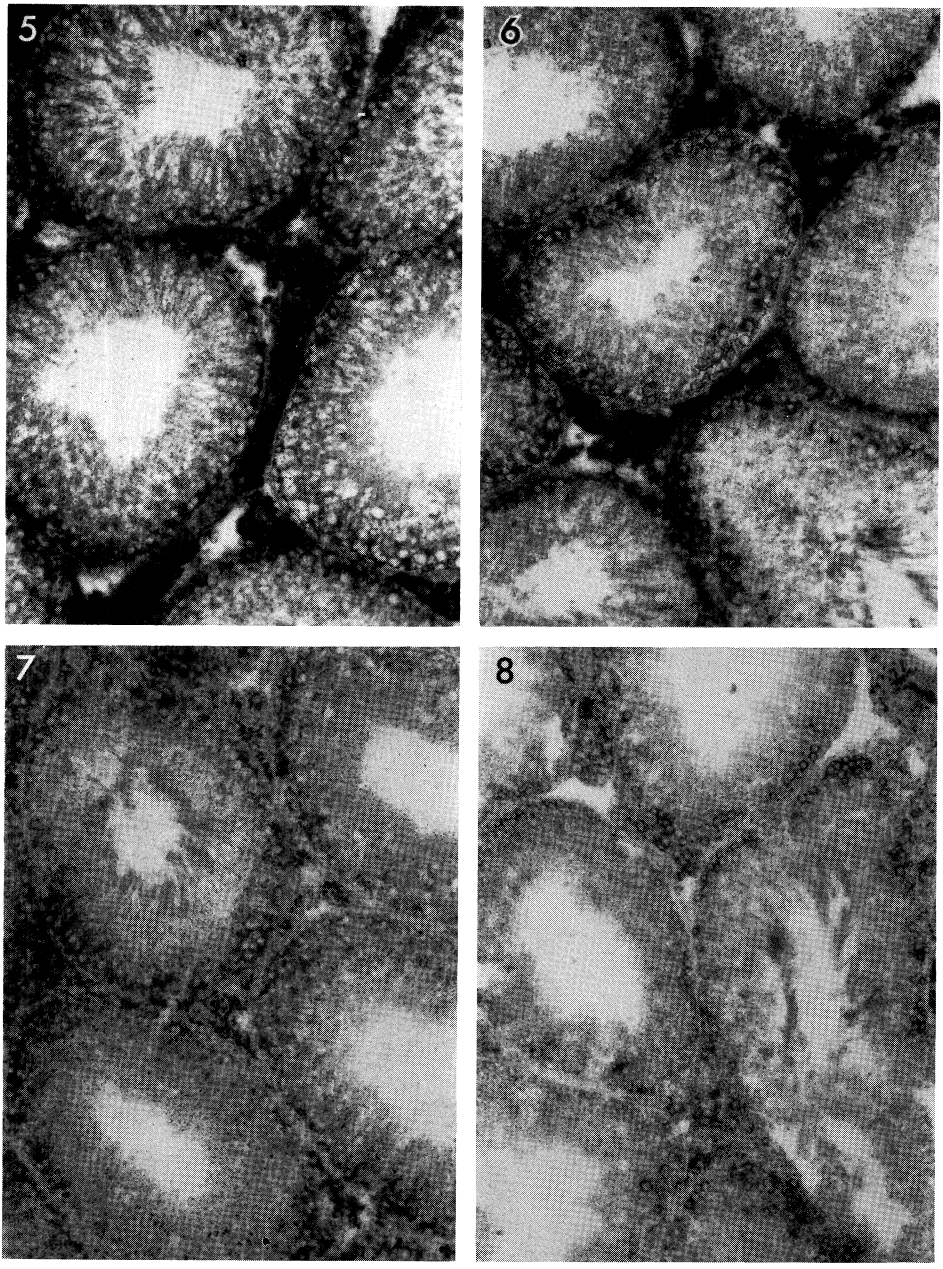
In the normal rat testis this enzyme was very active in the interstitial tissue with a moderate overall activity in the seminiferous tubules. Within the tubules, the pachytene spermatocytes were strongly active and there was good activity in the midpieces of the maturing spermatids (Fig. 5). After heating the testis the damaged spermatocytes were clearly outlined by formazan deposits within 4 hr (Fig. 6). As primary spermatocytes and round spermatids disappeared from the tubules the general staining reaction became more disordered, but maturing long spermatids stained normally.

(iii) *Succinate Dehydrogenase*

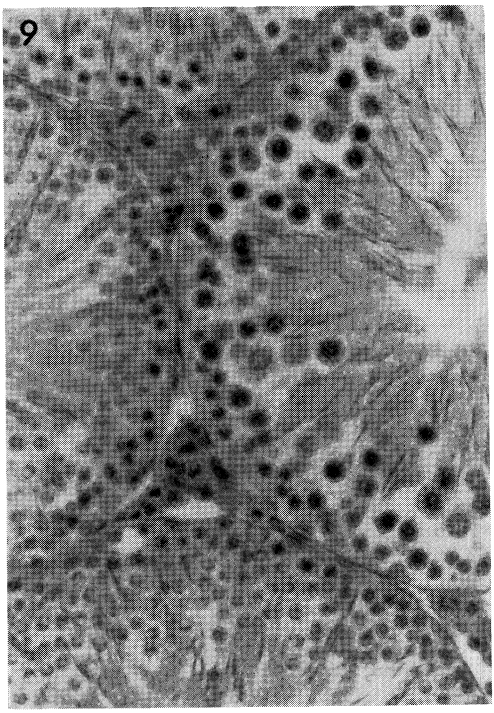
In the normal testis succinate dehydrogenase showed mainly tubular staining with strong activity in late spermatocytes and the midpieces of long spermatids. Activity about round spermatids was more diffuse and of lower intensity (Fig. 7). After heating, the primary spermatocyte reaction appeared more intense and persisted

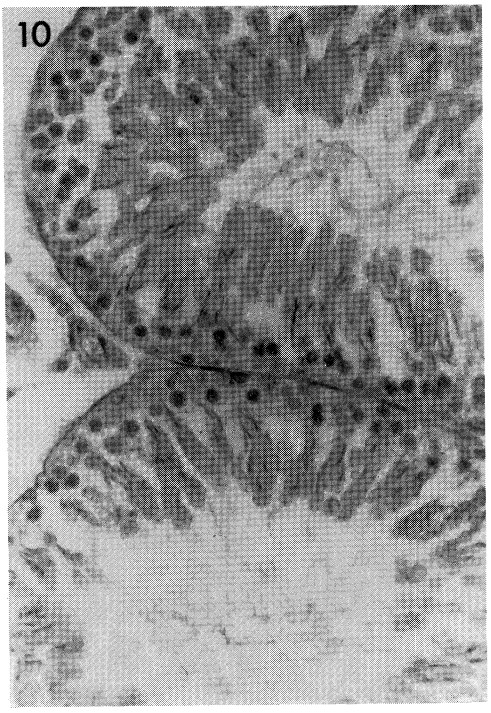


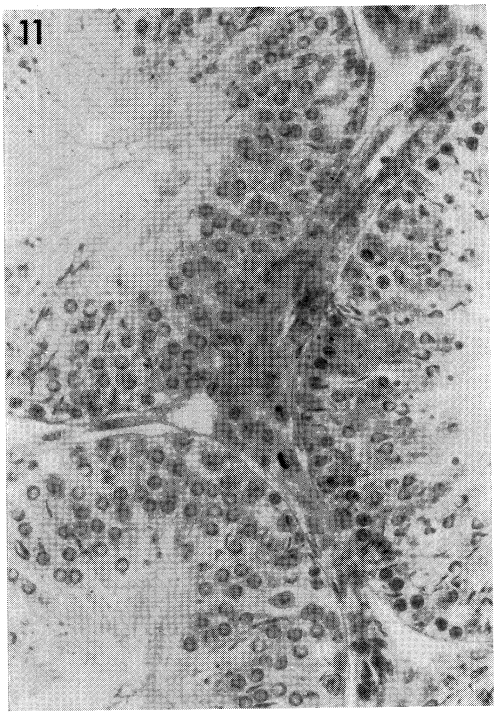
Figs. 1–4.—Histochemical changes in acid phosphatase activity (stained with α-naphthyl phosphate and fast blue BB) in the rat testis after heating to 42°C for 30 min. *1*, control testis (×104); *2*, 4 hr after heating (×90); *3*, 28 hr after heating (×90); *4*, 64 hr after heating (×90).

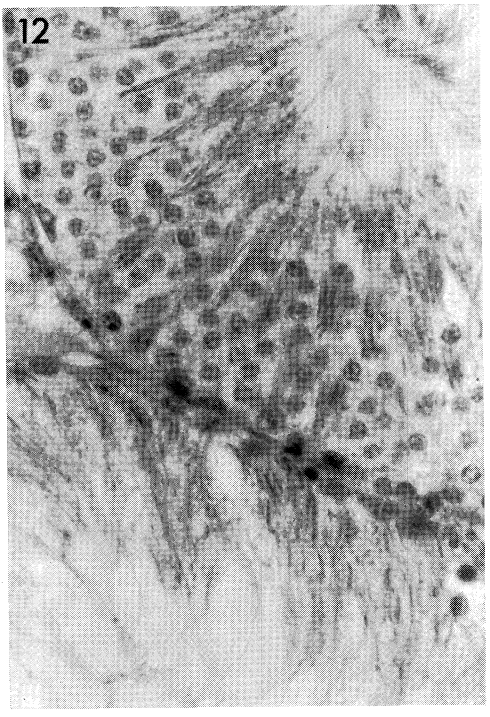


Figs. 5–8.—Histochemical changes in lactate and succinate dehydrogenase activities in the rat testis after heating to 42°C for 30 min (NBT reaction). All ×90. *5*, lactate dehydrogenase control; *6*, lactate dehydrogenase activity 4 hr after heating; *7*, succinate dehydrogenase control; *8*, succinate dehydrogenase activity 28 hr after heating.









Figs. 9–12.—Histological changes in the rat testis after heating to 42°C for 30 min (stained with haematoxylin and eosin). *9*, 4 hr after heating, showing eosinophilic pachytene primary spermato-

until after nuclear dissolution (28 hr; Fig. 8). At the end of the test period the reaction was patchy over most tubules but was maintained in the midpieces of long spermatids.

(*b*) *Qualitative Histology*

No histological changes were apparent in the tubules of testes heated to 36 or 39°C for 30 min but signs of seminiferous tubular damage were present within 1 hr of heating the rat testis to 42°C for 30 min. Up to 4 hr after heating, damage was most conspicuous in stages 1–4 of the cycle but there was extension of damage to stages 7 and 8 in some rats. The early cellular changes were restricted to the pachytene primary spermatocytes in these stages and consisted of a prominent chromophilia of the cytoplasm of the cells when counter-stained by eosin (Fig. 9) or Alcian blue. Nuclear degeneration of the pachytene spermatocytes was evident within 16 hr after heating and by 28 hr only cytoplasmic remnants of these cells remained (Fig. 10).

Frozen sections were also stained by the fluorescent protein probe TNS and examined for the characteristic fluorescence of the bound dye. In sections from testes removed 4 hr after heating, fluorescence was seen in cells identified by phase-contrast microscopy as pachytene primary spermatocytes. It was much reduced at 2 hr, and at 1 hr post-heating only occasional weakly fluorescing cells were visible. Fluorescence of spermatozoa also occurred but this was of lower intensity and could be removed by suitable filters.

At all periods from 4 to 6 hr after heating there were occasional early spermatocytes (leptotene and zygotene) showing cytoplasmic chromophilia in some testes (7 of 42). Nuclear vacuolation ("signet ring" nucleus) of early round spermatids in stage 5 was also present at all periods from 4 to 64 hr after heating. It was prominent in the testes of some rats but was noted in only 10 of 42 testes examined (Fig. 11).

Examination of the different cellular associations at the selected periods after heating showed that the progress of spermatogenesis in unshed cells was unaffected by the absence of many other germ cells in the epithelium. This was particularly noticeable in the later associations where almost mature spermatids were completing metamorphosis in the absence of all primary spermatocytes (Fig. 12).

(*c*) *Quantitative Histology*

The nuclear volumetric proportions of the elements of the germinal epithelium were estimated for the periods following exposure of the testes to heat. After testicular heating there was a highly significant linear decrease in the proportions of normal pachytene primary spermatocytes, but other cell types of the germ cell population were not affected over 1, 2, or 4 hr (Table 1). Sertoli cells showed an apparent increase in proportions but there was considerable variability in response.

Stage frequencies were compared, after angular transformation, and based on the total number of stages there were no significant treatment effects for any stage over 1, 2, or 4 hr post-heating. When the frequencies of normal stages were assessed

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cytes and "signet ring" round spermatids (×250); *10*, 28 hr after heating, showing remnants of pachytene primary spermatocytes (×250); *11*, 64 hr after heating, showing "signet ring" round spermatids and eccentric chromatin in elongating nuclei (×250); *12*, 64 hr after heating, showing long spermatids in the absence of primary spermatocytes (×320).

the pre-meiotic stages (2 and 3) showed significant decreases, but significant changes were absent in the post-meiotic tubules (stages 5–7) except for stage 8 (Table 2).

TABLE 1

EFFECT OF HEATING THE RAT TESTIS TO 42°C FOR 30 MIN ON THE NUCLEAR VOLUMETRIC PROPORTIONS OF THE CELLS OF THE GERMINAL EPITHELIUM MEASURED 1, 2, AND 4 HR AFTER HEATING

The mean nuclear volumetric proportions were calculated from mean angles. No. of animals in each group = 6

Cell types	Mean proportions (%) of control cells	Mean proportions (%) of cells at specified times after heating:			Summary of analyses of variance (angles)		
		1 hr	2 hr	4 hr	Error mean square (D.F. = 20)	Variance ratios: Linearity (D.F. = 1)	Deviations (D.F. = 2)
Spermatogonia	1·2	0·8	1·7	1·2	4·06	0·31	1·13
Spermatocytes							
Preleptotene, leptotene, zygotene	2·0	1·3	2·0	1·2	3·69	0·99	1·96
Early pachytene	3·2	2·7	3·1	2·8	3·40	0·13	0·23
Late pachytene							
Total	3·7	4·2	4·0	2·9	7·14	0·64	0·53
Normal	3·7	2·0	1·4	0·1	15·16	15·70**	0·00
Spermatids							
Round	10·1	8·4	10·3	8·4	3·62	0·73	2·31
Long	5·3	4·9	5·9	4·6	2·74	0·26	1·73
Sertoli cells	0·7	1·6	0·8	2·5	6·57	5·12*	5·73*

* $P < 0·05$. ** $P < 0·01$.

TABLE 2

EFFECT OF HEATING THE RAT TESTIS TO 42°C ON THE FREQUENCY OF NORMAL SPERMATOGENIC STAGES MEASURED 1, 2, AND 4 HR AFTER HEATING

Mean frequencies were calculated from mean angles. The number of animals in each group is given in parentheses

Treatment	Mean frequency (%) of spermatogenic stage:						
	1	2	3	5	6	7	8
Control (7)	3·8	4·7	18·2	5·1	36·5	13·7	15·4
Post-heating period (hr)							
1 (6)	3·8	1·2	7·5	4·0	40·7	15·8	8·2
2 (6)	1·6	0·6	1·1	3·3	19·5	13·1	8·0
4 (6)	0·8	0·2	0·5	2·4	36·8	14·0	4·8

Summary of analyses of variance (angles)

Source of variation	D.F.	Variance ratio for spermatogenic stage:						
		1	2	3	5	6	7	8
Effect of heating	1	1·84	15·84**	32·26**	0·80	0·73	0·06	15·15**
Response after heating								
1. Linearity	1	3·66	2·01	9·69	0·43	1·08	0·36	2·33
2. Deviations	1	0·09	0·00	1·43	0·01	0·06	0·50	0·61
Error mean square	21	32·36	20·59	43·03	46·60	14·62	17·20	20·34

** $P < 0·01$.

The results from the periods covering 4–64 hr after heating were analysed in the same way. It is clear (Table 3) that the pachytene primary spermatocytes were most vulnerable to heating and that quantitative changes in round spermatids mainly reflected cells shed from the epithelium.

TABLE 3

EFFECT OF HEATING THE RAT TESTIS TO 42°C FOR 30 MIN ON THE NUCLEAR VOLUMETRIC PROPORTIONS OF THE CELLS OF THE GERMINAL EPITHELIUM MEASURED AT VARIOUS TIMES AFTER HEATING

Mean nuclear volumetric proportions were calculated from mean angles. There were 10 animals in the control group and 7 in all other groups

Cell type	Mean proportions (%) of control cells	Mean proportions (%) of cells at specified times (hr) after heating:					
		4	16	28	40	52	64
I. Spermatogonia	0·9	1·0	1·0	1·1	0·7	1·2	1·0
II. Spermatocytes							
a. Preleptotene, leptotene, zygotene	1·9	1·2	1·1	1·4	1·7	2·0	1·3
b. Early pachytene							
(i) Total	3·1	2·6	2·5	2·7	2·2	1·2	3·0
(ii) Normal	3·1	1·9	1·4	2·5	1·7	0·8	2·6
c. Late pachytene							
(i) Total	3·5	2·3	1·0	0·8	0·5	0·4	1·1
(ii) Normal	3·5	0·3	0·2	0·7	0·4	0·3	1·0
III. Spermatids							
a. Round	9·6	7·9	9·0	7·6	7·4	6·9	5·7
b. Long	5·1	5·1	4·5	3·4	4·7	3·0	4·9
IV. Sertoli cells	1·1	2·4	2·4	1·9	2·5	2·4	1·9

Summary of analyses of variance (angles)

Source of variation	D.F.	Variance ratio for cell type:								
		I†	IIa	IIb(i)	IIb(ii)	IIc(i)	IIc(ii)	IIIa	IIIb	IV
Effect of heating	1	0·25	0·68	1·77	5·79*	14·53**	26·39**	6·18**	1·69	11·38**
Response after heating										
Linear	1	0·05	0·52	0·48	0·00	2·82	1·37	5·03*	0·74	0·40
Quadratic	1	0·07	0·25	0·79	0·47	4·09*	0·12	0·90	2·85	0·04
Deviations	3	0·49	0·28	1·73	2·82	0·39	0·63	0·32	1·80	0·53
Error mean square	45	6·65	10·94	9·62	9·24	14·94	14·43	6·99	6·07	5·00

* $P < 0·05$. ** $P < 0·01$. † Spermatogonia types A and B.

The overall frequencies of spermatogenic stages 3, 5, 6, and 7 showed significant decreases in the post-heating period (Table 4) coinciding with a big increase in the frequency of unclassified tubular profiles. When the frequencies of normal spermatogenic stages were analysed (Table 5) there were highly significant changes with time after heating. The frequency of normal stage 3 was very low at 4 hr but rose linearly over the remainder of the experimental period, whereas the frequencies of normal stages 8, 1, and 2 remained depressed over the 64-hr period. On the other hand, the frequencies of stages 5, 6, and 7 declined linearly over this time.

For comparative purposes the proportion of damaged tubular profiles was assessed using the mean values obtained from the histological sections and values

derived from similar counts made on frozen sections stained for acid phosphatase (Table 6). Correspondence between the two estimates was close.

TABLE 4

EFFECT OF HEATING THE RAT TESTIS TO 42°C FOR 30 MIN ON THE FREQUENCY OF ALL STAGES OF SPERMATOGENESIS MEASURED AT VARIOUS TIMES AFTER HEATING

Mean frequencies were calculated from mean angles. The number of animals in each group is given in parentheses

Treatments	Mean frequency (%) of spermatogenic stage:							
	1	2	3	5	6	7	8	Unclassified
Control (5)	4·1	3·7	20·5	15·2	26·6	7·4	19·6	0·0
Post-heating period (hr)								
4 (7)	2·5	4·0	21·3	9·7	35·9	15·0	6·4	0·0
16 (7)	4·6	4·0	23·6	12·5	29·1	14·0	8·6	0·0
28 (7)	3·9	3·1	15·4	6·2	18·4	14·3	11·5	19·0
40 (7)	3·7	5·2	10·3	4·4	15·7	8·4	15·3	29·6
52 (7)	2·8	2·6	6·1	2·6	13·1	5·5	11·0	47·6
64 (7)	2·8	4·5	8·2	2·5	9·9	5·0	9·6	49·7

Summary of analyses of variance (angles)

Source of variation	D.F.	Variance ratio for spermatogenic stage:							
		1	2	3	5	6	7	8	Unclassified
Effect of heating	1	0·34	0·03	2·18	8·63**	2·33	0·80	4·70*	16·14**
Response after heating									
1. Linearity	1	0·19	0·06	18·32**	15·76**	38·85**	17·41**	1·33	78·58**
2. Deviations	4	0·77	1·39	0·84	0·68	0·63	0·53	0·96	1·89
Error mean square	40	17·25	12·86	58·08	42·05	42·51	36·69	55·06	166·84

* $P < 0·05$. ** $P < 0·01$.

IV. DISCUSSION

It has been clear for some time that the most sensitive cells in the testis to acute heating above normal body core temperature are the pachytene primary spermatocytes. The response of these cells to heat is rapid, but only recent observations have shown that there is histological damage within 1–12 hr after heating (Waites and Ortavant 1968; Blackshaw and Hamilton 1970; Chowdhury and Steinberger 1970; Samisoni and Blackshaw 1971). Chowdhury and Steinberger (1970) have demonstrated a nuclear basophilia and enhanced PAS staining of the cytoplasm and in our hands there is a prominent cytoplasmic chromophilia to eosin or Alcian blue (Blackshaw and Hamilton 1970; Samisoni and Blackshaw 1971). Eosin is typical of the fluorescein dyes which are aromatic sulphonic acids and bind to hydrophobic sites of proteins (Dodd and Radda 1967; Flanagan and Ainsworth 1968). These changes are indicative of membrane and cytoplasmic damage and the former is confirmed by the use of the fluorescent probe TNS, which is practically non-fluorescent in water but fluoresces strongly when bound to protein (Weber and Laurence 1954). Edelman and Millette (1971) have also demonstrated that TNS binds strongly to the entire membrane of spermatozoa, the linkage being hydrophobic, to give a green fluorescence. Apart from late spermatids, fluorescence in normal tubules was

TABLE 5

EFFECT OF HEATING THE RAT TESTIS TO 42°C FOR 30 MIN ON THE FREQUENCY OF NORMAL STAGES OF SPERMATOGENESIS

Mean proportions were calculated from mean angles. The number of animals in each group is given in parentheses

Treatments	Percentage of normal spermatogenic stages:						
	1	2	3	5	6	7	8
Control (5)	4·1	3·7	20·5	15·2	26·6	7·4	19·6
Post-heating period (hr)							
4 (7)	0·8	0·7	0·1	4·8	33·6	11·5	4·2
16 (7)	0·3	0·2	1·3	6·4	26·0	5·2	1·4
28 (7)	0·8	1·0	3·3	2·1	12·6	5·1	3·7
40 (7)	0·4	1·2	4·3	2·8	4·7	3·8	7·7
52 (7)	0·1	0·8	3·4	0·7	1·3	2·5	3·5
64 (7)	0·3	1·4	6·5	0·6	0·2	1·5	3·8

Summary of analyses of variance (angles)

Source of variation	D.F.	Variance ratio for spermatogenic stage:						
		1	2	3	5	6	7	8
Effect of heating	1	13·03**	4·99**	16·45**	27·17**	17·37**	1·47	12·95**
Response after heating								
1. Linearity	1	0·90	1·01	7·68**	14·82**	139·25**	15·72**	0·26
2. Deviations	4	0·40	0·40	0·36	0·89	0·63	0·46	0·93
Error mean square	40	21·92	29·05	83·66	31·58	42·83	38·55	76·75

** $P < 0·01$.

TABLE 6

EFFECT OF HEATING THE RAT TESTIS TO 42°C FOR 30 MIN ON THE FREQUENCY OF NORMAL PRE-MEIOTIC (INCLUDING STAGE 4) AND POST-MEIOTIC STAGES OF SPERMATOGENESIS AND OF NORMAL STAINING FOR ACID PHOSPHATASE

Treatment of sections	Control tubular frequency (%)	Tubular frequency (%) at specified times (hr) after heating:		
		1	2	4
Haematoxylin and eosin staining				
Pre-meiotic stages	28·0	16·6	6·9	4·1
Post-meiotic stages	72·0	71·2	66·1	61·4
Total (all stages)	100·0	87·8	73·0	65·5
Stained for acid phosphatase (all stages)	100·0	89·0	71·0	67·0

absent, but damaged cells showed a pale fluorescence indicating binding of TNS which may be a sign of membrane damage.

The altered dye affinities of damaged cells were closely associated with changes in acid phosphatase and leucine aminopeptidase reactions. Lysosomal activation

and secondary lysosome formation was prominent around damaged primary spermatocytes and this reaction pattern persisted until dissolution of the injured cells had occurred.

Previous work (Blackshaw and Hamilton 1970) has shown that acute heat damage to the rat testis increases the *in vitro* fragility of testicular lysosomes and Samisoni (1972) confirmed this in the ram testis. Chapman (1967) has suggested that cellular damage after heating may be related to changes in the phospholipid components of cell membranes and Laufer and Davies (1969) proposed that injury to cell membranes leads to major ionic imbalance, mitochondrial damage, and then activation of lysosomes. Selective heat damage to the late pachytene spermatocytes indicates that the cytoplasmic membranes, at this critical stage of meiotic prophase, are unstable.

Metabolic and mitochondrial damage are also produced by fluoroacetamide (FA); the pachytene spermatocytes show similar lesions to heat damage (Novi 1968) and it is possible that oxidative metabolism in the Krebs cycle may be of major importance in the events leading to meiosis. Early round spermatids also show susceptibility to heat (Chowdhury and Steinberger 1970) and Novi (1968) has described late nuclear vacuolation of early round spermatids after FA treatment which is morphologically similar to that described after acute heat damage (Chowdhury and Steinberger 1970). The latter described very early nuclear changes leading to vacuolation whereas we have found a more delayed response, sometimes as late as 64 hr post-heat. These differences may be related to the temperature of heating which was 43°C (Chowdhury and Steinberger 1970) or 42°C in our experiments. The delayed response following treatment with FA (Novi 1968) and heating at 42°C suggests that metabolic changes in either the spermatids themselves or in the supporting Sertoli cells may be responsible for the nuclear abnormalities. Epididymal sperm in the caput region, examined 4 days after heating, showed marked nuclear distortion and it was evident that the control of metamorphosis of spermatids was severely upset (Elkington, unpublished data).

Detailed metabolic and enzymatic studies of individual cell types in the testis are now necessary to determine more closely the changes produced by heat and other injurious agents.

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