Acta Oncologica 27 (1988) Fasc. 4

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THE AGE DEPENDENCE OF RADIATION SENSITIVITY OF THE GONADS OF FEMALE MICE

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Abstract

Female CBA-mice were continuously gamma-irradiated with 0.9 or 2.4 Gy. The dose was given during a 4-day period with start at 50, 90, 135 or 190 days of age respectively. Fifty-six days after the irradiation the females were killed and the number of germ cells in their ovaries were compared with that from unirradiated control females of corresponding age. The total number of germ cells was, compared with the controls, reduced by about 70% in all age groups after the dose of 0.9 Gy and to between 3 and 8% after 2.4 Gy. Seven stages of oocyte development were observed as well as 2 stages in degeneration. The observations suggest a changing radiation sensitivity by age during an initial period of about 90 days followed by a period of fatal radiation effects.

Key words: Radiobiology, mice, gamma irradiation, female germ cells, age dependence.

Numerous investigations have been performed on the radiation sensitivity of the female gonads. Many of these investigations in rodents concerned the effects of external radiation on ovaries in foetuses as well as in juvenile females (2, 4, 8–13). Concerning internal emitters and especially ^{90}Sr, Nilsson & Henricson (6) reported on the effects of that nuclide given to pregnant mice on the 11th and 16th day post conception, and in later investigations the present author has studied the effects of radiation from ^{90}Sr on the foetal and juvenile ovaries in mice (14, 15). In these papers it was primarily reported a reduced number of germ cells as observed in the irradiated young female offspring. Moreover, disturbances of the reproductive capacity as well as an increased tumour incidence were observed in females treated in utero during the very last period of their foetal development (17).

The irradiation effects on the germ cells have been supposed to be age dependent with a decreasing sensitivity in higher ages. An interest therefore arose in a study of the injury of moderate gamma-doses on the ovaries of adult mice in different ages, and its significance for a presumptive incidence of ovarian cancer. The present investigation was designed to examine the radiation effects on the gonads of female mice aged between 50 and 190 days when irradiated.

Material and Methods

Female, non-pregnant CBA-mice were continuously gamma-irradiated during a 4-day period with the start at one of the following ages: 50, 90, 135 and 190 days respectively. In all age groups the females were irradiated with either 0.9 or 2.4 Gy respectively, and groups with non-irradiated females of the same age served as controls (Table 1).

The irradiation was performed with a ^{137}Cs-source with a dose rate of 0.0268 Gy/h (2.81 R/h) at a distance of 1 m from the source, measured in air with a Farmer 2570

Table 1

Experimental schedule

Age of females at irradiation (days)	No. of females Gamma-doses (Gy)		
	0 (Control)	0.9	2.4
50	10	10	10
90	7	15	15
135	9	15	15
190	9	15	15

Accepted for publication 29 January 1988.

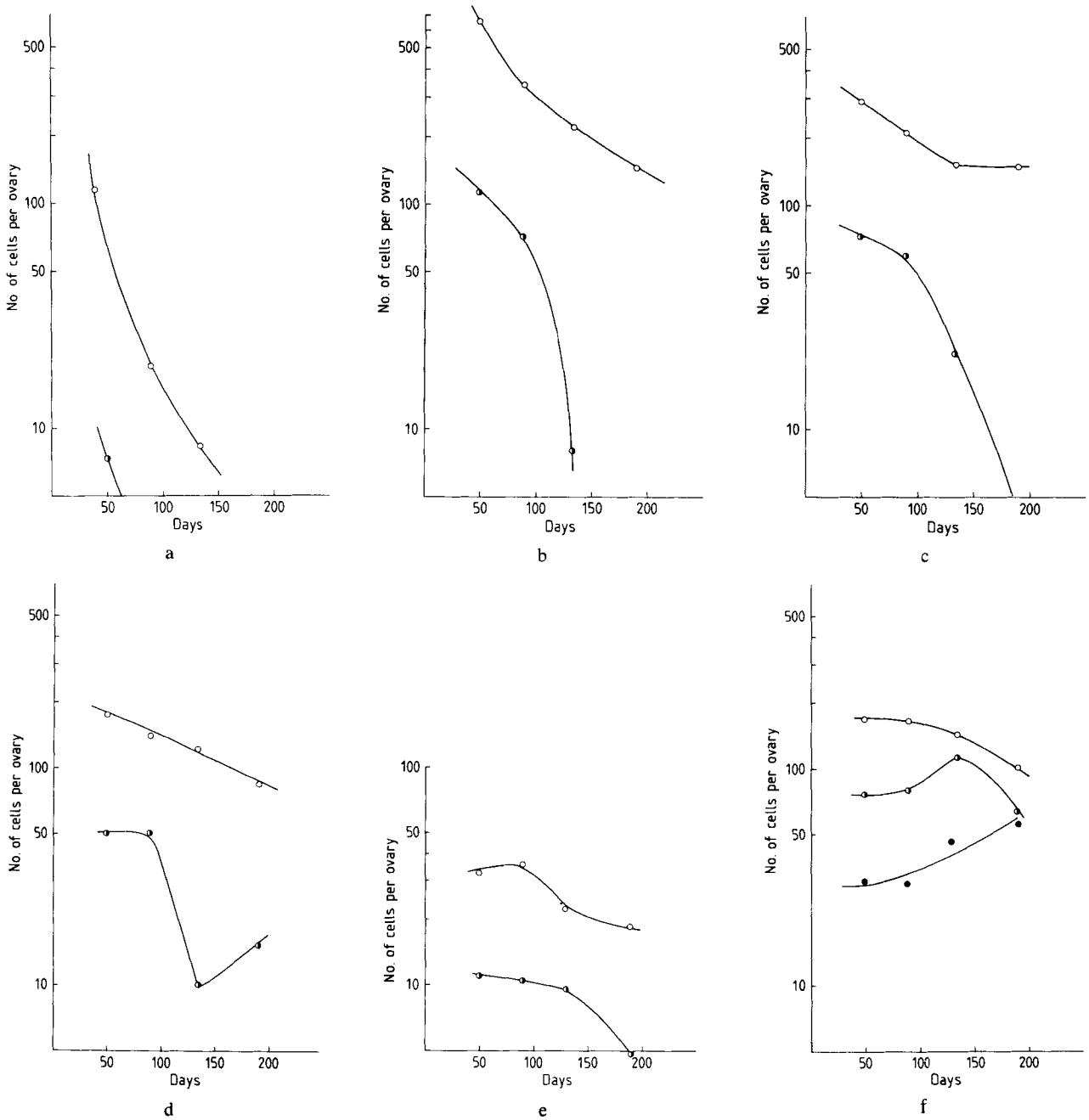

Fig. 1. Number of cells in different stages of development when scored 56 days from end of irradiation; a) oocytes type I, b) oocytes type II + III, c) primary follicles, d) growing + early antrum follicles, e) graafian follicles, and f) atretic follicles + corpora atretica. Non-irradiated controls (○), females irradiated with 0.9 Gy (◑) and females irradiated with 2.4 Gy (●) (only in 1f).

instrument. The ionizing chamber was placed 1 m above the floor, horizontally and perpendicular to the direction of the beam. The dose rates given by the instrument in roentgen (R) have been transformed into gray (Gy) according to EULEP (3). The factor for transforming 1 R, measured in air, into Gy in the case of gamma irradiation from ^{137}Cs, equalled 0.00955 according to ICRU Report No. 17, Table 6. 2b (5).

By adjusting the distance between the source and the animal cages proper dose rates were achieved to give the total doses of 0.9 and 2.4 Gy respectively, with an uncertainty not higher than 10%, mostly depending on the animals' moving in the cages. The irradiation was continuously given during 4 days except for a short period each day, when the animals were inspected and cared for.

After an interval of 56 days from the end of irradiation 5

Table 2A

Adult CBA-females γ-irradiated at different ages. Remaining number of germ cells 56 days after the end of the irradiation period

Age (days)	γ-dose (Gy)	No. of animals	Oocyte type I	Oocyte type II	Oocyte type III	Prim. foll.	Grow. foll.	Early antrum	Graaf. foll.	Atret. foll.	Corp. atret.	Total sum X̄±SE	Percentage of controls
50	–	4	115.0 ±32.0	299.4 ±18.3	358.4 ±10.7	287.7 ±26.3	162.6 ±19.3	12.0 ±6.3	33.3 ±6.3	94.1 ±11.7	74.3 ±5.6	1 436.8 ±45.3	–
50	0.9	5	7.3 ±5.0	46.0 ±7.7	66.0 ±10.0	73.3 ±10.0	42.6 ±4.3	6.9 ±2.6	11.3 ±3.5	33.9 ±2.0	42.1 ±2.0	329.4 ±15.2	22.9
50	2.4	5	0 –	0 –	2.0 ±1.3	0.7 –	0.4 –	0.4 –	0 –	0.9 ±0.4	29.0 ±5.7	33.4 ±5.9	2.3
90	–	3	19.0 ±9.7	101.0 ±7.7	241.0 ±30.7	210.0 ±10.0	128.3 ±1.3	6.5 ±2.6	36.3 ±5.0	80.4 ±5.7	82.5 ±7.2	905.0 ±36.2	–
90	0.9	5	0	14.7 ±4.3	56.0 ±9.0	59.3 ±7.3	46.5 ±8.9	3.5 ±1.1	10.4 ±2.6	26.5 ±3.5	52.9 ±7.1	269.8 ±17.4	29.8
90	2.4	5	0.7 –	0.7 –	1.3 –	0.7 –	0 –	0 –	0 –	0 –	28.5 3.4	31.9 –	3.5
135	–	4	8.3 ±6.3	71.7 ±21.0	148.4 ±28.3	151.0 ±10.0	109.4 ±5.0	10.4 ±2.0	22.4 ±3.9	75.0 ±17.6	65.3 ±11.2	661.9 ±43.1	–
135	0.9	5	1.3 –	0.7 –	7.3 ±3.4	22.0 ±3.4	3.7 ±8.7	6.1 ±2.4	9.6 ±2.0	20.9 ±5.2	90.6 ±4.7	162.2 ±12.6	29.5
135	2.4	5	0	0	0	0	0	0	0	0	45.4 ±11.2	45.4 –	6.9
190	–	4	1.0	34.3 ±7.7	108.4 ±12.7	150.0 ±16.0	78.3 ±10.9	5.4 ±1.3	18.5 ±5.7	45.2 ±15.4	55.4 ±8.2	496.5 ±30.6	–
190	0.9	5	0	0	1.3	3.3 ±1.5	13.5 ±3.6	1.3 ±0.5	4.4 ±1.5	7.8 ±3.1	55.5 ±8.2	87.1 ±9.7	17.5
190	2.4	5	0	0	0	0	0	0	0	0.9 ±0.4	55.6 ±16.3	56.5 –	11.4

Table 2B

The frequency (in percentage) of cells in different stages of development as compared to corresponding controls 56 days after the end of the irradiation period

Age (days)	γ-dose (Gy)	No. of animals	Oocyte type I	Oocyte type II	Oocyte type III	Prim. foll.	Grow. foll.	Early antrum	Graaf. foll.	Atretic foll.	Corp. atret.	All stages
50	0.9	4	6.4	15.4	18.4	25.5	26.2	57.5	33.9	36.0	56.7	22.9
50	2.4	5	0	0	0.6	0.2	0.3	3.3	0	1.0	39.0	2.3
90	0.9	5	0	14.6	23.2	28.2	36.2	53.9	28.7	33.0	64.1	29.8
90	2.4	5	3.7	0.7	0.5	0.3	0	0	0	0	34.6	3.5
135	0.9	5	15.7	1.0	4.9	14.6	33.8	58.7	42.9	26.7	138.7	29.5
135	2.4	5	0	0	0	0	0	0	0	0	69.5	6.9
190	0.9	5	0	0	0.9	2.1	17.2	24.1	23.8	17.3	100.2	17.5
190	2.4	5	0	0	0	0	0	0	0	2.0	100.4	11.4

randomly chosen females from each of the irradiation groups and 3 to 4 from corresponding control groups were killed. The excess number of females were left for study of survival time and tumour incidence. The ovaries of the sacrificed females were immediately fixed in Stieve's fluid and histologically prepared according to conventional methods. One ovary per female was serially sectioned at 5 μm and stained with haematoxylin-eosin. Microscopic analysis was performed of every 10th section including counting of oocytes and follicles in 9 different stages of development according to previous investigations (14).

The stages in question were: oocyte of type I without any surrounding granulosa cells; oocytes of types II and III with an increasing number of such cells; primordial oocytes where the granulosa cells form one complete layer; growing follicles with several layers but with no antrum formation; early antrum follicles with an antrum initiated and, as the seventh stage, the graafian follicles with a complete antrum formation in the follicular epithelium. Also 2 degenerative stages were counted: atretic follicles characterized by lytic and pycnotic cells in the follicular layers and often with a shrunken oocyte; and finally the corpora atretica, being the final stage of atresia in growing and graafian follicles.

By means of a correction formula (1) the observed number of germ cells was adjusted to give the total number in the ovary.

Results

Analysis of ovarian content. The number of germ cells in the different stages of development as well as the total number per ovary when scored 56 days after the end of the irradiation period, is given in Table 2 A and graphically in Fig. 1 a–f. They are presented in relation to the age of the females when being irradiated with either 0.9 or 2.4 Gy respectively. Table 2 B gives the frequencies (in percentages) of these cell numbers related to corresponding control values. In the unirradiated control group the total number of germ cells was strongly, negatively correlated to increasing age of the females. In females irradiated with 0.9 Gy the observations followed a similar pattern as concerns the rate of decrease. The reduction of the total number of germ cells was, however, about 70% of that of the control females. The dose of 2.4 Gy reduced the number of germ cells by about 92 to 97% compared with the control figures but, opposite of the other two groups, there seems to be a slight increase in the remaining, total number of the irradiated germ cells by increasing age.

The courses given at the individual germ cell stages are shown in Fig. 1 a–f as concerns the untreated females as well as those irradiated with 0.9 Gy. The number of cells in the highest dose group was strongly reduced and could be presented graphically only in Fig. 1 f, showing the degenerative stages.

The observations of oocyte types II and III have been added and so were those for growing and early antrum follicles, Fig. 1 b and d respectively. Also the 2 stages with cells in degenerative stages were united (Fig. 1 f).

The number of oocyte type I decreases very rapidly by increasing age in the untreated females (Fig. 1 a). There was, e.g. a reduction by about 93% units during the period of 85 days from the scoring of the 50-day group to that of the 135-day group. Irradiation with 0.9 Gy of the 50-day-old females caused the same effect giving a reduction of the oocyte number to only 6.3% of the control figure. In the older groups this cell stage was totally eliminated by the irradiation.

Unirradiated oocytes of types II and III showed a more moderate age-related decrease (Fig. 1 b). The gamma dose of 0.9 Gy reduced the number of such oocytes by about 80% in the 50- and 90-day-old females. In 135-day-old females only 4% of the control number of oocytes remained and irradiation at 190 days p.p. had totally eliminated in the initially low number of these oocytes.

Unirradiated primary follicles as well as growing and early antrum follicles were numerically reduced by age but at a lesser rate than were the oocytes. The dose of 0.9 Gy seems to have caused an accelerated reduction of the cell number, especially in 135- and 190-day-old females (Fig. 1 c and d). It should be noted, however, that the observations in the irradiated 135-day group, especially concerning the growing follicles (Fig. 1 d), suffered from unexpectedly great deviations around the mean. The lower part of that curve is therefore rather uncertain.

The unirradiated graafian follicles were numerically halved within the time interval studied (Fig. 1 e). After 0.9 Gy the animals showed only a faint decrease of the number of graafian follicles in the first 3 age groups, but a

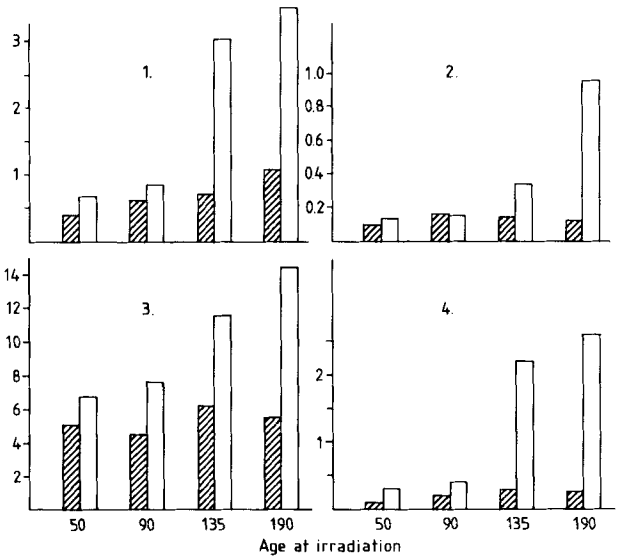

Fig. 2. Ratios No. 1–4, given for different ages of the females and formed by groups of germ cells in different development stages. (For details, see text and Table 3.) Non-irradiated females (■) and females irradiated with 0.9 Gy (□).

Table 3

Ratios between cell numbers in different stages of development in unirradiated as well as in gamma-irradiated mice. Studied in CBA-mice at four ages*

Ratios	Treatment**	Age at start of irradiation (days) (scored 56 days later)			
		50	90	135	190
1. Prim. + E. antr. foll./	C	0.388	0.600	0.702	1.081
Oocyte I–III	γ	0.672	0.888	3.022	3.538
2. Graafian foll./	C	0.111	0.168	0.140	0.119
Prim. foll. + E. antr.	γ	0.141	0.166	0.342	0.957
3. Degen. foll./	C	5.057	4.488	6.263	5.438
Graafian foll.	γ	6.726	7.635	11.615	14.386
4. Degen. foll./	C	0.133	0.220	0.269	0.254
Oocyte I–Graaf. f.	γ	0.300	0.417	2.199	2.660

* The actual number of cells are taken from Table 2.
** C: Nonirradiated control females. γ: Females irradiated with 0.9 Gy.

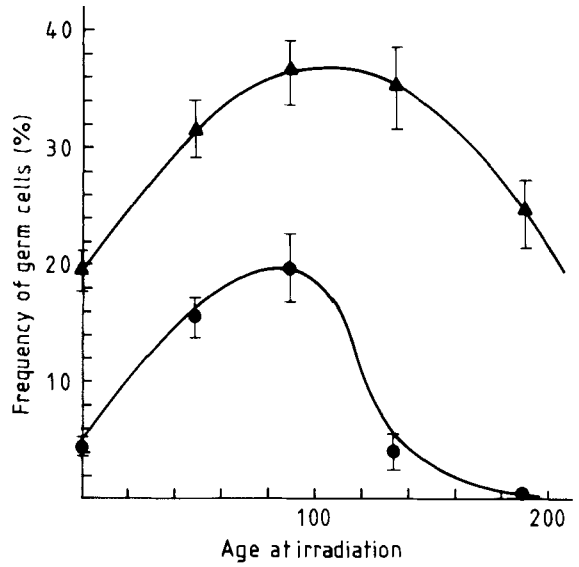

Fig. 3. Frequency of germ cells in percentage of corresponding control figure given for females gamma-irradiated with 0.9 Gy at different ages. The bars indicate Standard Errors. Oocytes type I–III (●) and germ cells, other than oocytes (▲).

Table 4

Frequency of remaining oocytes type I to III and remaining germ cells other than oocytes, in females irradiated with 0.9 Gy versus control females

Age at treatment (days)	Oocytes I to III		Germ cells other than oocytes	
	Mean ± SE	t-value[a]	Mean + SE	t-value[a]
0[b]	4.2±0.2	–	19.5±2.1	–
50	15.4±1.9	5.862***	31.6±2.5	3.706**
90	19.6±3.3	4.658**	36.6±2.8	4.886**
135	4.1±1.6	0.06	35.3±3.4	3.954
190	0.9	–	24.3±3.3	1.227

[a] t-values after comparisons with respective values in 0-day group.
[b] From: Rönnbäck (1983), Acta Radiol. Oncol. 22, 6, Table 3.
*** $p<0.001$. ** $0.001<p<0.01$.

sudden reduction of number was found in the 190-day group.

The number of germ cells in degenerative stages in the control females did also decrease by age (Fig. 1 f), but to a much lower extent than in most of the previously described groups. It could also be noted that in females irradiated with the lowest gamma dose the observations did not indicate any generally enhanced rate of the decrease by age. On the contrary, the findings in the highest dose group suggested an increase (though insignificant) of the number of cells by increasing age.

Survival time. The remaining number of females (about 10 in each treatment group) will be studied for survival time after the irradiation and for the presumptive incidence of cancer. These findings will later on be reported.

Discussion

The radiation sensitivity and consequently the injury to the mouse ovaries is assumed to decrease by increasing age. In previous investigations the author has observed that female CBA-mice, when 56 days old and gamma-irradiated with 0.9 Gy during the 4 days around their birth, had only about 10% of the total number of germ cells as found in unirradiated control animals of the same age. That gamma dose given around the birth eliminated the highly radiosensitive young oocytes by about 95% of the control figure. The corresponding figures for females irradiated when 85–90 days and scored when 165 days of age was about 50% and the frequency of oocytes was decreased to near 34% (16).

In the present investigation the ages at irradiation ranged from 50 to 190 days. The gamma doses chosen were 0.9 Gy, as in the previously mentioned studies, and 2.4 Gy as based on the assumption of a higher resistance in older females. The results obtained indicated that the latter dose was too high, since practically all germ cells were eliminated except for those in the stage of corpora atretica. It should also be underlined that after the 16th day of gestation, i.e. after the termination of the DNA-synthesis, no further formation of oocytes takes place in the mouse ovaries. After that time there is a successive depletion of oocytes. The age-related, numerical decrease of germ-cells in untreated female mice, as presented in Table 2, perfectly well followed previously observed courses. The gamma dose of 0.9 Gy depressed the ovarian content by about 75% and followed a similar course as in the controls. The observations after the dose of 2.4 Gy indicated, however, a different situation. Though a rather low number of germ cells remaining, a faint increase in that number was observed with increasing age of the females at the irradiation. This would suggest a seemingly lower radiation sensitivity in the ovaries by increasing age, probably depending on the fact that the early, more sensitive stages of germ cells at that time already were eliminated.

To investigate whether or not a change in the development rate of germ cells, as discussed by Henricson & Nilsson (4), could be verified in any groups of cell stages, ratios were set up for comparisons between the observed numbers in the controls versus the irradiated groups respectively:

Ratio No. 1:

$$\frac{\text{Primordial foll. + Early antrum foll.}}{\text{Oocytes stages I–III}};$$

Ratio No. 2:

$$\frac{\text{Graafian foll.}}{\text{Prim. foll.} + \text{Early antrum foll.}};$$

Ratio No. 3:

$$\frac{\text{Atretic foll.} + \text{Corpora atretica}}{\text{Oocytes type I} - \text{Graafian foll.}}.$$

The numerical values of the ratios, given in Table 3 and visualized in Fig. 2, show that in control females there is no important difference between the blocks of cells correlated to the age. On the contrary, the irradiated individuals show ratios more or less increasing by age. Ratio No. 1 (Fig. 2:1), comparing the two blocks of young germ cells, shows among the control females a slightly, though not significantly linear increase by age, mostly depending on the decreasing number of oocytes. In the irradiated ovaries this decrease is much more pronounced, especially in the 2 oldest groups, reflected by a nearly exponential increase of the ratios.

In next constellation (Fig. 2:2) the numbers from the non-irradiated females were of practically the same order of magnitude. A dose of 0.9 Gy caused a decrease of the number of primordial and early antrum follicles large enough to give a significantly exponential increase of the ratios ($0.01>p>0.002$).

In the comparison between follicles in degeneration stages versus graafian follicles (Fig. 2:3) no trend in the control females appeared by increasing age. In the irradiated females there was a significantly exponential increase ($0.01>p>0.002$) mainly depending on a 50% decrease of the number of graafian follicles within the age span investigated.

In the last comparison (Fig. 2:4) with cells at degeneration stages (atretic follicles and corpora atretica) versus all developing stages up to and including the graafian follicles, the decrease in cell number in the 2 groups of germ cells went in parallel with each other in the non-irradiated females. The irradiation with 0.9 Gy eliminated the sensitive young oocytes and thus lead to a more general decrease of the number of developing cells. This was most obvious in the 135- and 190-day groups and visualized by suddenly increased values of the ratio.

The seemingly lower radiation sensitivity in older female mice, often characterized by a higher number of germ cells remaining after irradiation, could thus partly be attributed to a situation with an ovary more or less depleted of the sensitive oocytes and young follicles, and consequently with an increasing proportion of cells in less radiosensitive stages. From that point of view the sensitivity depends on which fraction of germ cells being present in the ovaries at the time of irradiation. On the other hand, the observations in the irradiated mouse ovaries, as presented in Table 4 and Fig. 3, show an increasing frequency of remaining germ cells versus the untreated ovaries up to a maximum at about 100 days of age. The germ cells were here divided into 2 classes: oocytes type I–III and germ cells in all other stages.

The curves representing the 2 classes of germ cells have relatively parallel courses. The one showing the oocytes indicates a maximum of near 20% of the control figure whereas the curve reflecting the other cell stages (including the degenerative stages) has its maximum just above 35%.

This effect is also evident from Fig. 2:1 and 4, where the ratios formed for the irradiated germ cells were moderately affected when the irradiation had occurred in the ages of 50 or 90 days, but significantly accelerated in the 2 groups older than 100 days when irradiated. Evidently these increased ratios were mainly caused only by the elimination of oocytes.

These observations in the CBA-mouse suggest a changing radiosensitivity of the germ cells by age with a faintly increasing resistance during the first 3 months. Thereafter the fatal radiation effect seems more accentuated.

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