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STRONTIUM RETENTION IN MOUSE FOETUSES AT DIFFERENT INTERVALS AFTER CONTAMINATION OF THE DAM

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Abstract

Pregnant CBA mice were intravenously contaminated with radiostrontium (^{85}Sr) on either the 14th, 16th or 18th day post coitum. The radiostrontium content in the foetuses and corresponding placentas as well as that in the femurs of the mothers was measured separately and individually on the 19th day post coitum, i.e. after intervals of 5, 3 and 1 day, respectively. The retained amount of strontium in the foetuses successively increased by the time of contamination, i.e. the later during pregnancy the contamination occurred. Expressed as per cent of the injected amount of ^{85}Sr, the figures ranged from 4.6 per cent in foetuses contaminated on the 18th day to 0.7 per cent for those contaminated already on the 14th day after coitus, with a significantly negative correlation between time and retained amount of radiostrontium. The amount of strontium in the femurs of the mothers was reduced within the same intervals, due partly to decalcification of the skeleton of the mother during the latest part of the pregnancy.

Key words: Fetus; strontium-90, mice.

Internal ^{90}Sr contamination of pregnant CBA mice drastically reduces the number of germ cells in the foetal ovaries. The early oocyte stages in the foetuses, in particular, are extremely radiation sensitive, as was demonstrated after very low amounts of ^{90}Sr given to the dam during late pregnancy. Higher amounts of the nuclide affected the fertility conditions as well as the incidence of ovarian tumours in the in utero contaminated mice (6, 7, 10).

Owing to the complicated distribution of ^{90}Sr in the foetuses no estimations of the radiation doses to the foetal ovaries have yet been made. However, the effects on the number of germ cells after external gamma irradiation of the litters at the time around their birth have been compared with the effects from internally deposited ^{90}Sr (9), by which 'equivalent, external gamma doses' could be related to the amounts of ^{90}Sr administered to the dams. The very low, whole body gamma doses to the dams are not likely to induce any secondary effects of significance to the observed reduction of oocytes and follicles.

The subject of the present investigation was the retention of radiostrontium in mouse foetuses during late stages of development, as correlated to different intervals between the administration of radiostrontium to the dams, and sacrifice.

Material and Methods

Female CBA mice, aged about 85 days, were mated with untreated males, from the same strain. The onset of pregnancy was determined by the appearance of a vaginal plug and the day when a plug was observed was defined as day 1. The pregnant females were distributed to three treatment groups where the females (and their foetuses) were strontium contaminated on one of three occasions: on the 14th day, on the 16th day, or on the 18th day after coitus (Table 1). A total of 0.15 ml of an ^{85}Sr solution ($^{85}SrCl_2$, carrier-free water solution from Amersham International, England), diluted with physiologic saline to a strength of 370 kBq/ml giving an administered ^{85}Sr amount of 55 kBq/female was injected into a tail vein. The same aliquot (0.15 ml) of the ^{85}Sr solution was taken to a standard tube.

The pregnant dams were killed on the 19th day post coitum. Foetuses and corresponding placentas were prepared separately and their retained amounts of ^{85}Sr measured. The activity in the right femur from each dam was also measured. All measurements were performed with a scintillation spectrometer (Packard Auto Gamma Twin Scaler 5210) within 12 hours from sacrifice.

Each dam had previously been given an identification number, and, within each litter, the foetuses also were numbered in such a way that 'foetus (and placenta) No. 1'

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Table 1

Experiment schedule

Administration of ^{85}Sr (days after p.c.)	Interval (days) between injection and sacrifice (measurement)	No. of females (dams) injected	No. of foetuses analysed
14	5	7	54
16	3	11	89
18	1	8	57

were located in the upper end of the uterus next to the ovary. Also the positions, left and right side, were indicated. As every foetus thus was identified according to its uterine position a special analysis was made to investigate a possible correlation between the intra-uterine localisation of the foetus and its retention of the nuclide. The material obtained from the measurements was therefore divided into three classes: 1) from the foetuses in the uppermost position in the two uterine horns, 2) from those in the middle position and 3) from those in the lowest position. (In the case of an even number of foetuses in the horn the arithmetic mean was formed for the retention of the two centrally positioned foetuses.)

Results

The number of dams and foetuses investigated, as well as the survival time in each treatment group, appear in Table 1. The mean litter size, i.e. the number of living foetuses per fertile female, was observed to be almost the same in all three groups (Table 2). The occasional appearance of dead foetuses as resorption plates did not show any preference for any particular treatment group.

The retained amount of radiostrontium in the right femurs of the mothers on the 19th day after coitus was successively reduced with the decreasing interval of survival after an administration on the 14th through the 18th day post coitum. The retention in the foetuses and in the placentas demonstrated an opposite time dependence although the slope of the line calculated from the placenta measurements was only about one hundredth of that of the foetuses (Fig. 1). The ratio between the retention on the 19th day in the foetus versus that in the corresponding placenta consequently gave the lowest value for administration on day 14 after coitus and rose linearly with shorter time intervals (Table 2).

An increasing level of radiostrontium in the placenta was correlated to a very modest increase of the retention in the foetuses in the animals contaminated on the 14th day (Fig. 2). In animals contaminated on the 16th day as well as on the 18th day the two regression lines were almost parallel and had a significantly ($p<0.001$) steeper slope than the slope appearing after a contamination on the 14th day.

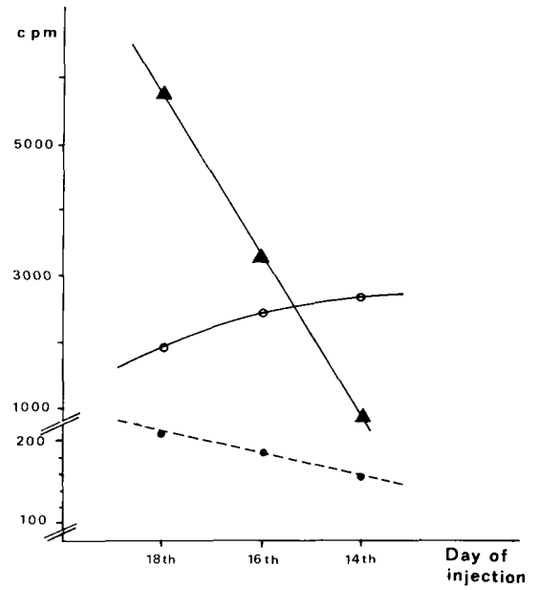

Fig. 1. Retention on the 19th day p.c. in the foetus (▲), the placenta (●) and the mother's femur (○) after ^{85}Sr contamination on either the 14th, the 16th or the 18th day of pregnancy.

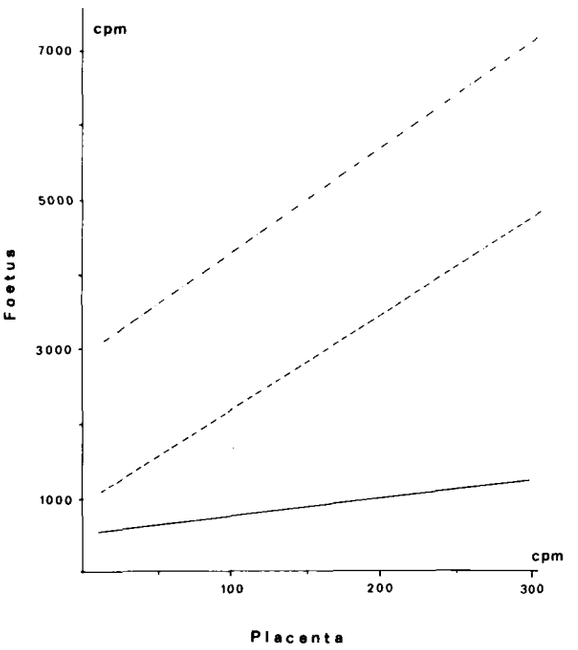

Fig. 2. Retention in the foetus on the 19th day p.c. as a function of the retention in the corresponding placenta. Contamination: 14 (——), 16 (– – –) and 18 (–·–) days p.c.

The retention values from the foetuses and placentas expressed in per cent of the injected amount of ^{85}Sr are presented in Table 3. Concerning the foetuses, the figures ranged from 4.6 per cent in foetuses contaminated on the 18th day to 0.7 per cent for those contaminated on

Table 2

Results of the activity measurements (in cpm). Mean ± SE. Number of females given in parentheses

Contamination periods (days p.c.)	No. of live foetuses per litter	Retention (cpm) in			Foetus/placenta
		Right femur of the mother	Individual foetuses	Corresponding placentas	
14th to 19th	7.7±0.3	2 657±187 (n=7)	880±14.9 (n=54)	158±2.7 (n=54)	5.45±0.16
16th to 19th	7.6±0.6	2 456±140 (n=11)	3 288±79.8 (n=89)	189±3.1 (n=89)	17.43±0.38
18th to 19th	7.5±1.0	1 898±184 (n=8)	5 749±21.7 (n=57)	210±6.1 (n=57)	27.79±0.96

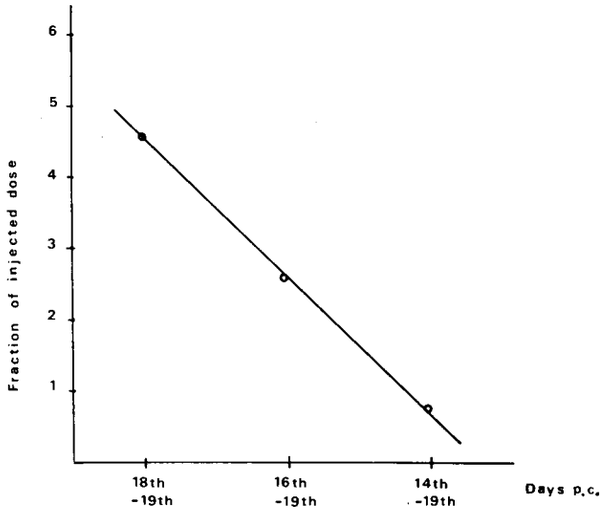

Fig. 3. Fraction (as per cent) of the injected amount of ^{85}Sr found in the foetuses at three intervals after contamination of the dam.

Table 3

*Retention on the 19th day in per cent of injected dose**

Contamination periods (days p.c.)	Right femur of the mother	Foetuses	Placenta
14th to 19th	2.1	0.7	0.13
16th to 19th	1.9	2.6	0.15
18th to 19th	1.5	4.6	0.17

* 0.15 ml of the ^{85}Sr solution as 'standards' (= the same amount that was administered to the dam corresponding to 55 kBq ^{85}Sr) gave 126 272±897 cpm.

the 14th day, with a significantly negative correlation ($0.01>p>0.002$) in this investigation between time and retained amount of radiostrontium (Fig. 3). The amounts retained in the placentas were very minute, about 0.15 per cent.

Discussion

The placental transfer of radionuclides has been intensively studied, and particularly the transfer of radiostrontium was early investigated by, among others, HOLMBERG et coll. (1), KRIEGEL (2) and NELSON et coll. (3). It was stated that the ability of the foetuses to retain any considerable amounts of that nuclide does not appear until the start of the ossification of the foetal skeleton (around the 15th day of foetal development in rodents). The retained amount of the nuclide has also been shown to be linearly correlated to the increasing weight of the foetus, i.e. of its skeleton (2).

In previous studies the litters were mostly lumped together and the results were given as for the whole litter, while in the present investigation the foetuses (and corresponding placentas) were measured individually. The analysis has given an estimate of the amounts of radiostrontium retained in the foetuses at an age of 19 days after coitus in relation to the amount administered to the dams on three different occasions during pregnancy. Nothing could, however, be ascertained from this material about the course of the retention during the time interval between administration and the measurement. The investigation also included presumptive effects on the strontium retention of the litter size as well as of the intrauterine position of the foetuses. However, the mean litter size in the three experiment groups deviated very little from one another and the deviations around the mean within each group were small, which excluded the opportunity to find any statistical correlation between litter size and radiostrontium content.

WARD et coll. (12) observed that after exposure to external gamma irradiation early in uterine life, embryos in the ovarian and cervical ends of the uterus had a higher mortality than embryos in a middle part of the uterine horn. No influence from the position in the uterus on the uptake of radiostrontium could, however, be demonstrated in the present investigation (Table 4).

It is thus most likely that the distribution of the radiostrontium to the foetuses was also even between the

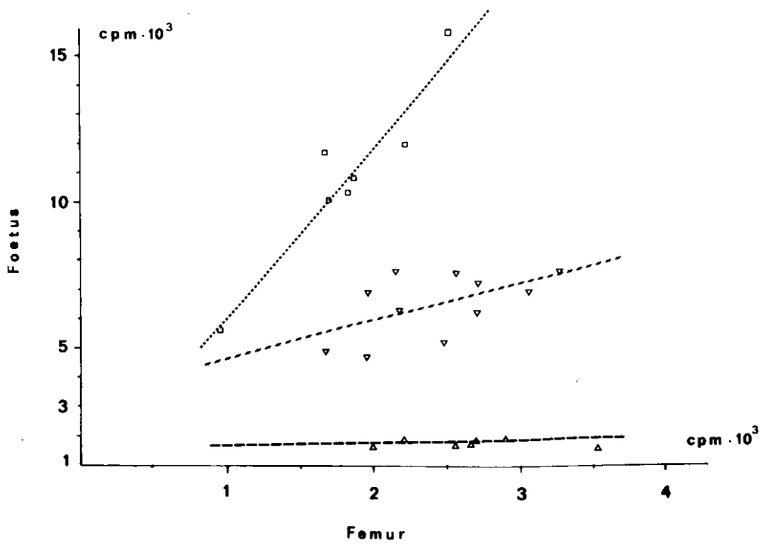

Fig. 4. Correlation between the activity measured in the foetuses and the activity in the corresponding femur of the dam. Intervals: 5 days (△), 3 days (▽) and 1 day (□).

individuals in the preceding experiments, which concerned injury to the foetal ovaries after a ^{90}Sr contamination of the dam (8). The deviations observed in the descendents might thus be due to individual differences in sensitivity.

The present measurements gave an estimate of the amount of the nuclide retained in each foetus (Table 3). The results might be used in similar strontium experiments to calculate approximately the amounts of ^{90}Sr transferred into the foetuses and thus the radiation doses to young CBA mice.

The injury to the ovaries of the in utero treated animals was histologically described as a decrease in the number of oocytes in early developmental stages, caused already by 11.1 kBq ^{90}Sr to the dam. This corresponds to about 0.5 to 0.6 kBq ^{90}Sr per foetus. A seriously disturbed reproductive capacity as well as an increased incidence of proliferative events were not noted until after an amount above 185 kBq ^{90}Sr, i.e. 8.5 kBq or more per foetus.

The negative correlation between the retained fraction of radiostrontium and the day of administration, as presented in Fig. 3, is so far in agreement with KRIEGEL's finding (2) of a linearity between the retention and the weight of the foetuses, i.e. the increasing weight of the foetal skeleton by age. However, the foetus incorporates seemingly very small amounts of strontium before the onset of ossification of its skeleton, considered to start on the 15th day of gestation in mice (13). The strontium content in the skeleton is thus not only a question of the foetal weight but is also dependent on the metabolic activity of the skeleton. After contamination of the mice on the 14th day of pregnancy or earlier most of the radiostrontium goes into the mother's skeleton, with only a minor part being absorbed by the foetuses, with a placental transfer that does not seem to exceed the needs of the growing embryo (11). Still lower retention values would therefore be expected in the foetuses contaminated as early as on the 14th day after coitus.

Table 4

Retention (as cpm) in foetuses with regard to their uterine position. Mean ± SE

Treatment periods (days p.c.)	Uterine position		
	Uppermost	Middle	Lowest
14th to 19th	859±34	883±30	867±27
16th to 19th	3 163±149	3 308±154	3 252±151
18th to 19th	5 449±323	5 765±499	5 560±400

Previous measurements of the radiostrontium content in blood from CBA mice contaminated intravenously as adults revealed a whole body retention, 24 hours after the injection, less than 5 per cent of that obtained 30 min after the injection, and after 48 hours the amount was almost negligible (5). A comparison with the results from a previous investigation on the radiostrontium content in CBA foetuses 90 min after administration of the nuclide to the dams (3) shows that the present figures are approximately 2.5 per cent higher. This higher level most likely depends on a continued strontium uptake in the foetal skeleton in spite of the very low concentration in the blood passing the placenta. This uptake, however, accelerates during the final part of the pregnancy by an increasing level in the blood, depending in its turn on a certain degree of decalcification of the maternal skeleton. It was shown by NILS-

son et coll. (4) that the strontium burden in parts of the skeleton of CBA females, e.g. the femurs, was significantly diminished in females that had had a number of pregnancies, even if the litters were not allowed to suck their mothers. Foetuses contaminated on the 14th day post coitum do not reflect varations in the activity level in the femur of the mother as indicated by the lowest line in Fig. 4. On the other hand, foetuses aged 16 and 18 days when contaminated show a positive correlation with the steeper slope obtained from the oldest individuals, reflecting an increasing rate of the ossification process and thus an increasing weight of the foetal skeleton.

The investigation thus gave an estimate of the amount of radiostrontium retained in the foetuses one day before birth as a consequence of an internal contamination of the dam during pregnancy. The injury previously observed in the foetal ovaries of contaminated CBA mice is a result of the very complicated irradiation situation including, among other things, the parameters of uptake and retention of the nuclide in the foetuses as well as in their mothers, together with the variations in radiation sensitivity during different stages of oocyte and follicular development.

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