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A Progress Report

to

The Atomic Energy Commission

Division of Biology and Medicine

Contract No. AT-(40-1)-1829

"The Metabolism of Alkaline Earth Metals by Bone"

Division of Chemistry

School of Biological Sciences

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Memphis

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Scope of the Investigations

The major emphasis has been given to comparing the deposition in and removal from bone of the various members of the alkaline earth group of elements. Throughout these studies *in vitro* cultures of embryonic bone were used as the test material. In order to compare the metabolic behavior with a degree of certainty, radio calcium was incorporated into the medium with the alkaline earth under investigation. The comparison of strontium metabolism to that of calcium received special emphasis. By culturing bones from 2 to 5-month old human fetuses some data was obtained on the behavior of these elements by human bone. In experiments with embryonic chick bone, the influence of various kinds of sera, temperature, and stable strontium upon the strontium-calcium ratio was determined. The removal and uptake of these nuclides was observed at several intervals within a 24-hour period to attempt to ascertain how soon the selective effect was operative and where the selection was occurring. The possible influence of enzymatic systems was investigated by comparing live and heat-killed bones.

Significant Results

A. Reinvestigation of Sr/Ca Ratios in Bone Growth *in vitro*

Preliminary work in this laboratory indicated that bone could select against strontium in the absence of other organs. Removal was implicated as the possible site of selection since strontium was found to leave bone at a ratio 1.2 times that of calcium. This has been rechecked using tibias from 9-day old chick embryos cultivated in a medium of dog serum. In addition the metabolism of the ends of the bones was contrasted to that of the shafts. The data obtained are:

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Table 1

Sr/Ca Ratio in Growing Bone

	Ends	Shaft
Labeled 7 days	0.69	0.75
Labeled 7 days then non-labeled medium for 4 days	0.59	0.69

The data bear out the selection against strontium and emphasize that the ends of the bone may select against strontium more than the shaft. By calculation it was determined that 1.12 times as much strontium as calcium left the ends of the bones. The corresponding figure for the shafts was 1.28. About 50% of the activity incorporated into the ends was lost during this period while only 20% was lost from the shafts.

B. Comparison of Barium and Calcium

In experiments similar to that above, the following data were observed:

Table 2

Ba/Ca Ratio in Growing Bone

	Ends	Shafts
Labeled 7 days	0.85	0.88
Labeled 7 days then non-labeled medium for 4 days	1.47	0.76

During the removal phase of the experiment, barium was selected against by the shafts of bone. The ends appeared to hold on to barium in preference to calcium. However, the counts were extremely low and reinvestigation would be helpful. Calculation of the data indicated that barium left the shaft at a ratio 1.35 times greater than calcium. For the ends of the bones only 0.81 times as much barium as calcium left during this period.

The 24-hour uptake of barium was investigated using tibias from 12-day old chick embryos. The data are graphed in Figure 1. Barium did not enter the ends of the bones as rapidly as did calcium; however, with time its accumulation increased, raising the Ba/Ca ratio. The total uptake of calcium and barium was very small. Barium appeared to enter the shafts at a rate

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FIGURE 1

Ba/Ca RATIO DURING UPTAKE

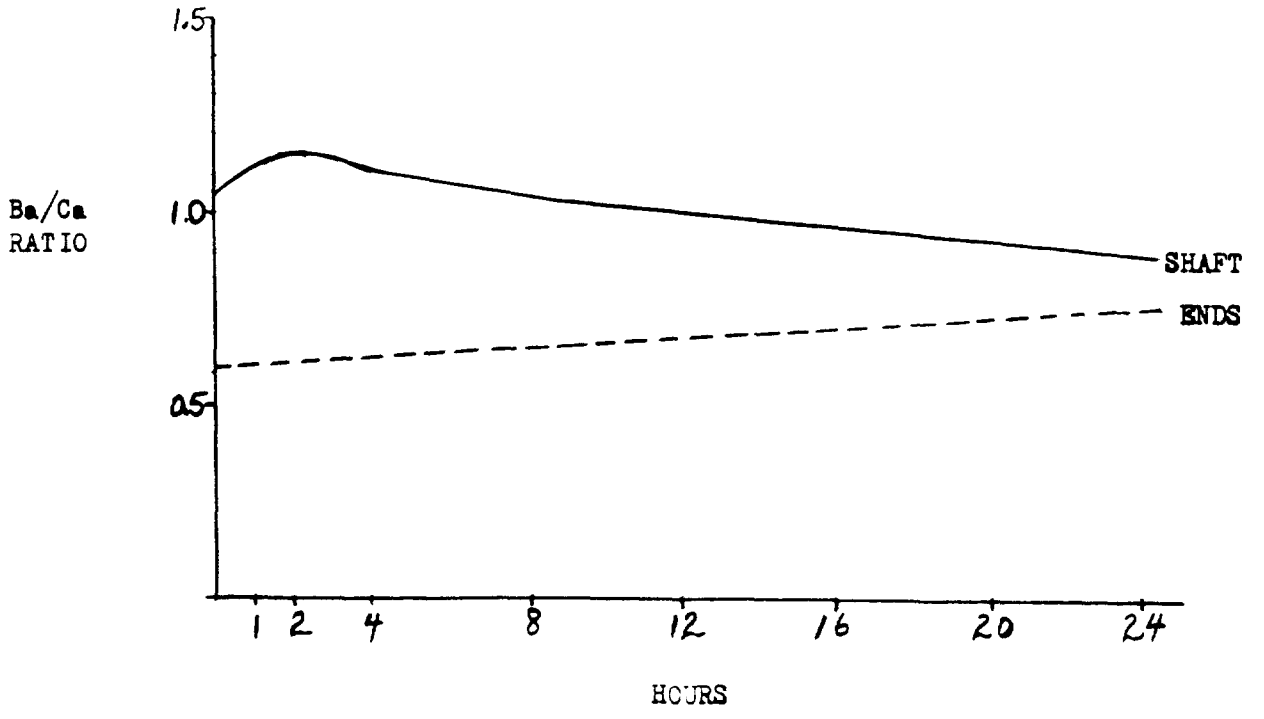
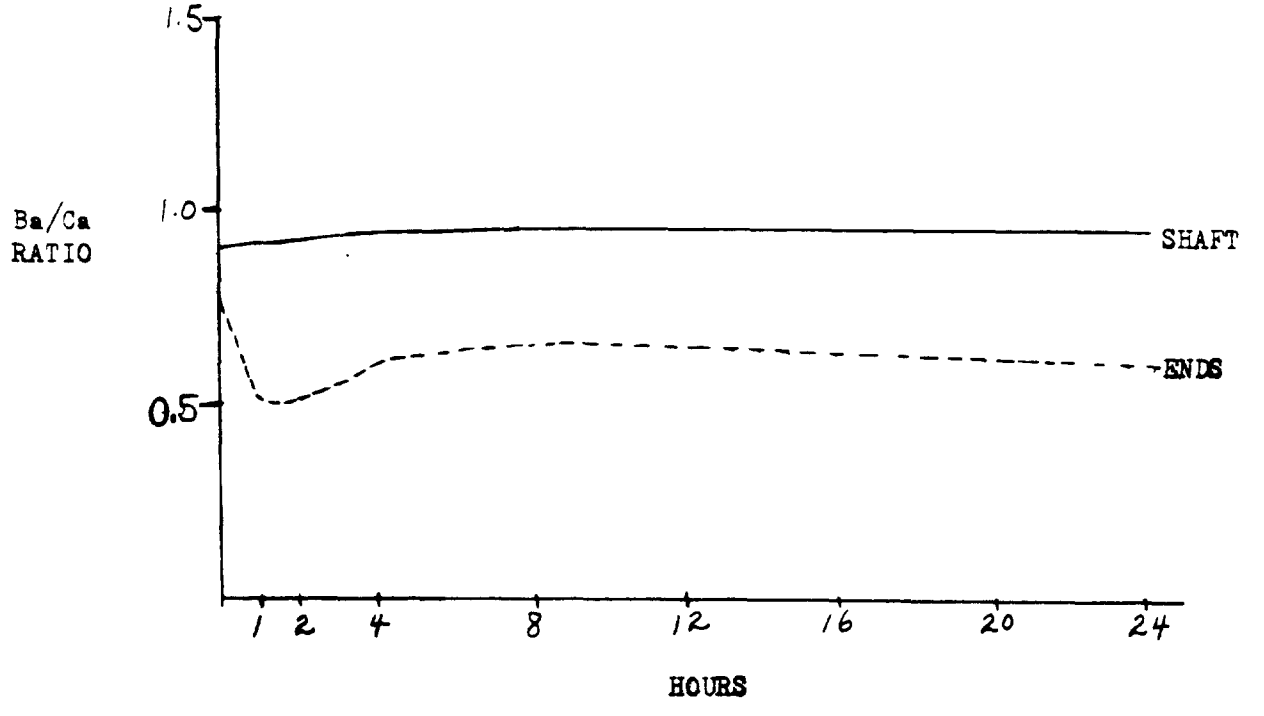


FIGURE 2

Ba/Ca RATIO DURING REMOVAL



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greater than that for calcium. With time the ratio decreased indicating a selection against barium was possibly occurring.

When bone was labeled for 24 hours and then removed to a non-labeled medium the process of removal could be studied. The data appear in Figure 2. The turnover in the ends of the bones was quite rapid and the selection against barium was maintained. For the shafts the calcium seemed more mobile than barium. At present, it is difficult to reconcile the results of the short-term and long-term studies.

C. Comparison of Beryllium and Calcium

The data for the long-term growth studies are presented in the following table:

Table 3

Be/Ca Ratios in Growing Bone

	Ends	Shafts
Activity for 7 days	15.76	2.18
Activity for 7 days then no activity for 5 days	43.50	2.42

The data indicate that tibias from 9-day old chick embryos accumulated beryllium to a greater extent than calcium. This was more evident in the ends than the shaft of the bone.

It was determined that beryllium left at a rate of 0.69 and 0.58 that of calcium for the shaft and ends of the bone, respectively.

In 24-hour experiments with bones from 12-day old embryos it was noted that the uptake of beryllium by the ends rapidly exceeded that for calcium (Figure 3). The shaft showed twice as much calcium taken up as beryllium. In studying removal (Figure 4) of the activity from bone, it was seen that calcium was mobilized from the ends more rapidly than the beryllium. For the shafts the ratio remained constant, indicating that the loss of beryllium was equal to that of calcium.

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FIGURE 3. Be/Ca RATIO DURING UPTAKE

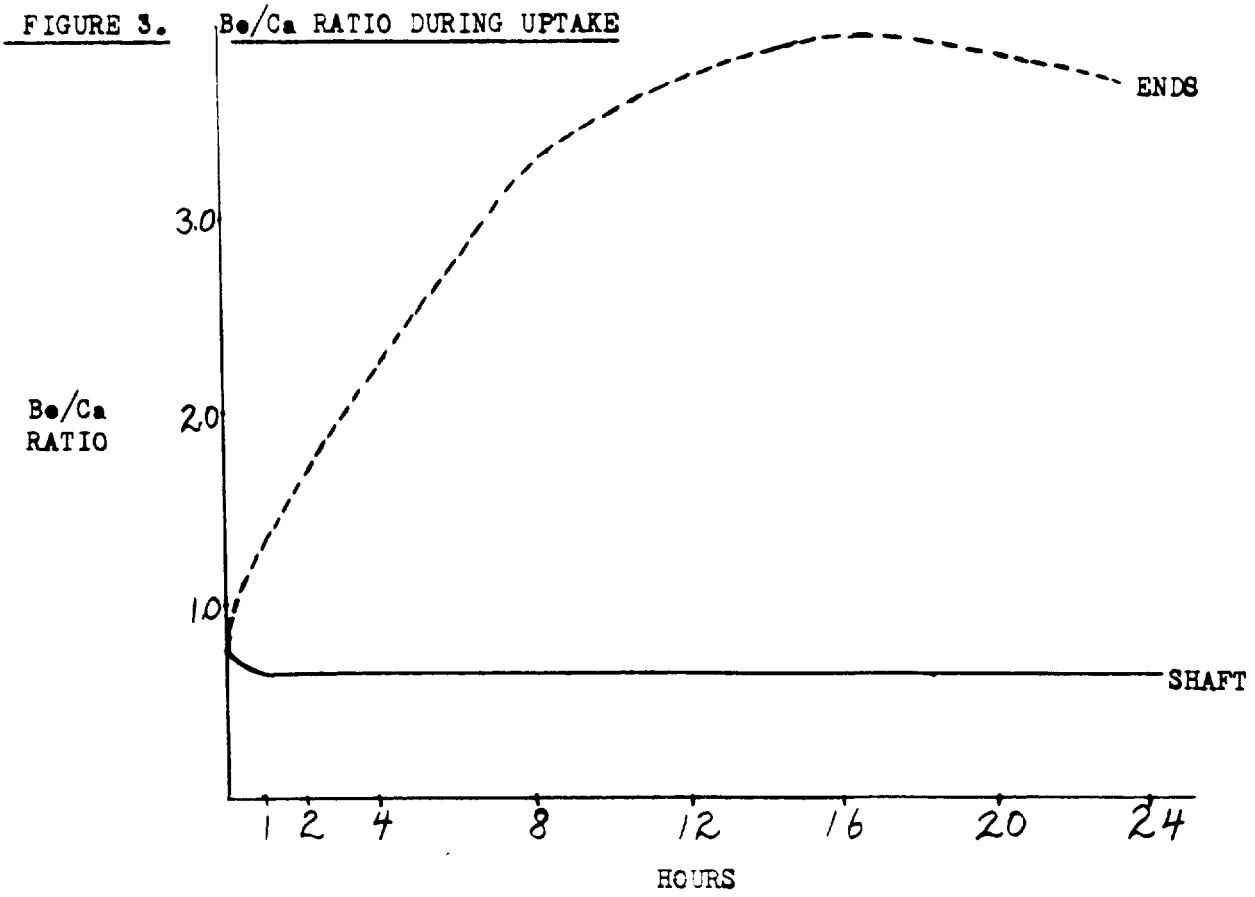
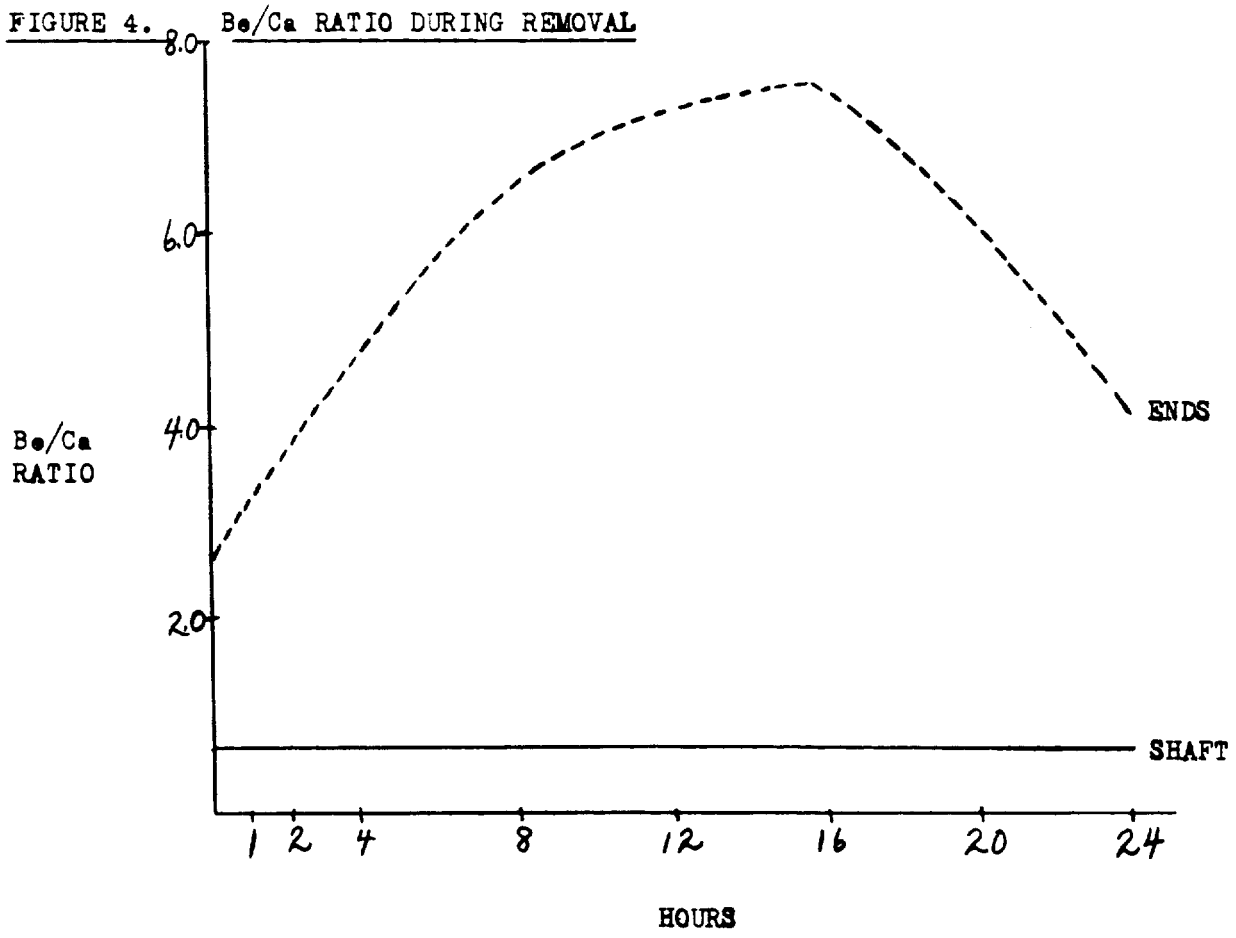


FIGURE 4. Be/Ca RATIO DURING REMOVAL



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The tremendous accumulation of beryllium by the ends of the bone fits in well with beryllium's known property of enzyme inhibition. Thus, the data for the ends does not depict the suitability of beryllium as a former of bone crystal. The data for the shaft probably expresses this suitability since the tissue is more highly calcified. However, more work is needed on this question.

D. Sr/Ca in Human Bone

The data for these experiments are contained in Table 4. Fetus No. 1 was 23 cm long and a negro female. The bones were large and well calcified so only metatarsal and metacarpal bones, and sections of R. B. were utilized. Fetus No. 2 was 12 - 14 cm in length and a negro male. Other estimates place the age as about 14 weeks. This was a surgical abortion and appeared in good condition. The bones were observed to increase in length during the culturing period. Fetus No. 3 was about 7 cm long and negro. The appearance was not good and the bones were not observed to grow in tissue culture.

For fetus No. 1 it can be seen that strontium left the bones at a ratio greater than that for calcium. However, the more calcified portions of the skeleton initially accumulated more strontium than calcium, while the less calcified portions selected against strontium. The data for fetuses 2 and 3 bear out the different metabolic behavior for the less calcified portions of the skeleton. However, fewer instances of selection against strontium during the removal phase were observed. Since it is in doubt that the bones from fetus 3 were viable few conclusions can be made from these data. Obviously, more experiments are needed.

E. Effect of Temperature on the Sr/Ca Ratio in Chick Bone

Table 5 contains the data relative to the Sr/Ca ratio in embryonic chick bone 24 hours after exposure to activity.

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Table 4

Sr/Ca Ratios in Human Embryonic Bones

Bone	Fetus 1			Fetus 2			Fetus 3		
	No. of Bones	Labeled 7 days	Labeled 7 days then 4 days in unlabeled medium	No. of Bones	Labeled 7 days	Labeled 7 days then 4 days in unlabeled medium	No. of Bones	Labeled 7 days	Labeled 7 days then 4 days in unlabeled medium
Metacarpal									
End	5	0.63	0.61	4	0.63	0.73		-	-
Shaft	5	1.14	1.03	4	1.07	1.06		-	-
Whole		-	-		-	-	4	1.16	0.85
Metatarsal									
End	4	0.77	0.72	4	0.84	0.64		-	-
Shaft	4	1.27	1.22	4	0.92	0.93		-	-
Whole		-	-		-	-	4	1.01	1.14
Rib									
End	6	1.16	0.99	3	1.02	1.03	3	1.02	1.05
Shaft	5	1.42	1.30	3	2.13	2.60	3	1.37	1.49
Cartilage		-	-	3	0.97	1.03	3	1.28	0.78
Ulna									
End				1	0.91	0.43	1	1.08	1.37
Shaft		no sample		1	2.32	2.10	1	0.96	1.03
Radius									
End							1	1.03	0.90
Shaft		no sample			no sample		1	0.95	1.01
Fibula									
End		no sample		1	0.89	0.97	1	1.21	0.93
Shaft				1	1.92	1.59	1	1.09	-
Tibia									
End		no sample			no sample		1	1.00	1.13
Shaft							1	0.97	1.20
Frontal		no sample			no sample		1	1.62	1.42
Femur									
End		no sample			no sample		1	1.11	1.00
Shaft							1	1.19	1.36

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Table 5

Temperature and the Sa/Ca Ratio

Temperature	Live Bone		Dead Bone	
	Ends	Shaft	Ends	Shaft
4°	0.59	1.00	0.52	1.00
25°	0.73	0.99	0.56	0.98
39°	0.69	0.85	0.63	1.05

It can be observed that selection occurred in the ends of live and dead bone at all temperatures. Selection in the shaft took place only in live bone at relatively physiological temperatures and indicates a possibility that other than physical processes are involved.

F. Effect of Stable Strontium on the Selective mechanism

For this experiment tibias from 12-day old embryos were put into the medium containing 1.0 mg % stable strontium for varying lengths of time. After this , activity was then added to the medium. At 24 hours the bones were harvested.

Table 6

Pre-Treatment	Stable Strontium and the Sr/Ca Ratio	
	Ends	Shaft
No stable strontium	0.51	0.75
Stable strontium simultaneously with activity	0.60	0.64
Stable strontium 1 hr. before activity	0.65	0.71
" " 3 hrs. " "	0.67	0.68
" " 6 " " "	0.67	0.71
" " 24 " " "	0.65	0.67

No effect was noted upon the total uptake of activity. Little effect could be ascribed to the stable strontium except that in the ends a lessening of selection may have occurred.

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G. Sr/Ca in Bone as Affected by the Serum Used in the Medium

Table 7

Type of Sera and the Sr/Ca Ratio

	Human		Dog		Chicken		Sheep		Horse	
	End	Shaft	End	Shaft	End	Shaft	End	Shaft	End	Shaft
30 min	1.36	0.99	1.35	0.92	0.83	0.91	1.04	0.98	1.37	0.87
7 days labeled	0.71	0.72	0.69	0.75	0.57	0.68	0.81	0.74	0.79	0.73
11 days labeled	0.71	0.68	0.73	0.73	0.54	0.70	0.96	0.85	0.89	0.79
7 days labeled and 4 days unlabeled	0.70	0.67	0.59	0.69	0.44	0.64	0.98	0.72	0.80	0.72

The data indicate that selection is relatively independent of the serum used for the medium and therefore must be a function of the bone itself. The initial entry of strontium into the shaft is at least equal to that of calcium and for the ends of bone is greater. The loss of strontium from bone shaft is larger than that of calcium in all sera, and this must be a function of bone metabolism.

H. The Short Time Uptake and Removal of Calcium and Strontium by Live and Dead Bone

For these experiments tibias from 12-day old embryos were labeled for various lengths of time and then assayed. Before labeling half of the bones were put into boiling isotonic saline for 2 minutes to kill the bones. Figure 5 presents the data of these experiments. As can be seen, the ends of the bones showed more selection against strontium than the shafts. The dead shafts displayed a constant Sr/Ca ratio throughout the experiment while the live shafts showed a continuous selection against strontium.

For studying removal the live bones were labeled for 24 hours. Before being put into non-labeled medium, half of the bones were put into boiling isotonic saline for 2 minutes. The bones were then put into unlabeled medium and groups of tibias were removed at predetermined intervals. As seen in Figure 6, the

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FIGURE 5

Sr/Ca RATIO DURING UPTAKE

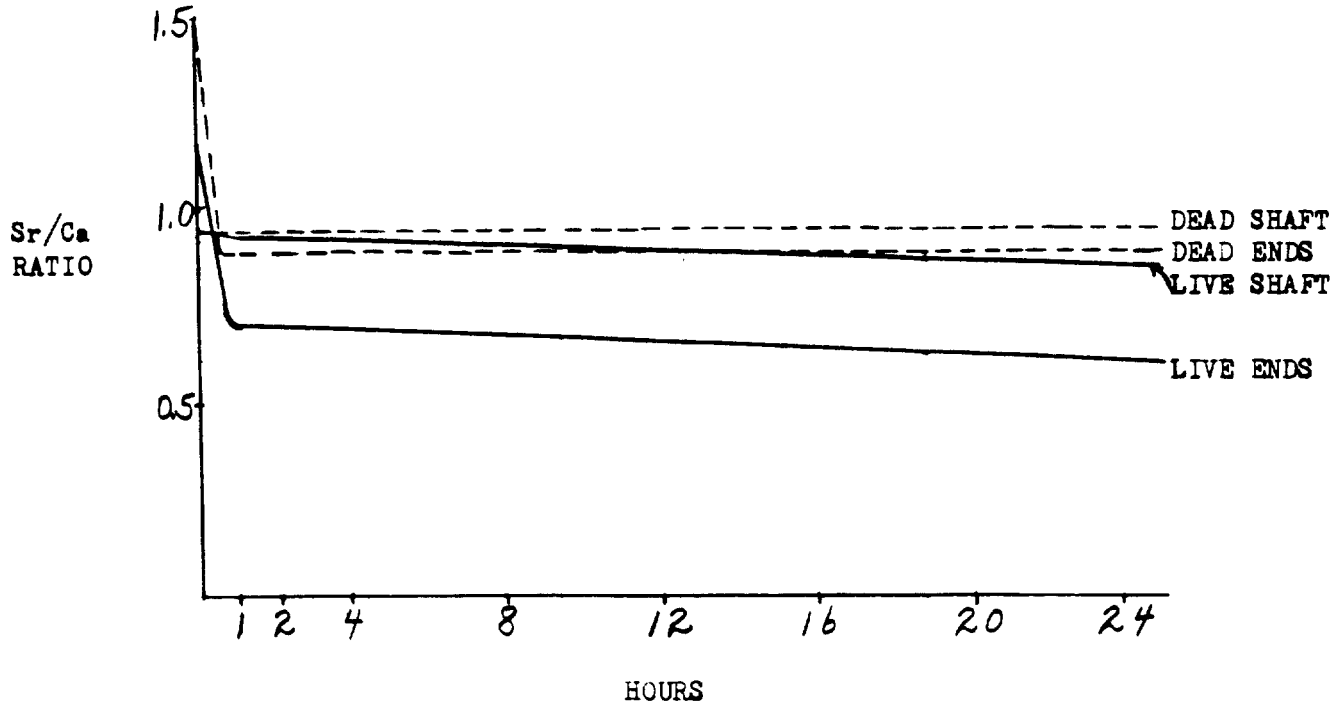
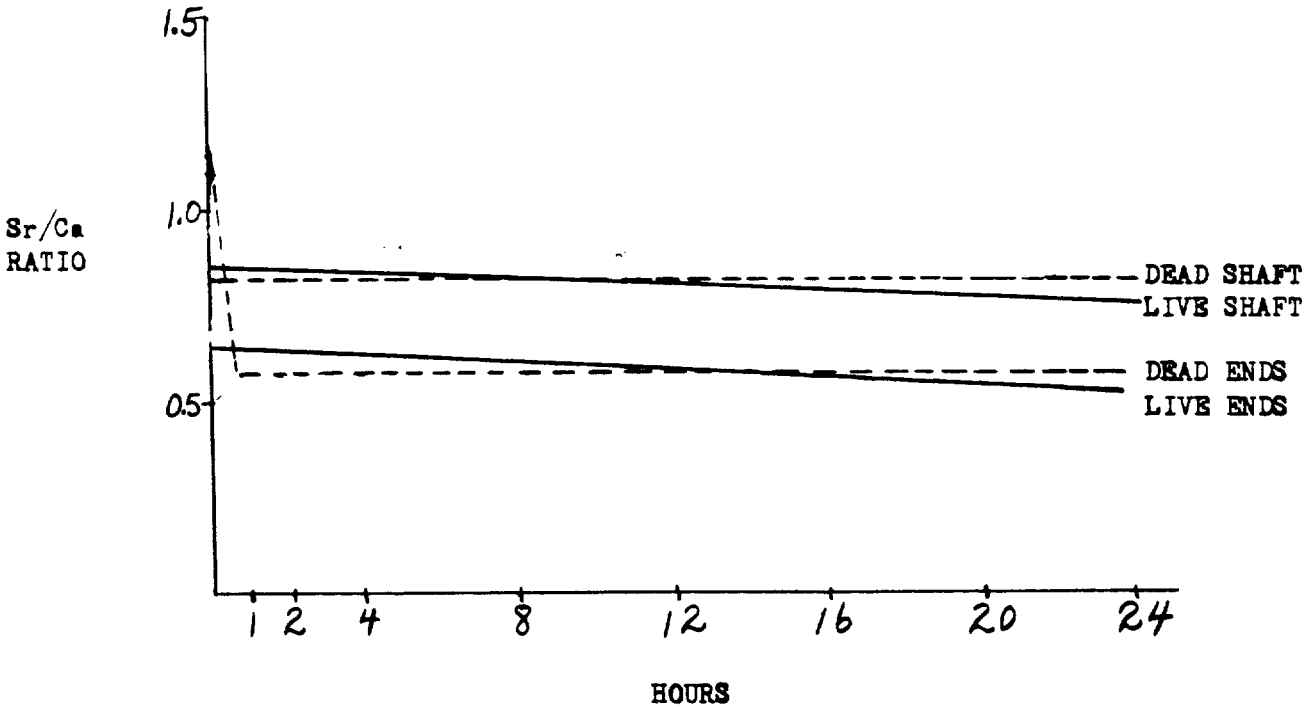


FIGURE 6

Sr/Ca RATIO DURING REMOVAL



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Sr/Ca ratio of the dead bones remained constant while the selection against strontium continued in the ends and shaft of the live bone. Much more activity was removed from the live bones than from the heat killed bone. Further analysis of the data is being carried out to determine what compartments are involved in these selections.

I. Long Time Removal of Strontium from Bone

For this study tibias from 9-day old chick embryos were cultivated in a labeled medium for 5 days. At this point they were changed to unlabeled medium and bones removed at intervals from 1 to 8 days. For both the ends and shaft of bone the major reductions in the Sr/Ca ratio came during the first 2 days (Figure 7). A very slight selection took place after that. Since live tissue was being used it is possible that new bone salt covered up the labeled bone salt and prevented its removal.

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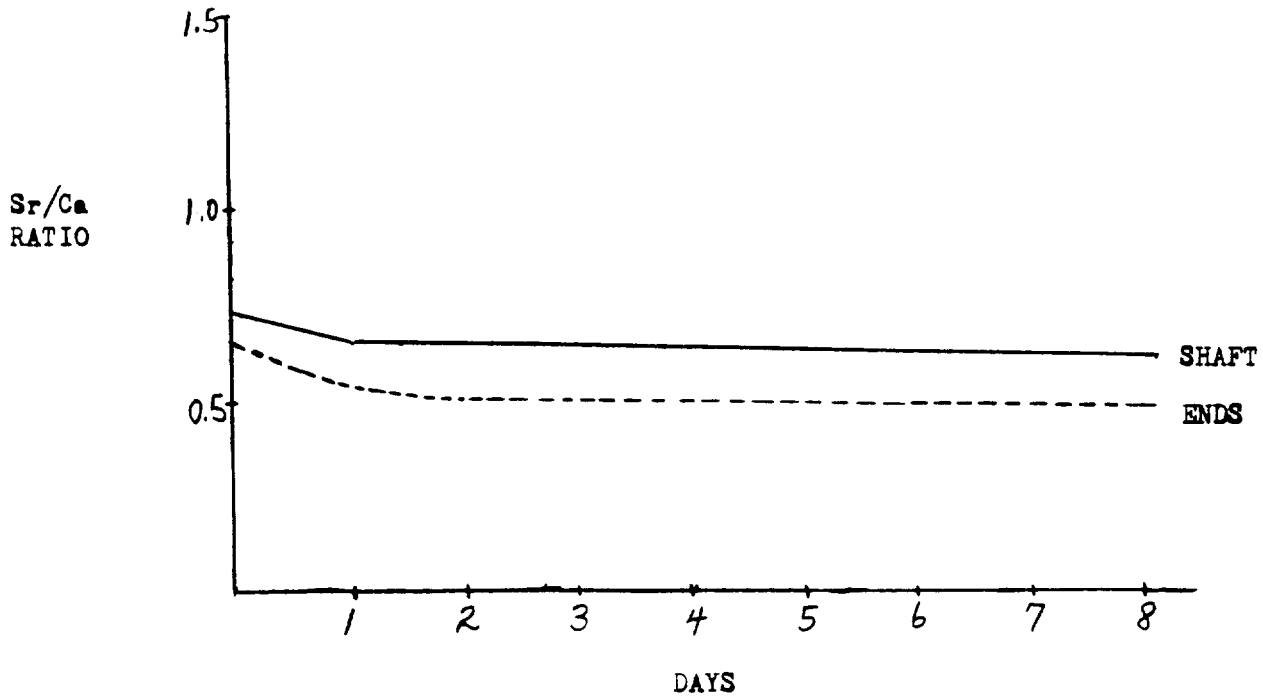
FIGURE 7

Sr/Ca RATIO DURING LONG TERM REMOVAL



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A FINAL REPORT

to

The Atomic Energy Commission

Division of Biology and Medicine

For the period

June 1, 1957 through March 23, 1959

Contract No. AT-(40-1)-1829

"The Metabolism of Alkaline Earth Metals by Bone"

Division of Chemistry

School of Biological Sciences

University of Tennessee

Memphis

Principal Investigator F. W. Lengemann

F. W. Lengemann
Assistant Professor
of Chemistry

Submitted, March 23, 1959

O. W. Hyman, Vice-President
in charge of Medical Units

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Scientific Scope

As stated in the original proposal, the major objectives of the research program were:

1. To compare the metabolism by bone of the members of the alkaline earth group of elements.
2. To investigate the phases of mineral metabolism by bone to determine what processes are enzyme influenced.
3. To determine the rates of strontium and calcium removal and to attempt to alter them.
4. To describe the mechanism by which stable strontium inhibits calcification and increases the discrimination against radioactive strontium.

This report details the results obtained in fulfilling these objectives.

Accomplishments

The major emphasis was given to comparing the deposition in and removal from bone of the various members of the alkaline earth group of elements. Throughout these studies *in vitro* cultures of embryonic bone were used as the test material. In order to compare the metabolic behavior with a degree of certainty, radiocalcium was incorporated into the medium with the alkaline earth under investigation. The comparison of strontium metabolism to that of calcium received special emphasis.

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Early work in this laboratory indicated that bone could select against strontium in the absence of other organs. Removal was implicated as the possible site of selection since more strontium was found to leave bone than calcium. The data of Table 1 demonstrate the selection against strontium and emphasize that the ends of the bone may have a different Sr/Ca ratio than the shaft.

Table 1

Sr/Ca Ratio in Growing Bone

	Ends	Shaft
Labeled 7 days	0.69	0.75
Labeled 7 days then non-labeled medium for 4 days	0.59	0.69

By calculation it was determined that 1.12 times as much strontium as calcium left the ends of the bones; the corresponding figure for the shafts was 1.28. About 50% of the activity incorporated into the ends was lost during this period while only 20% was lost from the shafts.

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Table 2

Sr/Ca Ratio the Shaft of Bone Grown in Various Sera

Sera	Human	Dog	Chicken	Sheep	Horse
30 minutes	0.99	0.92	0.91	0.98	0.87
7 days labeled	0.72	0.75	0.68	0.74	0.73
7 days labeled and 4 days unlabeled	0.67	0.69	0.64	0.72	0.72

The data of Table 2 indicated that selection was relatively independent of the serum used for the medium and therefore must be a function of the bone itself. The initial entry of strontium into the shaft was at least equal to that of calcium while the loss of strontium was greater than that of calcium for all sera: Again, the removal phase seemed to be responsible for the selection against strontium.

Figure 1 shows the Sr/Ca ratios of the ends and shafts of live and dead bone at various time intervals after immersion in the labeled medium. As can be seen, the ends of the bones showed more selection against strontium than the shafts. The dead shafts displayed a constant Sr/Ca ratio throughout the experiment while the live shafts showed a continuous selection against strontium. When labeled live bone was killed and then placed in unlabeled medium (Figure 2) the Sr/Ca ratio of the dead bones remained constant while the selection against strontium continued in the ends and shaft of the live bone.

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FIGURE 1

Sr/Ca RATIO DURING UPTAKE

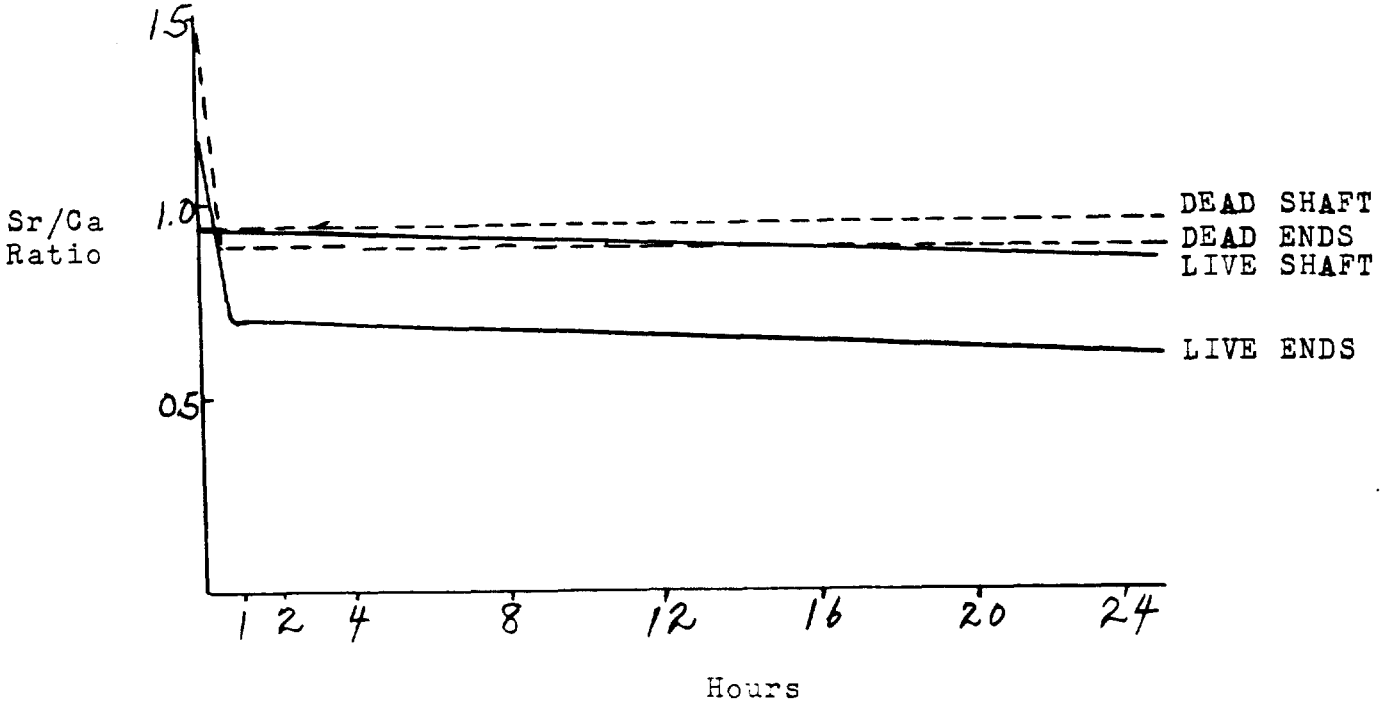
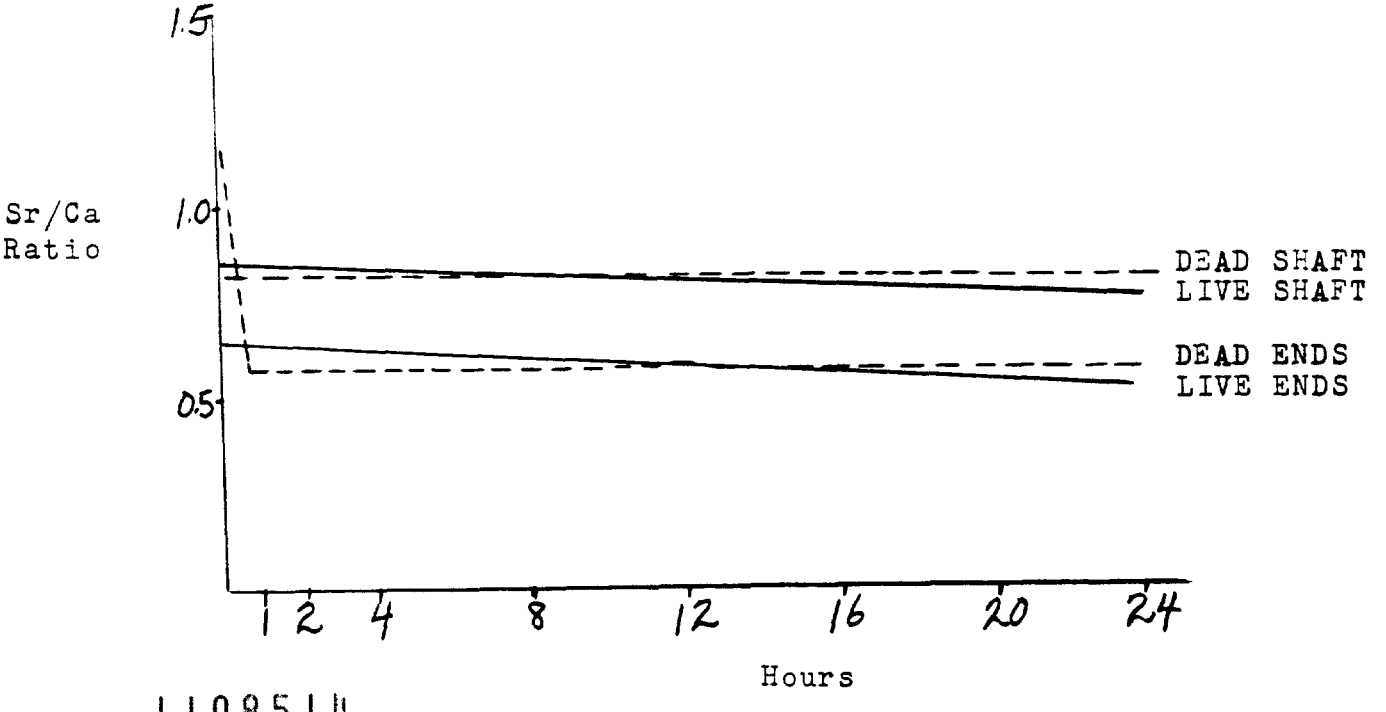


FIGURE 2

Sr/Ca RATIO DURING REMOVAL



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Much more activity was removed from the live bone than from the heat killed bone.

The total uptake of radioactivity was greater for the dead bone than for the live bone and since little selection was exhibited it is unlikely that selection occurs as these alkaline earths enter the mineral phase of the bone. The selection shown by live bone is most likely the result of turnover of the mineral phase with the resulting greater loss of strontium than calcium.

The selection against strontium has little significance if it occurs only in the avian species. Therefore, it was of interest to test embryonic bone from other species for its selective capacity. The data for this study are contained in Table 3.

Table 3

The Sr/Ca Ratio in Bone as a Function of Species

	Uptake		Removal	
	Ends	Shaft	Ends	Shaft
Chick	0.69	0.75	0.59	0.69
Rat	0.51	0.77	0.37	0.71
Human 1	0.70	1.21	0.65	1.12
Human 2	0.78	1.00	0.68	1.00
Human 3*	1.08		1.00	

* Sr/Ca Ratio for Whole Bone.

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The bones were cultured for 7 days in labeled medium and then some were continued in unlabeled medium for 5 days while the rest were removed. The chick bone (Tibias) came from embryo's indubated for 9 days; the rat bone was obtained from 20 day-old fetuses and consisted of femurs and tibias. Metacarpal and metatarsal bones were the human bone used and came from fetuses ranging in size from 23 cm. for Human #1 to 7 cm. for Human #3.

As can be seen, selection was quite similar for the chick and rat bone and the ends of the human metacarpal and metatarsal bones. The shafts of the human bone actually exhibited a selection in favor of strontium during the labeling experiment. However, when put into unlabeled medium some evidence was obtained of a selection against strontium during the turnover of bone mineral. This is good evidence that a selection against strontium does occur in bone of species other than the chicken.

The shaft of the human bone showed a preference for strontium during the labeling phase that was more marked than for the other species; the human bone seemed to be more calcified than that of the other species. Since selection was usually more intense in the ends of the bone when compared to the shaft, a possibility existed that the actual ratio exhibited by bone might vary with the degree of calcification. To test this, chick bone from progressively older embryos was cultured for 7 days in a medium containing strontium-89 and calcium-45.

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The data for this experiment are contained in Table 4.

Table 4

Sr/Ca Ratio in Bone as a Function of Age

Age of Embryos	Ends	Shafts
7 Days	0.66	0.75
9 "	0.77	0.79
12 "	0.78	0.79
14 "	0.62	1.02

Again, the ends of the bones tended to exhibit a different ratio than the shafts. More important, the bone excised from 14-day old embryos resembeled that of the human by showing a Sr/Ca ratio that was near unity at the end of a 7 day period of growth. The bones of 14 day old embryos are far more calcified than those of the younger embryos and the probability is increased of exchange becoming an important mechanism in the sequestering of radioisotopes by these tissues. The data of Table 2 and Figure 1 showed that the initial ratio of such a process is near unity. However, it was observed that the older bones removed a significant portion of the radioactivity of the medium. If selection was occurring to the same extent as

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present in the younger bones the Sr/Ca ratio of the medium would increase and this in turn would aid the movement of Sr* into the bone. All calculations were based on the premise that the Sr/Ca ratio of the medium remained constant and so the calculated ratios may be in error.

The experiment with live and dead bone tended to imply that a vital function was associated with the selective process. Since living material is sensitive to alterations of temperature, it was of interest to see if the selection could be modified by changes in the environmental temperature. Therefore, 12-day old bone was incubated in a labeled medium for 24 hours at various temperatures. The results are presented in Table 5.

Table 5

Temperature and the Sr/Ca Ratio

Temperature	Live Bone Shaft	Dead Bone Shaft
4°	1.00	1.00
25°	0.99	0.98
39°	0.85	1.05

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It should be noted that selection occurred only at 39°C with living tissues. This would indicate that selection is not merely a physical phenomenon but must be mediated by living tissue. In exploring the effect of temperature it was observed (Table 6) that the selection again appears to be most significant at the removal phase.

Table 6

Temperature and the Sr/Ca Ratio During Uptake and Removal

Uptake	4°	37°
24 hours	1.06	0.94
48 hours	1.11	0.90
Removal		
24 hours	0.79	0.81
48 hours	0.80	0.74

For this experiment 12-day embryonic chick bones were incubated for 24 and 48 hours in labeled medium and 4° and 37°C. Again selection against strontium was seen only at 37°C; the selective process appeared to be operative even at 48 hours.

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To be noted is that prolonging the time the bones were exposed to the 4° temperature did not change the results and indicated that the selective process was stopped and not just slowed down.

To study the effect of temperature upon the removal of the isotopes, bones were first labeled at 37° for 24 hours and then transferred to unlabeled medium at 4° or 37°C. The bones thus had an Sr/Ca ratio of 0.94 at the time of transfer. After 24 hours there was no difference in the ratio exhibited by bones at 4° as compared to those at 37°C. However, at 48 hours, the bones at 37° exhibited a lower ratio than similar bones at 4°C and bones incubated for 24 hours at 37°C. It should be noted that the ratios of bones kept at 4° and 37°C for 24 hours, though equal, were much lower than 0.94. The data can be interpreted as indicating the presence of 2 fractions in bone mineral. One fraction is extremely labile and has a much higher Sr/Ca ratio than the less labile phase. This labile portion is easily lost from bone by physical exchange processes and thus explains the lowering of Sr/Ca ratio seen when the labeled bone was incubated for 24 hours at 4°C. The less labile fraction needs the intercession of a living process in order to be lost from bone and so a continuation of the lowering of the Sr/Ca ratio was seen only in bone kept at 37°.

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A similar experiment was performed but this time bones labeled at 4° and 37° for 24 hours were placed in labeled medium and incubated for 48 hours more at both 4° and 37°C. The results are shown in Table 7.

Table 7

The Sr/Ca Ratio in the Ends and Shafts of Bone Kept at 37° and 4°C in Unlabeled Medium for 48 Hours

	Ends	Shaft	%Ca-45 Lost From Shaft
Labeled at 4°C			
Control	0.90	1.06	——
48 hrs. at 37°C	0.54	0.82	59
48 hrs. at 4°C	0.86	1.19	47
Labeled at 37°C			
Control	0.73	0.95	
48 hrs. at 37°C	0.55	0.76	48
48 hrs. at 4°C	0.73	0.90	10

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The results showed that selection occurred at 37° but not at 4°C for both the ends and shafts of bone. To be noted is the greater removal of Ca-45 from the shaft of bone labeled at 4° than at 37°C. If keeping bone at 4°C stopped a vital process in bone crystal turnover, then any activity that was removed must have been from a more accessable, more labile phase. When bone, labeled at 4°, was further incubated in unlabeled medium at 4°C a larger proportion of the total activity was removed than was the case of bone similarly handled but labeled at 37°C. Thus the size of the more labile compartment was greater at the lower temperature and implied that the conversion from the more labile phase to the less labile phase was temperature dependant as well as removal from the less labile compartments. It also was noted that bones incubated at 37° took up 1.65 times as much activity as bones at 4°C.

It was of interest to see if the temperature effect could be seen when bone was grown in a labeled medium for 5 days before being put into a non-labeled medium for 3 days at 4 or 37°C. The data are presented in Table 8.

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Table 8

The Sr/Ca Ratio in Growing Bone as Affected by Temperature During Labeling and Removal Studies

Groups	Temp. During Labeling	Temp. for Removal	Sr/Ca Ratio Ends	Sr/Ca Ratio Shaft
I	4°C	---	0.91	1.26
II	37°C	---	0.64	0.79
III	37°C	37°	0.47	0.66
IV	37°C	4°	0.52	0.70

These bones acted in the same manner as did bones incubated for much shorter periods. To be noted is the selection exhibited at 37° as opposed to 4° and that the ends showed more selection than the shafts. The apparent selection seen when the bones were labeled at 37° and then held at 4°C may again be explained on the basis of the existence of two compartments for bone mineral with one compartment being more labile, not temperature dependant, and having a much higher Sr/Ca ratio than the other. Again, the lower temperature decreased the uptake and removal of radioactivity from bone. The results were obtained using bone from 12-day old embryos and were duplicated using bone from 9-day old embryos.

1108523

The sensitivity of the selective process to temperature variation and the obliteration of the process by heat treatment suggested that somewhere in the calcification mechanism an enzyme system was operating. To get additional information bones from 9-day old chick embryos were grown for 5 days in labeled medium and the air above the medium was replaced by one of several gases. The results are shown in Table 9.

Table 9

The Effect of Various Gases upon the Sr/Ca Ratio and Calcium Uptake by Growing Bone

Gas	Ends		Shafts	
	Sr/Ca	Ca Uptake	Sr/Ca	Ca Uptake
Experiment 1				
Air	0.59	100	0.72	100
O_2	0.68	151	0.72	230
N_2	0.77	35	0.75	109
CO_2	0.72	7	0.71	46
Experiment 2				
Air	0.77	100	0.85	100
CO	0.69	50	0.73	78

1108524

It is obvious that the behavior of the bones was moderated by the gas to which the bones were exposed. Little effect was observed upon the Sr/Ca ratio but major effects were seen upon the amount of Ca-45 taken up. Oxygen increased the uptake of calcium markedly and thus suggested that an oxidative process could be involved with calcification. The depression of calcification by carbon monoxide, an inhibitor of the cytochrome system, reinforced this supposition. However, the block was not complete and since nitrogen did not have much effect, it is possible that an alternate pathway for the calcification mechanism exists.

The gases operated for the most part to slow or speed the process but probably had no effect upon individual members of the system. Other inhibitors might also have an effect and might possibly lead to a change in the selection ratio that would be beneficial. Therefore, a number of experiments were performed in which growing bone was exposed to various inhibitors at several concentrations. The results are shown in Table 10. Again, both the Sr/Ca ratio and the uptake of Ca-45 were used as the criteria of a change.

1108525

Table 10

The Effect of Several Concentrations of Various Inhibitors Upon the Sr/Ca Ratio and Calcium Uptake of Growing Bone

Inhibitor		Concentration 0	10^{-4} M	10^{-3} M	10^{-2} M
Fluoroacetate	Sr/Ca	0.85	0.83	0.74	1.12
	Ca Uptake	100	84	62	24
Iodoacetate	Sr/Ca	0.72	1.02	1.00	0.72
	Ca Uptake	100	184	165	174
Malonate	Sr/Ca	0.74	0.74	0.78	0.70
	Ca Uptake	100	83	106	73
Arsenite	Sr/Ca	0.75	1.00	1.00	1.02
	Ca Uptake	100	508	324	141
Fluoride	Sr/Ca	0.69	0.65	0.78	0.84
	Ca Uptake	100	200	391	84
Bisulfite	Sr/Ca	0.69	0.60	0.64	0.65
	Ca Uptake	100	90	101	80
Cyanide	Sr/Ca	0.77	0.71	0.85	0.88
	Ca Uptake	100	85	320	142
DL-Glyceraldehyde	Sr/Ca	0.77	0.76	0.72	0.80
	Ca Uptake	100	82	80	239
Azide	Sr/Ca	0.77	0.64	0.73	0.71
	Ca Uptake	100	60	95	91
Protamine	Sr/Ca	0.86	0.72	0.69	
	Ca Uptake	100	84	179	
Guanidine	Sr/Ca	0.71	0.69	0.72	0.82
	Ca Uptake	100	119	103	266

1108526

Table 10
(Continued)

Inhibitor		0	10^{-4} M	10^{-3} M	10^{-2} M
Phosphoethanol-amine	Sr/Ca	0.71	0.60	0.58	0.67
	Ca Uptake	100	92	40	45
Lactose	Sr/Ca	0.77	0.64	0.73	0.71
	Ca Uptake	100	60	95	91
Lithium	Sr/Ca	0.79	0.72	0.73	0.77
	Ca Uptake	100	98	81	96
Lead	Sr/Ca	0.72	0.78	0.62	
	Ca Count	100	72	9	
Barium	Sr/Ca	0.69	0.55	0.46	0.64
	Ca Count	100	53	37	15
Mercury	Sr/Ca	0.69	0.74	1.05	0.82
	Ca Count	100	80	124	100
Beryllium	Sr/Ca	0.85	0.83	0.74	1.12
	Ca Count	100	84	62	24

1108527

It is evident from the data that both the uptake of minerals and the Sr/Ca ratio of bone could be altered by inclusion of inhibitor of various sorts in the medium. Of those used, malonate, bisulfite, D-L glyceraldehyde, azide, guanidine, lactose, and lithium had little effect. It is of interest that iodoacetate, arsenite, arsenate, and fluoride, materials which inhibit the anaerobic breakdown of glucose between 3-phosphoglyceraldehyde and acetate, increased the uptake of activity and increased the Sr/Ca ratio; the increase in the ratio was not the same in all cases. This suggested that some enzymatic processes can influence the mineralization of bone and the preference for calcium. Fluoracetate and malonate, inhibitors of the Krebs cycle, had little or a slightly negative effect upon the deposition of calcium. Barium, beryllium, and phosphoethanolamine were interesting in that all decreased the Sr/Ca ratio and the total uptake of radioactivity as well. This may be important when related to the problem of reduction of the body burden of Sr-90. Barium appeared to be most effective but unfortunately is known to be very toxic; on the other hand, phosphoethanolamine has been found in the urine of some patients suffering from a peculiar form of osteoporosis.

The experiment with phosphoethanolamine was performed using long term cultures and so it was of interest to see if an effect could be seen at the end of a 24-hour period of incubation. The data, Table 11, show that phosphoethanolamine was effective even at this short interval.

1108528

Table 11

The Effect of Various Concentrations of Phosphoethanolamine (P-Et) on the Sr/Ca Ratio and Ca-45 Content of Bone after a 24-hour Labeling Period

Group	Ends		Shafts	
	Sr/Ca	Ca-45	Sr/Ca	Ca-45
Control	0.73	100	0.94	100
P-Et 10^{-4} M	0.78	64	0.92	87
10^{-3} M	0.78	64	0.88	76
10^{-2} M	1.12	84	0.94	52

The mechanism by which phosphoethanolamine acts is unknown as is the case for other inhibitors shown in Table 10. Also, further work is needed to determine if ethanolamine itself may be an active compound.

The effect of hydrocortesone, thyroxine, and parathormone upon the Sr/Ca ratio and the calcium content of the bone was also tested. Since a natural medium was used it must be understood that the amounts of the hormone stated were additions to what might normally be present in human serum.

When bones were labeled for 5 days and then shifted to unlabeled medium for 2 days it was observed that added parathormone at levels of 0.01, 0.1, 1.0 and 10.0 units per milliliter was without any significant effect. On the other hand, both hydrocortisone and thyroxine (Table 12) seemed to have an effect.

1108529

Table 12

The Effect of Added Thyroxine and Hydrocortisone Upon the Calcium Uptake and Sr/Ca Ratio of Embryonic Bone

	Ends		Shafts	
	Sr/Ca	Ca Uptake	Sr/Ca	Ca Uptake
7-Day Experiment				
Control	0.50	100	0.78	100
Thyroxine .01 µg/ml.	0.57	125	0.73	94
" 0.1 µg/ml.	0.55	110	0.63	62
" 0.3 µg/ml.	0.56	87	0.65	74
Hydrocortisone 0.0005 µg/ml.	0.60	108	0.72	74
" 0.005 µg/ml.	0.64	129	0.69	94
" 0.05 µg/ml.	0.63	114	0.64	117
24-hour Experiment				
Control	0.76	100	0.96	100
Thyroxine 0.01 µg/ml.	0.69	79	0.96	88
" 0.1 µg/ml.	0.65	47	0.86	98
" 0.3 µg/ml.	0.71	80	0.86	94
Hydrocortisone 0.0005 µg/ml.	0.95	76	0.83	84
" 0.005 µg/ml.	1.03	63	0.84	88
" 0.05 µg/ml.	1.03	81	81	87

1108530

The data demonstrate that thyroxine and hydrocortisone acted to increase the selection against strontium. Thyroxine, at the levels used in these experiments, was more effective than hydrocortisone in the long-term experiment while the reverse was true for the 24-hour study. In general, these hormones acted to decrease the mineralization of the bones. To be noted is the stimulatory effect of thyroxine, during the 7-day experiment, upon the calcium uptake of the ends of the bones while the same levels decreased the calcium uptake of the shafts. Again, the data imply that a living system is involved in the selection of calcium over strontium.

Information was also obtained on the selection by bone of the other alkaline earth metals. The ratios at which these materials left bone are shown in Table 13.

Table 13

The Removal Ratios for the Alkaline Earth Metals from Bone When Compared to the Removal of Calcium

	Ends	Shafts
Be/Ca	0.58	0.69
Mg/Ca		2.53
Sr/Ca	1.12	1.28
Ba/Ca	0.81	1.35
Ra/Ca	0.93	2.70

1108531

In general, for the shaft of bone, the larger the atomic number of an element the greater the selection against that element. An exception to this was Mg-28. The magnesium data were not conclusive since the specific activity was very low resulting in very low counts and a large increase in the milliequivalents of alkaline earths in the medium. The short half-life, 21 hours, necessitated shorter experiments than was the case for the other isotopes. For the other nuclides the different behavior of the ends and shafts of bone should be noted and may be indicative of a difference in the way the mineralization is carried out.

In the course of the study of Mg-28 a large amount of the isotope was available and so experiments were run on rats to compare the metabolism of magnesium to calcium in that species. Table 14 compares the distribution of an intraperitoneal injection of these isotopes 24 hours after dosing.

Table 14

The Distribution of an Intraperitoneal Injection of Equimolar Amounts of Ca* and Mg* 24 Hours after Administration to Young and Matur Rats

		Ca-45 (% of dose)	Mg-28 (% of dose)	Mg/Ca (% of dose)
Young ♀	Urine	0.24 ± 0.03[1]	17.0 ± 1.9	73.4 ± 10.2
	Feces	3.24 ± 0.38	9.4 ± 0.4	3.03 ± 0.78
	Bone	8.05 ± 0.13	3.38 ± 0.08	0.42 ± 0.01
	Muscle	0.026 ± 0.006	0.42 ± 0.009	16.7 ± 1.0
	Kidney	0.063 ± 0.005	0.89 ± 0.003	14.4 ± 1.1
	Liver	0.035 ± 0.007	1.03 ± 0.003	14.2 ± 2.9
	Spleen	0.127 ± 0.016	0.95 ± 0.003	8.25 ± 1.2
Mature ♂	Urine	0.50 ± 0.01	11.7 ± 1.1	23.4 ± 1.2
	Feces	11.6 ± 1.1	11.7 ± 0.5	1.04 ± 0.08
	Bone	4.40 ± 0.16	1.49 ± 0.08	0.34 ± 0.01
	Muscle	0.076 ± 0.003	0.23 ± 0.01	3.0 ± 0.2
	Kidney	0.087 ± 0.01	0.54 ± 0.02	6.7 ± 0.6
	Liver	0.068 ± 0.006	0.68 ± 0.03	10.0 ± 0.5
	Spleen	0.070 ± 0.006	0.70 ± 0.03	10.0 ± 1.0

[1] Mean ± standard error of the mean.

1108532

From the data (Table 14) it can be seen that the urine was a more important pathway of excretion for magnesium than for calcium; the feces also contained more magnesium than calcium. The soft tissues showed greater accumulations of magnesium than calcium while bone showed a marked preference for calcium.

The effect of increased age was to lower somewhat the urinary excretion of magnesium and increase its fecal excretion; however, the total retention of magnesium was greater than for the young rats. Fecal calcium increased markedly and the preference of bone for calcium was intensified in the older rats. The soft tissues, when the data was corrected for differences in body weight, showed a greater accumulation of both calcium and magnesium than was the case for the young animals. A larger increase in calcium resulted in a narrower Mg/Ca ratio in all soft tissues except the spleen.

Table 15

The Distribution of an Oral Dose of Equimolar Amounts of Ca* and Mg* 24 Hours after being Administered to Young and Old, Male and Female Albino Rats

			Ca-45 (% of dose)	Mg-28 (% of dose)	Mg/Ca
Young	♂	Urine	0.59 ± 0.26[1]	8.00 ± 1.22	21.2 ± 3.9
		Feces	45.3 ± 1.9	36.2 ± 2.1	0.69 ± 0.03
		Bone	4.58 ± 0.12	2.26 ± 0.12	0.49 ± 0.02
Young	♀	Urine	0.35 ± 0.24	12.1 ± 1.1	39.5 ± 4.8
		Feces	47.4 ± 3.9	32.4 ± 7.2	0.81 ± 0.06
		Bone	3.69 ± 0.32	2.04 ± 0.14	0.56 ± 0.04
Old	♂	Urine	0.62 ± 0.27	6.53 ± 0.60	20.5 ± 5.9
		Feces	60.1 ± 1.7	41.0 ± 1.3	0.69 ± 0.03
		Bone	2.04 ± 0.18	0.89 ± 0.10	0.44 ± 0.03
Mature	♀	Urine	0.81 ± 0.24	10.3 ± 1.5	16.7 ± 3.5
		Feces	62.2 ± 4.1	47.3 ± 5.0	0.75 ± 0.04
		Bone	2.19 ± 0.14	1.01 ± 0.05	0.46 ± 0.01

[1] Mean ± standard error of the mean.

110853

To obtain information on absorption from the intestinal tract the experiment described by Table 15 was performed. Six rats were used for each group. From the data it is evident that $MgCl_2$ was absorbed to a greater extent that $CaCl_2$; the amount of absorption was greater for the young animals than for the adults. Again, urinary magnesium exceeded the urinary calcium; however, the net retention of magnesium surpassed that of calcium in all groups. The bone mirrored the greater absorption of magnesium, and reemphasized the preference of the bone for calcium. Evident also was the increased selection for calcium by the bone of the older animals. The greater calcium-45 uptake by the femurs of the young male rats resulted in a Mg/Ca ratio wider than that for the young female rats. At this time it is not known if this will be reproducible.

The discrimination factors involved in these experiments were calculated from the data and are shown in Table 16.

Table 16

Magnesium-Calcium Observed Ratios (O.R.) and Discrimination Factors (D.F) for the Rat

Group	Route of Dose	O.R. retained-dose	D.F. fecal	D.F. Absorptive	D.F. Urinary
Young ♀	I.P.	0.77	0.94	----	0.82
Mature ♀	I.P.	0.87	1.00	----	0.87
Young ♂	Oral	1.03	----	1.26	----
Young ♀	Oral	1.06	----	1.30	----
Mature ♀	Oral	1.15	----	1.32	----
Old Males	Oral	1.33	----	1.52	----

1108534

From the factors it is evident that little selection was shown in the movement of the alkaline earths from the body into the intestine ($D.F._{fecal}$). Some selection against magnesium was evident in the young animals but not in the mature females. The kidney was a major site of selection against magnesium as indicated by $D.F._{urinary}$ values of 0.82 and 0.87. The value seemed to decrease with age. The most important selection was seen at the absorptive phase with the selection being in favor of magnesium over calcium.

Since the proportion of calcium to magnesium in the test dose was kept constant, it was of interest to see what effect there would be if the proportions were varied. The data of this experiment are shown in Table 17; for this study the total number of millimoles of the alkaline earths in the test dose were kept constant.

Table 17

The Effect of the Proportions of Calcium and Magnesium in the Test Dose Upon the Distribution of Ca-45 or Mg-28 in the Rat 24 Hours After an Oral Dose

Dosing Solution		Experimental			Calculated	
Ca	Mg	Femur	Urine	Feces	Femur	Urine
(Millimoles)		(% dose)	(% dose)	(% dose)	(% dose)	(% dose)
			Mg-28 Content			
0	0.26	1.19 ± 0.07	15.9	47.1	1.19	15.9
0.13	0.13	1.96 ± 0.10	14.1	38.0	1.54	9.3
0.195	0.065	1.75 ± 0.07	11.5	34.7	1.73	5.5
			Ca-45 Content			
0.26	0	3.40 ± 0.35	0.38	47.5	3.40	0.38
0.13	0.13	4.01 ± 0.18	0.70	45.9	3.51	0.20
0.065	0.195	4.30 ± 0.54	1.10	40.5	3.86	0.11

1108535

For interpretation of the results it was necessary to calculate what the femur uptake of the radionuclide would be based upon the improvement in retention seen when the specific activity was increased. Comparing the calculated femur content with the observed, it is seen that the inclusion of calcium in the test dose resulted in more Mg-28 being incorporated into bone than expected when the Ca: Mg ratio was unity. The inclusion of stable magnesium in the test dose also increased the uptake of calcium-45 by the bone. From the calculated urine values, it is evident that increasing the stable calcium causes more magnesium to be lost in the urine and vice versa. The data indicated that the calcium-magnesium ratio was very important and that it must be taken into account when discrimination factors are discussed.

This project was terminated at this point due to the resignation of the senior investigator.

Summary

The data developed during the course of this study demonstrated that bone itself was responsible for the selection against strontium in favor of calcium. The degree of selection was more pronounced in the ends of the bone than in the shafts. Discrimination appeared throughout the alkaline earth series of metals and it appeared that the element with the smaller atomic number was preferred to one with a higher atomic number.

1108536

Evidence was obtained that an enzyme system was directly or indirectly associated with the selection process. Heat killing of the bones, low temperatures, enzyme inhibitors, and hormones were found capable of interfering with the selection. The amount of calcium deposited as well as the compartment into which it was sequestered seemed to be affected. The initial entry of calcium or strontium seemed to be into an extremely labile phase and seemed to be a purely physical process. The subsequent movement into a less labile phase was heat sensitive. The removal of mineral from the less labile phase was temperature dependant while movement from the labile phase was not.

Other data were developed that described the distribution of calcium and magnesium in the rat and the discrimination factors of the various sites of selection.

Publications

The Metabolism of Magnesium and Calcium by the Rat. F. W. Lengemann submitted for publication to the Archives of Biochemistry and Biophysics (December, 1958).

Other publications developed from the data described herein are in progress or contemplated. The provision of funds by the A.E.C. to carry out this project will be acknowledged and copies of such publications submitted to the A.E.C.

1108537

Incident Report

In the period June1, 1958 through March 23, 1959 no incidents occurred.

Radioisotope Purchase Report

Nuclide	Mc	Catalog Price	Discount Price	Savings
Sr-89-P	5	10.00	2.00	8.00
Ca-45-P-2	5	25.00	5.00	20.00

In submitting the budget for this years work, the cost of radioisotopes was based on the use of the A. E. C. discount.

10 Percent Voucher

Since the project is being terminated short of the contracted date and since funds still remain in the account, it is contemplated that the 10 Percent voucher will not be submitted.

1108538

CONTRACT NO. AT-(40-1)-1829

STATUS OF TOTAL COST BUDGET

MARCH 1, 1959*

	ORIGINAL BUDGET	EXPENDITURES TO DATE	ESTIMATE THROUGH 3-23-59	TOTAL EXPEN-DITURES	REMAINING BALANCE 3-23-59
Salaries	$7,474.82	$4,808.70	$ 482.64	$5,291.34	2,183.48
Equipment	1,700.00	-0-	-0-	-0-	1,700.00
Supplies	1,700.00	734.42	194.00	928.42	771.58
Travel	400.00	127.51	-0-	127.51	272.4
Overhead and Indirect Costs (Including Retirement)	5,061.18	3,531.00	404.05	3,935.05	1,126.1
Totals	$16,336.00	$9,201.63	$1,080.69	$10,282.32	$6,053.6
Less: University Contribution as Per Statement--				6,481.25	1,274.7
Total Applicable to AEC				$3,801.07	$4,778.9
Less: Remaining 10% Not Billed--					858.0
AEC Funds Received to Date				7,722.00	
Estimated Refund as per AEC Statement				$3,920.93	3,920.9

* This statement was prepared by Mr. C. Q. Tipton and is an estimate of the expenditures through March 23, 1959. Mr. Tipton will later submit a final report to indicate the exact status as of March 23, 1959.

O. W. Hyman

O. W. Hyman, Vice-President
in Charge of Medical Units

1108539