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EFFECTS ON FOETAL OVARIES AFTER PROTRACTED, EXTERNAL GAMMA IRRADIATION AS COMPARED WITH THOSE FROM INTERNAL DEPOSITIONS

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Investigations by among others BEAUMONT (1962), JONES & KROHN (1961), MANDL (1964), OAKBERG (1966), PETERS (1961) and PETERS & LEVY (1964) have shown that foetal ovaries in rodents are highly sensitive to external ionizing radiation. Especially oocytes in the first stage of development, 'the naked oocytes' with no or very few surrounding granulosa cells, were also numerically reduced after very low amounts of ^{90}Sr given to the pregnant dam during late pregnancy (RÖNNBÄCK 1979, 1980). Owing to the complicated retention situation in the foetuses after ^{90}Sr had been administered to their mothers, no estimation of the radiation doses was available.

The present investigation therefore aimed at a comparison between the effect on the foetal ovaries by low doses of gamma radiation given continuously during four days around the birth and the injury caused by ^{90}Sr contamination of the dams previously observed.

Material and Methods

Irradiation facilities. A ^{137}Cs source was used, giving 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 instrument. The ionizing chamber was placed 1 m above the floor, horizontally and perpendicular to the direction of the beam.

The proper dose rates, and thus the doses ranging from 0.09 to 0.91 Gy, given during four days were achieved by adjusting the distance between the source and the animal cages. The irradiation proceeded continuously except for a short period each day when the animals were inspected and cared for. For doses and number of animals see Table 1.

The dose rates given by the instrument in roentgen (R) have been transformed to gray (Gy) according to EULEP (1981). The factor for transforming 1 R, measured in air, to Gy in the case of gamma irradiation from ^{137}Cs, equalled 0.00955 according to ICRU Report No. 17, Table 6.2 b.

Animals. Female CBA mice, aged 70 to 80 days, were mated to untreated males of the same strain. The onset of the pregnancy was controlled by the daily examination for the presence of a vaginal plug. The day when a 'plug' was noted was taken as Day 1. The pregnant females, separately caged, were placed in one of three irradiation groups or in a parallelly run, untreated, control group (Table 1).

The litters in the irradiation groups were chronically gamma irradiated during four days from the 19th day post coitum to the 2nd day post partum.

Analysis of ovaries. From the juveniles 7 randomly chosen females from each dose group were killed at 56 days of age. Their ovaries were immediately fixed in Stieve's fluid and histologically prepared according to conventional methods. The ovaries were serially sectioned at 5 μm and stained with haematoxylin-eosin. Every 10th section was taken for microscopic analysis including counting of oocytes and follicles in different stages of development.

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Table 1

Experimental schedule

Gamma doses (in Gy) continuously given during 19th day p.c. to 2nd day p.p.	No. of dams	No. of young*				No. of females used for	
		At birth		At weaning		Ovarian analysis at 56 days p.p.	Fertility test during 245 days
		F	M	F	M		
Control	16	24 (10)	36	22 (10)	33	7	14
0.09	8	24 (7)	21	24 (7)	21	7	14
0.20	17	49 (15)	46	44 (14)	39	7	12
0.91	11	29 (10)	37	23 (9)	30	7	16

* No. of litters within parentheses

Also 5 randomly chosen mothers from each dose group were killed and treated in the same way as their daughters. They were killed at an age of about 165 days, and had been exposed to gamma irradiation during the four-day period when between 85 to 90 days old.

All females were given a code number and the microscopic analysis was subsequently performed as a blind test to avoid any bias.

The cell material was divided into 7 classes as in previous investigations on the effects of ^{90}Sr on the ovaries (RÖNNBÄCK 1980): oocytes of type I without any surrounding granulosa cells, type II and III oocytes with an increasing number of such cells (but not forming a complete layer), primary oocytes with one complete layer of granulosa cells, and growing follicles with several layers but with no antrum formation, early antrum follicles with an antrum initiated and finally Graafian follicles with a complete antrum formation in the follicular epithelium. Also two degenerating stages were counted: atretic follicles and corpora atretica; the former characterized by lytic and pycnotic cells in the follicular layers and often with a shrunken oocyte, and the latter being the final stage of atresia in growing and Graafian follicles.

The observed number of germ cells was adjusted to give the total number in the ovary by means of a correction formula (ABERCROMBIE 1946). A comparison between the figures from the irradiated animals versus those from the controls gave an estimate of the injury caused by the irradiation.

Fertility test. Those of the females not suggested for ovarian analysis (Table 1) were mated when 65 to 70 days old to one untreated CBA male each during a period of 245 days. During that period the number of litters born per female were noted as well as the date for their births. Owing to an unusually high rate of mortality among the newborns no reliable recordings on litter size at birth and at weaning were possible. This non-radiation related loss of young appeared in all groups to be of about the same extent without any known reason. After the terminated fertility test, the females were caged for recording of survival time and presumptive tumour incidence.

Table 2

The production of young irradiated during 4 days from the 19th day post coitum to the 2nd day post partum

Dose group	Mean litter size*		Diff. (d)
	At birth	At weaning	
Control	6.0 (10)	5.0 (10)	−1
0.09	6.4 (7)	6.4 (7)	0
0.20	6.3 (15)	5.9 (14)	−0.4
0.91	6.6 (10)	5.9 (9)	−0.7

* No. of litters within parentheses.

Results

As appears from Table 2 the gamma irradiation apparently did not cause any lethal effects on the young notable during the weaning period, as the greatest loss in the litters was observed in the control group.

Table 3

Analysis of ovaries from 56 day old CBA mice, irradiated from the 19th day post coitum to the 2nd day post partum. Number of remaining germ cells per ovary. Mean ± SE

Irrad. dose	No. of females	(1) Ooc. type I	(2) Ooc. type II	(3) Ooc. type III	(4) Prim. foll.	(5) Grow. foll.	(6) E. antrum foll.	(7) Graafian foll.	(8) Atretic foll.	(9) Corp. atrec.	Total sum (±SE)	Total number in per cent of control
Control	7	233.1 ±41.1	394.5 ±35.9	398.7 ±30.1	209.1 ±14.4	115.2 ± 9.8	21.7 ±1.3	39.4 ±8.8	89.1 ±9.5	74.3 ±5.8	1 575.1 ±66.3	–
0.09 Gy	7	52.2 ±14.9	181.0 ±16.0	242.4 ±9.6	126.7 ±11.3	79.1 ±14.2	9.6 ±1.2	16.6 ±3.2	72.1 ±2.7	51.9 ±2.7	831.6 ±30.4	52.8
0.20 Gy	7	19.1 ± 9.7	28.1 ±13.1	96.7 ±39.0	83.4 ±23.2	41.3 ± 8.9	7.5 ±1.4	11.5 ±3.3	26.1 ±6.3	43.0 ±6.6	356.7 ±50.0	22.6
0.91 Gy	7	8.6 ± 4.3	11.4 ± 5.1	22.9 ± 5.3	28.6 ± 8.9	23.0 ± 2.1	3.1 ±0.4	5.6 ±1.6	14.0 ±2.7	32.9 ±5.0	150.1 ±13.8	9.5

However, the ovaries of the juvenile females were in fact injured by the irradiation and the degree of injury was shown to be clearly dose related. Table 3 gives the actual numbers of remaining oocytes and follicles in 56 day old females in the different treatment groups. The two columns to the right indicate a reduction of the total number of germ cells to about 50 per cent already in the lowest dose group (0.09 Gy), and the frequency of remaining cells in the two groups given 0.20 and 0.91 Gy had decreased to 22.6 and 9.5 per cent, respectively, all compared with the corresponding control value.

The table also gives information on the irradiation effects on the different stages of oocyte and follicular development (columns 1–9). Compared with the control figures, all stages in 0.20 and 0.91 Gy groups show a strong, statistically significant reduction in the number of germ cells ($p \leq 0.01$). After the highest irradiation dose all stages were reduced to a numerically very low level, strongly significantly different from that of the controls. No stages were, however, totally eliminated. Even at a dose level of 0.09 Gy statistically significant differences were revealed in practically all stages of development with only the growing and atretic follicles expected. Here too, the probability figures were less than 0.01. The most serious reduction occurred in the young stages, especially among the oocytes.

The microscopic investigation was extended also to include the 165 day old females, mothers of the in utero exposed young and irradiated during 4 days themselves at the delivery when 85 to 90 days old. The total number of germ cells as well as the figures obtained for the different stages are presented in Table 4. Though reduced by age, the number of germ cells in the different stages formed a 'profile' that resembled those presented from 56 day old females irradiated in utero. However, the initial number of oocytes type I was very low in the old females and was depressed to zero after gamma doses of 0.20 and 0.91 Gy. The other stages appeared less radiation sensitive in these females that were irradiated as adults, when compared with those irradiated around their birth. The total number of germ cells in the two groups exposed to 0.20 and 0.91 Gy, respectively, fell significantly below those of the controls ($p \leq 0.01$). In contrast, in the lowest dose group there was an obvious increase of the total number of germ cells compared with the controls ($0.02 > p > 0.01$). However, if the analysis in the latter dose group concerned individual stages of the oocyte and follicle development, the differences were not significant, probably due to large distributions around the mean.

Table 5 summarizes the observed reproductive capacity of in utero irradiated females after a 245-day mating period. All females but one (found in the 0.20 Gy group) gave birth to at least one litter. The mean number of litters per fertile female during the whole period evidently decreased with increasing radiation dose, though not linearly. The cumulative litter production during eight 30-day intervals from the start of mating up to 245 days is recorded in Fig. 1. It is apparent that there were no major deviations in the production of litters during the first three months in the different dose groups. During the

Table 4

Analysis of ovaries from 165 day old CBA mice. The females were exposed during 4 days together with their litters during the 2 last days of gestation and the 2 days after delivery at an age of 85 to 90 days. Number of remaining germ cells per ovary. Mean ± SE

Irrad. dose	No. of females	(1) Ooc. type I	(2) Ooc. type II	(3) Ooc. type III	(4) Prim. foll.	(5) Grow. foll.	(6) E. antrum foll.	(7) Graafian foll.	(8) Atretic foll.	(9) Corp. atrec.	Total sum (±SE)	Total number in per cent of control
Control	5	7.3 ±2.0	55.3 ±14.5	143.4 ±13.6	180.0 ±21.1	92.6 ± 8.4	12.6 ±2.1	16.5 ±2.0	49.6 ±8.6	93.0 ±6.2	650.3 ±32.2	–
0.09 Gy	5	15.3 ±4.3	89.4 ±14.2	198.0 ±15.9	213.4 ±17.3	118.3 ± 7.8	11.7 ±2.9	20.0 ±3.0	93.5 ±23.7	74.1 ±6.1	833.7* ±38.1	128.2
0.20 Gy	5	0	34.0 ±15.5	88.7 ±29.6	112.7 ±32.0	53.1 ±11.4	5.7 ±1.5	10.0 ±3.5	33.9 ±12.6	56.5 ±5.6	394.6 ±49.7	60.7
0.91 Gy	5	0	12.7 ± 8.9	56.7 ±25.2	76.7 ±27.1	45.7 ±17.8	4.4 ±1.8	9.6 ±2.0	31.7 ±12.0	77.5 ±9.1	315.0 ±44.7	48.4

* The increase versus control is significant: $0.02>p>0.01$.

Table 5

Reproductive capacity. Litter production

Dose (Gy)	No. of litters during 245 days	
	Total	Per fertile female, mean ± SE
Control	95	6.8±0.5 (14)*
0.09	68	4.8±0.6 (14)
0.20	39	3.3±0.6 (11)
0.91	43	2.9±0.3 (15)

* Figures within parentheses indicate the numbers of fertile females.

Table 6

Reproductive capacity. Time intervals between litters

Dose (Gy)	Interval between mating and 1st litter (days), mean ± SE	'Fertility span',* mean ± SE
Control	23.1±0.9 (14)	161.3±15.9 (14)
0.09	23.0±1.3 (14)	99.1±14.1 (14)
0.20	26.5±2.9 (11)	78.4±15.6 (9)
0.91	22.1±0.4 (15)	53.5± 6.4 (13)

* Fertility span = interval between birth of the first and last litter (12).

following period of three months, on the other hand, the decrease in litter production was manifest, and during the last two months practically no more litters were born except in the control group.

The time interval between the start of mating and birth of the first litter did not seem to be affected by the irradiation (Table 6). On the other hand, the interval between birth of the first and the last litter, 'the fertility span' according to PETERS (1969) seemed to be negatively, exponentially correlated to increasing radiation dose (Fig. 2).

Discussion

Radiostrontium (^{90}Sr) has been shown to seriously affect the oocytes and follicles during ovarian development in mice contaminated as foetuses. The later in development the contamination occurred, the worse was the injury. Even very minute amounts of ^{90}Sr (11 kBq, i.e. 0.3 μCi) given to the dam on the 19th day of pregnancy reduced the number of oocytes in the young ovaries to about 50 per cent (RÖNNBÄCK 1980). Greater amounts led to disturbances of fertility conditions as well as an increased rate of ovarian tumours (RÖNNBÄCK 1981, RÖNNBÄCK & NILSSON 1982).

No direct determination of the radiation doses to the foetal ovaries was made in these investigations as the retention situation is very complex. However, the distance between the pelvic bones of the mother and the ovaries in the foetuses has been estimated to be too large to give a notable radiation dose to the ovaries of the young. On the other hand, the mineralization of the foetal skeleton (which starts around the 15th day post coitum; ZORZOLI 1948) leads to an increasing degree of deposition of radiostrontium into the bones. This means that a strontium-contamination after that time will constitute a permanent, internal radiation source near the ovaries of the foetuses. For natural reasons, the

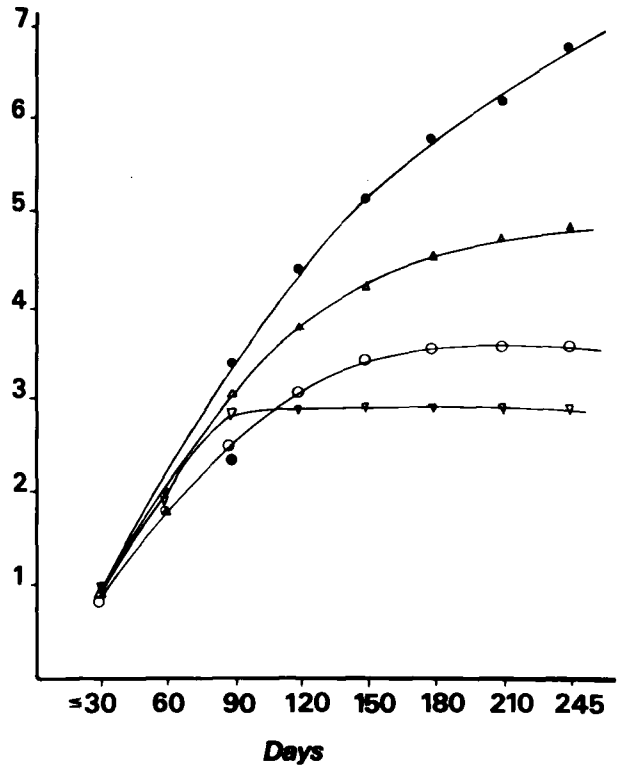

Fig. 1. Litter production during eight 30-day intervals. Untreated controls (●). From pre- and neonatally irradiated females: 0.09 Gy (⊿) 0.20 Gy (○) and 0.91 Gy (▽).

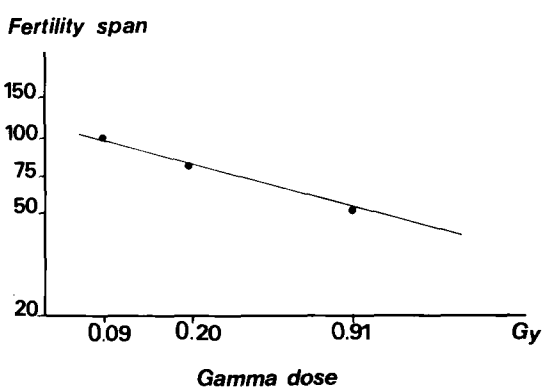

Fig. 2. Time interval (in days) between birth of the first and the last litter (fertility span).

intensity of exposure decreases with increasing age and dimensions of the young. The injury to the ovaries, expressed as a significant decrease in the number of young oocytes, increases the later during pregnancy the contamination of the dam has occurred. The radiation sensitivity of the oocytes decreases from the 15th day of gestation to about one day before partus, when most oocytes are in a late pachytene stage (BEAUMONT & MANDL 1962). After birth the sensitivity increases when the oocytes enter the dictyate stage, and it shows a peak about 72 hours after birth, when most oocytes have reached this very sensitive stage (OAKBERG 1979).

The period with external, continuous gamma irradiation from ^{137}Cs in the present investigation was chosen to approximately cover the same period around the birth when internal irradiation from ^{90}Sr had been shown to be most deleterious (RÖNNBÄCK 1979). The three dose levels were arbitrarily chosen and ranged from 0.09 to 0.91 Gy.

As in previous investigation with 1.5 Gy given to CBA mice during the 14th to 18th day of gestation (RÖNNBÄCK & SHERIDAN 1979), no obvious effect of the irradiation on litter size or on the survival of the litters was observed during the weaning period nor during the period up to the adult age.

The examination of the ovaries of 56 day old pre- and neonatally irradiated females revealed a dose related decrease of the number of remaining oocytes and follicles with increasing doses (Table 3). The total number of germ cells in the untreated controls agreed very closely with the figures found in previous investigations with ^{90}Sr contamination of the foetuses. Also the pattern of decrease in the different stages of oocyte development resembled that of earlier findings. The youngest oocyte stage was now, as usually found, subjected to the most serious numerical reduction. Only one fourth of the control value remained at 56 days p.p. even after the very low dose of 0.09 Gy delivered during the four days. This observation corresponds to that made in females contaminated by ^{90}Sr in utero with about 22 kBq given to the dams on the 19th day p.c. (RÖNNBÄCK 1980).

The same effect, though to a lesser degree, was observed when the ovaries of the older females (mothers) from the two higher dose groups were analysed . These mice were killed at about 165 days of age, and the survival time after the irradiation period was about 75 days, i.e. two and a half months compared with approximately one month for the younger females. An age-related loss of germ cells normally occurs in females and the figure of 650 as the total number observed in the control group (Table 4) coincides very well with the previous findings for CBA mice within the range of 14 to 330 days of age (RÖNNBÄCK 1982).

More astonishing were the results from the group of mothers exposed to the lowest radiation dose (0.09 Gy): in nearly all stages of development, except for the early antrum follicles and Corpora atre-

tica, far higher numbers of cells were found than those in the controls. The number of 'naked oocytes' (type I) were more than twice as high as in the control, and in oocyte type II a corresponding figure of 1.6 was noted.

In these experiments on irradiation effects on young ovaries most irradiation groups consisted of at least five animals. This had throughout the series given a statistically acceptable distribution around the mean. On the other hand, when the figures from the separate development stages in the ovaries from 165 day old mice were analyzed, the numbers of germ cells were found to be too extensively scattered to allow any prediction as to significant differences between the means. However, with all stages taken together the total number of germ cells in the 0.09 Gy group showed an increase, compared with controls, at a significance level of $0.05>p>0.01$, an effect never seen before.

There is no obvious explanation of the real increase of the cell number in this dose group. An analysis of the frequency of cells in each development state, expressed as per cent of the total sum of germ cells per individual, gives no indication of an enhanced rate of the development process in the irradiated females. The curves from the irradiated animals did not demonstrate any significant deviations from those of the controls (Fig. 3), which would have been the case had the process been speeded up.

In the two groups exposed to higher radiation doses the total cell number (Table 4) was significantly decreased ($p<0.001$), but less pronounced than in females killed and examined at 56 days p.p. Thus, in spite of an age correlated decrease in the radiation sensitivity, a gamma dose of 0.20 Gy totally eliminated the 'naked' oocytes and caused a numerical reduction of the other cell stages to a level between 50 and 60 per cent of the control figure.

The injury to the in utero gamma irradiated ovaries of the 56 day old females in the present investigation was compared with that previously reported for females ^{90}Sr contaminated in utero on the 19th day p.c. (RÖNNBÄCK 1980). The effect after 0.20 and 0.91 Gy approximately equalled what was found after 195 and 355 kBq ^{90}Sr, respectively, administered to the dam. Based on these figures calculations by WALINDER (1982) indicated that the effects of an amount of 11.1 kBq ^{90}Sr given to the dam would approximately correspond to those seen after doses to the foetal ovaries of between 0.010 and

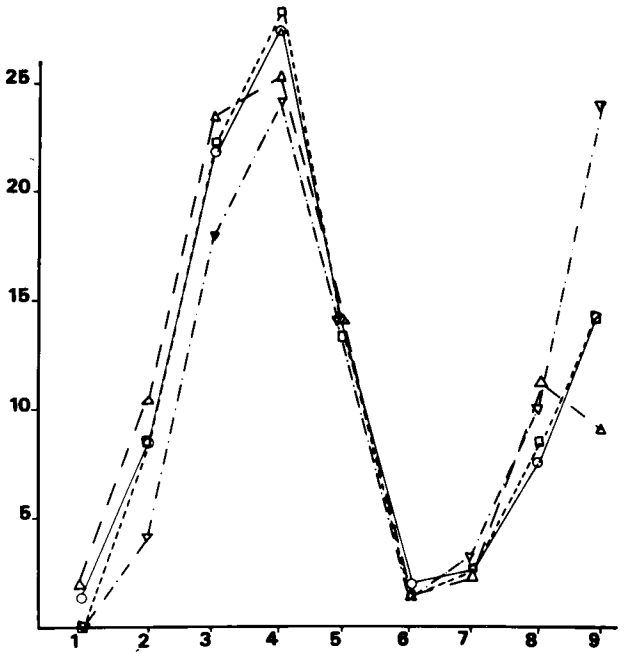

Fig. 3. CBA females, 165 days of age, irradiated when 85 to 90 days old. The frequency of germ cells (in per cent, calculated on the total number per individual female) plotted against the nine stages of oocyte development, as given in Table 3.

0.013 Gy. If the foetuses were presumed to have recieved the gamma dose (D) continuously during a 4-day irradiation period, it would correspond to an acute dose (D_{ekv}) ranging from 0.0054 to 0.0067 Gy, according to the formula: $D_{ekv}=0.8 \cdot D \cdot 4^{-0.29}$, given by KIRK et coll. (1972) and modified by WALINDER (1979).

The severe histologic effects observed on female germ cells in the CBA mouse and especially on oocytes in early development stages has been supposed to depend on a very high radiation sensitivity rather than on high radiation doses delivered. The calculations above seem to confirm this assumption.

In a previous paper the author reported on a decreased reproductive capacity in females ^{90}Sr treated in utero, when the nuclide was given to the dam on the 19th day p.c. (RÖNNBÄCK 1981). The ^{90}Sr amounts ranged between 46.3 and 740 kBq, and in spite of an obvious histologically detected reduction of the number of germ cells also after the lowest ^{90}Sr dose, the effect on the litter production was not apparent until a dose level of 185 kBq. With higher doses the effect increased rapidly. Very roughly this amount of the nuclide would correspond to a total radiation dose of 0.2 Gy to the foetal ovaries, according to the figures given above.

In the present experiment the litter production

decreased already after 0.09 Gy (Table 5), a nearly significant difference ($0.2>p>0.01$). This 'threshold' around 185 kBq of ^{90}Sr, above which the decrease of the reproductive capacity accelerated in the nuclide experiment (RÖNNBÄCK 1981), seems thus to be lowered in the present experiment. A dose level of 0.09 Gy is only about half of the presumptive radiation dose from 185 kBq ^{90}Sr, but nevertheless it affects the ovaries to such a high degree.

On the other hand, the effect of the gamma irradiation expressed in terms of the 'fertility span' seems contradictory, as the figures from the highest gamma irradiation group did not significantly differ from those obtained after 370 kBq ^{90}Sr, whereas the figure from the 185 kBq group, 128.2 (RÖNNBÄCK 1982), was significantly higher ($p\approx0.01$) than what was found in the present 0.20 Gy group, the nearest dose level to which it is to be compared.

Whether or not these differences in the observations within the field of reproductive capacity are of some dignity may be doubtful. However, it is a fact that the young oocytes are extremely sensitive to low doses of ionizing radiation from internally deposited radiostrontium as well as from external, protracted gamma irradiation. A very rough estimation of the effects of ^{90}Sr versus those from the gamma irradiation gave the result that a figure of 2 to 5 kBq ^{90}Sr corresponded to 0.01 Gy from ^{137}Cs under the present circumstances.

SUMMARY

Effects of low doses of protracted gamma irradiation on pre- and neonatally exposed ovaries in CBA mice have been compared with those previously obtained by ^{90}Sr contamination of the dam. The total number of germ cells at 56 days of age was reduced to about 50 per cent after a dose of 0.09 Gy given during four days from the 19th day post coitum to the 2nd day post partum. The highest dose level used (0.91 Gy) reduced the frequency to about 10 per cent. Females exposed to the same dose levels at an age of 85 to 90 days showed a lesser degree of injury. The reproductive capacity in females exposed around their birth (expressed as the number of litters per female) was negatively correlated to the dose of gamma irradiation. The effects on the foetal ovaries of 2 to 5 kBq ^{90}Sr given to the dam on the 19th day of gestation seemed to correspond to those from 0.01 Gy from ^{137}Cs under the present circumstances.

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