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RELATIONSHIP BETWEEN GONADAL DEVELOPMENT AND RADIATION SENSITIVITY IN THE MALE GOLDEN HAMSTER*

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ABSTRACT

Nai-Hsien Chao and Eugene W. Hupp (1971). *Relationship Between Gonadal Development and Radiation Sensitivity in the Male Golden Hamster.* Bull. Inst. Zool. Academia Sinica **10**(2): 59-67. Male golden hamsters were irradiated with 220 R of x-rays on 13 and 15 days prenatal and 1, 3, 5, 6, 7, 8, 11, 15, 18 and 22 days postnatal. Mean testes weights in all groups were significantly suppressed by radiation. The mean number of seminiferous tubules of hamsters irradiated on all days except day 18 postnatal differed from the control group significantly ($p<0.05$). Suppression in testes weights and mean number of seminiferous tubules was greatest in animals irradiated on day 3 postnatal. There was a good correlation ($r=0.74$, $p<0.05$) between testes weight and the number of tubules.

Control hamsters had the greatest number of fully active seminiferous tubules. Animals irradiated during the sensitive peroid, days 1, 3, 5 and 6 postnatal had the least fully active seminiferous tubules. This corresponded to the minimum testes weight and the greatest reduction of the number of tubules per cross section. A strong correlation ($r=0.95$, $p<0.05$) was found between testes weight and the number of fully active seminiferous tubules.

Fetal and neonatal hamsters were killed on proper days for the study on normal development. The present results indicate that there is a relationship between the qualitative and quantitative changes in the testes with increasing age and radiosensitivity. Maximum radiosensitivity in the male hamsters was associated with gonocytes that were larger in size and which had little or no mitotic activity.

Studies of the effects of irradiation on the gonads of mammals have been largely restricted to the rat and the mouse (6, 10). Recently, use of the hamster as an experimental animal has increased (1, 12). The first objective of this research was to establish the radiosensitivity pattern of hamsters. A second objective was to determine non-irradiated fetal and neonatal gonads if changes in the radiosensitivity of germ cells could be correlated with stages of major change in morphogenesis.

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MATERIALS AND METHODS

Random-bred golden hamsters were ordered from the Lakeview Hamster Colony, Newfield, New Jersey. They were maintained in an animal room in which temperature and light were controlled (75°F, and 12-hour day, 12-hour night). The hamsters were kept in clean wire cages and were fed on Wayne Lab-Blox and water.

Female hamsters were mated and checked daily for litters. Postnatally irradiated hamsters were obtained by irradiating the litters at each proper day. Prenatally irradiated hamsters were obtained by irradiating pregnant females of a known stage of gestation. A 250 KV General Electric Therapy Unit was used as a source of radiation. The unit operated at 250 kVp at 15 ma, with 0.5 mm copper and 1.0 mm aluminum filtration. The target-to-subject distance was adjusted to obtain a dose rate of 55 R per minute, totalling 220 R for four minutes.

A group of ten males from each irradiated day with approximately five litters being represented in each group was kept. They were weighed and killed at 50 days of age. The testes were removed and weighed together, then fixed in Bouin's fixing solution. Histological sections were prepared by the paraffin technique and stained with Hematoxylin and Eosin. Sectioning and analyzing of the testes were done. The system of classification of the seminiferous tubules will be described in the result section. With the objective to relate radiosensitivity to the normal development of fetal and neonatal hamsters, two male hamsters were killed at the age similar to that at which radiation was applied. Histological serial sections were prepared and treated as above. Polaroid pictures were taken to aid in the comparisons between the various stages of development. Statistical analysis, using Duncan's multiple range test (2), was applied to weights and counts to aid in interpretation of the results obtained.

RESULTS

Testes weight:

Means of testes weights were significantly decreased by radiation (Table 1). Hamsters irradiated on day 3 of postnatal life had the smallest mean testes weight, only 13% of the control testes weight; while those irradiated on day 15 of postnatal life had the greatest mean testes weight, 87% of the control weight. Suppression in the weight of testes was almost as great in animals irradiated on days 1, 5, and 6 of postnatal life as day 3 with testes weight of 14, 15, and 16% of control, respectively. Statistical analysis showed that mean testes weight in these four groups and the group

TABLE 1
Weight of Both Testes of Hamsters X-Irradiated with 220 R and Killed at 50 Days of Age

Treatment	Mean ± Standard Deviation (g.)	Percent of Control (%)
Control	2.296 ± 0.377	100.0
Day Irradiated		
13 Prenatal	$1.687 \pm 0.297^{ab*}$	73.5
15 Prenatal	1.131 ± 0.734^{d}	49.3
1 Postnatal	0.320 ± 0.070^{f}	13.9
3 Postnatal	0.296 ± 0.036^{f}	12.9
5 Postnatal	0.332 ± 0.050^{f}	14.5
6 Postnatal	0.360 ± 0.171^{f}	15.7
7 Postnatal	0.454 ± 0.151^{ef}	19.8
8 Postnatal	0.681 ± 0.337^{e}	29.7
11 Postnatal	1.521 ± 0.743^{bc}	66.3
15 Postnatal	1.996 ± 0.427^{a}	83.9
18 Postnatal	1.713 ± 0.220^{ab}	74.6
22 Postnatal	1.265 ± 0.218^{cd}	55.1

* Means with the same superscript do not differ at the 5% level of significance.

irradiated on day 7 postnatal did not differ from each other at 5% level of significance.

Animals irradiated on days 8, 11, and 15 of postnatal life as well as on day 15 of prenatal life showed a greater variability in testes weight than did the animals that were irradiated on other days. They ranged from

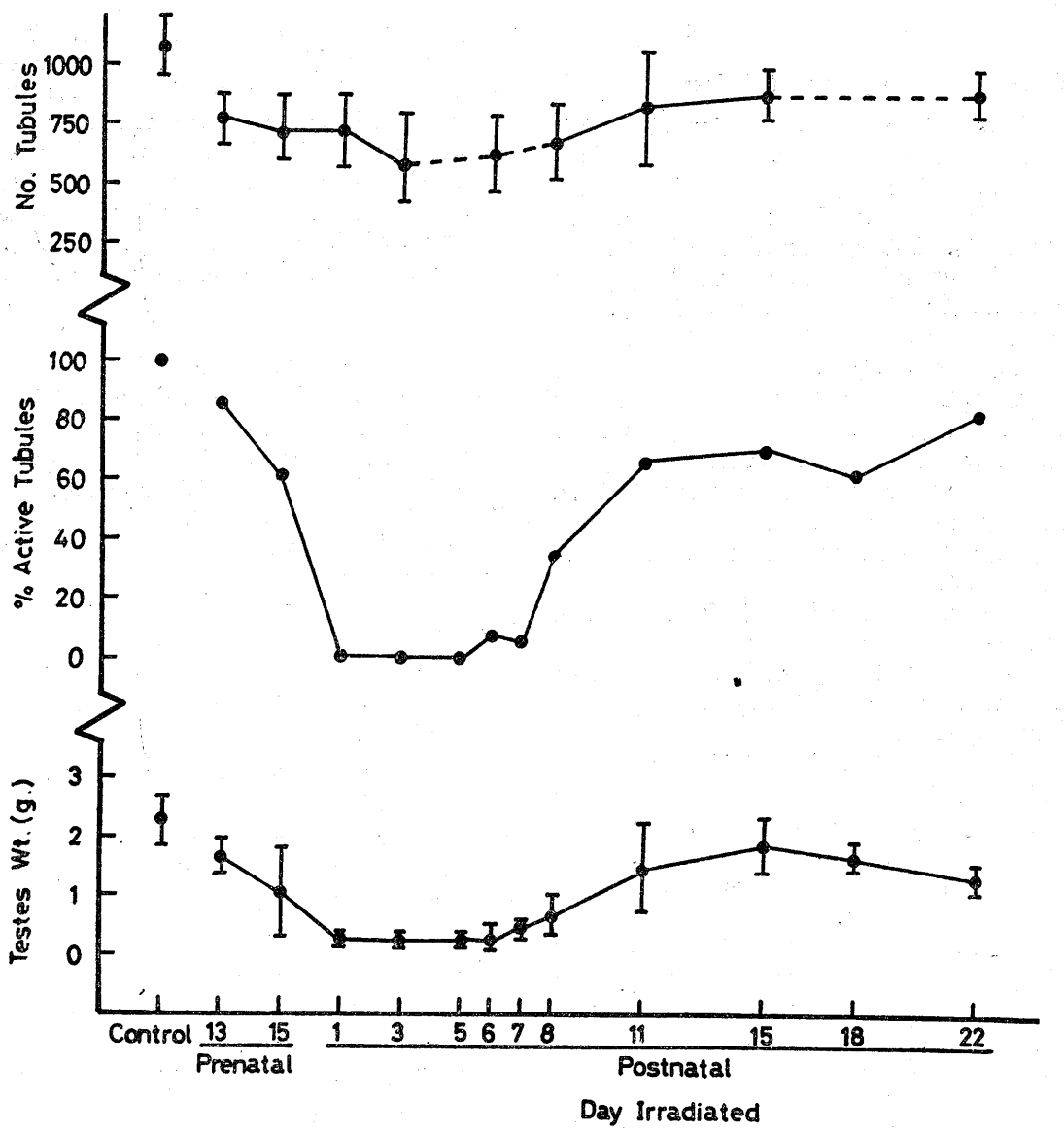

Fig. 1. Comparison of Means and Standard Deviation of Testes Weight, Total Number of Seminiferous Tubules, and Percentage of Active Tubules of Hamsters X-Irradiated with 220 R and Killed at 50 Days of Age.

1.298 g to 0.288 g; 2.370 g to 0.271 g; 2.778 g to 1.624 g; and 1.976 g to 0.330 g, respectively. Postnatal irradiation had less effect on testes weight as age at irradiation increased; however, there appeared to be a secondary depression in animals irradiated at 18 and 22 days postnatal (Figure 1).

Histological analysis of tubules:

The method of classification of seminiferous tubules of male hamsters was based on a preliminary examination of the testes slides and was the same method used by Curvey (1). The system of classification of seminiferous tubules was started by Pace *et al.* (8), it was then modified and improved gradually by Ricks and Hupp (10), Gates (3), Hall (4), Partlow (9), Teng (11), and Curvey (1).

Class 1 consists of seminiferous tubules with a wrinkled cell membrane, vacuoles and few disorganized Sertoli cells. No germinal elements such as spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, and mature spermatozoa are present.

Class 2 consists of seminiferous tubules with Sertoli cells in an orderly arrangement around the periphery of the seminiferous tubules. Sometimes a few spermatogonia or vacuoles are observed.

Class 3 consists of seminiferous tubules with Sertoli cells, spermatogonia, primary spermatocytes and secondary spermatocytes but no spermatids or spermatozoa. Most seminiferous tubules of Class 3 do not have many spermatocytes.

Class 4 consists of seminiferous tubules with more advanced germinal elements than Class 3. All cellular elements are in order but are less in numbers. The tubules classified as Class 4 range from those with only a few terminal elements to those with 50 sperm or terminal elements.

Class 5 consists of the fully active seminiferous tubules with all cellular elements and reasonable number (more than 50) of terminal germinal elements. Nevertheless, some of the tubules classified as Class 5 are probably not fully active.

The number of tubules per cross section was reduced in all irradiated groups (Table 2). Irradiated animals had from 55 to 90% as many tubules as control hamsters. The mean number of seminiferous tubules of hamsters irradiated on all days except day 18 postnatal differed from the control group at 5% level of significance. Animals irradiated on days 3 and 6 postnatally had mean numbers of tubules which differed from the majority of other groups (Table 2).

The reduction of the number of tubules

TABLE 2
Mean and Standard Deviation of Total Number of Tubules Per Testis Cross Section in Hamsters X-Irradiated with 220 R and Killed at 50 Days of Age

Treatment	Mean±Standard Deviation (no.)	Percent of Control (%)
Control	$1072 \pm 111^{a**}$	100.0
Day Irradiated		
13 Prenatal	762 ± 87^{cdef}	71.7
15 Prenatal	749 ± 121^{def}	69.9
1 Postnatal	736 ± 125^{ef}	68.7
3 Postnatal	589 ± 199^{g}	55.0
5 Postnatal*	870 ± 52^{bcd}	81.2
6 Postnatal	614 ± 164^{g}	57.3
7 Postnatal*	818 ± 88^{cde}	76.3
8 Postnatal	680 ± 163^{fg}	63.4
11 Postnatal	819 ± 240^{cde}	76.4
15 Postnatal	868 ± 94^{bcd}	81.0
18 Postnatal*	968 ± 48^{ab}	90.3
22 Postnatal	890 ± 84^{bc}	83.0

* Thess tissues also obtained at 50 days but in a later experiment; unevaluated factors may have been responsible for apparent increased number of tubules.

** Means with the same superscript do not differ at the 5% level of significance.

per cross section was greatest in animals with minimum testes weight. There was a good correlation ($r=0.74$ $p<0.05$) between testes weight and the number of tubules. The radiation damage was reflected in the reduced number of tubules. It is also indicated that some degenerative changes were responsible for the apparent shortening of the severely damaged tubules, causing a decrease in the number of tubules. A considerable amount of variation among individuals of each age group in the number of tubules comprising each of the five classes as well as testes weight was observed.

Control hamsters had the greatest number of Class 5 tubules (Table 3). Animals irradiated on day 13 prenatal and day 22 postnatal had 88 and 82% tubules of Class 5 respectively, while controls had 86% (Table 4). The proportion of active tubules in these two groups was as great as in the control group and was least radiation-affected. Animals irradiated on days 3 and 5 of

TABLE 4
Percentage of Tubules Per Class in Hamsters X-Irradiated with 220 R and Killed at 50 Days of Age

Treatment	Percentage of Tubules Per Class				
	1	2	3	4	5
Control	0.0	0.0	1.5	11.1	86.2
Day Irradiated					
13 Prenatal	0.0	1.0	2.2	9.1	87.7
15 Prenatal	0.1	23.7	3.5	11.4	61.2
1 Postnatal	0.0	97.6	1.1	0.5	0.9
3 Postnatal	0.0	97.9	0.9	0.3	0.8
5 Postnatal	0.1	98.1	0.7	0.2	0.2
6 Postnatal	0.1	87.0	2.0	2.8	8.1
7 Postnatal	0.0	83.7	4.5	5.5	6.3
8 Postnatal	0.0	50.1	5.3	9.5	35.2
11 Postnatal	0.3	13.8	4.7	12.5	68.7
15 Postnatal	0.0	0.1	2.6	25.7	71.6
18 Postnatal	0.0	0.1	2.2	35.2	62.5
22 Postnatal	0.0	3.0	1.6	13.6	81.8

TABLE 3
Mean and Standard Deviation of Number of Tubules Per Class in Hamsters X-Irradiated 220 R and Killed at 50 Days of Age

Treatment	Mean ± Standard Deviation Per Class				
	1	2	3	4	5
Control	0±0	0±0	16±12	119± 73	923±294
Day Irradiated					
13 Prenatal	0±0	8± 11	17± 5	69± 33	669±103
15 Prenatal	1±2	178±116	26±26	86± 32	459±448
1 Postnatal	0±0	719±123	8± 6	4± 6	6± 10
3 Postnatal	0±0	577±191	5± 4	2± 3	5± 6
5 Postnatal	1±3	854± 52	6±10	2± 3	2± 2
6 Postnatal	0±1	534±168	12± 7	17± 27	50± 93
7 Postnatal	0±0	684±149	36±29	45± 45	51± 62
8 Postnatal	0±0	340± 50	36± 5	64± 10	239± 35
11 Postnatal	2±4	113±175	38±32	103± 79	563±553
15 Postnatal	0±0	1± 1	23±15	223±150	622±148
18 Postnatal	0±0	1± 2	21±15	341±345	605±208
22 Postnatal	0±0	27± 82	14±12	121± 72	728±174

postnatal life had the least Class 5 tubules (0.83 and 0.19%), respectively, followed by those irradiated on days 1 and 7 of postnatal life (Table 4).

This phenomenon corresponded to the minimum testes weight and the greatest reduction of the numder of tubules per cross section. There was a strong correlation ($r=0.95$ $p<0.05$) between testes weight and number of active, Class 5 tubules. The most radiation-affected period showed by suppression of both testes weight and number of Class 5 tubules was during days 1, 3, 5, and 6 of postnatal life (Tables 1 & 3). Low sensitivity in the animals irradiated in prenatal and later postnatal life was indicated by increased testes weight and the increased number of active tubules of Class 5.

Normal testes development:

The mean number of tubules per testis section and the mean diameter of the tubules are listed in Table 5. Both the number and the size increased as the age increased. Histological analysis (5) showing the distinguishing features of the progressive age is as follows:

TABLE 5

Mean Number and Diameter of Seminiferous Tubules in Testis Cross Sections of Fetal and Neonatal Hamsters

Age	Mean Number	Diameter (μ)
Day 13 Prenatal	60	130
Day 15 Prenatal	85	130
Day 1 Postnatal	95	135
Day 3 Postnatal	169	145
Day 5 Postnatal	322	120
Day 7 Postnatal	596	145
Day 10 Postnatal	607	135
Day 14 Postnatal	728	145
Day 18 Postnatal	801	160
Day 22 Postnatal	823	210

Day 13 Prenatal

The testis contains a considerable amount of undifferentiated epithelial tissue, especially at one end. Tubules were generally well organized so that their outline could be recognized, but in most cases a basement membrane could not be readily identified. The cells within the tubules looked similar and were generally the same size; however, usually there was a row of cells around the periphery that tended to be smaller than those in the center. The small cells resembled those observed in a similar location at later ages.

Day 15 Prenatal

The tubules were relatively uniform. The basement membrane was fairly established. The organization of smaller cells around the outside and larger cells in the center was more regular than that of day 13 prenatal.

Day 1 Postnatal

The basement membrane of the tubules was well organized with small cells usually arranged around the periphery of the tubules and the large cells in the center. The cells were more the same size than that of later stages and had a somewhat random appearance. No mitotic activity was apparent. A few pycnotic cells were observed.

Day 3 Postnatal

The tubules became more definitely established and greatly increased in number. A definite arrangement of supporting cells and gonocytes was observed. A single layer of small cells were arranged around the periphery. The nuclei of the large cells increased in size and these cells were located in the center of the tubules as those of 1 day of age. Large cells stopped dividing in this stage. This stage showed the greatest number of large cells, afterwards they progressively decreased in number. No spermatogonia were present.

Day 5 Postnatal

The tubules increased in number. The large cells were more prominent at this stage and appeared to have increased in size, but decreased in total number. This was a start of a widespread vacuolation of the cytoplasm of the large cells which would subseqently lead to their disappearance and the appearance of the lumen. An occasional spermatogonium was observed. The smaller cells were in the same arrangement as before. Chromatin material was deeply stained.

Day 7 Postnatal

The tubules resembled those in previous stage but were more twisting and convoluted and increased in size. The large cells were still prominent, but significantly reduced in number. The chromatin lost its affinity for hematoxylin stain and therefore showed only a slightly deeper tint than the cytoplasm. The cytoplasm was somewhat vacuolated. Spermatogonia were present in all tubules, but were more numerous in some than in others. Differential maturation of the tubules was indicated in this stage.

Day 10 Postnatal

The center area in the tubules was just beginning to become lightened. Large cells almost disappeared. There was an increased number of spermatogonia present, but these had not yet differentiated into primary spermatocytes.

Day 14 Postnatal

This stage was distinguished by the first appearance of a few primary spermatocytes in some tubules. The number of germinal elements was much greater than those at 10 days of age. The large cells completely disappeared. The small cells formed two or three layers around the periphery, but were not as well arranged as at later ages,

Day 18 Postnatal

The tubules increased in size. The lumen became more prominent and was present in almost all the tubules. There was a considerable increase in the number of tubules with a high portion of germinal elements which formed approximately three layers around the periphery. Some tubules contained numerous round spermatids.

Day 22 Postnatal

There was an additional increase in tubule size and the lumen was very prominent in the majority of the tubules. There were more than three layers of germinal elements. In many tubules primary spermatocytes were very numerous with less secondary spermatocytes and spermatids.

DISCUSSION

Tests weights were suppressed by radiation on all days selected in this study. The smallest mean testes weight of irradiated animals was only 12.5% of the control testes weight and the least suppressed testes weight was 84% of control weight. Mean testes weights in all irradiated group in the present work were significantly suppressed. Curvey (1) irradiated hamsters of the same strain with the same dose on the same days. However, mean testes weight of animals irradiated on day 15 of postnatal life did not differ from the control group significantly. But in both experiments, hamsters irradiated on days 15 and 3 of postnatal life had the largest and the smallest testes weights respectively among the irradiated groups. A similar radiosensitivity pattern based on testes weights was thus established. For some unexplained reason, the total number of seminiferous tubules in the testis cross section of hamsters irradiated on days 5, 7, and 18 in the later study was greater than those in the earlier study (Table 2 and Figure 1). Except for this, smooth curves based on total number

of tubules and number of active tubules are obtained with day 3 of postnatal life being the most radiation-affected stage.

Fertility tests of male hamsters irradiated with 220 R (1) indicated that the lowest fertility occurred following irradiation on days 1 and 3 of postnatal life. Thus low testes weights, low number of tubules, and a minimum number of active tubules in this study all coincide with the lowest fertility on day 1 and 3 of postnatal life.

The maximum reduction in testes weights noted by Hupp *et al.* (6) in rats was on day 18 to 22 of prenatal life. Such a period found in hamsters and the stage of development appears to correspond to that in rats. The testes of hamsters irradiated on days 1, 3, 5, and 6 of postnatal life are the most sensitive to irradiation and, since the hamster has an average gestation length of 16 days, are also 17, 19, 21, and 22 days from conception. Animals irradiated on these days also exhibited a decreased number of active tubules. Radiation sensitivity was less in the animals irradiated prenatally; following the most sensitive period, testes weights and the number of active tubules increased toward the control values as the age increased. Hupp *et al.* (6) also found that rats irradiated on days 17 prenatal and 1 postnatal exhibited reduced fertility. Such stages of development in both rodent are also similar.

From the study of development of testes of fetal and neonatal hamsters, it was observed that the primitive type of germ cells (gonocytes) were present during days 13 and 15 of prenatal life and also days 1, 3, and 5 of postnatal life and then became gradually spermatogonia from day 7 of postnatal life on. The low radiosensitivity noted at days 13 and 15 of prenatal life was associated with gonocytes that were smaller in size and mitotically active. The high radiosensitivity in animals irradiated on days 1, 3, and 5 of postnatal life was associated with gonocytes that were larger in size and had little or no mitotic activity. At later days of postnatal life in hamsters, radioresistance increased corresponding with the presence of more spermatogonia, spermatocytes, and spermatids (7).

Observations of the irradiated testes have been compared with those made in the study of normal gonadal development. The present results indicate that there is a relationship between the quantitative and qualitative changes in the gonads with increasing age and radiosensitivity.

The method of classification of seminiferous tubules is not ideal and needs to be improved. Curvey (1) indicated that many Class 2 tubules in irradiated animals other than those irradiated on days 1 and 3 postnatally contained some sperm, indicating that the entire tubule was not inactive as was the case in the absolute Class 2 tubules of animals irradiated on days 1 and 3 postnatally. Similar observations were made in the present study. In addition, the tubules classified as Class 4 ranged from those with a few terminal elements to those with less than 50 sperm or terminal elements and it is sometimes very difficult to make a distinction between Classes 4 and 5. Some of the tubules classified as Class 5 are probably not fully active. Some of normal diameter have somewhat less cells; also, some smaller in diameter than normal have a normal concentration of cells but would contain less total cells. Thus improvements in the technique of classifying tubules should be worked out.

Since the gestation length in hamsters is rather short (16 days), and animals achieve sexual maturity at an early age, the development of testes progresses very rapidly. A study on developing testes at every day during the period of rapid development instead of every few days would provide a more complete picture of the process of development.

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