Studies on Inhibition of Intestinal Absorption of Radioactive Strontium

I. Prevention of Absorption from Ligated Intestinal Segments

STANLEY C. SKORYNA, M.D., Ph.D., T. M. PAUL, M.B., B.S., M.Sc.* and DEIRDRE WALDRON EDWARD, Ph.D., *Montreal*

ABSTRACT

A method is reported which permits selective suppression of absorption of radioactive strontium from ingested food material, permitting the calcium to be available to the body. Studies were carried out *in vivo* by injection of Sr^{89} and Ca^{45} in the presence of inert carrier into ligated intestinal segments in rats, and the amount of absorption was measured by standard monitoring techniques. The pattern of absorption of both ions is very similar but the rate of absorption is different. It was found that the polyelectrolyte, sodium alginate, obtained from brown algae (Phaeophyceae), injected simultaneously with radiostrontium effectively reduces the absortion of Sr^{89} from all segments of the intestine by as much as 50-80% of the control values. No significant reduction in absorption of Ca^{45} was observed in equivalent concentrations. The reduction in blood levels of Sr^{89} and in bone uptake corresponded to the absorption pattern. The difference in the effect on strontium and calcium absorption may be due to differences in the binding capacity of sodium alginate from the two metal ions under the conditions present *in vivo.*

SOMMAIRE

Une méthode permettant la suppression sélective de l'absorption de strontium radioactif provenant d'aliments ingérés, est rapportée. Cette méthode n'empêche pas la disponibilité du calcium pour l'organisme. Les présentes recherches ont été entreprises, au moyen d'injection de Sr^{89} et Ca^{45} *in vivo* avec la présence de porteur inerte dans les segments intestinaux ligaturés chez des rats. La quantité d'absorption a été mesurée par les techniques normales de contrôle. Le mode de distribution d'absorption des deux ions est bien semblable, mais le taux d'absorption est différent. Il a été démontré que l'alginate de sodium polyélectrolyte obtenu des algues brunes (Phaeophyceae), injecté simultanément avec le strontium radioactif, réduit effectivement l'absorption de Sr^{89} de tous les segments de l'intestin autant que 50-80% des séries de contrôle. Il n'a pas été observé de réduction importante de Ca^{45} dans les concentrations équivalentes. La réduction du niveau de Sr^{89} dans le sang et de dépôt dans l'os, correspondait avec le mode de distribution d'absorption. Les raisons de la différence dans l'effet de l'alginate de sodium sur l'absorption de strontium et de calcium, peuvent être attribuables à la différence dans la capacité de fixation de l'alginate de sodium dans les conditions présentes *in vivo.*

THE prevention of absorption of radioactive products with a long half-life resulting from the inadvertent release of materials in an industrial atomic plant or from atomic explosions remains of great importance, although the danger of fall-out from fission tests has abated. Strontium and barium are absorbed to a significant degree from the gastrointestinal tract; other fission products with a relatively long half-life are less dangerous in this respect.[1] Radioactive strontium is rapidly deposited in the crystalline lattice of bone.[2] The potential hazards of exposure to this isotope include dysfunction and depletion of bone marrow and its products, interference with normal growth, and initiation of malignant tumours.[3, 4] Many attempts have been made to remove the isotope from bone, but no major modification in the amount of incorporated material has been possible.[5] Several investigators have successfully removed radioactive strontium from milk, replacing calcium which would normally be removed simultaneously.[6-9] However, these methods not only are laborious but also produce some undesirable changes in the nutritional value of the milk. It is also probable that other foods, including all plant material, as well as meat derived from areas of contamination would contain radioactivity.

With these considerations in view, we have attempted to devise a method which would permit selective inhibition of absorption of radioactive strontium from the gastrointestinal tract, permitting the calcium to be available to the body. The problem also presented an opportunity to study the mechanism of specificity of ionic absorption.

From the Gastro-Intestinal Research Laboratory and the Department of Experimental Surgery, McGill University, Montreal.
Supported by a grant from the Medical Research Council of Canada.
*Medical Research Fellow, Medical Research Council of Canada.

It was considered that an *in vivo* method of study would reflect more closely the physiological conditions existing in the gastrointestinal tract, as well as other factors affecting ionic absorption.

We have previously suggested that inhibition of the carcinogenic action of radioactive strontium might be effected by interference with the intestinal absorption of the isotope.[4] Several naturally occurring macromolecular substances with ion-exchange properties have been studied.[10, 11] A compound polymer of mannuronic and guluronic

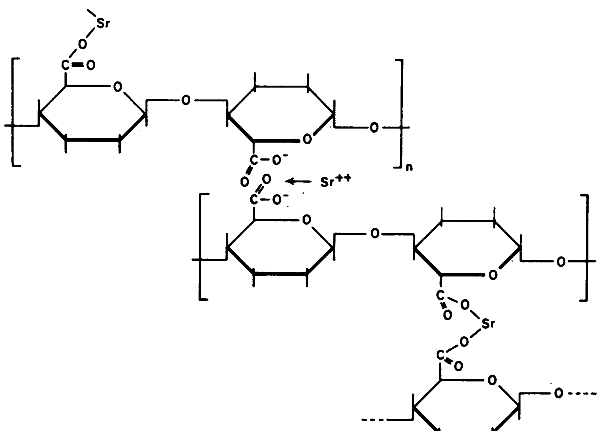

Fig. 1.—Proposed structure of strontium salt of polymannuronic acid.

acids known as alginic acid, which is found in various species of brown algae (Phaeophyceae), has been found to be the most effective of the preparations tried (Fig. 1). A method involving the use of ligated intestinal segments has proved to be most useful in screening tests for efficacy of new compounds and dosage levels.

EXPERIMENTAL METHODS AND MATERIALS

Radioactive Strontium and Calcium

The isotopes strontium-89 and calcium-45, in dilute hydrochloric acid (HCl), were supplied by Atomic Energy of Canada Ltd. Sr^{89} had a high specific activity with no carrier strontium chloride ($SrCl_2$); Ca^{45} had an appreciable amount of inert isotope present. As an increased concentration of the non-radioactive salt suppresses the percentage absorption of radioisotope,[8] small amounts of carrier calcium chloride ($CaCl_2$) or $SrCl_2$ were added to the radioactive solution to give a final concentration of 1.1. μM. calcium chloride per ml. or 0.9 μM. of strontium chloride per ml. Final activity of each solution was 4 microcuries (μc.) per ml.

Preparation of Ligated Segments

Young rats of R.V.H. strain, of either sex, weighing 100-120 grams were used. The animals were fasted for 24 hours and then anesthetized with pentobarbital (Nembutal). The anterior abdominal wall was shaved and laparotomy performed. The total length of the small intestine was divided into nine segments of approximately 5 cm. each. The

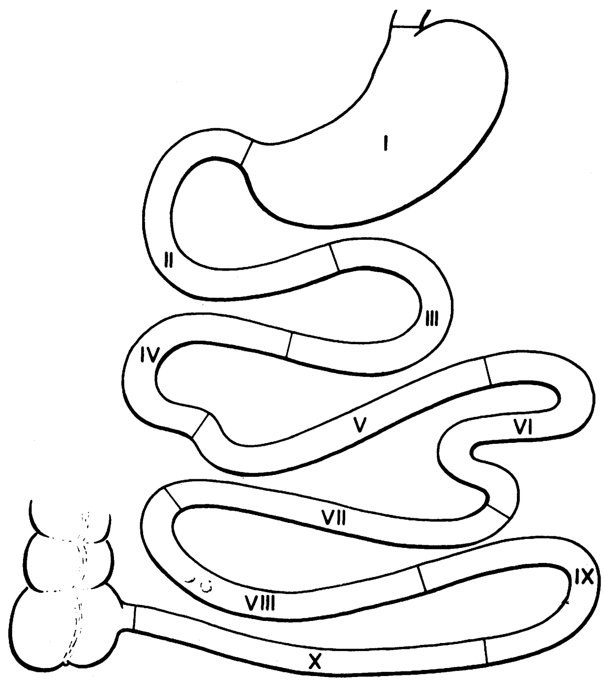

Fig. 2.—Schema of division of small intestine by ligation into nine segments (II — X). The stomach has been designed as segment I.

duodenal segment was numbered II and the rest in serial sequence, the terminal ileal segment being designated as segment X, and the stomach as segment I (Fig. 2). Each segment was ligated at both ends without disturbing the vascular or lymphatic supply, or detaching the segment from the remainder of the intestine (Fig. 3). Only one segment was used in each animal.

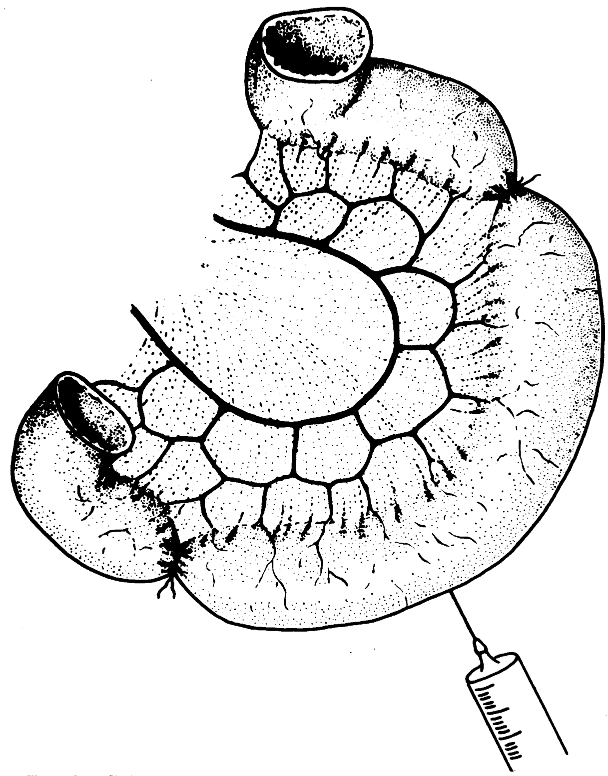

Fig. 3.—Schematic drawing of a lighted intestinal segment. The segment was left in continuity with the remaining small bowel.

Methodology of Absorption from Ligated Segments

An amount of 0.5 ml. of test solution containing 2 μc. Sr^{89} plus 0.45 μM. strontium chloride or 2 μc. Ca^{45} plus 0.55 μM. calcium chloride was injected into each ligated segment using a disposable tuberculin syringe with a fine (27 gauge) needle. Twelve rats were used to estimate absorption from each segment. Intestinal segments were excised after 30 minutes with ligatures intact, rinsed and placed in 3 ml. 38% HCl for 24 hours, by which time the intestinal wall was completely dissolved. One femur from each animal was removed, denuded of soft tissue and dissolved in concentrated HCl. One ml. of blood was collected into a tube containing 2 ml. of sodium citrate solution. The acidic solutions of bone and intestinal segments were neutralized with 30% sodium hydroxide (NaOH), and diluted. Aliquot samples were dried in metal planchets in an oven, and counted in standard Geiger-Müller monitory equipment. All counts were corrected for self-absorption.

The above process was repeated using radiocalcium and radiostrontium in the presence of 1 mg. of the sodium salt of alginic acid. These were injected simultaneously into the ligated segment. Absorption was estimated as the *difference* between the total radioactivity administered and the radioactivity remaining in the lumen plus that of the intestinal wall.

RESULTS AND DISCUSSION

Pattern of Absorption from Ligated Intestinal Segments

All experiments were carried out over a 30-minute period. This time interval was selected because it was shown previously[12] that the absorption rate had reached its maximum. It was also demonstrated that during this time no gross morphological or microscopically detectable changes occur in the intestinal mucosa. Throughout this time the animals remained under the constant influence of anesthesic.

The absorption of strontium and calcium mainly takes place from the duodenum, in the ligated segment procedure (Fig. 4 and Table I). Sixty-six per cent of the calcium and 33.5% of the strontium administered were absorbed during the 30 minutes of the experiment. In the segment immediately distal to the duodenum (segment III), the absorption rate for both elements dropped by more than 50%. Thereafter, there was a very slow decline towards the distal end of the ileum. No statistically significant differences were observed in absorption between the distal segments (segments IV to X). In the stomach, 10% of the administered Sr^{89} was absorbed, whereas no detectable amount of Ca^{45} was absorbed from this region.

The blood levels and bone uptake of Sr^{89} and Ca^{45} correspond to the amount of the isotope absorbed from each segment (Figs. 5 and 6; Tables

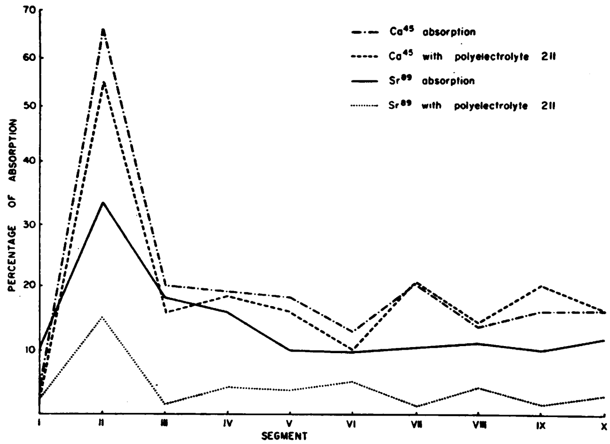

Fig. 4.—Effect of sodium alginate (Polyelectrolyte 211) on intestinal absorption of radioactive strontium and calcium. The solutions used were as described in text.

II and III). Although in the 30-minute period nearly twice as much calcium as strontium was absorbed from the duodenum, the amount of the

TABLE I.—INFLUENCE OF SODIUM ALGINATE ON Sr^{89} AND Ca^{45} ABSORPTION FROM RAT'S GASTROINTESTINAL TRACT

	2 microcuries Sr^{89} with 0.45 micromole of inactive $SrCl_2$ for 30 minutes		*2 microcuries Ca^{45} with 0.55 micromole of inactive $CaCl_2$ for 30 minutes*	
Segment	*Control*	*with 1.5 micromoles of sodium alginate*	*Control*	*with 1.5 micromoles of sodium alginate*
I	10.0 ± 4.95	2.3 ± 2.19	4.1 ± 3.6	4.4 ± 2.89
II	33.46 ± 5.89	15.2 ± 4.17	66.2 ± 11.53	55.1 ± 18.02
III	18.3 ± 7.11	1.7 ± 3.24	20.3 ± 10.1	15.9 ± 4.76
IV	16.7 ± 7.61	4.3 ± 5.00	19.2 ± 5.65	19.7 ± 5.56
V	9.7 ± 5.1	5.5 ± 4.40	12.9 ± 5.91	10.7 ± 6.85
VI	10.4 ± 6.96	1.5 ± 2.32	20.9 ± 6.92	22.8 ± 7.07
VII	11.2 ± 5.59	4.0 ± 3.01	13.5 ± 5.29	14.6 ± 3.60
VIII	10.4 ± 5.66	1.6 ± 1.48	16.6 ± 6.77	20.9 ± 8.02
IX	11.8 ± 4.52	2.9 ± 1.61	16.1 ± 5.74	16.8 ± 5.19

Each figure represents mean percentage of absorption and standard deviation in studies on 12 rats.

latter radionuclide deposited in the bone was considerably higher (Fig. 6 and Table III). The rapid deposition of strontium was previously observed by Nilsson and Ullberg.[13]

It is apparent from the foregoing data that although the pattern of absorption of calcium and

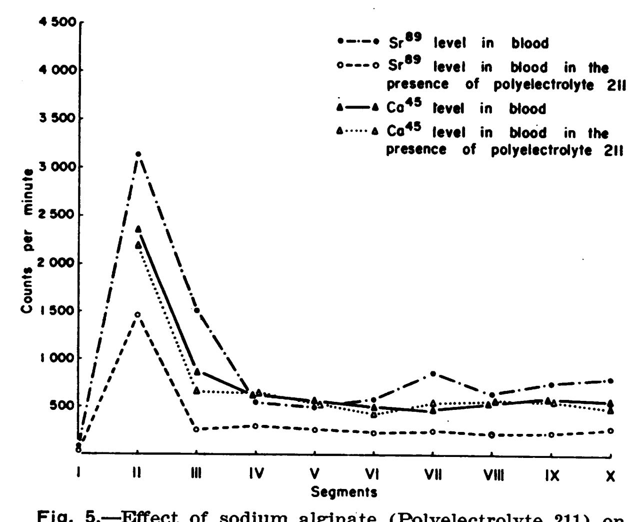

Fig. 5.—Effect of sodium alginate (Polyelectrolyte 211) on blood level of radioactive strontium and calcium following injection into an intestinal segment. The solutions used were as described in text.

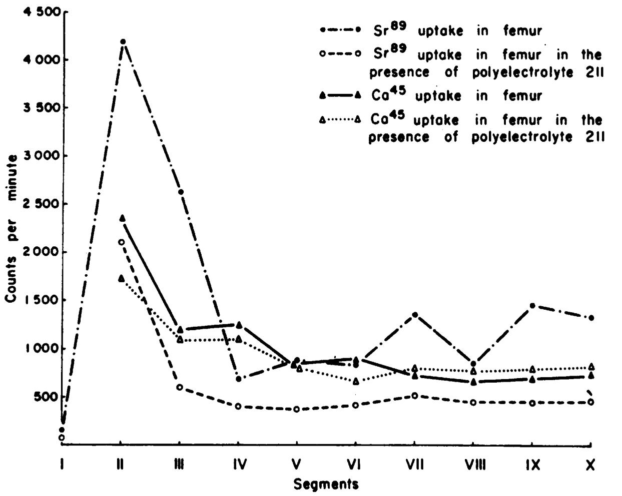

Fig. 6.—Effect of sodium alginate (Polyelectrolyte 211) on bone uptake of radioactive strontium and calcium after injection into an intestinal segment. The solutions used were as described in text.

strontium from ligated intestinal segments is very similar, the rates of absorption differ markedly, showing physiological preference towards calcium.

TABLE II.—INFLUENCE OF SODIUM ALGINATE ON SR^{89} AND CA^{45} LEVELS IN RAT'S BLOOD

Segment	*2 microcuries of Sr^{89} with 0.45 micromole of inactive $SrCl_2$ for 30 minutes* Control	*with 1.5 micromoles of sodium alginate*	*2 microcuries Ca^{45} with 0.55 micromole of inactive $CaCl_2$ for 30 minutes* Control	*with 1.5 micromoles of sodium alginate*
I	86 ± 32	30 ± 21	———	———
II	3133 ± 1328	1476 ± 409	2351 ± 436	2187 ± 770
III	1504 ± 781	255 ± 46	856 ± 226	651 ± 280
IV	548 ± 195	293 ± 79	612 ± 156	624 ± 200
V	497 ± 116	265 ± 71	553 ± 195	504 ± 139
VI	578 ± 159	232 ± 74	492 ± 119	431 ± 114
VII	862 ± 594	246 ± 58	465 ± 131	541 ± 141
VIII	624 ± 250	214 ± 44	534 ± 185	551 ± 163
IX	748 ± 258	240 ± 81	578 ± 163	556 ± 139
X	805 ± 375	278 ± 108	551 ± 146	495 ± 165

Each figure represents mean counts per minute and standard deviation for studies on 12 rats.

The excretion of calcium and strontium ions into the intestinal lumen is not accounted for in the above experiments; these studies are in progress.

The Effect of Sodium Alginate on Absorption of Calcium and Strontium

Sodium alginate administered in quantities somewhat in excess of that required for two uronic acid residues to bind one atom of the divalent metal

TABLE III.—INFLUENCE OF SODIUM ALGINATE ON SR^{89} AND CA^{45} UPTAKE BY RAT'S FEMUR

Segment	*2 microcuries Sr^{89} with 0.45 micromole of inactive $SrCl_2$ for 30 minutes* Control	*with 1.5 micromoles of sodium alginate*	*2 microcuries Ca^{45} with 0.55 micromole of inactive $CaCl_2$ for 30 minutes* Control	*with 1.5 micromoles of sodium alginate*
I	164 ± 81	86 ± 38	———	———
II	4180 ± 1865	2095 ± 570	2333 ± 1123	1714 ± 980
III	2625 ± 1034	593 ± 189	1185 ± 285	1090 ± 214
IV	692 ± 186	403 ± 120	1228 ± 190	1090 ± 290
V	888 ± 529	379 ± 117	814 ± 214	771 ± 142
VI	826 ± 324	421 ± 124	833 ± 176	661 ± 119
VII	1353 ± 473	510 ± 164	723 ± 180	780 ± 242
VIII	859 ± 289	452 ± 156	652 ± 133	766 ± 180
IX	1457 ± 506	449 ± 79	685 ± 138	771 ± 157
X	1326 ± 551	460 ± 130	714 ± 119	804 ± 228

Each figure represents mean counts per minute and standard deviation in 12 rats.

effectively reduced the absorption of strontium from all intestinal segments and from the stomach by as much as 50-80% of the control values (Fig. 4 and Table I). Reduction in blood level and in bone uptake followed the same pattern (Figs. 5 and 6; Tables II and III). No significant reduction in the absorption of calcium was observed, and these results were confirmed by the unchanged calcium blood levels and bone uptake. It was not possible to increase the dosage of alginate (1 mg. per 0.5 ml.) in these experiments because of the higher viscosity of the injected solutions. Experiments in administration by means of orogastric tube, which will be reported later, have shown that greater amounts of sodium alginate are capable of depressing calcium absorption to some extent; however, this effect was observed not to interfere with the nutritional balance of the animal. The amount of sodium alginate required to affect strontium absorption from intestinal segments is very small compared with the amount of citric acid or versene used to suppress calcium absorption in similar experiments described by Thomas, Litovitz and Geschikter.[14]

SUMMARY

The pattern of absorption of strontium and calcium was studied in rats by an *in vivo* technique, using ligated intestinal segments with Sr^{89} and Ca^{45} as tracers. The pattern of absorption of both ions was very similar but the rates of absorption differed. The distribution of Sr^{89} and Ca^{45} in blood and bone uptake was found to correspond with the absorption pattern. Polyelectrolyte sodium alginate, when injected simultaneously and in excess with the radiostrontium, effectively reduced the absorption of Sr^{89} from all segments of the intestine by as much as 50-80% of the control values. The reduction in blood levels of Sr^{89} and in bone uptake was comparable. No significant reduction in absorption of Ca^{45} was observed.

The technical assistance of Mr. Michael Farrell, Mr. Serge Podymov and Miss Margaret Evans is gratefully acknowledged.

REFERENCES

1. MCLEAN, F. C. AND URIST, M. R.: Bone; an introduction to the physiology of skeletal tissue, 2nd ed., University of Chicago Press, Chicago, 1961.
2. BOURNE, G. H., *editor*: Biochemistry and physiology of bone, Academic Press, Inc., New York, 1956.
3. SKORYNA, S. C. AND KAHN, D. S.: *Cancer*, **12:** 306, 1959.
4. KAHN, D. S., MAKHANI, J. S. AND SKORYNA, S. C.: *Laval Méd.*, **34:** 169, 1963.
5. ZANDER-PRINCIPATI, G. E. AND KUZMA, J. F.: *Radiat. Res.*, **13:** 489, 1960.
6. MIGICOVSKY, B. B.: *Canad. J. Biochem. Physiol.*, **37:** 1287, 1959.
7. GEORGE, W. H. S.: *Nature (London)*, **195:** 155, 1962.
8. EDMONDSON, L. F. *et al.*: *Journal of Dairy Science*, **45:** 800, 1962.
9. SILVERMAN, J., GHOSH, D. AND BELCHER, R. L.: *Nature (London)*, **198:** 780, 1963.
10. PAUL, T. M., WALDRON, E. D. AND SKORYNA, S. C.: Proceedings of the Canadian Federation of Biological Societies, **1:** 40, 1964.
11. SKORYNA, S. C., PAUL, T. M. AND WALDRON, E. D.: *Rapp. 7ème Cong. Int. de Gastroentérologie (Bruxelles)*, **3:** 291, 1964.
12. PAUL, T. M. *et al.*: Factors affecting absorption of calcium and strontium, to be published.
13. NILSSON, A. AND ULLBERG, S.: *Acta Radiol. (Stockholm)*, **58:** 81, 1962.
14. THOMAS, R. O., LITOVITZ, T. A. AND GESCHIKTER, C. F.: *Amer. J. Physiol.*, **176:** 381, 1954.