Comparative Effects of Elevated Temperature and 1000 r X-rays on the Seminal Epithelium of the Mouse¹

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Introductions

In two previous studies (1960a and b) we have examined the effect of localized acute X-irradiation on the mouse testis. From this work and a perusal of the literature, the mode by which various noxious agents act specifically on the process of spermatogenesis appeared insufficiently clear. It is well known that the degeneration of the seminal epithelium in the undescended testicle differs from that produced by acute irradiation. This paper represents a qualitative description of preliminary observations of the effect of elevated temperature on mouse spermatogenesis.

The literature on elevated temperature and its histological and cytological effect on mammalian spermatogenesis is sparse. Hardberger in 1950 used a temperature of 32.2°C, which is three degrees lower than that used in the present study, but he was mainly concerned with the effects of elevated temperature on fertility and on gross histological aspects. Stieve (1930) in his monumental textbook mentions no experimental work with elevated temperature. The study of Ehrenberg *et al.* in 1957 was concerned with the genetic effects of elevated scrotal temperatures in man. In the clothed human male scrotal temperatures are raised to 34°C as compared with 30.7°C in the nude male. The genetic effect is said to have a Q_{10} of 6.5 for increased mutation rate. Cytological detail is not given in this paper. Temperature effects on mitosis proper are fairly well documented and described.

Danielli (1950) considers mitosis as consisting of at least eight main steps. It is a fair assumption that changes in temperature have differential effects on most of the eight processes which are normally in delicate balance. In addition to such intracellular changes one is, in the testis, dealing with severe changes of intercellular arrangement which in the case of the seminiferous tissue are more marked after heat treatment than after X-irradiation. There is a large body of literature on injury to the testes, of which we shall mention only two examples. Mason (1933) has studied the effects of Vitamin A and E deficiencies and inanition on testicular injury and repair. Maximow (1899) investigated the effects of mechanical injury on the testes of several

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mammals and amphibia, noting the formation of spermatid and spermatocyte giant cells. Extensive literature reviews may be found in these papers.

Materials and methods

Four C57 black male mice approximately 100 days of age were subjected to 35°C and 70% humidity for a total of 61 hours according to the following schedule :

Day	Hours of treatment
1	7
2	8
3	24
4	22
Total	61

The mice when not at the elevated temperature were returned to the normal temperature and humidity of the animal quarters (22°–24°C and 40%–50% humidity) and were sacrificed individually on days 7, 9, 11, and 14 (3, 5, 7, and 10 days after the end of the heat treatment). The testes were fixed in a mixture of glacial acetic acid and alcohol (1:3), embedded in paraffin, sectioned at 7 μ and stained with Feulgen and fast green. All the tubules cut obliquely or at right angles in one testis cross section of each mouse were observed (an average of approximately 135 tubules per mouse); counts were made to determine the percentage of tubules with giant cells (large multinucleated cells), of "empty" tubules, of tubules containing no spermatogonia, of tubules with "blocked M_I" divisions (or necrobiotic first meiotic metaphases), and of tubules in "recovery" (see below). These counts were compared with control material and with tubular cross sections from mice sacrificed 10 days after acute localized irradiation with 1000 r X-rays. Next, cell categories seen 10 days after heat treatment were compared with those seen 10 days after X-irradiation and with control material.

Observations

The results of the survey of tubular cross sections observed after heat treatment and irradiation are presented in Tables 1 and 2.

Giant cells. These do not occur in normal testicular tissue and are likewise absent in the tubular cross sections from testes of irradiated mice. They are very common and striking in heat-treated material. The term giant cell is used to describe any agglomeration of multiple nuclei within an apparently common cytoplasm. Thus they may result from repeated karyokinesis without cytokinesis or from a regrouping of cells that lose their individual cell surfaces and appear to be enclosed in a new and common sphere. Where such new structures involve groups of spermatozoa it is strongly suspected that a number of spermatozoal heads with imperfect tails are

clumped together in some of the cytoplasm which was originally part of the Sertoli syncytium. In no case however were Sertoli nuclei observed to be included in these "spermatozoal giant cells".¹ Giant cell spermatids may have arisen by agglomeration, by incomplete C-meiosis or by a combination of both processes. In giant cell spermatocytes agglomeration is suggested as the more common mode of origin. No single giant cell was observed to include more than one nuclear category. With reference to the control testes it may be stated that we have never seen any giant cells in a total of 30 control mice of the C57 black strain aged about 100 days. In contrast to this the LAF₁ white male mouse "normally" forms many giant cells containing spermatid nuclei, hence it cannot be used for experimental work of the present type.

The percentage of tubules containing giant cells (Table 1) showed great variation from animal to animal and no quantitative statement can be made

Table 1. Changes seen in tubular cross sections of mouse testes after heat treatment and X-irradiation

Days after treatment	Percentages of tubules				
	with giant cells	"empty"	without spermatogonia	with "blocked M ₁ "	in recovery
Heat treatment					
3	8.5%	10.0%	6.2%	10.0%	0
5	25.2%	13.0%	2.4%	6.5%	10.6%
7	7.8%	15.1%	1.8%	1.2%	11.4%
10	4.8%	11.1%	6.3%	0	19.8%
Control	0	.6%	2.6%	0	0
1000 r X-irradiation					
10	0	.5%	99%	76%*	0

* This figure includes visibly blocked M₁ and restituted spermatocytes; some of these may have started regression before ever coming up fully to M₁. Most of them appear to be "floating", headed for elimination; at 10 days only a few have taken up positions beside the Sertoli cells on the basement membrane.

concerning a trend, but it appeared that during the 10-day period of observation the giant cells were slowly being eliminated. The most common type of giant cell was that composed of spermatozoal heads with indistinct tails mentioned above. Giant cell spermatids and giant cell spermatocytes together were approximately equivalent in number to the first type.

¹ According to Leblond and Clermont (1952), the germinal cells after two reduction divisions should be called spermatids until they are released from the testicular tubules; after this they should be termed spermatozoa. However, we prefer the classical nomenclature whereby these "late spermatids" become young spermatozoa as soon as a tail is well developed and the head becomes opaque with basophilic dyes.

Empty tubules. These are defined as containing only one layer of cells along the basement membrane. Usually this layer consisted of Sertoli cells and spermatogonia though at times it also contained some abnormal spermatocytes. Spermatogonia are very rare in X-irradiated testes. The percentage of empty tubules showed no marked decline with time (Table 1) and thus even at 10 days after heat treatment it was markedly higher than in control and X-irradiated testes. However it seems likely that over a longer time period, e.g. 6 weeks, 1000 r of irradiation would result in more permanently "empty" tubular cross sections than did the present heat treatment. In the normal C57 black male mouse, occasional empty tubular cross sections are seen. Stieve (1930) states that empty tubular cross sections in man increase with age, with great individual variability, and also as a consequence of systematic debilitating diseases.

Absence of spermatogonia. This feature, observed in 2.6 % of the tubular cross sections in control tissue on the basis of quantitative determinations (Table 1), does not imply a true absence, since spermatogonia could always be found in the same tubule in an adjacent section. The percentage of tubular cross sections "without spermatogonia" in heat-treated material varied but remained relatively low as compared to the effect of X-irradiation. Heat in contrast to 1000 r X-rays did not cause extensive death of spermatogonia.

Blocked M_1 . In heat-treated testes, there is a significant decrease with time of the percentage of tubules showing "blocked M_1 " (Table 1). "Blocked M_1 " in heat-treated material refers to apparently necrobiotic cells arrested at or very near to the first meiotic reduction division (c.f. Nebel and Murphy 1960a). We believe that heat may occasionally induce a true restitution division or C-division in place of the first reduction division. By this process tetraploid nuclei would arise. This process is, however, rarer after heat treatment than after X-irradiation. Heat may also cause a regression of meiosis, e.g. following diakinesis, leading to large nuclei with diffuse chromatin indistinguishable from true restitution nuclei (c.f. the cells marked $\times$ in Fig. 1). This regression of late spermatocytes is far more common, and the products of the regression are far more persistent, after X-irradiation. At 10 days after treatment, the effects of heat on M_1 differ greatly from those of 1000 r X-irradiation. After heat treatment, no blocked M_1 metaphases or restitution cells are seen. After X-irradiation 76 per cent of all tubules show regressed, restituted or blocked meiotic spermatocytes (Table 2).

Recovery. With time after heat treatment, an increasing percentage of tubular cross sections show "recovery" (Table 1); the term here means a heavy and continuous ring of dividing spermatogenic cells, either spermatogonia or young spermatocytes, with no other cells of older generations present. Here the contrast between heat and X-ray treatments is especially marked at 10 days. After heat treatment, recovery is in full swing after

Table 2. Comparison of contents of tubular cross sections of mouse testis in terms of cell categories, observed at 10 days after heat treatment and at 10 days after 1000 r of acute localized X-irradiation, expressed as percent of tubules

Treatment	Cell categories								Sertoli
	sperma- togonia	spermatocytes					sperma- tids	sperma- tozoa	
		early	med.	late	resti- tution	regress- ed			
Heat, 35°	100 ¹	38	23	4	0	0	0	11	100
Irradiation 1000 r	<1 ²	0	0	34	66 ³	17	0	100	100
Control	97	55	42	33	0	0	87	95	100

¹ Not only did all tubules contain spermatogonia, but within each tubule these were very plentiful.

² Less than 1% of the tubular cross sections contained any spermatogonia and those found were always single.

³ The number of cells of a given category within the tubule was generally fairly high when the tubular percentage was large. Where it was small relatively few cells of this category were usually present.

10 days; after 1000 r X-irradiation it has not yet begun (c.f. Nebel and Murphy 1960a). Extrapolating beyond the present observations, the heat treatment applied here may yield a final increment of 10 % of empty tubular cross sections, while 1000 r X-irradiation yields nearly 50 % empty tubules after 6 weeks and later. All the other tubular cross sections seem to recover completely or nearly so after either treatment.

The characteristic cell population of control, heat-treated, and X-irradiated testis, as observed at 10 days after treatment, are compared in Table 2. In control tissue, all cell categories are fully represented in all tubular cross sections according to the stages observed. Borrowing a term from hematology, heat-treated material observed after 10 days shows a relative "shift to the left," with spermatogonia and young spermatocytes the most prevalent cell categories. Late spermatocytes are nearly absent, spermatids are missing, and spermatozoa are sparse. In contrast to this, at 10 days after X-irradiation there is a "shift to the right"; spermatozoa are present in all tubular cross sections. Restituted and regressed spermatocytes are common, spermatogonia are very rare, spermatids are absent.

The reasons for these differences may be summarized as follows:

1. *Spermatogonia* are killed by X-rays but survive the heat treatment used here.

2. *Spermatocytes* are also differentially affected but the difference is more subtle. Under heat treatment, late spermatocytes are destroyed at about the first reduction division. This leads to an absence of spermatids at 10 days. Younger spermatocytes are meanwhile recruited from the plentiful spermatogonia and are thus in full recovery. After X-irradiation, late sperma-

tocytes are blocked or restituted, or they regress thus not permitting spermatids to be formed. However, there is at 10 days no recruitment of young spermatocytes, since the spermatogonia have not yet begun to recover.

3. *Spermatids*, and especially completion of the processes of tail formation are severely affected both by heat and by X-rays. After X-irradiation, however, a relatively greater number of spermatid nuclei develop into independent spermatozoal heads. Finally, the differences seen at 10 days are greatly dependent on the differential ability of tubules to discharge or propel the damaged spermatogenic contents into the epididymis. The emptying mechanism appears to be partially paralyzed by irradiation but to be less impaired by heat treatment.

4. Thus

Spermatozoa,

numerous but often scattered, are uniformly present after irradiation but not after heat treatment.

5. The *Sertoli* nuclei seem to withstand the two types of insult equally well.

Fig. 1. Seminiferous tubule of mouse sacrificed 3 days after heat treatment. Notice the pycnotic first meiotic metaphases, the M_1 restitution cells (x), the disoriented early spermatozoa (z), some of which are forming giant cells (gz), and the small number of spermatids (t). 548 $\times$. Symbols used in Figs. 1-6. x, M_1 restitution cell. g, spermatogonia. S, Sertoli cell. pl, preleptotene spermatocyte. ic, immature spermatocyte. c, spermatocyte. gc, spermatocyte giant cell. t, spermatid. gt, spermatid giant cell. iz, immature spermatozoa. z, spermatozoa. gz, spermatozoa giant cells. et, empty tubule. L, Leydig areas.

Cytological observations on the testes of mice subjected to elevated temperature are illustrated in Figs. 1-6. Fig. 1, a micrograph of testis from a mouse sacrificed 3 days after heat treatment shows especially well the restituted or regressed spermatocytes (x), of which we do not yet know the ultimate fate (c.f. Nebel and Murphy 1960a). The slightly pycnotic M_1

spermatocytes are obvious.

In Fig. 1 are also seen some early disoriented spermatozoa (z) apparently forming giant cells (gz). These spermatozoa lack proper tails. In this picture there are almost no spermatids (t) in the central tubule. Some disoriented spermatid nuclei are seen at 8 o'clock near the left margin of the photograph. At the left margin at 10 o'clock is what appears to be part of a spermatid giant cell. This as well as the other micrographs

illustrates that the giant cells in this material are formed from

Fig. 2. Seminiferous tubule of mouse sacrificed 5 days after heat treatment. Notice the giant cells containing spermatozoa (gz) and the lack of other cell types except Sertoli cells (S) and occasional spermatogonia (g). 548 $\times$.

groups of cells of the same category. Nuclei of different categories are not mixed in any given giant cell in spite of their topical propinquity. This may indicate that only cell surface membranes of the same molecular structure may reconstitute effectively to make this type of a giant cell. Spermatogonia and early spermatocytes unlike late spermatocytes, spermatids and spermatozoa, have not been observed in this study to form giant cells.

Fig. 2, a micrograph of testis from a mouse sacrificed 5 days after the end of the heat treatment, shows a tubular cross section with very extensive damage. The giant cells seen here contain late spermatids (gz). Except for the cells on the basement membrane (Sertoli cells (S) and occasional spermatogonia (g)) no other cell types are seen. The presence of spermatogonia indicates that the prognosis for recovery of this tubule is excellent.

In Fig. 3, a micrograph of the same mouse testis, can be seen a giant cell containing spermatocyte nuclei (gc). The tubule also contains pycnotic late spermatocytes (pc) *not* agglomerated as giant cells, and many scattered late spermatids (z). In terms of recovery, however, the most important elements are the numerous spermatogenic cells lining the basement membrane, which are probably preleptotene spermatocytes or late type B spermatogonia (g).

Figs. 4 and 5 represent different

Fig. 3. Seminiferous tubule of mouse sacrificed 5 days after heat treatment. Notice the giant cell containing spermatocyte nuclei (gc), the pycnotic late spermatocytes (pc), the scattered spermatozoa (z), and the numerous spermatogenic cells around the basement membrane (late type B spermatogonia or preleptotene spermatocytes). 548 $\times$.

magnifications of the same area of a slide from the same testis Figs. 2 and 3. The low power micrograph (Fig. 4) illustrates the wide variation in pathological events occurring in adjacent tubules. No two of the many tubular cross sections observed showed identical content, partly because of the variation in stage at the time of treatment, and partly because of vari-

ation in the reactions elicited by heat. Several examples of these variations follow. An empty tubule can be seen to the left of the center (et). At one o'clock in the upper half of the photograph are two tubules containing predominantly scattered immature spermatozoa (iz) lacking proper tails. On the right side of the lower margin at five o'clock is half a tubule containing

Fig. 4. Seminiferous tubules of mouse sacrificed 5 days after heat treatment. Notice the wide variation of pathological events between adjacent tubular cross sections: an empty tubule (et), immature spermatozoa (iz), and immature spermatocytes (ic). 168 $\times$.

only regenerating spermatogonia or immature spermatocytes (ic). In several tubular cross sections giant cells are visible. This figure also illustrates well that many tubules contain young regenerated spermatocytes which have begun to fill the lumina before the degenerate elements have been completely removed.

ing pachytene spermatocytes (c), occasional spermatogonia (g), and some apparently disoriented spermatozoa (z). Tail structures are seen near the center of Fig. 5 at 10 o'clock. The general orientation of the respective spermatozoa is quite disorderly.

Throughout these observations we have seen very little if any damage to the Leydig cells and no marked hypoplasia or hyperplasia of Leydig

Fig. 5 is a higher magnification of the tubule (cut longitudinally) in the center of Fig. 4. There are three giant cells containing spermatid nuclei (gt), a large group of what seem to be surviving

areas. Three typical Leydig areas (L) are shown in Fig. 6. In the center of this figure is an empty tubule lined only with Sertoli cells (S). We cannot on this evidence predict a lack of recovery in this tubule, since close inspection of such material has always revealed surviving spermatogonia, which may quite possibly be present in the adjacent sections. At the lower margin of the photograph we see a tubule with an abundance of spermatogenic cells (late type B spermatogonia (g)) which we interpret as signifying active recovery. At the upper margin is a tubule in which only a few heavily damaged spermatocytes (c) are seen near the Sertoli lining of the basement membrane.

Summary

C57 black male mice about 100 days of age were exposed to elevated temperature of 35°C and 70 % humidity in four exposure periods totaling 61 hours distributed over 4 days. Animals were sacri-

ficed at 3, 5, 7 and 10 days after the end of the heat treatment. Results of the heat treatment were compared with those following 1000 r X-irradiation. The damage inflicted on the mouse testis by this heat treatment differed fundamentally from that induced by 1000 r acute localized X-irradiation. Comparison at 10 days after treatment was especially revealing. Elevated

Fig. 5. Seminiferous tubule of mouse sacrificed 5 days after heat treatment (higher magnification of tubule in center of Figure 4). Notice the three giant cells containing spermatid nuclei (gt), surviving spermatocytes (c), occasional spermatogonia (g), and spermatozoa (z) apparently disoriented. 548 $\times$.

temperature induced the formation of giant cells, originating either from karyokinesis without cytokinesis or from agglomeration. The latter condition was most predominant when spermatozoal heads with indistinct tails were involved. No giant cells were seen after X-irradiation. Spermatogonia survived the heat treatment so that after 10 days, recovery was in full swing.

Fig. 6. Seminiferous tubule from mouse sacrificed 10 days after heat treatment. In the central tubule notice the complete absence of spermatogenic cells and the presence of Sertoli cells (S). In the tubular cross section of the lower margin late spermatogonia (g) are plentiful. Leydig areas are present (L). In the tubular cross section near the upper margin are spermatocytes (c). 548 $\times$.

X-Rays killed spermatogonia with the exception of occasional type A cells. Recovery had not started after 10 days. Late spermatocytes became pycnotic as a result of heat treatment; of these, some regressed, and a few underwent restitution or C-division. Normal spermatids were not formed. Late spermatocytes were blocked by 1000 r X-irradiation; the block was so persistent and so severe that 76 % of the tubular cross sections seen at 10 days contained some form of restituted or regressed spermatocytes.

Ten days after heat treatment no restituted or re-

gressed spermatocytes were seen. This may be related to the fact that heat seems to be less damaging than X-rays to the emptying mechanism of tubules. Spermatids appear equally damaged by heat and by X-rays. Spermatozoal heads are more common at 10 days after X-rays than after heat treatment;

this may result from the lesser effect of heat on the emptying mechanism as well as from a qualitative difference in the damage inflicted.

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