XIII. DEPOSITION OF STRONTIUM SALTS IN HYPERTROPHIC CARTILAGE *IN VITRO*.

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EXPERIMENTS on the deposition of barium, strontium and magnesium salts in bone slices *in vitro* have been previously reported [Robison and Rosenheim, 1934] and have shown that the calcifying mechanism is not entirely specific for the calcium salt of bone, although the properties of this salt are very specially appropriate to the function which it fulfils. Deposits of the phosphates (or carbonatophosphates) of these allied metals were produced in the matrix of the hypertrophic cartilage by agency of the phosphatase mechanism. The behaviour of strontium showed further resemblance to that of calcium in that its salts could also be deposited in the cartilage from supersaturated inorganic solutions, in absence of added phosphoric ester, that is, by the agency of the second mechanism.

This reaction of hypertrophic cartilage towards strontium salts gained interest as a result of the experiments of Sobel *et al.* [1934] who showed that the substitution of SrCO_3 for CaCO_3 in a rickets-producing diet caused the development in rats of a severe type of rickets which was not cured by vitamin D therapy. Bone slices from these rats also failed to become calcified *in vitro* in artificial sera which produced good deposits in bone slices from rats suffering from rickets of the usual type. It was subsequently shown [Sobel *et al.*, 1935, 1, 2] that there is a marked diminution, but not a complete destruction, of the calcifying power of the bones of animals suffering from strontium rickets and that the activity of the calcifying mechanism can be restored both *in vivo* and *in vitro* by bathing the bone cells in fluids free from strontium. It was shown that Sr^{++} strongly inhibits calcification *in vitro* but does not affect the activity of phosphatase even in concentrations as high as 80 mg. Sr/100 ml. These authors suggest that Sr combines with a factor in the bone whose concentration plays a part in calcification.

In view of these results it appears worth while to describe briefly some further experiments which confirm and extend our previous findings although they do not provide the full information for which we sought, namely the exact composition of the deposits. We first investigated the possibility that the deposits obtained in the earlier experiments and presumed to consist of strontium salts were in reality calcium salts, the calcium being derived either from the strontium chloride used in preparing the solutions or from the calcified portions of the bone slices themselves. Specimens of the strontium chloride previously used were submitted to spectrographic analysis and were found to contain appreciable amounts of calcium; the deposits produced in hypertrophic cartilage by solutions prepared from this salt were however shown to contain strontium. It is a reasonable assumption that even if the values of the product $\text{Ca} \times \text{P}$ in these solutions were high enough for calcification *in vitro* in absence of Sr (and this is very unlikely), calcification would have been completely inhibited by the high concentration of Sr.

Other specimens of strontium chloride, supplied by well-known firms, were also found to contain calcium; but a satisfactory specimen ("Specpure") was

obtained from Messrs A. Hilger, Ltd. The degree of purity of this salt was 99.993 %; it contained only 0.0013 % Ca reckoned on its Sr content. With this salt our further experiments were carried out.

The technique was similar to that previously described. Slices from the heads of the tibiae and humeri of rachitic rats were immersed for periods of 10–22 hours in the solutions at 37° and p_H 7.2–7.3; this p_H was slightly lower than usual in order to lessen the marked tendency of the strontium solutions to spontaneous precipitation. With 0.01 *M* or 0.005 *M* Sr, dense deposits extending through most of the zone of hypertrophic cartilage were uniformly obtained with solutions containing glycerophosphoric ester equivalent to 30 mg. P/100 ml. but no inorganic phosphate. Deposits were also obtained with regularity in absence of ester with solutions containing Sr and inorganic phosphate in suitable concentrations. The Sr $\times$ P product (500–900) was much higher than the Ca $\times$ P product required for calcification *in vitro*. For deposition in absence of ester a basal solution containing 0.6 % NaHCO₃ and 0.6 % or 0.4 % NaCl was again found to give the best results. Basal solution B, similar to that used for calcification *in vitro* [Robison and Rosenheim, 1934, p. 685] and containing 0.22 % NaHCO₃ but no sulphate, gave rather poor deposits with general precipitation. Typical results of these experiments are shown in Table I.

Table I. *Deposition of Sr salts in hypertrophic cartilage in vitro.*

Concentration of SrCl ₂ ("Specpure")	g. per 100 ml. NaHCO ₃	mg. per 100 ml.		Deposition in cartilage (max. 10 ≡)	Sr $\times$ P
		P inorganic	P organic		
0.01 <i>M</i>	0.6	7	0	8 ≡, 9 ≡	616
0.01 <i>M</i>	"	8	0	9 ≡, 6 ≡	704
0.005 <i>M</i>	"	12	0	6 ≡, 5 ≡	524
0.005 <i>M</i>	"	16	0	7 ≡, 7 ≡	706
0.0025 <i>M</i>	"	30	0	8 =	660
0.0025 <i>M</i>	"	40	0	7 ≡	880
0.01 <i>M</i>	0.22 (B)	9	0	1 =	792
0.01 <i>M</i>	"	10	0	5 -	880
0.005 <i>M</i>	"	16	0	2 -	706
0.0025 <i>M</i>	"	30	0	2 -	660
0.0025 <i>M</i>	"	40	0	3 -	880
0.01 <i>M</i>	0.05	0	30	9 ≡, 9 ≡, 10 ≡	
0.05 <i>M</i>	"	0	30	5 ≡	

Deposition in hypertrophic cartilage free from calcified tissue.

In order to eliminate as far as possible the objection that calcium might be derived from the bone slice itself, experiments were also carried out with segments of cartilage dissected from the metaphyses of tibiae and free from all calcified tissue. Deposition occurred in these segments as regularly and as heavily as in the usual bone slices. Spectrographic analysis showed that these cartilage segments, when removed from the bone, contained only traces of calcium, in amounts of the order 0.001–0.003 mg.

Nature of the deposits formed in hypertrophic cartilage in vitro with the experimental strontium solutions.

The spectrographic examination of the deposits obtained in the earlier experiments was carried out by the method of Ramage [1929; Sheldon and Ramage, 1931]. In the later experiments a method of spark spectrum analysis was employed, using copper electrodes. The zone of hypertrophic cartilage containing the deposit was dissected under a binocular microscope and placed in 0.5 ml. *N* HCl; after 16–20 hours 0.5 ml. H₂O was added. Inorganic phosphate

was estimated colorimetrically in 0.5 ml. of this extract. Tests carried out by ashing the residual cartilage showed that extraction was complete. For the spectrographic examination, 0.01 ml. of the extract was pipetted on to the electrode and evaporated by gentle warming; three successive exposures, each of 15 sec., were made. The standard solutions contained Sr, Ca, inorganic phosphate and HCl in concentrations as close as possible to those present in the cartilage extracts. The presence of phosphate had a very marked effect in reducing the intensity of the Ca and Sr lines.

Table II shows some typical results obtained with segments of hypertrophic cartilage from which all calcified cartilage and bone had been dissected away. Three of these segments were immersed for 17 hours in a solution containing

Table II. *Composition of deposits formed in hypertrophic cartilage.*

Composition of solution			Composition of deposits (HCl extracts of cartilage)		
Sr (M)	Inorganic phosphate mg. P/100 ml.	Deposition in cartilage	Sr (mg.)	Ca (mg.)	P (mg.)
0.0025	10	—	0	0.003	0.001
"	"	—	0	"	"
"	"	—	0	"	"
"	40	+	0.26	"	0.040
"	"	+	0.20	"	0.022
"	"	+	0.24	"	0.030

0.0025 M Sr, 40 mg. P/100 ml. as inorganic phosphate, 0.6 g. NaHCO₃ and 0.6 g. NaCl per 100 ml. The other three segments, which served as controls, were immersed for a similar period in a solution containing too low a concentration of phosphate (10 mg. P/100 ml.) to yield any deposit in the cartilage. Great accuracy cannot be claimed for the values for Sr so far obtained by this method, but they undoubtedly prove that the deposits formed in cartilage consist largely of strontium phosphate or carbonatophosphate. The values of the ratio Sr : P suggest that the proportion of carbonate may be higher in these deposits than in the bone salt, but a definite opinion on this point cannot be expressed until more reliance can be placed on the results of the spectrographic analyses. It would seem that the cartilage itself firmly retains a trace of calcium which is not removed by immersion in the strontium solutions.

SUMMARY.

1. The deposition of strontium salts in hypertrophic cartilage *in vitro*, by immersion of the cartilage in solutions containing strontium, inorganic phosphate and carbonate ions, has been confirmed, using spectroscopically pure strontium salts and cartilage dissected away from all calcified tissue.

2. The deposits so formed have been shown by spectrum analysis to consist largely of a strontium phosphate or carbonatophosphate.

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