seminal vesicle proteins rather than in the vesiculase preparations used, since coagulation induced by partially purified vesiculase, which had undergone repeated precipitation and dialysis, is not influenced by the addition of Mn^{++} or Ca^{++} ions to the reaction mixture (1).

Summary. Studies are presented on the influence of temperature and of a number of chemical substances upon coagulation of proteins of the seminal vesicle secretion by the prostatic enzyme vesiculase. Experiments involving delayed addition of metal chelating agents and of heavy metal ions have shown that the coagulation process can be separated into two distinct stages.

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Comparative Metabolism of Sr^{89} and Ca^{45} by Bone Grown *in vitro*.* (22856)

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The comparative metabolism of calcium and strontium has been of recent interest because of the possible hazards arising from ingestion of radioactive strontium. Calcium has been shown to be preferentially absorbed from the digestive tract(1) and strontium to be excreted to a greater extent than calcium by the kidney(1,2); the net result of both processes favors the retention of calcium over strontium. Evidence is lacking, however, that bone metabolism distinguishes between these two alkaline earth metals. The large discriminations of the intestinal tract and the kidney could mask any selective capacity of bone when the intact animal is used as the experimental subject. Therefore, embryonic chick bones were cultured *in vitro* in order to study the comparative metabolism of Sr^{89} and Ca^{45} by bone uninfluenced by the action of the other organs.

Most experiments have compared calcium to carrier-free radiostrontium. It seemed advisable for present purposes to include the uptake of radiostrontium in the presence of significant levels of carrier strontium. This would also have clinical aspects since stable strontium salts have been advocated as therapeutic agents to aid in the remineralization of bone(3).

Methods. The procedure used was essentially that of Fell(4) except that the bones, rather than resting in a plasma clot, were bathed by a liquid medium while in a roller-tube apparatus. Tibias and femurs were dissected from 7-day-old chick embryos and adhered to the walls of 18 x 150 mm pyrex test tubes in a chicken plasma clot. The medium consisted of 0.5 ml chick embryo extract, 2. ml of dog serum, 50 μl of Tyrode's solution containing about 0.1 and 0.05 μc of Ca^{45} and Sr^{89}, respectively, 100 units of penicillin, and 100 μg of streptomycin. The total amount of medium was increased by 50 μl in the experiments testing the effects of carrier strontium. The additions were made to the medium from

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0, 0.00037, 0.0037, and 0.037 M solutions of $SrCl_2$. The calcium content of the media ranged from 9.7 to 12.7 mg %. The prepared tubes were incubated at 37°C while being rotated at 20 revolutions per hour. The medium was changed every 2 or 3 days. Each bone was individually assayed for radioactivity. To prepare the samples, the bone was placed in a 1-inch diameter stainless steel planchet, dissolved in 1 ml of 6 N nitric acid, and dried under infra-red lamps. Self-absorption corrections were unnecessary since there was less than 1 mg of residue. The samples were counted, after a 21-day waiting period, under a thin window G. M. tube by means of a differential absorption of the β emissions(5). The results are expressed as the strontium-calcium Observed Ratio ($OR_{bone\text{-}medium}$) as suggested by Comar *et al.* (1).

$$OR_{bone\text{-}medium} = \frac{Sr^*/Ca^* \text{ of bone}}{Sr^*/Ca^* \text{ of medium}} \quad (1).$$

Results. Experiment A presents the data on the Observed $Ratio_{bone\text{-}medium}$ (O.R.) of embryonic chick bone after immersion in the labeled medium for a 2-hour period. No distinctions were made between tibias and femurs since their relative metabolism of strontium and calcium were identical. In this early phase of mineral uptake, 1.08 times as much strontium-89 entered the bone as calcium-45. It will be noted that the carrier strontium at the 0.1 mg % level had no effect upon the total amount of Ca^{45} accumulated. About 0.16% of the Ca^{45} present in the medium was deposited in the bones under these conditions.

When bone was grown in the presence of the labeling isotopes for a period of 14 days an O.R. of 0.83 resulted (Exp. B). In preliminary experiments, culture periods as short as 7 days were sufficient to produce this ratio, indicating that equilibrium had been established between bone and the medium. When bone was grown in the labeled medium for 9 days and followed by 5 additional days in a radioisotope-free medium, the O.R. fell from 0.83 to 0.76. These ratios were shown to be different ($P<0.05$) by statistical analysis.

In another experiment employing a labeling period of 6 days, the O.R. was found to be 0.80, 0.71, and 0.66 at 0, 4, and 6 days, respectively, after being placed on the non-labeled medium. A plot of the Observed Ratios versus Time suggested that during this short period of observation the rate of decline was linear.

TABLE I. Deposition of Ca^{45} and the Strontium-Calcium Observed Ratio of Embryonic Chick Bone Grown *In Vitro*.

Carrier strontium added, mg %	Time period	No. of bones	Observed Ratio	Avg % of Ca^{45} dose in bone
		Exp. A		
.0	2 hr	24*	1.08 ± .02†	.16
.1	2	24	1.05 ± .01	.15
		Exp. B		
.0	14 days	30	.83 ± .02	4.95
.0	9	18	.84 ± .01	4.78
.0	14 ‡	30	.76 ± .01	3.00
		Exp. C		
.0	9 days	18	.84 ± .01	4.78
.001	9	18	.80 ± .02	4.38
.01	9	18	.80 ± .02	3.48
.1	9	18	.77 ± .02	2.23

* Composed of equal numbers of femurs and tibias assayed individually.
† Mean ± stand. error.
‡ Radioactivity omitted from the medium during the last 5 days.

In 9 days the bones (Exp. B) accumulated an average of 4.78 and 3.98%, respectively, of the radiocalcium and radiostrontium present in the medium. After 5 subsequent days without activity, only 3.00% of the Ca^{45} and 2.27% of the Sr^{89} remained. The Sr^{89}/Ca^{45} ratio of the lost activity was 0.96. If it is assumed that the ratio in bone declined linearly during this period, then by dividing 0.96 by the average strontium-calcium ratio of bone $\left(\frac{0.83 + 0.76}{2}\right)$ it can be calculated that 1.2 times as much strontium-89 left bone as calcium-45.

The data of Exp. C demonstrated that even with relatively high levels of non-radioactive strontium in the medium, bone retained more calcium-45 than strontium-89. Only at the 0.1 mg% level of carrier strontium was the O.R. depressed sufficiently to be significantly

different ($P<0.05$) than the O.R. of the carrier-free group.

The addition of 0.1 mg% of inert strontium produced markedly lower ($P<0.01$) uptakes of Ca^{45} than did a level of 0.001 mg % of strontium or the carrier-free group. The bones grown in a medium to which carrier strontium had not been added contained twice the radioactivity of the 0.1 mg% strontium group. In another experiment, 1.0 mg % strontium lowered the Ca^{45} uptake by 80% and indicated that stable strontium can markedly interfere with the calcification of bone when present in large enough quantities.

Discussion. Like the kidney and the digestive tract, bone, in forming its solid phase, has exhibited an over-all discrimination against strontium in favor of calcium. The system used for these experiments was artificial and therefore the results may not quantitatively represent what occurs *in vivo*. There are no reasons, however, to assume that embryonic and post-natal bone differ in their processes of bone crystal formation; thus, the qualitative events depicted can be expected to take place in bone metabolism *in vivo*.

The data in this paper show that strontium is favored in the initial uptake by bone by a ratio of 1.08, but that in the removal processes more strontium is lost than calcium by a factor of 1.2. Using the preceding discrimination factors and measurements showing that 4.78% Ca^{45} and 3.98 Sr^{89} were in the bones, it can be calculated by simultaneous equations that the accretion of Ca^{45} and Sr^{89} was 14.78 and 15.98%, respectively, of the available radioactivity. Major portions, 10.0 and 12.0% of the Ca^{45} and Sr^{89}, were removed during this 9-day period by resorption. The net result of these 2 processes would give an O.R. of 0.83. The bones were estimated to have added 0.015 mg of calcium during the 9-day period. From this it was determined that the accretion rate for calcium was 0.0145 mg calcium/hour/mg bone calcium and for resorption was 0.010. In 3-month-old rats, the corresponding rates for the tibias were 0.0026 and 0.0020(6). The values for the embryonic chick bones were about 5 times higher than those for 3-month-old rats. Since the rates do diminish with age, the *in vitro* data presented are in reasonable agreement with that seen *in vivo*.

The addition of large amounts of carrier strontium had very significant effects upon the extent of calcification of bone. This had been encountered before in both *in vitro* and *in vivo* studies(3,7). The addition of 0.1 mg % strontium increased the number of equivalents of calcium plus strontium in the medium by only 0.4% while the amount of Ca^{45} deposited in bone decreased 53%. This would rule out the possibility that strontium acted by competing with calcium for sites of deposition.

Summary. An *in vitro* culturing technic was used in comparing metabolism of calcium-45 and strontium-89 in bone. The results of a large number of determinations indicated: a) that at 2 hrs bone contained 1.08 times as much strontium-89 as calcium-45; b) when exposed to constant levels of Sr^{89} and Ca^{45}, the Observed $Ratio_{bone\text{-}medium}$ equilibrated at 0.83 within 7 days; c) strontium-89 was released from bone at a ratio of about 1.2 times that of calcium-45; d) addition of carrier strontium markedly inhibited the long term deposition of calcium in bone but the discriminatory mechanisms were unaltered.

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