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REDUCTION IN STRONTIUM ABSORPTION IN PREGNANT, LACTATING AND SUCKLING RATS

by

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Previous work has indicated that the absorption of radioactive strontium from the gut may be reduced by various dietary additives. The best results were obtained by increasing the calcium, phosphate and alginate content of the diet (KOSTIAL et coll. 1967 a). This also proved successful in reducing strontium absorption from the intestine in lactating rats (KOSTIAL et coll. 1969 a) without interfering with its greatly increased absorption due to lactation (KOSTIAL et coll. 1969 b, DURAKOVIĆ & KOSTIAL 1969). The addition of sodium alginate to the milk of artificially fed suckling rats also caused a reduction in the absorption of strontium from the gastrointestinal tract without interfering with the high absorption of calcium (KOSTIAL et coll. 1969 a).

The purpose of the present work was to estimate the absorption of calcium and strontium from the intestine over the entire reproductive period (pregnancy and lactation) as well as to evaluate the effect of dietary additives on strontium absorption during this period. We also tried to obtain more data on the effect

Submitted for publication 8 February 1971.

of dietary additives on strontium absorption from the gut in 5—7 day-old rats by using a simpler experimental technique than in the previous experiments (KOSTIAL et coll. 1969 a).

The results obtained indicate that alginates may be successfully used for reducing strontium absorption without affecting the higher calcium absorption during the reproductive period and at a very early age.

Methods

Determination of strontium and calcium absorption in pregnant rats. The absorption of calcium and strontium was determined in two groups of pregnant albino rats fed on a normal diet during the earlier (7—10th day) and later (16—19th day) stages of pregnancy and in another group of pregnant rats given a diet with calcium, phosphate and alginate additives during the later stage of pregnancy. The absorption of these cations was also determined in two groups of virgin rats fed on normal and experimental diets, respectively. The rats were between 15 and 16 weeks old at the time of mating. The beginning of pregnancy was determined from daily evaluation of vaginal smears. The number of animals in each group varied from 5 to 11.

The calcium, strontium and phosphorus content of the control diet was 1.2 g, 1.86 μg and 0.8 g per 100 g dry food, respectively. The addition of calcium as chlorides, phosphates as potassium dihydrogen phosphate, and alginates as sodium alginate (Manucol SS(LD)2, Alginate Industries Ltd, London) to the control diet increased the calcium phosphate and alginate content or the experimental diet to 2.1 g Ca, 1.1 g P and 10 g alginates per 100 g dry food; both diets were fed ad libitum. The food consumption was determined during the earlier and later stages of pregnancy over a period of three days while keeping the animals in metabolic cages for measuring the absorption of calcium and strontium from the intestine. The calcium, strontium and phosphate content of the diet was determined by standard methods (COMAR 1965, HARRISON 1958, LUCENA-CONDE & PRATT 1957).

Carrier free ^{47}Ca or ^{45}Ca or ^{85}Sr, as supplied from the Radiochemical Centre, Amersham, England, were given to animals in drinking water for two days. About 0.5 μCi of calcium-47 and 0.3 μCi of strontium-85 were added to 10 ml of drinking water containing about 10 mg of calcium per 100 ml; no additional carrier was therefore required. The water bottles were supplied with stainless steel balls to prevent spillage of the radioactive solution. The amount of radioisotope received by each animal was calculated from the difference between the weight of the radioactive solution at the beginning and end of the two-day period. The percentage of radioactivity recovered in the carcass, urine and feces

under such experimental conditions amounted to 95 per cent, with an average standard error of 3—4 per cent. The technique used produced the same results as in experiments in which radioactive isotopes were added to the diet, so that uniform mixing of the radioactive isotopes in the drinking water with the calcium and strontium in the diet was assumed. The animals were killed 24 hours later by an overdose of ether. All animals were in metabolic cages over this period and separate collections of urine were made.

The carcass of the mother rat (the gastrointestinal tract removed), the uterus with fetuses and the pooled three-day urine samples were ashed in a muffle furnace. Calcium-47 and strontium-85 in dissolved samples were determined in a well type scintillation counter connected to a single channel analyzer (KOSTIAL et coll. 1964). Calcium-45, precipitated as oxalate, was estimated in an end-window counter. All results were expressed as percentages of the oral dose. The absorbed dose was recorded as the sum of the radioactivity in the carcass, fetuses and urine in pregnant animals and in the carcass and urine in controls.

The experimental methods used were essentially the same as in the previous work in lactating animals (KOSTIAL et coll. 1969 a, b).

Determination of calcium and strontium absorption in new-born rats. The experiments in young rats were performed by a similar experimental technique of artificial feeding as described in our previous paper (KOSTIAL et coll. 1967 b). The baby rats, 5—6 days old, were fed artificially for 8—10 hours on cow's milk with tracer amounts of ^{47}Ca and ^{85}Sr and additives of calcium, phosphate and alginates. After this artificial feeding period they were returned to their mothers and killed 40 hours later. The baby rats were fed by means of the dropper, each receiving about 18 drops in a volume of 0.4 ml. The control group had cow's milk with $CaCl_2$ and KH_2PO_4 additives to reach the normal calcium and phosphate content of rat's milk (400 mg Ca and 230 mg P per 100 ml of milk) (SPRAY 1950). The experimental group received an additional amount of sodium alginate O.G.1 (HUMPHREYS 1967) of 2 g per 100 ml. Radioactive calcium and strontium were determined in dissolved ashed samples of the carcass in the same way as in the mother rats.

Results

The results of calcium and strontium absorption in pregnancy are presented in Table 1 and Figs 1 and 2 together with the previous data obtained during the earlier (2—5th day) and later (14—17th day) stages of lactation for rats on control (KOSTIAL et coll. 1969 b, DURAKOVIĆ & KOSTIAL 1969) and experimental diets (KOSTIAL et coll. 1969 a). All experiments were performed by practically the same experimental technique.

Table 1

Calcium and strontium absorption from the intestine in pregnant, lactating and virgin rats on control and experimental diet. Each value represents the arithmetic mean and standard error of the mean

Experimental group	Diet	No. of anim.	Amount of food consumed g/day	Dietary Ca g/ 100 g
7—10th day of pregnancy	Control	11	17.1 ± 0.7	1.200
Virgins	Control	8	16.1 ± 1.0	1.200
16—19th day of pregnancy	Control	10	20.6 ± 0.5	1.200
	Alginate	5		2.100
Virgins	Control	10	16.1 ± 1.0	1.200
	Alginate	8		2.100
2—5th day of lactation	Control	12	24.5 ± 0.8	1.200
Virgins	Control	12	16.1 ± 1.0	1.200
14—17th day of lactation	Control	27	30.9 ± 1.2	1.200
	Alginate	16		2.400
Virgins	Control	41	16.1 ± 1.0	1.200
	Alginate			2.400

The daily calcium and strontium intakes appear in columns 4 and 7 of Table 1. The values were calculated from the result of daily food consumption (column 2) and the calcium 1.2 g, 2.1 g and 2.4 g per 100 g of food, respectively) and strontium (18.6 μg per 100 g) content of the diet. The daily calcium and strontium absorptions from the intestine are presented in columns 6 and 9 and were calculated from the daily intake values and the percentage radioactive calcium and strontium absorptions (columns 5 and 8).

The results obtained during the earlier and later phase of pregnancy and lactation in animals on experimental diets, presented in Table 1, are always followed by data obtained in virgin-control animals on control and experimental diet for the same time intervals.

Food consumption appears gradually to increase during the reproductive period, being only slightly higher during pregnancy to reach the highest values during the later phase of lactation. All virgin rats consumed approximately the same amount of food on control and experimental diets so that the same figure of 16 gr per day was used for calculations in Table 1. The percentage calcium absorption was also higher in pregnancy, reaching the highest values towards the end of the lactation. The increase in the percentage strontium absorption during

Table 1 *(cont.)*

Daily Ca intake mg	^{45}Ca, ^{47}Ca % absorption	Daily Ca absorption mg	Daily Sr intake μg	^{85}Sr % absorption	Daily absorption μg
205	16.9±0.7	34.7±1.4	318	8.1±0.3	25.6±0.9
193	13.9±1.1	27.0±2.2	300	8.6±1.0	25.7±3.0
248	16.4±0.4	40.5±1.0	383	7.0±0.2	26.8±0.8
433	10.6±0.3	45.9±1.3	383	2.6±0.1	9.8±0.4
193	13.7±0.5	26.4±0.9	300	6.0±0.2	18.0±0.7
338	8.8±0.6	29.8±1.9	300	2.2±0.2	6.6±0.5
294	18.7±1.1	54.9±3.3	456	7.4±0.6	33.8±2.1
193	12.4±1.3	22.1±2.8	300	6.4±0.8	19.3±2.2
371	29.5±1.3	109.3±5.0	575	10.7±0.6	61.4±3.5
742	14.2±0.5	105.4±3.9		2.9±0.2	16.6±1.2
194	11.4±0.5	22.2±1.0	300	5.0±0.4	14.8±1.0
386	7.4±0.4	28.4±1.4	300	2.5±0.2	7.2±0.7

the reproductive period was always less marked. The percentage radioactive calcium absorption was always lower in animals on experimental diets but the daily absorption of calcium from the gut remained practically unchanged. The percentage strontium absorption and the daily strontium absorption, in rats on experimental diet was, however, always decreased.

The results in Fig. 1 demonstrate the increase in the amount of strontium absorbed from the intestine over the reproductive period. Pregnant and lactating animals with calcium, phosphate and alginate additives in the diet maintained, however, the same level of strontium absorption from the gut as the rats in the control group.

The results presented in Fig. 2 disclose the high increase in the absorption of calcium from the gut during the reproductive period compared to the virgin controls. The addition of calcium, phosphates and alginates to the diet failed to prevent the increased absorption of calcium during the reproductive period.

The selective action of these dietary additives on strontium absorption from the gut during the reproductive period caused a great reduction in the $^{85}Sr/^{45}Ca$ or ^{47}Ca ratio in the carcass of the mother, fetus and suckling (Fig. 3). The values were corrected for the calcium content of the diet.

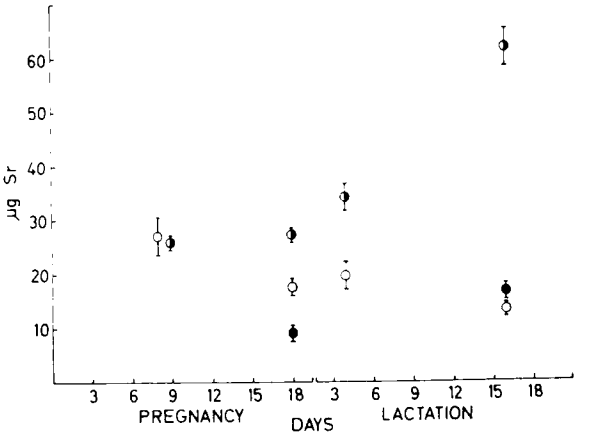

Fig. 1. Intestinal absorption of strontium in pregnant and lactating rats on control diet (◑) and diet with calcium, phosphate and alginate additives (●) as compared to intestinal absorption in virgin rats on control diet (○). Mean ± SE.

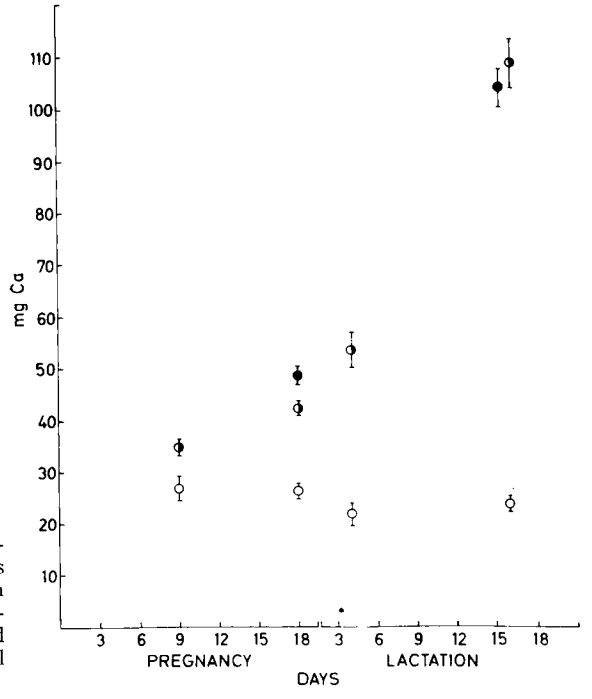

Fig. 2. Intestinal absorption of calcium in pregnant and lactating rats on control diet (◑) and diet with calcium, phosphate and alginate additives (●) as compared to intestinal absorption in virgin rats on control diet (○). Mean ± SE.

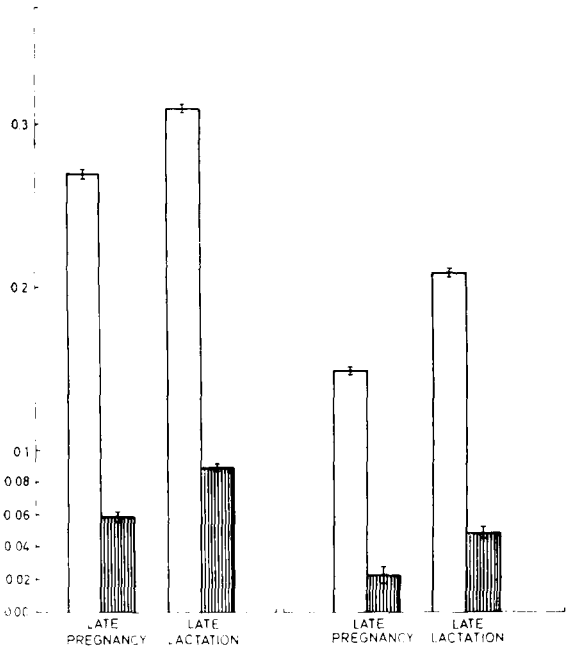

Fig. 3. Ratio of radioactive strontium to calcium in the carcass of the mother (to the left) and fetuses or sucklings (litter) (to the right) during the later stage of pregnancy and lactation in animals on control diet (open fields) and diet with calcium, phosphate and alginate additives (hatched fields). Mean ± SE.

The effect of dietary additives on strontium and calcium absorption in newborn rats. The retention of radioactive calcium and strontium in the carcass in sucklings fed artificially on milk with and without alginate additives appears in Table 2. The previous data obtained in 10-day feeding experiments are presented in the same table for comparison (KOSTIAL et coll. 1969 a). Both sets of the results are in good agreement. The addition of alginates to milk caused a decrease of about 50—60 per cent of the radiostrontium retention without influencing the high calcium absorption from the intestine.

Discussion

The data of calcium requirements during the reproductive period are usually calculated from the amount of calcium provided by the mother for the fetus or infant and from calcium losses in the mother's urine, presuming an intestinal absorption rate of 25 per cent (WHO, Technical Report Series 1962, 1965). The authors have however previously been able to indicate both with in vitro and in vivo techniques that the absorption rate from the intestine greatly increases in lactating rats. This increased absorption is assumed to be mainly due to an increased passive transport of these ions but also to an enhanced active transport of calcium through the intestine (KOSTIAL et coll. 1969 b). The changes in the alimentary tract of lactating animals observed by other authors provide

Table 2

Influence of alginate additives on the retention of ^{47}Ca and ^{85}Sr by 5—7 day-old rats fed artificially on rats' milk for 1 and 10 days

Milk (mg/100 ml)			Days of artificial feeding	No. of rats	Retention in carcass percentage oral dose mean ± SE		
Ca	P	Alg.			^{85}Sr	^{47}Ca	$^{85}Sr/^{47}Ca$
400	230	—	1	136	63.50±0.84	67.11±0.80	0.927±0.004
400	230	2 000	1	86	26.31±0.58	60.68±1.20	0.439±0.007
400	230	—	10	36	69.99±0.76	73.52±0.84	0.96±0.01
400	230	2 000	10	36	33.26±0.57	71.72±1.22	0.47±0.04

morphologic and histologic bases for explanation of these results (BOYNE et coll. 1966). The present experiments reveal that an increased absorption of calcium through the gut wall starts by the pregnancy period and reaches maximum values during the later phase of lactation. The rats also balance their higher needs for calcium by increasing their consumption in food during the reproductive stage. The present results of food consumption are in good agreement with data published by KUMARESAN & TURNER (1968) for pregnant and by ANDERSON & TURNER (1963) for lactating rats.

The absorption of strontium is also increased during the reproductive period, although not to the same degree as that of calcium.

Several other changes in calcium metabolism due to pregnancy and lactation may also affect the deposition of strontium in bone. The acceleration of calcium turnover (STERNBERG 1968, STERNBERG et coll. 1969) may cause the removal of bone seekers incorporated in the deeper parts of the bone before the reproductive period. This has been demonstrated for radiostrontium in various experimental investigations (MOMČILOVIČ et coll. 1969. KOLLMER & KRIEGEL 1965, RÖNNBÄCK et coll. 1968) and constitutes another way in which radioactive strontium may be transferred to the fetus and in nursing mothers to their infants.

The best way of avoiding radiostrontium uptake in the fetus and suckling would be to prevent radiostrontium absorption from the mother's intestine during pregnancy and lactation. This might justify the use of dietary additives as a means of decreasing radioactive strontium retention in both mother and offsprings during the reproductive period. Alginates have already been successfully employed in human subjects and experimental animals for reducing radiostrontium absorption from the intestine (HARRISON et coll. 1966, HESP & RAMSBOTTOM 1965). An addition of calcium, phosphates and alginates to the diet has been proved to be the most effective method of lowering radiostrontium retention in experimental animals (KOSTIAL et coll. 1967 a). Rats fed on diets with alginate additives for one year developed no changes in food consumption,

growth rate or calcium and phosphate content in their skeletons (HARRISON 1967). Similar results were obtained in rats fed calcium, phosphate and alginate supplemented diets for six months in our experiments (ŠLAT & KOSTIAL 1967).

The present data on the effect of these dietary additives in pregnancy fully confirm the previous results obtained during the lactation period; they indicate a successful means of decreasing radiostrontium absorption from the intestine without influencing the greatly increased calcium absorption from the gut during the reproductive period. Their inclusion results in a three to four times lower intestinal strontium absorption and in a four to six times decreased strontium to calcium ratio in the carcass of the mother, fetus and suckling.

The other possible way of decreasing strontium absorption from the gut in artificially fed infants or animals would be a direct addition of alginates to the milk. The present and previous results indicate that the addition of alginates to the milk reduces strontium absorption from the gut by 50—60 per cent without decreasing the very high calcium absorption. Alginates seem to be the only additive to the milk that causes a decrease in strontium absorption since an increased calcium or phosphate content in the milk had no effect on strontium metabolism in new-born rats (KOSTIAL et coll. 1967 b, KOSTIAL et coll. 1969 a).

Acknowledgements

The authors wish to express their thanks to the MRC Radiobiological Research Unit, Harwell for the supply of sodium alginate (O.G.1) and to Messrs M. Buben, M. Landeka and M. Vnučec for their skilful technical assistance. This work was supported by a grant from the Federal and Republic Scientific Research Council, Belgrade and Zagreb.

Some of the results were briefly presented at the Fourth Yugoslav Symposium on Radiological Protection, Baško Polje 1969 and the Second International Congress of the International Radiation Protection Association, Brighton 1970.

SUMMARY

The absorption of oral radioactive calcium and strontium was determined during the earlier and later stages of pregnancy and lactation in rats. A gradual increase in their absorption from the gut occurred, maximum values being reached at the end of lactation. The addition of alginates to the milk of new-born rats, fed artificially, decreased the absorption of strontium without affecting that of calcium.

ZUSAMMENFASSUNG

Die Absorption oral verabreichten radioaktiven Calciums und Strontiums wurde während der früheren und späteren Stadien der Gravidität und Laktation bei Ratten bestimmt. Ein gradueller Anstieg der Absorption vom Darm trat auf, maximale Werte wurden am Ende der Laktation erreicht. Der Zusatz von Alginaten zur Milch von künstlich ernährten neugeborenen Ratten verminderte die Absorption von Strontium ohne die des Calciums zu beeinflussen.

RÉSUMÉ

L'absorption de calcium et de strontium administrés par voie bucale a été étudiée pendant les premiers et les derniers stades de la gravidité et de la lactation chez des rates. On a observé une augmentation graduelle de leur absorption intestinale, les valeurs maximales étant atteintes à la fin de la lactation. L'addition d'alginates au lait donné à des rats nouveaux-nés alimentés artificiellement a diminué l'absorption du strontium sans modifier celle du calcium.

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