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PLACENTAL TRANSFER OF STRONTIUM 85 IN MICE

by

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The high radiosensitivity of the foetus has made the placental transfer of radiostrontium of particular interest. A number of investigations have been performed. The transfer of ^{90}Sr from mother to foetus has been investigated in our laboratories and reported by HOLMBERG, NELSON & WALLGREN (1960). The investigation included the time-course of uptake by the end of gestation, the uptake at different stages of gestation, and the foetal uptake in matings at different time-intervals following injection of the females. The results disclosed that the ability of the foetal skeleton to take up ^{90}Sr appeared after the 14th day of gestation, and it was further demonstrated that ^{90}Sr injected into the mother was available to the foetus in undiminished quantity for at least 4 weeks.

The accessibility of ^{85}Sr, which is a γ-emitter, and of a 'small-animal counter' with a plastic scintillator, have made it possible for us to repeat the investigation with greater accuracy and to obtain supplementary data.

Material and Methods. A total number of 560 pregnant CBA female mice, all approximately 75 days old at the start of the experiment, were used.

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Table 1

Experimental scheme

Number of litters at different time-intervals between injection and sacrifice:	Day of pregnancy	15	45	90	180	240	min
Series I	12	15	15	15	15	15	15
	14	15	15	15	15	15	15
	16	15	15	15	15	15	15
	18	15	15	15	15	15	15
		90 min	2 days	4 days	6 days	8 days	10 days
Series II	12	10	15	15	15	—	15
	14	10	15	15	—	15	—
	16	10	15	—	15	—	—
	18	10	—	15	—	—	—
Number of litters from females injected at different time-intervals before mating		1 week		2 weeks			4 weeks
Series III	18	20		20			20

The radiostrontium used consisted of ^{85}Sr nitrate in nitric acid solution diluted with physiologic saline. The injection volume was in all cases 0.3 ml and contained about 0.3 μCi of ^{85}Sr. This activity gave a counting rate of approx. 59 000 cpm at the initial measurement in the animal injected.

The animals were mated and the day of pregnancy was determined by controlling the vaginal plug. The females were injected in a tail vein on different days of pregnancy (the 12th, 14th, 16th and 18th) with ^{85}Sr solution and were measured within three minutes in the 'small-animal counter' (NILSSON 1961). This counter consists of a large well-shaped plastic crystal detector having a length of 240 mm and a diameter of 90 mm; attached to the scintillator is a photomultiplicator tube connected to a conventional counter unit. The animals were put in a cylindric plastic box and placed in the detector in such a way that the center was on the central axis of the crystal. After a certain time the activity of the females was measured again and then they were sacrificed; the weight and activity of each foetus was determined.

The experiment was carried out in three stages as indicated in Table 1.

Table 2

A — Results of series I

Day of pregnancy	Time between inj. and sacrifice	Mean litter size	Initial measurement, cpm in thousands (mean ± SE)	Measurement at sacrifice, cpm in thousands (mean ± SE)	Activity of foetus*, cpm in thousands (mean ± SE)	Activity of foetus in % of that of pregnant mother**
12	15 min	7.67	54.03 ± 1.41	52.80 ± 1.35	0.02 ± 0.01	0.03 ± 0.01
	45 »	6.87	51.11 ± 1.94	51.55 ± 1.98	0.03 ± 0.01	0.05 ± 0.01
	90 »	7.80	53.56 ± 2.10	49.18 ± 1.62	0.03 ± 0.01	0.05 ± 0.01
	180 »	7.13	53.35 ± 2.06	51.93 ± 1.26	0.02 ± 0.01	0.04 ± 0.01
	240 »	5.73	50.29 ± 2.15	44.26 ± 2.71	0.02 ± 0.01	0.05 ± 0.01
14	15 min	6.93	59.33 ± 1.84	57.82 ± 2.17	0.06 ± 0.01	0.10 ± 0.02
	45 »	7.67	62.59 ± 1.99	56.08 ± 1.87	0.10 ± 0.02	0.18 ± 0.03
	90 »	7.00	58.29 ± 1.50	52.28 ± 2.08	0.09 ± 0.02	0.18 ± 0.03
	180 »	7.20	55.12 ± 2.52	48.03 ± 1.82	0.08 ± 0.01	0.17 ± 0.03
	240 »	7.06	52.92 ± 2.11	48.23 ± 2.23	0.06 ± 0.01	0.14 ± 0.02
16	15 min	6.95	61.12 ± 2.82	58.61 ± 2.40	0.18 ± 0.01	0.31 ± 0.02
	45 »	7.00	55.28 ± 1.76	53.30 ± 1.72	0.26 ± 0.02	0.49 ± 0.04
	90 »	7.13	59.51 ± 2.33	53.50 ± 2.28	0.37 ± 0.01	0.71 ± 0.04
	180 »	6.80	58.29 ± 2.62	53.46 ± 2.89	0.43 ± 0.03	0.81 ± 0.01
	240 »	6.87	60.55 ± 1.99	53.76 ± 2.49	0.51 ± 0.02	0.96 ± 0.01
18	15 min	7.20	55.71 ± 1.33	56.49 ± 1.58	0.58 ± 0.04	1.03 ± 0.06
	45 »	7.73	55.51 ± 1.12	53.82 ± 1.51	0.96 ± 0.05	1.81 ± 0.11
	90 »	7.66	55.89 ± 0.78	52.07 ± 1.40	1.21 ± 0.05	2.33 ± 0.09
	180 »	6.64	54.33 ± 1.52	52.33 ± 1.74	1.45 ± 0.05	2.80 ± 0.11
	240 »	6.47	59.81 ± 1.45	55.92 ± 3.50	1.61 ± 0.08	2.92 ± 0.14
			B — Results of series II			
12	90 min	5.70	53.14 ± 3.11	49.37 ± 2.76	0.05 ± 0.01	0.10 ± 0.02
	2 days	7.07	54.82 ± 2.47	24.23 ± 0.94	0.04 ± 0.01	0.15 ± 0.03
	4 »	6.81	70.24 ± 2.97	30.33 ± 1.42	0.04 ± 0.04	0.15 ± 0.04
	6 »	7.56	62.78 ± 2.96	22.42 ± 1.38	0.15 ± 0.01	0.68 ± 0.05
14	90 min	7.50	61.09 ± 2.46	57.40 ± 3.20	0.16 ± 0.02	0.27 ± 0.03
	2 »	7.07	69.80 ± 2.24	32.64 ± 1.75	0.13 ± 0.02	0.42 ± 0.07
	4 »	7.21	73.48 ± 1.71	32.18 ± 1.55	0.29 ± 0.01	0.90 ± 0.06
16	90 min	7.00	61.51 ± 3.77	57.72 ± 3.90	0.50 ± 0.04	0.87 ± 0.05
	2 days	7.25	67.50 ± 3.44	34.87 ± 1.39	1.46 ± 0.10	4.21 ± 0.28
18	90 min	7.90	75.87 ± 3.39	78.81 ± 2.81	1.42 ± 0.06	1.82 ± 0.08
			C — Results of series III			
	1 week	7.35	71.30 ± 0.91	18.67 ± 0.62	0.10 ± 0.01	0.53 ± 0.08
	2 weeks	7.25	81.93 ± 4.77	20.42 ± 0.79	0.10 ± 0.02	0.47 ± 0.07
	4 »	6.74	77.78 ± 1.43	13.81 ± 0.96	0.05 ± 0.02	0.42 ± 0.06

*Activity of foetuses = mean activity (and SE) of the mean foetus of each litter.

**The mean of the mean activity of the foetuses in each litter in percent of the activity of its pregnant mother.

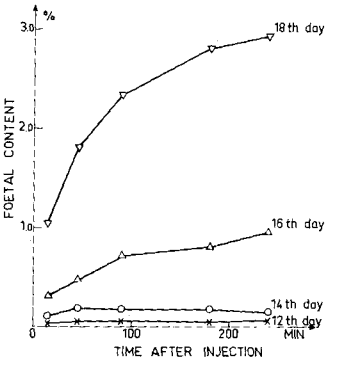

Fig. 1. Rate of uptake of ^{85}Sr (15 to 240 min) administered at various days of gestation.

Results

The results of the investigation are shown in Table 2 and Figs 1 and 2. The short time-intervals (Table 2-A) were chosen in order to give an idea of the time-course of the foetal uptake of strontium on different days of gestation, i.e. the distribution rate after administration.

Equilibrium seems to occur very early on the 12th, 14th and 16th day of gestation, but on the 18th day it takes a little longer. At 240 min after the administration, however, the slope appears to be levelled. Because of the difficulties in isolating embryos macroscopically before the 12th day, no measurements were made earlier, and due to the 2-day intervals between examinations, the 18th day has been the last one. On the 20th, and sometimes on the 19th day, the mice begin to bring forth young.

The longer time-intervals (Table 2-B) indicate the transfer of ^{85}Sr from mother to foetus due to the growth of the foetuses and their ossification.

The administration of strontium before mating was undertaken in order to investigate the effect of gestation on the strontium incorporated in the skeleton of the mother.

It may be noted that the results of the initial measurements are relatively constant. The observed distribution is in part due to the difficulties in achieving constant geometry, since the mice were not anaesthetized, and in part to the method of measurement. The same applies to the results of measurements at sacrifice.

The differences between the initial measurements and the measurements at sacrifice are insignificant for the short time-intervals in series I. The excretion from the mothers seems to be too small. In series II and III the differences are obvious, the greatest being evident between measurements at 90 min and at 2 days. After this time the differences seem to be fairly consistent. The

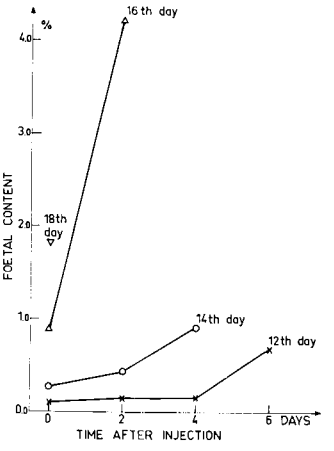

Fig. 2. Rate of uptake of strontium 85 (90 min to 6 days) administered at various days of gestation.

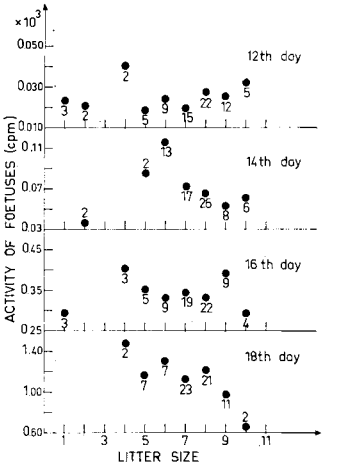

Fig. 3. Relation between mean foetal activity and litter size; figures at the points indicate number of litters.

values representing changes with time do not deviate from the well-known strontium excretion curve in non-pregnant animals.

The 'activity of the foetuses' recorded in Table 2 is the mean of the mean foetal activity within each litter. A decrease in activity per foetus with increasing litter size could have been expected but such a tendency was not observed (see Fig. 3); this confirms results obtained by NEUMANN & KRIEGEL (1961). The apparent effect at the 18th day of examination is due to two extreme values. However, only two litters in each group were observed; hence the great distribution.

The values recorded in the last column of Table 2, 'activity of foetus in % of that of the pregnant mother', are the means of the mean activity of the foetuses in each litter as a percentage of the activity of the pregnant mother. After administration of radiostrontium on the 12th day of gestation, a very low and constant percentage was observed up to and including the 16th day. On the 18th day, however, it increased 4 to 6 times. Administration on the 14th day produced similar results even if the percentage was a little higher from the beginning; also in this case there was an increase on the 18th day. Injection on the 16th day increased the percentage of activity already for the short time-intervals, and a very high increase was noted on the 18th day.

Administration of the radiostrontium on the 18th day produced a very different picture. The uptake was high from the beginning and the curve made a steep slope up to 180 min, at which point it seemed to become level.

When radiostrontium was administered before mating, the uptake on the 18th day of gestation was 0.4 to 0.5 %, with no significant difference between 1 and 4 weeks.

Comments

The placental transfer during the second half of gestation is dependent on at which day of gestation the radiostrontium has been administered. It was found, when the foetal content was determined 90 min after injection, that the mean foetal weight on the 12th day of gestation was ~ 0.03 g, with an uptake of radiostrontium from the mother of only 0.05 to 0.10 %; on the 14th day the weight was ~ 0.10 g and the uptake 0.2 to 0.3 %; on the 16th day the weight was 0.3 g and the uptake 0.7 to 0.9 %, and on the 18th day the values were 0.7 g and 1.8 to 2.3 %, respectively.

These results confirm the findings of KRIEGEL (1960) in rats, and of STERNBERG (1960) in guinea-pigs. These authors reported a linear correlation between the weight of the embryos and the strontium content, but according to KRIEGEL it was valid only for the development during the last 3 to 4 days of gestation. Our results, however, indicate a correlation between the weight and the short-term uptake during the whole second half of the gestation period. In those cases in which strontium was administered on the 12th, on the 14th, and on the 16th day of gestation, and the activity measured on the 18th day, the foetal uptake was observed to be much greater on the 18th day than on previous days. This observation corresponds with the results obtained by HOLMBERG et coll. (1960). The small discrepancies noted are probably due to different methods of measurement and to the fact that different gestation days were used in the two investigations.

The significant increase in the foetal uptake on the 18th day is obviously due to ossification of the foetal skeleton, which seems to start on the 15th day (ZORZOLI 1948, HOLMBERG et coll. 1960). KOLLMER & KRIEGEL (1963) assumed that the initial incorporation of strontium into the females' skeleton was finished before the third day after injection, the amount of strontium in the blood being less than 0.001 % of the dose injected. This observation has been confirmed by the present authors in an investigation (unpublished) which showed that no ^{85}Sr could be measured in the blood after 48 hours. Thus, the radiostrontium taken up by the foetuses derives mainly from the maternal skeleton, and the rate of placental transfer does not seem to exceed the needs of the growing embryo (STERNBERG 1960). If radiostrontium is administered a relatively short time before mating, the foetal uptake by the 18th day of gestation is low and the radiostrontium originates from the maternal skeleton.

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SUMMARY

The placental transfer of ^{85}Sr in mice has been examined. If the strontium is administered before mating the foetal uptake is low. The rate of uptake by the foetuses after foetuses injection on the 12th, 14th or 16th day of gestation is dependent on the growth of the foetus, i.e. the size. The high uptake on the 18th day is due to the ossification of the foetal skeleton.

ZUSAMMENFASSUNG

Die Übertragung von ^{85}Sr durch die Placenta von Mäusen wurde erforscht. Die Aufnahme des Strontiums durch den Fötus ist gering wenn das Strontium vor der Copulation verabgereicht wird. Wenn man das Strontium am 12ten, 14ten oder 16ten Tag der Schwangerschaft einspritzt, hängt die Aufnahme des Strontiums in den Fötus von dessen Entwicklungsstadium ab. Die am 18ten Tage beobachteten hohen Aufnahme hängt mit der Knochenentwicklung zusammen.

RÉSUMÉ

Les auteurs ont étudié la traversée placentaire de ^{85}Sr chez les souris. Si le strontium est administré avant l'accouplement, la fixation foetale est faible. Le taux de fixation par les foetus après injection aux 12^e, 14^e ou 16^e jour de la gestation dépend de la croissance du foetus, c'est-à-dire de sa taille. La forte fixation au 18^e jour est due à l'ossification du squelette foetal.

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