Effects of continuous low dose irradiation on mice. II. Quantitative histologic analysis of the effects of continuous gamma irradiation on mouse testes.*

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マウスへおよぼす低線量γ線連続照射の影響 II. 精巣へおよぼされた影響

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CF#1-JCL 雄マウスを受精から出生をへて 195～196日令にいたる期間 1R/day および 6R/day の線量率で Co^{60} γ線の連続照射を行い，精巣におよぼされる影響を組織学的に研究した．蓄積線量は1R群で 214.5R，6R群で1290.0Rであつた．

精巣重量は6R群で対照群の $^1/_2$ に減少しており，その差は統計的に有意であつたが，1R群では有意な減少はみられなかつた．精巣の断面積，細精管の面積にも重量変化に並行した変化がみられた．

Chalkley の方法にしたがつて組織の定量的な観察を行つた結果，精原細胞と精母細胞は両照射群とも著しく減少し，それに起因した精細胞，精子の減少がみられた．これは細精管あたりの Type A 精原細胞，Intermediate と Type B 精原細胞，細糸期の第一精母細胞の数からもうらづけられた．これとは逆にセルトリ—細胞，間細胞，精巣内の間隙，細精管内の間隙の割合の増加が認められた．

これらの結果から，1R群では精巣重量の変化が少ないにもかかわらず精子形成を行なう細胞群が著しく影響をうけていること，また6R群では精子形成を行なう細胞群の減少が精巣重量にも影響していることが判つた．

It is recognized that the testes are highly sensitive to ionizing radiations. Numerous investigations have been published on the radiation effects of mammalian spermiogenesis. These changes vary in intensity, and depend on the age of animals when exposed, dose-rate, and the way of dose delivery, single acute or continuous irradiation.

Lorenz *et al.* (1947), Eschenbrenner *et al.* (1948), and Eschenberenner and Miller (1946, 1954) have demonstrated that the alteration of testicular weight and decrease of spermatogenic elements were induced by the low dose continuous irradiation on adult mice. The Lorenz group described the effects of 1.1, 4.4, and 8.8 R/day. Recently Nebel *et al.* (1963) and de Boer (1964) reported the similar effects of continuous irradiation on mouse testes described by Lorenz group.

*This paper is dedicated to professor Sajiro Makino, Zoological Institute, Hokkaido University, Sapporo, in honor of his sixtieth birthday, June 21, 1966.

However, there are few reports that deal with the effects of continuous irradiation on mouse testes through their whole reproductive period, i.e., from conception until fully grown. Previously Muramatsu *et al.* (1965) reported that when mice were bred under the influence of continuous low dose gamma irradiation from conception until fully grown the postnatal development of sucklings and the growth after birth were affected. The investigation reported herein deals with the effects of continuous whole-body Co^{60} gamma irradiation on the mouse testes through their whole reporductive period.

Experimental procedure

1. Materials

Animals used were the CF#1-JCL mice supplied by the Zikken Dobutsu Chuo Kenkyujo. Each group consists of ten male mice.

2. Irradiation

The female mice with vaginal plug after mating were segregated and entered into the gamma field. Male and female mice delivered from these mothers were irradiated with Co^{60} gamma rays during their whole reproductive period, namely 215—216 days from conception until fully grown. In this manner, mice were exposed to total doses of 214.5 R, and 1290.0 R at dose-rate of 1 and 6 R per 22 hr-day. Control series were bred in the same manner as in the irradiated series without irradiation.

3. Autopsy

At 195—196 days old, the animals were killed, and testes were removed, weighed, and fixed in Allen's P.F.A. III fluid. After embedding in paraffin, sections were cut at 5 μ from the center of the testis at right angles to the long axis. The sections were stained by the PAS technique (Hotchkiss 1948) and with Mayer's haemalum.

4. Measurements

The testis area and tubular areas of each animal were determined by the method of Nebel *et al.* (1963).

Quantitative histologic analysis of the testis was carried out according to Chalkley's method (Chalkley 1943). Fourhundreds "hits" were recorded for each animal. Hits were recorded in the following categories: spermatogonia, spermatocytes, spermatids, immature spermatozoa, Sertoli cells, inner space (intratubular space), and outer space (extratubular space, interstitial cells, blood vessels, and etc.). The hits of each element were summed and the mean proportions were calculated.

Furthermore, the Chalkley's counts were supplemented by direct counts on type A spermatogonia, intermediate and type B spermatogonia, and leptotene primary spermatocytes, because the Chalkley's method will tend to miss cells that are presented in very few frequency. For the counts of type A spermatogonia, sixty tubules from the testis cross section of each animal were selected randomly, and the number of type A spermatogonia were counted. Intermediate and type B spermatogonia were counted in twenty tubules of spermiogenic stage II, III, IV, V, or VI on each animal. The leptotene spermatocytes were counted in twenty tubules of spermiogenic stage VIII, IX, or X on each animal. The stage of spermiogenesis was determined by the classification of Leblond and Clermont (1952) and Oakberg (1956). The direct counts data points represent an average value from five animals in each group.

Results

1. Testes weight, testis area, and tubular areas.

Table 1. Accumulated dose and testes weight for the three series of mice.

Series	No. of animals used	Age (days)	Accumulated dose (R)	Body weight * (g)	Testes weight (mg) *,**
0R/day	10	195.5	0.0	37.05±1.88	248.33± 8.39 ′
1R/day	10	195.5	214.5	37.80±1.92	223.63± 25.40 ″
6R/day	10	196.0	1290.0	36.54±1.46	122.48± 16.49‴

* : Mean ± 95% fiducial limit.

** : Statistical significance

′ vs ″ $0.60 > P > 0.50$
′ vs ‴ $P > 0.001$
″ vs ‴ $0.05 > P > 0.02$

The effects of continuous irradiation on mouse testes may be seen in Table 1. It was observed that right testis weight is larger than that of left ones in most cases, but there is no statistical difference between right and left testis wieght. Then, the testes weight in three series were compared with the mean weight of the pair of testes. Reduction of testes weight was observed at all exposure levels. In 6R series, testes weight decreased markedly, and the difference between the control and 6R series is statistically highly significant ($P<0.001$). Weight of 1R series lies intermediate between control and 6R series, and the weight loss is not significant.

Table 2. Testis area and tubular areas for the three series of mice.

Series	Testis area (mm^2)	Tubuar areas (mm^2)	Proportion of tubular areas (%)
0R/day	13.52	11.82	87.45
1R/day	12.73	10.71	84.10
6R/day	8.70	6.74	77.52

Testis area and tubular areas follow closely that of testes weight as shown in Table 2. In both irradiated series, these areas lowered with correspond to the decrease of testis weight. It is suggested that decrease of these areas is associated with a loss in testes weight.

2. Chalkley's counts.

The spermatogenic elements of the testes of mice continuously exposed to gamma radiation have been analysed quantitatively by Chalkley's method. Table 3 shows the mean proportions of each elements.

As shown in Table 3, a marked decrease of the total quantity of spermatogenic elements in the testis of both irradiated series were observed. Proportion of spermatogonia in irradated series was reduced to half or less of the control. Also spermatocyte counts reduced in the irradiated groups. Nevertheless spermatids and spermatozoa show a higher radioresistance, these cells were also reduced to half of the control in the irradiated series. Thus it seems that the reduction of these cells reflects a cutoff in spermatogonia and spermatocytes.

On the other hand, Sertoli cells increased by three times of the control in the 6R series. In the 1R

Table 3. Chalkley's counts (*) of cell types and of inner and outer spaces for the three series of mice.

Series	0R/day	1R/day	6R/day
No.of animals used	10	10	10
Total Chalkley's hits	4000	4000	4000
Spermatogonia	10.83±1.35	3.90±0.64	3.82±0.53
Spermatocytes	18.10±1.28	13.25±1.34	11.28±1.37
Spermatids	21.13±2.16	14.13±1.53	12.83±1.45
Immature spermatozoa	13.80±3.08	6.70±0.77	6.18±1.06
Sertoli cells	2.18±0.55	2.85±0.40	7.20±0.80
Inner space	20.70±4.78	41.90±3.59	33.65±3.77
Outer space	13.27±1.99	17.29±2.26	25.05±3.55

* : Mean proportion ± 95% fiducial limit.

Table 4. Direct counts per tubule for the three series of mice.

Series	0R/day	1R/day	6R/day
Type A spermatogonia	2.81	2.34	1.51
Intermediate and type B spermatogonia	11.98	9.62	6.87
Leptotene primary spermatocytes	46.40	41.52	22.56

series, a little changes were observed. And inner and outer spaces were markedly increased in two irradiated series. Further, in 6R series, interstitial cells (Leydig cells), which refers to outer space, increased by two times of the control, i.e., 4.2 and 8.4 in the control and 6R series respectively, but mice of 1R series showed the similar proportion as control (4.1).

3. Direct counts.

The direct counts of spermatogonia and primary spermatocytes are tubulated in Table 4. From these results, the decrease of all cell types was observed in both irradiated series.

In 6R series, direct counts of type A spermatogonia, intermediate and type B spermatogonia and leptotene spermatocytes decreased by about one-half of the control. However, in 1R series, the decrease of these cells was not remarkable as the 6R series.

Discussion

The present results seem to indicate that the radiation effect on the testis even at low dose-rate (1R or 6R/day) is cumulative for the whole reproductive period, from conception until fully grown. Of cource, the radiation effect on the testicular cellularity accompanied to the accumulation of effective gamma doses and the degree of effects depends on the intensity of daily doses.

At 195—196 days old, the testes weight loss of both irradiated series were remarkable. And it has been demonstrated from the quantitative histological observations that these weight losses were fundamentally caused by the decrease of spermatogenic elements in the seminiferous butules. As shown in Table 3 and 4, number of spermatogonia and spermatocytes decreased by half or one-third of the control in 1R and 6R irradiated series. The decrease of testicular cellularity was ascribed to the cutoff or decrease of these radiosensitive cells. On the other hand, normal mitotic figures of these cells were frequently

observed in the irradiated mice after accumulation of 1290 R with 6 R daily dose. Further, the cross section of tubule, which was emptied of all spermatogenic cells by cell loss, was scarely observed after 1290 R accumulation. Nebel *et al.* (1963) reported that the continuously irradiated mice with low dose-rate show survival of type A spermatogonia apparently at a reduced functional level which represents an adaptation to ionizing radiation. Recently many similar papers have appeared which deal with adaptation at cellular and organismal levels. However, further study is required to consider these problems in the present investigation.

Contrary to the decrease of spermatogenic elements, the proportion of Sertoli cells and interstitial cells increased in the irradiated series, Sertoli cells were not destroyed by acute irradiation and the increased proportion were reported in the testis of continuous irradiated mice (Nebel *et al.* 1963). Also the apparent increase of interstitial tissues in the testis of irradiated mice was reported by Eschenbrenner and Miller (1946, 1954) in the case of continuous irradiation. Since the gross changes in the histological appearance of the irradiated testis, the increase in the proportion of Sertoli cells and interstitial cells observed after irradiation was considered primarily due to shrinkage of the seminiferous tubules in the irradiated mice.

When the adults were irradiated continuously with low dose-rate, the testes weight loss and histological damages were induced in mice (Lorenz *et al.* 1947; Eschenbrenner and Miller 1946, 1954; Eschenbrenner *et al.* 1948; Nebel *et al.* 1963; de Boer 1964), and the induced effects were larger in the higher daily doses than that in the lower daily doses. Also the late effects on the testis were obesrved by the foetal irradiation of mice (Rugh and Jackson 1958; Rugh and Wohlfromm 1964). In the male albino rats, the continuous exposures from 15th day of gestation to the 23rd day of postnatal life produced the significant changes in the testes weight and histological evaluations when the rats were fully grown (Pace *et al.* 1964). Unfortunately the present results could not be compared exactly to the results of the above papers for the differences of dose-rate used, the age when irradiated, and irradiated period. However, the similar effects reported by these authors were observed in the present investigation. Sterility and partial sterility were observed in the exposed mice group under continuous irradiation with low dose gamma- or X-rays (Lorenz *et al.* 1947: Charles 1950; Deringer *et al.* 1954; Carter *et al.* 1954; Charles *et al.* 1960, 1961). Carter *et al.* (1954) reported that at dose-rate of 8.0 R/week a 200 R dose induced sterility in 39 per cent of the adult male mice ($P<0.001$). In the present study, the testicular cellularity was markedly affected in the 1 R/day series which brought about similar cumulative dose as in the experiment of Carter *et al.*, whereas the testes weight loss was slightly lower than that of the control series. Therefore, it seems that the fertility was reduced by the continuous irradiation with low dose-rate through their whole reproductive period, although these effects on fartility were not observed in the present study.

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