# THE EFFECT OF X-RAYS ON GONOCYTES IN THE TESTIS OF THE RAT

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*Summary*

After treatment with X-rays (300 r) at 1 or 4 days of age, the majority of gonocytes in the testes of rats failed to divide and by 8 days of age these cells, together with their nuclei and nucleoli, had markedly increased in size. A number of gonocytes still present in the testes of normal rats at 8 days of age had also undergone this generalized enlargement.

Determinations of the surface areas of germ cell nuclei confirmed the occurrence of the hypertrophy of gonocyte nuclei in normal and irradiated testes, and showed that the period during which gonocyte nuclei in irradiated testes became enlarged was the same irrespective of the age of irradiation. This period coincided with the one during which gonocytes in normal testes had begun to divide. These results led to the suggestion that irradiation, whilst suppressing the visible manifestations of mitosis, had not similarly affected the earlier synthetic phase, and that it was the products of this synthetic phase that had caused nuclei to enlarge. An inherent incapacity to complete the final stages of mitosis could likewise have resulted in the nuclear hypertrophy observed in some gonocytes in the testes of normal rats at 8 days of age.

## I. Introduction

A number of recent papers have shown that, in the rat, the gonocytes or male germ cells of the testis of the foetus and newborn are readily destroyed by X-irradiation (Beaumont 1960; Harding 1961; Hughes 1962).

Gonocytes do not divide in late foetal life and this resting phase continues for a short time in the newborn animal. A burst of mitotic activity in the middle or towards the end of the first week of postnatal life terminates the resting phase and produces the first spermatogonia. The latter are smaller than the parent gonocytes, which by 12 days of age have almost completely disappeared from the testis tubules.

It was Stschegolew (1934*a*, 1934*b*) who first observed that X-irradiation of neonatal rats had the specific effect of suppressing the postnatal mitotic activity of gonocytes. No spermatogonia were produced, gonocytes degenerated, and testes remained sterile. In similar experiments, Sapsford (1959) found that irradiation of the testes of rats at 1 day of age with 300 r did not completely suppress the mitotic activity of gonocytes, and careful searching of sections of testes from rats of 12 days of age revealed the presence of a few spermatogonia. At this age almost all gonocytes had degenerated. In the testes from rats of 17–50 days of age, the majority of tubules were sterile. Some, however, were producing cells of the spermatogenic line. These results therefore confirmed Stschegolew's (1934*a*, 1934*b*) observations concerning the relationship between gonocytes and the first spermatogonia.

One change not described by Stschegolew, however, was that prior to their degeneration gonocytes became markedly increased in size. This enlargement was

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particularly noticeable in the nuclei and nucleoli. The present paper gives details of a further series of experiments in which the occurrence of the nuclear hypertrophy was confirmed. Possible causes of the hypertrophy are discussed.

## II. Materials and Methods

The animals irradiated were randomly bred Wistar rats, the strain used in a previous survey of the changes in the size of germ cell nuclei during the development of the testis (Sapsford 1964).

Animals to be irradiated were strapped down in the supine position and a lead shield was placed over the body in such a way as to expose the region just caudal to the umbilicus. The dose of X-rays for each individual was 300 r (85 kV, 5 mA, at 12 in. for 2 min 44 sec).

Rats were irradiated either 24 hr (1-day rats) or 96 hr (4-day rats) after birth. Three 1-day irradiated rats, together with an equal number of normal litter mates, were killed at 5 days of age. Similar numbers of irradiated and normal litter mates were killed at 8 days of age. These 12 rats will henceforth be referred to as belonging to group 1. Three 4-day irradiated rats and three normal litter mates were killed at 5, 6, or 8 days of age. Two irradiated and two normal litter mates were also killed at 19 days of age. These 22 rats comprised group 2. The body and testis weights of all rats were determined at time of death.

Testes were fixed in Stieve's fluid and embedded in paraffin. Sections cut at 8 $\mu$ and stained with Groat's haematoxylin and phloxine were examined for histological changes in the testes of normal and irradiated rats. These sections were also used for the determination of the surface areas of the plane of maximum size (henceforth referred to as the surface areas) of germ cell nuclei by the method described by Sapsford (1964). The major and minor diameters of 50 nuclei in the testes from each of the following individuals were measured: two normal and two irradiated rats of group 1 at 5 and 8 days of age; two normal and two irradiated rats of group 2 at 5, 6, and 8 days of age.

## III. Observations

### (*a*) *Changes in Body and Testis Weight*

Table 1 gives the mean body and mean testis weights of normal and irradiated rats in groups 1 and 2 at age of slaughter. Increases in body weight with age were comparable in both normal and irradiated rats, indicating that the irradiation had no general deleterious effect. Testis weights in irradiated rats, however, increased at a slower rate than in normal rats. Since the diminished growth rate was reflected equally in both testes of all individuals, these results are considered as being indicative of the effectiveness of the irradiation.

### (*b*) *Histological Changes in Normal Testes*

The histological changes observed in normal testes were essentially the same as those recorded by Sapsford (1962). Some mitotic activity was noted in gonocytes in testes of 5-day rats. This activity was much more evident in the testes of 6-day

rats. In the testes of 8-day rats, resting and dividing spermatogonia were present in considerable numbers, and the number of gonocytes had considerably diminished. Some of the latter, together with their nuclei and nucleoli, had become very enlarged.

Spermatogenesis was proceeding in all tubules of rats killed at 19 days of age.

### (c) *Histological Changes in Irradiated Testes*

During the histological examination of sections of irradiated testes, attention was mainly confined to germ cells. The effects of irradiation on the other cells of the testis were briefly as follows. Connective tissue and Leydig cells showed no obvious changes. Within the tubules, however, varying numbers of indifferent cells contained

TABLE 1

BODY AND TESTIS WEIGHTS OF NORMAL AND IRRADIATED RATS

| Age (days) | Treatment | Mean Body Weight (g) ± S.E. | | Mean Testis Weight (mg) ± S.E. | |
|---|---|---|---|---|---|
| | | Group 1 | Group 2 | Group 1 | Group 2 |
| 5 | Normal | 10·7±0·6 | 9·6±0·2 | 8·7±0·3 | 7·2±0·4 |
| | Irradiated | 10·8±0·4 | 10·1±0·3 | 6·5±0·3 | 6·1±0·4 |
| 6 | Normal | — | 11·2±1·8 | — | 8·7±0·6 |
| | Irradiated | — | 11·2±1·3 | — | 6·3±0·8 |
| 8 | Normal | 11·8±0·6 | 14·3±2·8 | 13·2±0·3 | 13·9±1·4 |
| | Irradiated | 12·1±0·2 | 14·3±2·1 | 8·4±0·3 | 8·0±0·7 |
| 19 | Normal | — | 24·1±1·6 | — | 61·4±3·8 |
| | Irradiated | — | 23·2±0·2 | — | 27·0±1·5 |

pycnotic nuclei. Small clumps of chromatin were also found lying free of any definitive cells. The number of degenerating indifferent cells was greatest in the testes of group 2 rats killed at 5 or 6 days of age, and least in the testes of group 1 rats killed at 8 days of age. Loss of indifferent cells no doubt was an important factor contributing to the reduced weight of irradiated testes. Mitotic activity amongst indifferent cells was present at all ages. No close examination was made to determine whether all mitoses were normal. It is likely that some divisions were normal since testis weight increased between 5 and 8 days of age and the only cells in this organ concurrently undergoing obvious proliferation were indifferent cells.

At 5 days of age no obvious cytological differences between gonocytes in normal and irradiated testes could be detected. At 8 days of age, however, considerable enlargement of gonocytes in all irradiated testes had taken place. As in previous observations the enlargement was particularly noticeable in the nuclei and nucleoli. At 8 days degenerative changes were beginning to appear in some of the

enlarged gonocytes. These changes were characterized by the development of an intense acidophilia of both nucleus and cytoplasm. Chromatin, normally evenly dispersed in a fine granular form, was beginning to appear in irregularly shaped clumps.

Gonocytes in mitosis were rare at all ages and no spermatogonia were found in the sections examined from rats up to 8 days of age. Presumably because of the failure of the majority of gonocytes to divide and produce spermatogonia, gonocytes at this age were more numerous in irradiated testes than in normal testes.

In rats of 19 days of age the majority of tubules contained only the precursors of Sertoli cells. All gonocytes had disappeared. The presence of cells of the spermatogenic line in a small proportion of tubules indicated that some normal mitoses of gonocytes, resulting in the production of spermatogonia, must have taken place in parts of the testes of irradiated rats.

### (*d*) *Quantitative Assessment of the Size of Germ Cell Nuclei*

The mean surface area of the nuclei measured in the testes of individual rats and the means for each normal and irradiated pair of the same age in groups 1 and 2 are given in Table 2.

TABLE 2

SURFACE AREAS OF GERM CELL NUCLEI

| Age (days) | Treatment | Mean Surface Area ($\mu^2$) ± S.E. | | | |
|---|---|---|---|---|---|
| | | Group 1 | | Group 2 | |
| 5 | Normal | 83·99 ± 2·31<br>80·37 ± 1·22 | 82·18 ± 1·31* | 82·97 ± 2·19<br>84·08 ± 2·26 | 83·53 ± 1·56* |
| | Irradiated | 79·86 ± 1·59<br>77·31 ± 1·85 | 78·59 ± 1·22* | 76·72 ± 1·62<br>86·33 ± 2·04 | 81·53 ± 1·38* |
| 6 | Normal | —<br>— | | 93·38 ± 2·68<br>90·97 ± 3·35 | 92·17 ± 2·14* |
| | Irradiated | —<br>— | | 116·25 ± 3·85<br>112·58 ± 3·18 | 114·42 ± 2·49* |
| 8 | Normal | 72·44 ± 2·90<br>72·55 ± 3·66 | 72·50 ± 2·32* | 77·28 ± 3·86<br>71·53 ± 3·75 | 74·41 ± 2·69* |
| | Irradiated | 154·56 ± 4·41<br>144·91 ± 4·57 | 149·73 ± 3·20* | 163·34 ± 4·84<br>150·24 ± 4·40 | 156·79 ± 3·32* |

* Mean (± S.E.) of pair.

(i) *Distributions of the Surface Areas of Germ Cell Nuclei in Normal Testes.*— Analyses of variance or, where appropriate, *t*-tests demonstrated that there were no significant differences between the means for all normal individuals of the same age. In order, therefore, that the form of the distributions of the surface areas of nuclei at different ages could be more clearly defined and compared, all surface area values from all normal individuals of the same age were pooled. The distributions, expressed

as cumulative percentages plotted on a logarithmic scale on probability paper, are given in Figure 1. A logarithmic scale was used because Sapsford (1964) had found that in homogeneous populations the distributions of the surface areas of germ cell nuclei in rats of the same strain approached normality more closely on this type of scale than on an arithmetical one.

In this previous survey it was found that in rats of 5 days of age and younger, populations of germ cell nuclei were homogeneous. In the present survey, however, the distribution of surface areas of germ cell nuclei in the testes of normal rats at

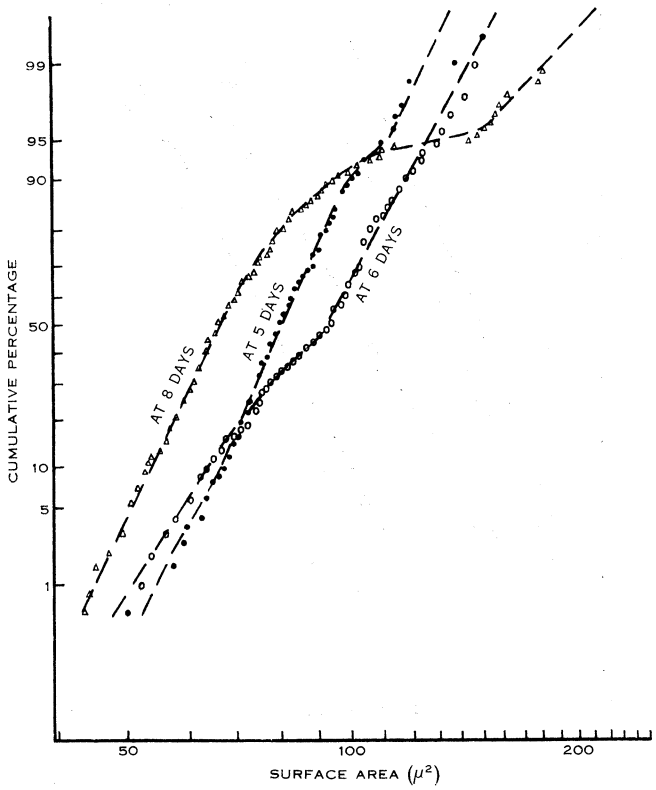


Fig. 1.—Distributions, on logarithmic scale, of the surface areas of germ cell nuclei in the testes of normal rats at 5, 6, and 8 days of age.

5 days of age showed a tendency to depart from normality above the 90% and possibly below the 10% levels (Fig. 1). At 6 and 8 days of age, distinct departures from normality can be seen (Fig. 1). The population at 8 days in the present study resembles those for rats of 8 to 9 days in the previous survey, in that the greater proportion of nuclei were smaller than the nuclei of gonocytes at 5 days of age and were thus spermatogonial in type. The remainder of the nuclei which had surface areas in excess of the values expected in a normal distribution were interpreted in the previous survey as being those of gonocytes which had not yet divided. The same interpretation applies to the nuclei comprising approximately the upper 10–15% of the population

at 8 days in the present study. The distribution of the surface areas at 6 days of age (Fig. 1) is interpreted as being an early stage in the splitting of the germ cell nuclei into spermatogonial and gonocyte types. It is not impossible that in the present survey a few spermatogonial nuclei had been encountered in the testes of 5-day rats. These nuclei could have produced the tendency of the distribution to depart from normality below the 10% level.

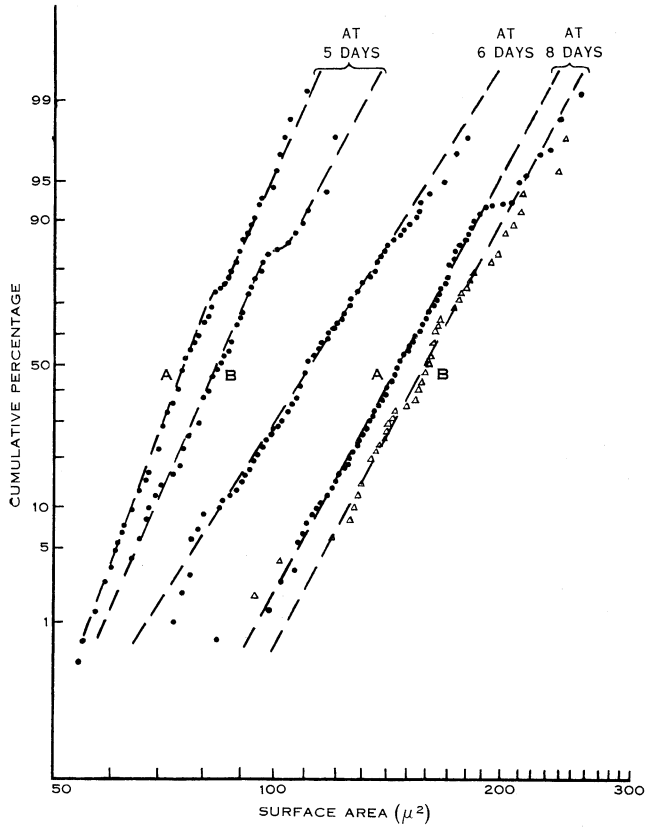


Fig. 2.—Distributions, on logarithmic scale, of the surface areas of germ cell nuclei in the testes of irradiated rats at 5, 6, and 8 days of age. *A*, distribution obtained by pooling data from three rats; *B*, distribution from remaining rat of same age.

(ii) *Distributions of the Surface Areas of Germ Cell Nuclei in Irradiated Testes.*—Pooling of the surface areas of nuclei in the testes of all irradiated individuals of the same age was only permissible in the case of 6-day rats. At 5 and 8 days of age, the distributions for rats with mean nuclear surface areas of $86 \cdot 33\ \mu^2$ and $163 \cdot 34\ \mu^2$ were plotted separately from those obtained by pooling the surface areas from the three other individuals of the same age. At each age, the means of these other individuals constituted a homogeneous group and their pooled mean differed significantly from that of the fourth individual.

The distributions of the surface areas of nuclei in testes of irradiated rats are given in Figure 2. At 5 days of age, a tendency to depart from normality, similar to that noted in the distributions for normal rats of this age, can be observed above the 80–85% levels. At later ages, departures from normality were less obvious in that they involved smaller proportions of nuclei. At 8 days, for example, the first few nuclei had surface areas considerably less than those which might have been anticipated if the populations had been homogeneous. At the same age departures from normality could also have occurred above the 93–94% level.

The division of the populations of nuclei into spermatogonial and gonocyte types observed in the case of normal rats did not take place at any age in irradiated rats.

(iii) *Comparison of the Mean Surface Areas of Germ Cell Nuclei in Normal and Irradiated Testes.*—A measure of the increase in the size of gonocyte nuclei in the testes of irradiated rats can be obtained from the data in Table 2. At 5 days of age only one mean ($86 \cdot 33\ \mu^2$) obtained from irradiated rats was greater than the means from normal rats of the same age. The difference between this mean and that of the pair of normal litter mates was not significant ($P > 0 \cdot 1$, $t = 1 \cdot 06$ on 148 d.f.). At 8 days of age, however, the means of the two pairs of irradiated rats had become almost twice those of normal or irradiated rats at 5 days of age. In the testes of irradiated rats of 8 days of age, the absence of spermatogonial nuclei and the enlargement of gonocyte nuclei were responsible for means which are more than twice those from normal rats of the same age.

## IV. Discussion

### (*a*) *Hypertrophy of Gonocyte Nuclei*

The determinations of the surface areas of germ cell nuclei confirmed the impression gained from histological examination that gonocyte nuclei in the testes of irradiated rats had become markedly increased in size. These quantitative surveys also established the fact that spermatogonia were first produced in the testes of normal rats in the interval between 5 and 8 days after birth. In demonstrating that spermatogonia did not appear in detectable numbers in the testes of irradiated rats, the results confirmed the rarity of mitoses in gonocytes after irradiation. They showed that the period in which the nuclei of irradiated gonocytes became abnormally enlarged was between 5 and 8 days after birth and was seemingly independent of the age of irradiation. The coincidence of the period of abnormal enlargement with the period in which mitoses in irradiated gonocytes were being suppressed indicated that these two phenomena could have been linked.

There were at least two possible ways in which the suppression of mitotic activity could have brought about the nuclear hypertrophy. The first of these was a simple continuation of the process of enlargement characteristic of gonocyte nuclei during the resting phase in late foetal and early postnatal life (Sapsford 1964). That this was probably not the principal reason for the hypertrophy, however, was indicated by the fact that the rate of increase in nuclear size during the early stages of development was considerably less than that observed in irradiated gonocyte nuclei. In the interval between 16 days after conception and 5 days after birth, the

mean surface area of gonocyte nuclei increased by a factor of 1·42 (Sapsford 1964). In the much shorter interval of time between 5 and 8 days after birth, the mean surface area of irradiated gonocyte nuclei increased by a factor of 1·88.

The second explanation for the nuclear hypertrophy was that whilst irradiation had obviously suppressed the visible manifestations of mitosis beginning with prophase, it may not have similarly affected the earlier synthetic phase. It was possible that as a result of this synthesis and because of its prolongation over a period of days, or perhaps re-occurrence after an interphase, nuclei had become hypertrophied.

Some very enlarged gonocyte nuclei are found in the testes of normal rats of 8 and 9 days of age (Sapsford 1964). In the present study, a small group of these at 8 days of age had surface areas varying from 113 to 187 $\mu^2$ (Fig. 1). These surface areas lie within the range of surface areas of gonocyte nuclei in the testes of irradiated rats at 8 days of age—83–293 $\mu^2$ (Fig. 2). It was possible that the enlarged nuclei found in normal testes belonged to gonocytes which had been intrinsically incapable of completing the division process. The nuclear hypertrophy in these cells could have been brought about in the same way as the nuclear hypertrophy in irradiated gonocytes, i.e. it could have been the result of the synthesis of nuclear substances.

These possibilities are supported by the well-recognized fact that, in normal tissues, a change in ploidy is accompanied by an increase in nuclear size. Alfert, Bern, and Kahn (1955) have shown that in naturally occurring polyploidy, increases in nuclear volume are accompanied by comparable increases in both DNA and nuclear protein. These substances constitute 98–99% of the dry weight of a variety of nuclei (Mirsky and Osawa 1961). Further investigation of the nuclear hypertrophy which had taken place in gonocytes in normal and irradiated testes would therefore entail estimations of the DNA and protein content of the nuclei of these cells. An account of such determinations is given in the following paper (Sapsford 1965).

### (*b*) *Departures from Normality in the Distributions of Surface Areas of Germ Cell Nuclei*

It has been pointed out that in a previous survey (Sapsford 1964), populations of germ cell nuclei in the testes of normal 5-day rats were found to be homogeneous when the distributions of their surface areas were plotted as cumulative percentages on a logarithmic scale. In the present survey, similarly plotted distributions from normal rats of this age showed possible departures from normality below the 10% and above the 90% levels (Fig. 1). These discrepancies could have been due to the fact that the rats used in the present study were slightly more mature than those examined previously.

It has already been suggested that the nuclei causing the departure from normality below the 10% level were spermatogonial in type. The presence of this type of nucleus in small numbers would indicate that the first gonocyte divisions had just been completed. It might be anticipated that this would not have been the case in the testes of more immature rats and spermatogonial nuclei would not have been present.

The onset of mitotic activity in gonocytes might not be synchronous throughout the testis and in the normal 5-day rats examined in the present study only a proportion of gonocytes may have been preparing to divide. Nuclear hypertrophy resulting from the premitotic synthesis of DNA and nuclear protein in these cells could have produced the departure from normality above the 90% level (Fig. 1). This synthesis may not have begun in gonocytes in the testes of the conceivably more immature rats examined in the previous survey (Sapsford 1964).

Departures from normality in the distributions of the surface areas of gonocyte nuclei in the testes of irradiated rats at 5 and 8 days (Fig. 2) could have been due to variation in the degree of nuclear hypertrophy associated with variation in the degree of nuclear synthetic activity. At 5 days, enlargement of the nuclei of part of the gonocyte population, induced in the same way as in normal testes at the same age, could have produced the departures from normality above the 80–85% levels. At 8 days, the inhomogeneity with respect to nuclear surface area could have been due to the presence of nuclei of different ploidy classes.

These matters will be further discussed in the following paper dealing with the DNA and protein content of gonocyte nuclei (Sapsford 1965).

## V. Acknowledgments

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