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THE EFFECT OF STABLE STRONTIUM ON THE ALKALINE PHOSPHATASE ACTIVITY OF RAT TISSUES—*IN VITRO* STUDIES

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Abstract—*In vitro* studies on the effect of various amounts (10^{-6}–10^{-3} M) of stable strontium (Sr^{2+}) on the alkaline and acid phosphatase activities of rat tissues have shown a diverse response of the enzymes depending on the Sr^{2+} level and the tissue. Although both the enzymes manifest reduced activities at 10^{-3} M Sr^{2+} in almost all the tissues studied only those of liver alkaline phosphatase and small intestine acid phosphatase were significantly affected. There was, however, no change at lower Sr^{2+} concentrations. The Sr^{2+}-induced apparent inhibition of liver alkaline phosphatase, was found to be nonspecific and reversible by the concomitant addition of magnesium probably due to the manifestation of the Mg^{2+}-dependent enzyme. The implications of the inhibitory action of Sr have been discussed with reference to tissue Sr content regarding the possible therapeutic use of stable Sr for minimizing the retention of Sr-90.

Stable strontium has been used therapeutically in experimental animals [1–5] to minimize the retention of Sr-90 by virtue of isotopic dilution and in man for Sr-85 removal [6]. This mode of treatment involves feeding or injection of stable strontium (Sr) the levels of which may at times be high and unphysiological since this element is present in trace amounts only in the tissues [7]. The effects of such high levels are not fully known, there being reports about its beneficial [8] as well as deleterious effects [9, 10]. Although the toxicity of Sr has been studied, [11, 12] there are, however, only a few reports about the effect of Sr on the enzyme activities of tissues. Sobel *et al.* [13] did not find any significant change in the alkaline phosphatase activity of rat bones due to Sr feeding. Hofstee [14] in his studies on the mechanism of the action of Zn^{2+} and Mg^{2+} on calf duodenal alkaline phosphatase observed loss of activity at 10^{-3} M Sr^{2+}, when the crude extract of the duodenum powder was pretreated with versene. Neumann [15] demonstrated that the pH optimum of intestinal alkaline phosphatase does not change due to Sr^{2+}.

The therapeutic use of stable Sr necessitates a detailed study of the action of various amounts of Sr^{2+} on the tissue enzymes for a proper understanding of the possible side effects and for devising suitable remedial measures against them. This paper describes the results of *in vitro* studies on the effect of Sr^{2+} under various conditions on the activities of alkaline phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1) and acid phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2) of rat tissues. Both these enzymes seem to be a group of enzymes having perhaps different substrate specificities, rather than a single enzyme. Also there are Mg^{2+}-dependent and Mg^{2+}-independent alkaline phosphatases. As this investigation is a gross study of the activities of the enzymes, the terms such as liver or intestinal enzymes used subsequently in the text actually connote the enzyme activity of the tissue and not necessarily single enzymes.

MATERIALS AND METHODS

Adult male Wistar rats were used for the experiment. They were fed *ad lib.* the stock diet of our colony. After overnight fasting (to reduce the intestinal contents) the rats were killed by stunning. The tissues were weighed after wiping off the blood with blotting paper and homogenized in ice-cold 0·9% saline using a blendor (Braun) at high speed for 2 min. This coupled with prior freeze–thawing 3 times fully solubilized the latent (lysosomal) acid phosphatase activity [16]. Prior to homogenization, the small intestine was split open and washed gently twice with ice-cold saline. The homogenates were filtered through gauze. To obviate the influence of endogenous electrolytes and diffusible substances the homogenates were dialyzed (in ion specificity studies) against distilled water for 36 hr in the cold with replacement of water after every 10–12 hr. The rat liver alkaline phosphatase enzyme was prepared as described by Fishman *et al.* [17]. The alkaline phosphatase activity was determined by the method of King and Armstrong [18] which was modified as follows: an aliquot (about 0·5 ml) of the tissue homogenate, serum or enzyme solution was incubated at 37° for 30 min with 3 ml 50 mM veronal buffer (pH 9·2), 2 ml of the substrate-disodium phenyl phosphate (concentration 10 mM, unless stated otherwise) and with or without added ions. Mg^{2+} (10^{-3} M as $MgSO_4$)

was added to the assay medium unless stated otherwise. The enzyme action was stopped with 2 ml dilute Folin–Ciocalteu reagent and the mixture filtered. Aliquots of the filtrate were treated with 2 ml 0·5 M NaOH to develop the blue colour which was read at 580 nm. Acid phosphatase was determined in the same way as above except that a 200 mM citrate buffer (pH 4·9) was used in place of veronal buffer and that no Mg^{2+} was added. Solutions of $SrCl_2$, $Sr(NO_3)_2$, $BaCl_2$, $CaCl_2$ or $MgSO_4$ (all of analytical grade) were added to the reaction medium to give the required ion concentration. The pH was checked with a glass electrode at the beginning and the end of the enzyme action in trial runs and was found to be unchanged. The substrate was rejected if it showed any free phenol or PO_4^{3-} as the latter is an inhibitor of alkaline phosphatase. Bone alkaline phosphatase was assayed after extraction of the enzyme as described by Sobel *et al.* [13]. The enzyme activity was expressed as mg (μmole × 10·6) of phenol liberated during 30 min incubation period at 37° by 100 g of fresh tissue, unless stated otherwise.

RESULTS

Alkaline phosphatase activity of different tissue homogenates. The results of the experiment with different tissues, given in Table 1, show that only the activity of the liver enzyme was affected significantly ($P < 0·01$) at 10^{-3} and 10^{-4} M Sr^{2+}, the losses being 80 and 40% respectively. The lowering of enzyme activity in other tissues, seen only at 10^{-3} M Sr^{2+}, was not significant. Mg^{2+} was added during the assay, the concentration being 10^{-3} M.

In view of the greater susceptibility of the liver enzyme the influence of Sr^{2+} on it was studied in detail.

Liver alkaline phosphatase activity and the time of Sr^{2+} addition. Experiments with 10^{-3} M added Mg^{2+} were carried out to ascertain if the observed loss of liver alkaline phosphatase activity was due to the effect of Sr^{2+} on the enzyme action itself, by adding Sr^{2+} before and after the enzyme action. The results (Table 2) reveal some loss of activity at 10^{-3} M Sr^{2+} although Sr^{2+} was added after the enzyme action. In this regard it may be mentioned here that occasionally some precipitates were formed at this high Sr^{2+} concentration. These precipitates may perhaps adsorb some of the coloured complex formed due to the reaction of liberated phenol with the Folin–Ciocalteu reagent leading to the apparent loss of activity. Nevertheless, the highly significant net loss of 52% and 27% of enzyme activity at 10^{-3} and 10^{-4} M Sr^{2+} respectively is due to the lowering effect of Sr^{2+} on the enzyme action itself.

Ion specificity studies. The specificity of stable Sr in alkaline phosphatase inhibition was tested by a comparative study, using dialyzed liver homogenates, with Sr^{2+}, Ba^{2+}, Ca^{2+}, NO_3^- and Cl^-, the latter two ions to see if anions have any modifying influence on the action of Sr^{2+}. The results (Table 3) demonstrate that both $SrCl_2$ and $Sr(NO_3)_2$ lower the enzyme activity to the same extent (60%) in the presence of added Mg^{2+} indicating that the inhibition is due to cation alone, the anion having no influence. Ba^{2+} and Ca^{2+} also lower the activity, though Ca^{2+} is somewhat less deleterious than Sr^{2+} or Ba^{2+}. Results of the experiments without Mg^{2+} show that there is a 50 per cent reduction in the phosphatase activity at 10^{-3} M Sr^{2+} level only and none at lower levels.

Enzyme specificity studies; acid phosphatase. Whether the Sr^{2+}-induced inhibition is specific to alkaline phosphatase was investigated by studying the action of Sr^{2+} on the acid phosphatase activity of liver, small intestine and kidney under similar conditions. A significant loss of 37 per cent ($P < 0·05$) was discernible (Table 4) in the case of intestinal acid phosphatase compared with the control at 10^{-3} M Sr^{2+} concentration whereas the losses in liver and kidney enzyme at the same Sr^{2+} level were not statistically significant. The enzyme activity remained unaffected at lower Sr^{2+} concentrations. The deleterious effect of Sr^{2+} is thus not specific to alkaline phosphatase.

Lability of tissue alkaline phosphatase. The possibility of the preferential reduction by Sr^{2+} of the alkaline phosphatase activity of liver which has relatively lower enzyme activity than small intestine or kidney was examined by appropriately adjusting the enzyme concentration (in the reaction medium) of the latter tissues so as to be comparable with that of liver, at a fixed Sr^{2+} level (10^{-3} M) and with 10^{-3} M added Mg^{2+}.

Table 1. Alkaline phosphatase activity of tissues at different concentrations of Sr^{2+}

Tissue	No. of rats	Concn of added Sr^{2+} (as $SrCl_2$)				
		0 Control	10^{-6} M	10^{-5} M	10^{-4} M	10^{-3} M
Liver	17	52 ± 4·5	50 ± 3·9	42 ± 3·7	30 ± 2·4	10 ± 2·4
Small intestine	8	452 ± 55·0	451 ± 51·8	450 ± 51·2	440 ± 54·4	397 ± 44·5
Kidney	7	429 ± 13·0	426 ± 15·0	421 ± 17·0	419 ± 17·5	351 ± 36·5
Bone	12	250 ± 23·4	262 ± 26·5	246 ± 26·7	243 ± 19·8	203 ± 24·4
Serum	12	31 ± 6·9	31 ± 6·9	31 ± 7·3	31 ± 5·6	27 ± 8·5

Enzyme activity (mean ± S.E.) is expressed as mg (μmoles × 10·6) phenol liberated in 30 min at 37° by 100 g fresh tissue or 100 ml serum. Substrate, 10 mM $C_6H_5Na_2PO_4$; 50 mM veronal buffer (pH 9·2); 10^{-3} M Mg^{2+}

Table 2. Liver alkaline phosphatase activity and the time of Sr^{2+} addition

	Concn of added Sr^{2+} (as $SrCl_2$)								
	0	10^{-6} M		10^{-5} M		10^{-4} M		10^{-3} M	
	Control	Before	After	Before	After	Before	After	Before	After
Mean ± S.E. (10 rats)	48 ± 5·8	47 ± 5·1	46 ± 6·0	40 ± 4·7	46 ± 5·7	32 ± 3·0	45 ± 3·9	8 ± 3·0	33 ± 4·9
Loss of activity (%)	—	2	4	16	4	33	6	83	31
Net loss of activity (%)	—	—	—	12	—	27	—	52	—

Enzyme activity is expressed as mg (μmoles × 10·6) phenol liberated in 30 min at 37° by 100 g fresh tissue. Substrate, 10 mM $C_6H_5Na_2PO_4$; 50 mM veronal buffer (pH 9·2); 10^{-3} M Mg^{2+}. $SrCl_2$ was added before or after the enzyme action.

Table 3. Effect of Sr^{2+}, Ba^{2+}, Ca^{2+}, Cl^- and NO_3^- on liver alkaline phosphatase activity

	Concn of added ion (M)							
	0 Control	Sr^{2+} (as $SrCl_2$) 10^{-6} M	10^{-5} M	10^{-4} M	10^{-3} M	Sr^{2+} [as $Sr(NO_3)_2$] 10^{-3} M	Ba^{2+} (as $BaCl_2$) 10^{-3} M	Ca^{2+} (as $CaCl_2$) 10^{-3} M
Mean (with 10^{-3} M Mg^{2+})	100	96	80	63	39	37	36	50
Loss of activity (%)	—	4	20	37	61	63	64	50
Mean (without Mg^{2+})	56	55	56	56	30	—	—	—
Loss of activity (%)	—	—	—	—	46	—	—	—

Enzyme activity is expressed as mg (μmoles × 10·6) phenol liberated in 30 min at 37° by 100 g fresh tissue. Substrate, 10 mM $C_6H_5Na_2PO_4$; 50 mM veronal buffer (pH 9·2). Dialysed liver homogenate was used. Number of rats = 9.

Table 4. Acid phosphatase activity of tissues at different concentration of Sr^{2+}

Tissue	No. of rats	Concn of added Sr^{2+} (as $SrCl_2$) 0 Control	10^{-6} M	10^{-5} M	10^{-4} M	10^{-3} M
Liver	6	191 ± 17·2	162 ± 13·4	162 ± 13·4	181 ± 18·3	139 ± 20·3
Small intestine	6	108 ± 13·8	102 ± 13·4	100 ± 11·9	98 ± 11·0	68 ± 6·5
Kidney	6	230 ± 10·8	233 ± 13·4	230 ± 15·3	244 ± 14·5	200 ± 10·4

Enzyme activity (mean ± S.E.) is expressed as mg (μmoles × 10·6) phenol liberated in 30 min at 37° by 100 g fresh tissue. Substrate, 10 mM $C_6H_5Na_2PO_4$; 200 mM citrate buffer (pH 4·9).

The results, depicted in Fig. 1, show that over a hundred-fold dilution, the loss in the enzyme of small intestine and kidney remains practically unchanged. At the same enzyme concentration, the losses suffered by alkaline phosphatase of liver, small intestine and kidney are about 55, 16 and 13 per cent, respectively, suggesting the lability of liver enzyme towards Sr^{2+}.

Studies with Mg^{2+}. The effect of concomitantly added Mg^{2+}, a known activator of alkaline phosphatase, was analysed to see if the inhibition of the enzyme in liver by Sr^{2+} could be reversed, thus affording protection by Mg^{2+}. An overall enhancement of 100, 50 and 30% of the enzyme activity was seen (Table 5) in the presence of 10^{-2}, 10^{-3} and 10^{-4} M Mg^{2+}, respectively.

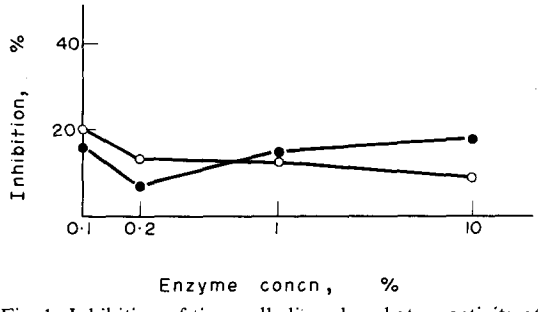

Fig. 1. Inhibition of tissue alkaline phosphatase activity at different enzyme concentrations (homogenate %) by Sr^{2+} (10^{-3} M). (●) Small intestine; (○) kidney. Homogenates were incubated with 10 mM $C_6H_5Na_2PO_4$, 50 mM veronal buffer (pH 9·2), 10^{-3} M Mg^{2+} and with or without added Sr^{2+} (10^{-3} M) for 30 min at 37°. Results expressed as inhibition (%) compared with the controls (without added Sr^{2+}).

The effect of Sr^{2+} on the enzyme was nearly the same, however, there being a loss of about 45 and 25 per cent at 10^{-3} and 10^{-4} M Sr^{2+}, respectively, in the presence or absence of added Mg^{2+}, when comparisons were made with corresponding Mg^{2+} controls. Furthermore the loss of activity due to Sr^{2+} was overcome by the concomitant addition of Mg^{2+} the concentration of which was about ten times that of Sr^{2+}. This suggests that the inhibition of enzyme activity by Sr^{2+} is apparent and that the restoration of activity is possibly due to the manifestation of the Mg^{2+}-dependent enzyme.

Studies with the partially purified liver alkaline phosphatase. Studies with partially purified liver alkaline phosphatase, the results of which are shown in Table 6, demonstrate the enzyme inhibition, albeit on a reduced scale, at 10^{-3} M Sr^{2+} only, in sharp contrast to those with the homogenates. Mg^{2+} does not alter the pattern of the results. In order to find out if the decrease in the enzyme activity is a sequel to the binding of the enzyme by Sr^{2+}, the enzyme preparation was preincubated for half an hour with various amounts of Sr^{2+} with or without Mg^{2+}, before the addition of the substrate. Pre-incubation, as seen from Table 6, enhanced the loss of activity by 15 per cent either in the presence or absence of added Mg^{2+} at 10^{-3} M but

Table 5. Effect of Sr^{2+} and Mg^{2+} on liver alkaline phosphatase activity

Concn of added Mg^{2+} (as $MgSO_4$)	Concn of added Sr^{2+} (as $SrCl_2$)		
	0	10^{-4} M	10^{-3} M
0	58 ± 6·2	44 ± 6·5	29 ± 8·8
10^{-4} M	67 ± 12·1	54 ± 13·4	42 ± 9·2
10^{-3} M	82 ± 7·9	59 ± 10·0	46 ± 7·1
10^{-2} M	110 ± 28·1	76 ± 14·1	66 ± 12·4

Enzyme activity (mean ± S.E., 10 rats) is expressed as mg (μmoles × 10·6) phenol liberated in 30 min at 37° by 100 g fresh tissue. Substrate, 10 mM $C_6H_5Na_2PO_4$; 50 mM veronal buffer (pH 9·2).

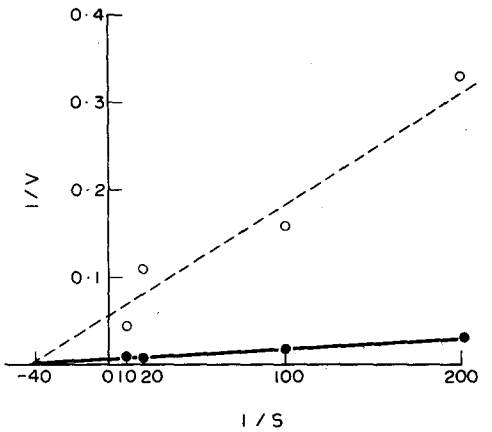

Fig. 2. Lineweaver–Burk plot showing the non-competitive inhibition of the partially purified liver alkaline phosphatase activity by Sr^{2+}.

V is expressed as mg (μmoles × 10·6) phenol liberated by 100 ml enzyme solution in 30 min at 37°. [S] is expressed as the molarity of the substrate ($C_6H_5Na_2PO_4$). (●) Enzyme without added Sr^{2+}, no inhibition. (○) Enzyme with added Sr^{2+}, 10^{-3} M. Enzyme solution was incubated with different concentrations (5–100 mM) of the substrate ($C_6H_5Na_2PO_4$) 50 mM veronal buffer (pH 9·2), 10^{-3} M Mg^{2+} with or without added Sr^{2+} (10^{-3} M).

failed to induce inhibition at even 10^{-4} M Sr^{2+}. This probably indicates the existence of a threshold level of Sr^{2+} for the manifestation of its inhibitory action. The nature of the inhibition, whether competitive or non-competitive, was investigated by experiments with various concentrations (5–100 mM) of the substrate in the presence or absence of added 10^{-3} M Sr^{2+}, the highest Sr^{2+} concentration, and 10^{-3} M added Mg^{2+}. This study was undertaken since it is known that metals bind with the substrates to form a complex [19]. It is possible that such a complex formation may render the substrate unavailable for the enzyme action. In Fig. 2 the reciprocal of the velocity of enzyme action is plotted against the reciprocal of the substrate concentration (Lineweaver–Burk plot). It may be seen that the inhibitory effect of Sr^{2+} was not abolished even at high substrate concentration. The curve shows that the inhibition was of the noncompetitive type.

DISCUSSION

This investigation has shown the diverse response of the alkaline and acid phosphatase activities of various tissues to different levels of stable Sr. Differences in the response of rat tissue alkaline phosphatase towards various levels of the inhibitors were also observed by Cochran *et al.* [20] in their studies on Be poisoning and by Fishman *et al.* [17] who studied the inhibition of the enzyme by L-phenylalanine. Human dental alkaline phosphatase is either unaffected or activated by Sr^{2+} [21].

Specificity studies described above have demonstrated that the inhibition is specific neither to Sr^{2+}, as Ba^{2+} and to a lesser extent Ca^{2+} also decrease the enzyme activity, nor to alkaline phosphatase as the intestinal acid phosphatase is likewise affected. While the effect of Sr^{2+} on these enzymes is adverse, Ba^{2+} ions, are known to cause a slight activation at pH 3·75–5·60 of rat liver acid phosphatase [22]. Sr^{2+} thus appears to be more deleterious in its action than Ba^{2+} or Ca^{2+} as far as these enzymes are concerned. That the inhibition is reversible and apparent has been shown by studies with Mg^{2+} and will be discussed later.

The possible mechanism of the alkaline phosphatase inhibition by Sr^{2+} may involve (a) its effect on the enzyme protein itself, (b) competition with the substrate or (c) reversal of enzyme activation by factors like Mg^{2+}. The results of these studies do not permit any speculation on the effect of Sr^{2+} on the enzyme protein. However, the preincubation studies and the complete restoration of activity by Mg^{2+} do not suggest any sizable effect of Sr^{2+} on the enzyme protein unless there are some conformational changes of the type as seen by Brunel and Canthala [23]. The lowering of this enzyme activity by Sr^{2+} is non-competitive with regard to the substrate. The studies with Mg^{2+}, Sr^{2+} and liver enzyme, provide some basis, for considering the possible mechanism of the Sr^{2+}-induced alkaline phosphatase inhibition by way of reversal of the enzyme activation due to Mg^{2+}. The addition of equimolar Mg^{2+} and Sr^{2+}, leads to partial protection of the enzyme against Sr^{2+} and that of ten times the

Table 6. Effect of Sr^{2+} on the partially purified liver alkaline phosphatase preparation

	Concn of added Sr^{2+} (as $SrCl_2$)								
	0 Control	10^{-6} M Preincu-bation*	10^{-6} M Before†	10^{-5} M Preincu-bation*	10^{-5} M Before†	10^{-4} M Preincu-bation*	10^{-4} M Before†	10^{-3} M Preincu-bation*	10^{-3} M Before†
Without added Mg^{2+}	71	71	75	75	71	75	94	16	29
With added Mg^{2+} (as $MgSO_4$) 10^{-3} M	89	93	89	72	83	89	89	32	42

Enzyme activity is expressed as mg (μmoles × 10·6) phenol liberated in 30 min at 37° by 100 ml of the enzyme solution. Substrate, 10 mM $C_6H_5Na_2PO_4$; 50 mM veronal buffer (pH 9·2). Number of rats = 8.

* Enzyme solution preincubated with Sr^{2+} for 30 min prior to the substrate addition.

† $SrCl_2$ added before the enzyme action.

Yield of activity in the preparation 78%; purification 10-fold; tissue equivalent 11 mg N per 100 ml of the enzyme solution.

concentration of Sr^{2+} results in not only complete protection but some increase also in the enzyme activity. This would seem to indicate that Sr^{2+} may have greater affinity for the enzyme than Mg^{2+} on a molar basis. Aldridge [24] has shown that a molar ratio of Mg^{2+}:Be^{2+} of about 40,000 was required to reduce the Be^{2+} inhibition of rabbit kidney alkaline phosphatase from 80 to 30 per cent. The extent of liver enzyme inhibition is somewhat less after dialysis and particularly in the enzyme preparation which was affected only at 10^{-3} M Sr^{2+}. It is worthwhile to note that in the dialyzed homogenate the loss of phosphatase activity increases gradually with the rise in Sr^{2+} concentration in the presence of Mg^{2+} than in its absence; in the latter case the loss, on a reduced scale, is discernible only at 10^{-3} M Sr^{2+}. This attenuated inhibition possibly suggests that the action of Sr^{2+} on liver alkaline phosphatase is not operating primarily through the effect on enzyme protein *per se* but rather through the reversal of activation by factors like Mg^{2+} which are gradually removed in the process by dialysis and partial purification. This contention would seem to be supported by the observed ability of Mg^{2+} to counteract completely the inhibition by Sr^{2+} and also by the enhancement of loss of activity on preincubation with Sr^{2+} presumably due to competition by Sr^{2+} with Mg^{2+} for the same sites in the enzyme molecule. It would be relevant to mention here that Sobel *et al.* [25] had observed that the intense inhibition of calcification *in vitro* by Sr^{2+} ions required the presence of Mg^{2+} ions. Moreover rat liver has been shown to contain alkaline phosphatases which are either activated by Mg^{2+} [26] or not activated by it [27]. Admittedly the fact that Mg^{2+} completely overcomes the Sr^{2+}-induced diminution of enzyme activity cannot be said to constitute the conclusive evidence in support of the mechanism of alkaline phosphatase inhibition by reversal of the enzyme activation. This is so because there are indications that "Mg may act by increasing the number of active sites rather than enhancing a particular rate" [28]. This total protective action of Mg^{2+} at such high concentration as 10^{-2} M, the ionic strength of the reaction medium being 0·112, makes it difficult to explain the fall in the enzyme activity in many tissues at 10^{-3} M Sr^{2+} (ionic strength 0·072) on the basis of ionic strength effect. It is even possible that the reduction in alkaline phosphatase also involves competition with some dialysable cofactor. The existence of a dialysable organic cofactor has been shown by Lehninger [29].

It would be of interest to discuss the possible implications of the inhibitory action of Sr^{2+} with reference to the Sr and Mg levels in the diet and tissues concerning the practical application of the Sr therapy. The stable Sr concentration (due to tissue Sr content) in the reaction medium for enzyme assay containing aliquots of tissues is of the order of 10^{-7} M as computed from a separate study (to be published). The addition of stable Sr in the range 10^{-6}–10^{-3} M raised the total Sr^{2+} concentration in the reaction medium from 10 to 10,000 times of that in the tissue itself. The Mg^{2+} concentration in the medium under similar conditions is estimated to be about 10^{-5} M using the figures available in the literature [30, 31]. Thus the Mg^{2+} concentration is nearly 100 times that of Sr^{2+} and extraneous addition of Mg^{2+} raised it from 10 to 1000 times the normal tissue content (liver). It is noteworthy that whereas the intestinal and kidney alkaline phosphatases can withstand, without any appreciable change, Sr^{2+} levels as high as 1000 times the usual tissue level, the more vulnerable liver enzyme is affected even at 100 times the normal Sr^{2+} concentration in the tissue. In view of the known lack of homeostasis the tissue Sr levels, unlike those of Ca, may rise promptly as well as proportionately [32] and remain at very high, perhaps toxic, levels if the dietary Sr intake is unusually high for longer periods, notwithstanding the rapid removal of Sr from the soft tissues as shown by the metabolic studies with radioactive Sr. The normal dietary Sr level varies considerably but is about 0·0001–0·004 per cent [33–35]; in the stock diet used in this experiment it was found to be about 0·001 per cent (unpublished data). Stable Sr levels as high as 1–2 per cent in the diet have been used for various periods in therapeutic studies [36–39]. These levels (1000-fold the normal dietary ones) may increase the tissue Sr concentration proportionately i.e. from 10^{-7} to 10^{-4} M Sr^{2+} in the reaction medium, to cite an example. At this high level of 10^{-4} M Sr^{2+} obviously a significant deleterious effect of Sr^{2+} on the liver alkaline phosphatase activity (albeit in the homogenate) was seen. Indeed, serum Sr values as high as 4·9 mg Sr per 100 ml in rats fed 1·5% Sr diet for 2 weeks were reported by Storey [40] as against the normal plasma concentration of 0·004 mg% for Sr [32]. This *in vitro* study, therefore, if it has a bearing on the *in vivo* conditions, would serve to illustrate the possible damage due to prolonged therapy with large amounts of stable Sr to alleviate Sr-90 poisoning.

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