Arh. hig. rada, *19* (supl. 1) (1968) 61.

SOME FACTORS INFLUENCING CALCIUM AND STRONTIUM METABOLISM IN THE BODY

KRISTA KOSTIAL

In this article some of the results obtained at the Institute for Medical Research in the field of the comparative metabolism of calcium and strontium are presented. Special attention has been paid to the effect of dietary additives on strontium and calcium absorption from the gastrointestinal tract. New chelating agents have been tested for their toxicity and efficiency in radiostrontium removal. Changes in calcium and strontium metabolism due to special physiological conditions were studied in newborn and lactating rats. Investigations of the effect of estrogen hormones on bone were also performed.

Studies of calcium metabolism in humans include data on the role of plasma proteins in calcium transport as well as data on the kinetic analysis of calcium metabolism in normal subjects.

Studies of strontium as a substitute for calcium in synaptic transmission are included.

Calcium is one of the most important inorganic elements of the body, essential for the maintainance of most physiological functions. Strontium, however, is only a trace element whose physiological function is not yet known.

Recently the interest in strontium metabolism has greatly increased because of its radioactive isotope, strontium-90, which appeared in the human environment. The protection against strontium-90 received a particular scientific interest since strontium-90 proved to be one of the most toxic fission products. Beside similarities between calcium and strontium metabolism many differences in their behaviour have been observed. This applies particularly to the passage of these cations through biological membranes. For calcium an active transport system has been found, while the passage of strontium seems to be only passive. This probably represents the physiological basis for the well known discrimination against strontium in the body (1). This discrimination mainly occurs during the absorption of these cations from the gastrointestinal tract, as well as during their passage through the placenta, mammary gland and kidney.

It is hoped that a better understanding of these metabolic processes will permit enhancement of the discrimination against strontium and thereby reduction of the hazard of strontum-90 for man.

At the Institute for Medical Research a group of scientists has been studying the effect of various factors on the comparative metabolism of calcium and strontium for several years. This article is a survey of the results they have obtained.

EFFECT OF DIETARY ADDITIVES ON STRONTIUM AND CALCIUM ABSORPTION FROM THE DIGESTIVE TRACT

In studying factors which might influence the comparative metabolism of calcium and strontium special attention has been paid to the effect of the dietary factors on the absorption of these cations from the digestive tract. The gastrointestinal tract represents the main route of entry of radioactive strontium into the body. When present in the diet, strontium is deposited primarily in the skeleton, but following a complex chain of metabolism at a Sr/Ca ratio lower than in the diet. It is hoped that in some of this processes its metabolism can be influenced by various dietary additives.

In earlier work special attention was paid to increasing dietary calcium as a way to reduce strontium absorptian from the gut (2), neglecting to a great extent the important effect of dietary phosphates.

The scope of our work was to investigate how the changes in the dietary phosphates within the physiological range influence the comparative metabolism of calcium and strontium. We have been able to show that skeletal retention of radioactive strontium is strongly affected by the phosphate level of the diet (3).

Phosphate ions influence the comparative Sr/Ca metabolism at two sites: in the intestine by reducing strontium absorption and in the kidney by lowering its excretion. Higher phosphates preferentially reduce strontium absorption and strontium excretion as compared to calcium. The former effect on strontium absorption which tends to reduce body contamination with strontium is stronger than the latter on strontium excretion whose tendency is opposite. An increase of dietary phosphates from 0.5 to 1.2 % P has the net effect of reducing skeletal retention of strontium almost to one half leaving skeletal calcium almost unaffected. Phosphates exert this action if present in the gut at the moment of radioactive strontium contamination and previous feeding with high phosphate diets does not improve this effect (4, 5, 6). The effect of phosphates seems to be independent of the function of the parathyroid gland and of smaller variations in the vitamin D content in the diet (7, 8). The phosphate effect is also independent of the chemical form of phosphate additives (9).

Systematic investigation of two important variables which were shown to affect the relative absorption and retention of calcium and strontium, i.e. the dietary levels of calcium and phosphates showed that the effect of phosphates can be improved by increasing at the same time the calcium content of the diet. The minimum body burden of

strontium-90 would be attained if the calcium level of the diet were increased to a maximum by use of uncontaminated calcium and if, at the same time, the phosphate level were also increased to a maximum (10, 11).

Later on a comparison was made of the effect of other substances which proved to be efficient in decreasing radioactive strontium absorption from the intestine i.e. of sulphates (12), phosphates and alginates (13). The combined therapy with alginate and calcium phosphate supplementation produced the highest reduction of the absorbed or retained strontium that we have so far obtained with any dietary supplementation (6–7 times) (14, 15). Rats fed this diet for 4 months showed no changes in growth increment rates. On dissection no pathological changes were observed. Histological findings in the kidney were normal (16).

EFFECT OF AGE ON CALCIUM AND STRONTIUM METABOLISM

It is well known that age is one of the important physiological factors which influence calcium and strontium metabolism. We have been able to prove that the labile fraction of bone changes with age. In young animal (9–10 weeks) the labile fraction of the skeleton amounts to 23% of the bone calcium while in adult rats (23–24 weeks) to only 6% (17).

Recently considerable interest has been taken in the turnover of strontium and calcium in the newborn. It has been postulated that in the very young the turnover of body stores of calcium and strontium must be relatively rapid compared with that in adults, that is, the calcium and strontium are much more labile. Our experiments on new born rats lend considerable support to this hypothesis. By labelling rats in utero we were able to show that the Sr/Ca ratio of the infant bone existing at birth as well as that of the new bone being formed assumes a value determined by the phosphate content of the mother's diet (18).

We also showed that strontium deposited in the fetus during embryonic development was as available for exchange and removal from the body as that administered parenterally. In other words essentially all of the fetal strontium was available to enter into exchange equilibrium with the circulating fluids. It is thus to be expected that the Sr/Ca ratio of the bone formed in utero rapidly changes to become identical with that of the new bone being formed (19, 20).

Using data from these animal experiments calculations can be made to anticipate gains or losses of stable strontium under varying conditions (20).

The results of these experiments suggest that an increase in the phosphate intake of nursing mothers could provide an effective means of decreasing the uptake of radioactive strontium in the newborn. This effect could be caused by either the action of the higher phospate con-

tent in mother's milk on calcium and strontium absorption from the gastrointestinal tract in newborn rats or by a decreased ratio of Sr/Ca in the milk caused by the influence of phosphates on calcium and strontium metabolism in the nursing mother. To clarify this point we introduced a method of artificial feeding of 5-day-old rats. The results obtained show a high absorption efficiency and an almost complete lack of discrimination against strontium in animals of this age. They also indicate that an increase in the phosphate content of the milk does not influence calcium or strontium absorption from the gastrointestinal tract of young animals. The reduction of radioactive strontium retention observed in baby rats in our previous experiments was therefore due to a decreased Sr/Ca ratio in mother's milk influenced by the increased phosphate content of the diet. It is also interesting to note that an increase of the calcium and phosphate content of the milk caused a 5–6 times greater total absorption of calcium from the intestine (21).

THE EFFECT OF PREGNANCY AND LACTATION ON CALCIUM AND STRONTIUM METABOLISM

Changes in calcium and strontium metabolism are known to occur during pregnancy and lactation (22, 23). We were mainly concerned to find out which part of skeletal minerals is being mobilized during lactation and to study changes in the kinetics of calcium metabolism due to lactation.

By marking the maternal skeleton at two different time intervals before the beginning of the lactation period we were able to prove that during the first phase of lactation (0–10 day) only minerals from the superficial i.e. exchangeable fraction of the skeleton are being mobilized. During the second phase of lactation a small fraction of minerals from the deeper i.e. unexchangeable fraction of the bone are also mobilized (24). Maximal mobilization of calcium and strontium from the skeleton occurs between the 15th and 20th day of lactation (25).

By measuring the specific activity of calcium-47 in the serum and tibiae of rats during lactation we were able to prove an increase in the size of the exchangeable fraction of bone calcium and an increased calcium accretion rate into bone (26).

THE INFLUENCE OF SOME HORMONES ON CALCIUM AND STRONTIUM METABOLISM

Numerous data have been published about the influence of estrogenic hormones on bone tissue. It is however still uncertain whether estrogens affect bones directly or whether the estrogenic action on bones should be attributed to the activation of adrenal glands caused by estrogens (27). Our experiments were undertaken to elucidate the mode of action of exogenous estrogen hormones on mineral metabolism of bone tissue.

Estradiol administered to male rats in the course of 20 days caused a reduction of radioactive calcium and strontium retention in the bone. This effect of estradiol was non-specific for 2 reasons: chronic formalin stress – acts in an analogous way, and adrenalectomy inhibits this effect of estradiol. Our results therefore strongly support the hypothesis that the effect of estrogens on bones should be attributed to the action of the adrenal gland (28).

Studies of calcium and strontium metabolism in rats with a mammotropic tumor of the hypophysis undertaken to estimate the effect of various hormones on calcium and strontium metabolism also indicate that the reduction of calcium and strontium retention in bones of these rats is mainly due to the action of adrenal hormones. While adrenalectomy inhibits the effect of the mammotropic tumor on bone tissue an addition of cortizol to adrenalectomized animals restores this effect (29).

THE EFFECT OF SOME COMPLEXING AGENTS ON CALCIUM AND STRONTIUM METABOLISM

Chelation therapy with polyaminopolycarboxylic acids such as EDTA (ethylenediamine tetraacetic acid) has not proved very useful in removing radiostrontium from the body since formation constants of these complexing agents with calcium are higher than those with strontium. In order to find a chelating agent more effective for radiostrontium removal, chelating agents with a higher relative complexing power for strontium than those already in use are being synthetized. The only chelating agent which was found to be efficient in removing radioactive strontium from the body was BADE (2:2'–bis/di(carboxymethyl)amino/diethylether) (30, 31). We tested the efficiency of some newly synthetized chelating agents such as CPDTA (cyclopentanediamine tetraacetic acid), DIMEDTA (dimethylethylenediamine tetraacetic acid), and PDTA (propylenediamine tetraacetic acid) in enhancing radioactive strontium elimination from the body. All these chelating agents proved to have higher relative complexing power for strontium than EDTA. Their toxicity was found to be of the same order as that of EDTA (32, 33).

Of all the chelates tested only the strontium chelate of CPDTA applied intraperitoneally at the time of radioactive strontium application caused a 20% reduction of skeletal retention of radiostrontium. Strontium chloride injected intraperitoneally was equally efficient in removing radioactive strontium from the body as equimolar amounts of strontium chelate of BADE or CPDTA. Chelation therapy proved to be not only inefficient in the case of oral contamination with radioactive strontium, as assumed by other authors, but caused a statistically significant increase in the skeletal retention of radioactive strontium (34).

STRONTIUM AS A SUBSTITUTE FOR CALCIUM IN SYNAPTIC TRANSMISSION

At neuromuscular junction as well as at neuronal synapses calcium plays an essential part in the process of synaptic transmission (35, 36). The question is raised whether strontium ions can replace calcium in this process. Conflicting reports regarding the capacity of strontium or barium to restore the indirect excitability of neuromuscular preparation have been reported (37, 38).

We used the isolated perfused superior cervical ganglion of the cat to study the effect of strontium ions on synaptic transmission. When calcium chloride was substituted with equimolar amounts of strontium chloride in the perfusion fluid, contractions of the nictitating membrane to preganglionic nerve stimulation were well maintained for a period of over 60 mins. (39). In experiments in which acethylcholine output was determined, we proved that strontium ions could well replace calcium ions in releasing acethylcholine from preganglionic nerve terminals. The output of acethylcholine remained unchanged while perfusing the ganglion with Locke's solution containing equimolar amounts of strontium instead of calcium.

These results were confirmed by *Miledi* who found that strontium can replace calcium in the process of transmitter release by a nerve impulse at the neuromuscular junction and that the transmitter is still released in quantal fashion (40).

STUDIES OF CALCIUM METABOLISM IN HUMANS

The role of plasma proteins in the process of calcium transport in body fluids is still uncertain (41, 42). We performed electrophoretic investigations of the calcium transport in plasma to obtain more data on the subject. Labelled plasma electrophoresis was carried out in a barbiturate medium with the aid of discontinual and continual technique. At these conditions it was shown that albumins, beta- and gamma-globulins may take part in the transport of calcium (43).

A direct method of assessing calcium accretion rates in humans was introduced by using forearm radioactivity measurements of calcium-47. This method avoids daily collections of urine and faeces. The radioactivity in the forearm was determined by means of two scintillation counters connected to a single-channel analyser. By using the modified Wendeberg's kinetic model (44) a linear correlation was found between values of accretion rates obtained from human forearm measurements and whole body measurements (dose – excreta). The main advantage of this method is its technical simplicity and reliable results (45, 46).

Kinetic analysis of calcium metabolism in humans was performed in 9 normal subjects using Wendeberg's model system. Values for accretion rates were found to be 0.57 ± 0.12 g/day and the amount of calcium in S and E pools 2.01 ± 0.32 and 2.86 ± 0.39 g respectively (47).

This work was performed by the members of the staff of the Laboratory for Mineral Metabolism and Laboratory for Human Metabolism of the Institute for Medical Research, Yugoslav Academy of Sciences and Arts. Some experiments were performed in collaboration with Dr. G. E. Harrison and his assistants from the M. R. C. Radiobiological Research Unit, Harwell, England, and with Prof. C. L. Comar, Department of Physical Biology, Cornell University, Ithaca, New York, U. S. A.

References

1. *Comar, C. L., Wasserman, R. H., Nold, M. M.:* Strontium-Calcium Discriminatory Factors in the Rat, Proc. Soc. Exptl. Biol. Med., *92* (1956) 859.
2. *Comar, C. L., Wasserman, R. H.:* Strontium, in Comar, C. L., Bronner, F.: Mineral Metabolism, IIa, Academic Press, New York, 1964, str. 523.
3. *Kostial, K., Lutkić, A., Gruden, N., Vojvodić, S., Harrison, G. E.:* The Effect of Dietary Phosphorus on the Metabolism of Calcium and Strontium in the Rat, Int. J. Rad. Biol., *6* (1963) 431.
4. *Vojvodić, S., Kadić, M., Maljković, T., Kostial, K.:* Some Methods of Reducing Radioactive Strontium Retention in the Body, Proc. Intern. Congress of Occup. Health, Vienna, 1966, p. 169.
5. *Kostial, K., Vojvodić, S., Gruden, N., Lutkić, A.:* Skeletal Uptake of Calcium–47 and Strontium–85 as Influenced by the Phosphorus Content of the Diet, in Bone and Tooth, Pergamon Press, London, 1964, str. 111.
6. *Kostial, K., Gruden, N., Lutkić, A., Vojvodić, S.:* The Influence of Phosphates on Calcium and Strontium Absorption from the Gut, Biochem. Pharmacol. Suppl., *12* (1963) 205.
7. *Gruden, N., Rabadija, L., Kostial, K.:* The Effect of Phosphates on Strontium and Calcium Metabolism in Control, Parathyroidectomized and Parathormone Treated Rats, Arh. hig. rada, *19* (1968) 25.
8. *Kostial, K., Maljković, T., Gruden, N., Duraković, A.:* The Influence of the Vitamin D and Phosphate Content on the Diet on Calcium and Strontium Absorption from the Gastrointestinal Tract, Arh. hig. rada, *19* (1968) 5.
9. *Harrison, G. E. Howells, G. R., Pollard, J., Kostial, K., Manitašević, R.:* Effect of Dietary Phosphorus Supplementation on the Uptake of Radioactive Strontium in Rats, Brit. J. Nutr., *21* (1966) 561.
10. *Manitašević, R.:* Djelovanje fosfata na metabolizam kalcija i stroncija, Magistarski rad, Sveučilište u Zagrebu, (M. Sc. Thesis, University of Zagreb), 1967.
11. *Kostial, K., Vojvodić, S., Comar, C. L.:* Effects of Dietary Phosphorus and Calcium on the Comparative Behaviour of Strontium and Calcium, Nature, *208* (1965) 1110.
12. *Volf, V., Roth, Z.:* Retention of Sr–85 in Rats. Effect of Sodium, Magnesium, Calcium, Strontium, and Barium Sulphate, Acta Radiologica, *3* (1965) 216.
13. *Waldron-Edward, D., Paul, T. M., Skoryna, S. C.:* Studies on the Inhibition of Intestinal Absorption of Radioactive Strontium, Canad. Med. Assoc. J., *91* (1964) 1006.
14. *Kadić, M.:* Utjecaj fosfata, sulfata i alginata na apsorpciju radioaktivnog stroncija iz probavnog trakta, Magistarski rad, Sveučilište u Zagrebu, (M. Sc. Thesis, University of Zagreb) 1967.

15. *Kostial, K., Maljković, T., Kadić, M., Manitašević, R., Harrison, G. E.:* Reduction of the Absorption and Retention of Strontium in Rats, Nature, *215* (1967) 182.

16. *Šlat, B., Kostial, K.:* Djelovanje hrane s povišenim sadržajem kalcija, fosfata odnosno alginata na metabolizam kalcija i stroncija, II jugols. kongres za med. rada, Split, 1967, Sadržaji saopćenja, 5–7.

17. *Harrison, G. E., Kostial, K., Howells, G. R.:* Turnover of Calcium and Strontium in the Skeleton of Growing Rats on High Strontium Diet, Int. J. Rad. Biol., *4* (1962) 623.

18. *Kostial, K., Gruden, N., Harrison, G. E.:* Turnover of Radioactive Strontium in Newborn Rats, Nature, *201* (1964) 1240.

19. *Gruden, N.:* Utjecaj nekih faktora na mineralni metabolizam skeleta, Disertacija, Sveučilište u Zagrebu, (Ph. D. Thesis, University of Zagreb), 1965.

20. *Comar, C. L., Kostial, K., Gruden, N., Harrison, G. E.:* Metabolism of Strontium in Newborn, Health Physics, *11* (1965) 609.

21. *Kostial, K., Šimonović, I., Pišonić, M.:* The Effect of Calcium and Phosphates on Gastrointestinal Absorption of Strontium and Calcium in Newborn Rats, Nature, *215* (1967) 1181.

22. *Collmer, W. E., Kriegel, K.:* Influence of Lactation on the Retention of a Single Dose of Strontium-90 in Rats, Nature, *200* (1963) 187.

23. *Kollmer, W. E., Kriegel, K.:* Das biologische Verhalten von Radiostrontium bei Ratten im Verlauf der Laktation, Int. J. Rad. Biol., *9* (1965) 369.

24. *Duraković, A., Kostial, K.:* Utjecaj laktacije na demineralizaciju skeleta štakora, IV Kongres Jugoslavenskog društva za Fiziologiju Ljubljana 1965, Zbornik kratkih sadržaja referata, str. 17.

25. *Duraković, A., Kostial, K.:* The Influence of Lactation on Skeletal Demineralization of the Rat, Iugoslav. Physicol. Pharmacol. Acta, *1* (1965) 150.

26. *Blanuša, M., Duraković, A., Kostial, K.:* Effet de la lactation sur le métabolism du calcium, Arh. hig. rada, *19* (1968) 39.

27. *Frost, H. M.:* A Model of Endocrine Control of Bone Remodelling Henry Ford Hosp. Med. Bull., *10* (1962) 119.

28. *Rabađija, L., Gruden, N., Kostial, K.:* Effect of Large Doses of Estradiol on Skeletal Retention of Radioactive Calcium and Strontium in Mature Male Rats, Iugoslav. Physiol. Pharmacol. Acta, *2* (1966) 21.

29. *Rabađija, L., Milković, S., Kostial, K.* (1968) – Unpublished work.

30. *Catsch, A.:* Untersuchungen über die Dekorporation von Radiostrontium, Atomkernenergie, 7 (1962) 65.

31. *Catsch, A.:* Untersuchungen über die Dekorporation von Radiostrontium durch Chelatbildner, Int. J. Rad. Biol., *4* (1961) 75.

32. *Kostial, K., Maljković, T., Šlat, B., Weber, O.:* Toxicity of Some New Chelating Agents for Radiostrontium Removal, Arh. hig. rada, *13* (1962) 295.

33. *Weber, O., Kostial, K., Paulić, N., Purec, Lj., Vojvodić, S.:* Physical-Chemical and Biological Properties of a New Chelating Agents for Radiostrontium Removal, Biochem. Pharmacol. Suppl., *12* (1963) 205.

34. *Kostial, K., Vojvodić, S., Maljković, T.:* Djelovanje nekih kompleksona na metabolizam radioaktivnog stroncija, Arh. hig. rada, *18* (1967) 111.

35. *Hutter, O. F., Kostial, K.:* Effect of Magnesium Ions Upon the Release of Acethylcholine, J. Physiol., *120* (1953) 53.

36. *Hutter, O. F., Kostial, K.:* The Relationship of Sodium Ions to the Release of Acethylcholine, J. Physiol., *129* (1955) 159.

37. *Edwards, C., Lorković, H., Weber, A.:* The Effect of the Replacement of Calcium by Strontium on Excitation-Contraction Coupling in Frog Skeletal Muscle, J. Physiol., *186* (1966) 295.

38. *Brutsaert, D. L.:* Frequency Potentiation and Paired Stimulation Potentiation of Cat Papillary Muscles in Calcium and in Strontium Containing Media, Arch. internat. Physiol. Bioch., *75* (1967) 229.

39. *Šlat, B., Kostial, K.:* The Effect of Some Bivalent Ions on Synaptic Transmission, Symp. on Current Problems of Neurophysiol., Beograd, 1964, Proc., p. 15.

40. *Miledi, R.:* Strontium as a Substitute for Calcium in the Process of Transmitter Release at the Neuromuscular Junction, Nature, *212* (1966) 1233.

41. *Neuman, W. F., Neuman, M. W.:* The Chemical Dynamics of Bone Mineral, University Chicago Press, Chicago, Illinois, USA, 1958, p.

42. *Guerin, M. T. H., Guerin, R. A.:* Liaisons aux protéines sériques du radiocalcium (Ca–47) et du radiostrontium (Sr–85), Pathologie–Biologie, *13* (1965) 297.

43. *Jovanović, V., Šimonović, I.:* Elektroforetsko ispitivanje transporta kalcija u plazmi, Arh. hig. rada, *18* (1967) 147.

44. *Wendeberg, B.*, u: Medical Uses of Ca–47, I. A. E. A., Technical Report Series, 10, Beč, 1962, p. 51.

45. *Harmut, M.:* The Measurement of Calcium–47 in the Forearm as an Indication of Calcium Metabolism in Humans, II Symposium on Health Physics, Pecs, 1966, Proc., p. 171.

46. *Simonović, I., Jovanović, V., Harmut, M., Latković, I., Popović, S.:* Internal Report IMI–SF–XII_1–13b.

47. *Jovanović, V., Popović, S., Latković, I., Šimonović, I.:* Kinetics of Calcium–47 in Man, Arh. hig. rada, *19* (1968) 11.