Effect of Time on Distribution of Injected
Radiostrontium in the Skeleton of Six-Month-Old
Swine and Its Relation to Calcium Accretion and Bone Growth

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ABSTRACT

The distribution of ^{85}Sr was examined in 13 selected bones of the skeleton of 8 Hampshire pigs, intravenously dosed at 6 months of age. The pigs were killed from 24 hours to 180 days post-injection, at which time bones were removed, cleaned, ashed and assayed for ^{85}Sr as well as total stable calcium. The calculated % of injected dose per gram of calcium showed not only the expected decrease with time but also a remarkable constancy in the selected bones of a given animal. Grouping of the bones according to type, I (head bones), II (long bones) and III (vertebrae), revealed differences in net calcium accretion, which was greatest (132%) in long bones 180 days post-injection, and in ^{85}Sr retention, which was also greatest in the long bones 180 days post injection. Accretion and resorption of the three bone groups are discussed with respect to calcium accretion and ^{85}Sr removal during the period from 6 months to one year of age; and it appears that two types of bone growth exist during this period: (1) accelerated accretion with normal resorption; and (2) normal accretion with decreased resorption.

Introduction

A previous study (1) showed that the distribution pattern of ^{85}Sr in the skeleton of young growing pigs at 24 and 50 days of age was not uniform. The largest percentages of ^{85}Sr per gram of calcium at 50 days of age was found in the tibia-fibula, the skull and mandible and the scapula, whereas radioactivity in the vertebrae was much less. When the per cent of radiostrontium retained following injection of 1 μCi at 2 days after birth was compared in the two pigs, the skull and mandible ranked first, followed by the long bones, and lastly by the vertebrae.

Blair et al. (2) found that retention of calcium in the skeleton as a whole was substantial in pigs from birth to 8 weeks of age. Bone growth, as a function of fresh wet weight, was determined in swine from birth to 28 weeks of age by McMeekan (3, 4) and Cuthbertson and Pomeroy (5, 6). Results from these studies pointed to increased rates of growth in the vertebral bones and less in the skull and mandible. The rate of growth of the long bones appeared to be scattered between the vertebrae and the skull, including the mandible. Other studies (1, 7) emphasized the accumulation of radiostrontium in the skull (and mandible) in swine dosed at an early age. Finally, Moulton (8) demonstrated in 1928 that the ash content of total body composition, though largely in bone, of swine leveled off at a value of about 4% at 150-300 days of age, the time designated as chemical maturity which also happens to be the approximate time of reproductive maturity.

To elucidate the relationship of bone growth to calcium accretion and radiostrontium retention in individual bones of sexually mature swine from six months to one year of age, this study was undertaken. In addition,

initial bone growth from birth to six months of age was compared to growth beyond six months following a single intravenous injection of radiostrontium. A better understanding of the skeletal metabolism of the potentially deleterious radiostrontium as a function of age in swine as a model for man is the overall objective of these continuing investigations.

Methods

Each of 8 male Hampshire pigs was injected intravenously with 15 μCi of ^{85}Sr at 6 months of age. They were housed in a single outdoor pen with shelter and fed an identical ration (Ralston Purina Groweena, St. Louis) and offered water ad libitum. At 1, 10, 28, 60, 90, 120, 137, and 180 days after injection, a pig was killed. Thus, the last pig killed was one year of age. Selected bones (see Table I) were cleaned and ashed in a muffle furnace at 400°C for 24 hours and then at 600°C for 48 hours. The ash was dissolved in a 1:1 $\text{HCl}:\text{H}_2\text{O}$ solution to final volumes varying from 100 to 2000 ml, depending upon the size of the bone. From these solutions, duplicate 15 ml aliquots were removed for gamma spectrometric counting of ^{85}Sr and duplicate 1.0 ml samples were withdrawn for gravimetric determination of total calcium by oxalate precipitation. From the percent of the injected ^{85}Sr dose measured in each bone and the grams of stable calcium determined in each bone, the per cent of injected dose of ^{85}Sr per gram of calcium (specific activity) was calculated.

Results

The per cent injected dose of ^{85}Sr , quantity of calcium per bone, and calculated specific activity are presented in Tables I, II, and III, respectively, for thirteen parts of the pig skeleton. It is noteworthy that even with the variation and non-uniformity in the quantity of calcium and the per cent of injected dose retained in each animal, the specific activity was relatively constant for a particular animal in the bones examined (Figure 1).

If the bones are generally grouped according to type of formation and/or relative amounts of cortical and trabecular bone as listed in Table IV, and the per cent change is noted from sample 1 to sample 8, the bones of Group II (long bones) not only had the largest per cent increase in calcium per bone (132%), but also had the smallest percentage decrease of the originally injected dose of ^{85}Sr (41%) over the six month period. The average initial retention (day 1) of ^{85}Sr of the bones of Group II was 1.44 (Table I).

The bones of Group I (head bones) and Group III (vertebrae) had similar losses of radiostrontium, 61% and 70%, but yet the per cent increase in calcium accretion was quite different from the two groups (Table IV). Group III bones retained the least radioactivity initially (day 1), 0.72, but continued to lose it as rapidly as those of Group I, which had the highest initial retention, 8.63 (Table 1). The similar rate of loss of ^{85}Sr was not compatible with the rate of gain in calcium occurring during growth since the bones of Group I had almost three times the rate of gain of calcium as did the bones of Group III (128% versus 47%). Yet Group II bones with the lowest loss in radioactivity had the highest rate of calcium accretion.

Discussion

Initially, little correlation appeared to exist between net calcium accretion and ^{85}Sr retention, as presented in Table IV. The increase in calcium accretion was marked in the bones of both Group I (skull and mandible) and Group II (long bones) but was much less in the bones of Group III (vertebrae). It should be mentioned that the retention data do not necessarily have to correlate with the data for net increase in calcium per bone since the retention data only suggest the way in which net increase in bone mineral was obtained and not how much was actually laid down.

In an earlier study (1), where net calcium accretion and retention of ^{85}Sr in young pigs (Table V) were also measured, the findings do not correlate closely with those from the present experiment. As previously noted, the ^{85}Sr retention data in Table IV are listed in terms of per cent decrease in retention from original, whereas those of Table V are given simply in terms of per cent of injected dose retained. The percent of injected dose retained in selected bones of the older animals was much less than that of the younger animals. Data of initial ^{85}Sr retention on day 1 give a rough estimate of accretion rates between different groups of animals according to age. Even the retention on day 1 of the older animals was considerably lower than the day 50 post-injection retention of the pig injected at two days of age. Yet the more mature animals had not ceased growing as pig bones continue to grow for at least 3 1/2 years after birth (9).

The net calcium accretion data corroborate these earlier findings. The net rate of accretion of young animals was about 40% per month, whereas in the sexually mature pigs the rate decreased to approximately 20% per month.

The radiostrontium retention data in Table I are used to estimate the resorption of bone which had already been laid down. Assuming that the ^{85}Sr labels largely remained at the sites of bone incorporation attained by 24 hours injection, the rate of resorption would have to have been low through 180 days post-injection. In other words, once the radionuclides ions were integrated at a particular bone locus with a low resorption rate, the probability of their remaining in a given bone would be high unless resorption occurred. Conversely, if the labels were lost rapidly from the bone, the rate of resorption would have to have been high. These retention data and net calcium accretion percentages are used in postulating a model of early and maturing bone growth in swine.

Arbitrary numbers on a scale from 1 to 10 have been assigned to bones in Group I to III in order to estimate rates of accretion and resorption and they, thus, serve to illustrate the net changes observed physiologically in the bones examined. Since more mature animals, compared to neonates, had lower initial retentions, the numbers assigned were relative to rates within a given age group at the time of injection (Table VI). Two general types of bone growth were apparent in terms of net calcium accretion. In one type accretion was accelerated whereas resorption was

average. This type of bone growth was represented by a more rapid loss of the radioactive label as measured six months after injection. In the second type accretion was average whereas resorption was decreased. This type demonstrated a greater retention of the label six months post-injection.

Data from the younger animals (Table V) and the arbitrarily assigned rate scale of Table VI, showed that the bones of Group I were of the second type of growth pattern, i.e., average accretion with decreased resorption. (The term average is used here as a relative term for descriptive purposes only.) Those of Group II were intermediate, having an average accretion and a slightly decreased resorption. The bones of Group III were of the first type of bone growth, i.e., the accelerated accretion and average resorption. These estimates are summarized in Table VI. Similar types of bone growth could be postulated for the more mature bones, but there seemed to be a larger relative decrease in accretion than in resorption in the case of vertebrae.

The results of the present study (Table IV and VI) indicate that changes in the type of bone growth after the onset of sexual maturity in the pigs most likely occurred in the period from six to twelve months of age. Comparing accretion relative to resorption, i.e., net increase in bone mineral, skull and the mandible seemed to have enhanced accretion and reduced resorption, though not as much as for swine injected at birth. The long bones as a group had a similar accretion but a decreased resorption compared to newborn swine. Thus, the long bones had the largest net accretion of calcium as a result of decreased resorption. The vertebrae demonstrated a

markedly decreased net lay down of bone mineral which is accounted for by a decrease in accretion and an increase in resorption rates. In early life the vertebrae appeared to increase in calcium content more than the other bones, but after sexual maturity was reached the long bones retained more calcium. One might therefore state that porcine animals initially grow more in length than height and then beginning at about 6 months of age more in height than length in an ordered developmental sequence. Such a sequential pattern of skeletal growth is apparently not the case in man when growth in width and height occur more nearly concurrently.

It is of note that our use of data of ^{85}Sr retention and net increase in bone calcium served as a better index of the type of bone growth than the use of the simple method of McMeekan of measuring the changes in wet weight and demensions of bones.

Further investigations are necessary to determine what portions of the pig bones, such as cortical and trabecular bone components, are changing in growth from 6 months of age and on and what influences are impinging on the bone growth patterns around the time of sexual maturity. Similar studies of swine injected at birth and of swine injected at one year of age are planned in order to establish the age at which certain bones and their radiosensitive marrow contents are most likely to receive higher dose rates from fallout of ^{90}Sr incorporated in bone mineral.

JJBA:csc

7/21/69

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TABLE I
Per cent of Injected Dose of ^{85}Sr in Selected Bones of Swine
at Several Times (Days) Post-Injection

	Pig Number							
	1	2	3	4	5	6	7	8
Days Post-Injection	1	10	28	60	90	120	137	180
Group I								
Skull	9.35	7.24	7.19	6.53	5.32	5.66	5.38	4.51
Mandible	7.92	5.00*	3.00	3.16	3.02	2.54	2.57	2.35
Group II								
Right Scapula	1.86	1.20	1.22	0.91	1.18	1.01	1.04	0.89
Right Humerus	1.51	1.64	1.60	1.25	1.68	1.33	1.38	1.08
Right Radius-Ulna	2.11	1.25	1.10	0.94	1.24	1.10	1.02	1.02
Right Femur	1.95	1.86	1.76	1.25	1.17	1.42	1.50	1.43
Right Tibia	2.01	1.18	1.15	0.76	0.86	1.04	0.95	1.01
Right Fibula	0.22	0.21	0.16	0.11	0.13	0.11	0.13	0.16
7th Thoracic Rib	0.39	0.31	0.32	0.22	0.26	0.17	0.18	0.19
Group III								
1st Cervical Vert.	0.81	0.50	0.43	0.31	0.41	0.36	0.34	0.26
2nd Cervical Vert.	0.59	0.40	0.32	0.17	0.30	0.40	0.20	0.14
7th Thoracic Vert.	0.63	0.28	0.30	0.20	0.23	0.17	0.16	0.17
4th Lumbar Vert.	0.83	0.49	0.48	0.32	0.29	0.41	0.27	0.29
Sum:	30.18	21.56	19.03	16.13	15.89	15.72	15.13	13.50
	<u>Means</u>							
Group I	8.63							
Group II	1.44							
Group III	0.72							

*Estimated Value

TABLE II

Grams of Calcium Per Bone of Swine at Selected Times (Days)

Post-Injection

	Pig Number							
	1	2	3	4	5	6	7	8
Days Post-Injection	1	10	28	60	90	120	137	180
Group I								
Skull	81.26	113.20	126.20	119.10	130.30	154.20	193.70	166.70
Mandible	34.56	45.00	58.40	57.25	67.70	63.20	85.35	86.70
Group II								
R. Scapula	18.13	15.58	22.63	18.84	27.95	28.73	33.43	33.10
R. Humerus	20.32	21.55	30.85	26.37	38.05	37.40	40.28	44.75
R. Radius-Ulna	14.78	18.33	22.78	21.37	29.15	29.15	32.80	34.98
R. Femur	13.00	28.83	32.55	27.83	27.85	40.38	41.58	46.53
R. Tibia	17.22	19.68	23.25	20.89	22.10	29.33	29.40	36.60
R. Fibula	2.65	2.98	3.33	6.04	3.28	3.18	4.38	5.02
7th Thoracic Rib	3.35	4.73	5.18	5.09	6.59	4.86	7.10	7.40
Group III								
1st Cervical Vert.	7.17	6.39	7.15	6.75	9.98	10.70	11.28	11.94
2nd Cervical Vert.	4.86	5.08	5.45	4.07	6.81	11.42	7.25	7.58
7th Thoracic Vert.	5.99	3.54	4.71	5.16	4.83	5.14	5.55	6.84
4th Lumbar Vert.	<u>8.51</u>	<u>7.28</u>	<u>8.35</u>	<u>7.33</u>	<u>7.00</u>	<u>11.83</u>	<u>12.04</u>	<u>12.73</u>
Sum:	231.80	282.17	350.83	326.05	381.59	429.52	505.14	504.87

TABLE III

Per cent of Injected Dose of ^{85}Sr Per Gram of Calcium in Selected Bones
of Swine at Selected Times (Days) Post-Injection

	Pig Number							
	1	2	3	4	5	6	7	8
Days Post-Injection	1	10	28	60	90	120	137	180
Group I								
Skull	0.115	0.0639	0.0570	0.0548	0.0408	0.0367	0.0278	0.0271
Mandible	0.229	0.1111*	0.0513	0.0553	0.0446	0.0402	0.0301	0.0271
Group II								
R. Scapula	0.103	0.0769	0.0540	0.048	0.0421	0.0353	0.0311	0.0268
R. Humerus	0.0744	0.0762	0.0517	0.0475	0.0441	0.0356	0.0343	0.0241
R. Radius-Ulna	0.143	0.0680	0.0484	0.044	0.0424	0.0378	0.0311	0.0292
R. Femur	0.150	0.0644	0.0541	0.0448	0.0421	0.0351	0.0361	0.0307
R. Tibia	0.117	0.0600	0.0495	0.036	0.039	0.0354	0.032	0.0276
R. Fibula	0.083	0.071	0.047	0.019	0.040	0.036	0.031	0.032
7th Thoracic Rib	0.12	0.065	0.061	0.044	0.040	0.036	0.025	0.025
Group III								
1st Cervical Vert.	0.11	0.078	0.061	0.045	0.041	0.034	0.030	0.022
2nd Cervical Vert.	0.12	0.075	0.059	0.042	0.048	0.035	0.027	0.019
7th Thoracic Vert.	0.11	0.079	0.064	0.039	0.041	0.033	0.029	0.025
4th Lumbar Vert.	<u>0.098</u>	<u>0.067</u>	<u>0.057</u>	<u>0.044</u>	<u>0.041</u>	<u>0.035</u>	<u>0.022</u>	<u>0.023</u>
Mean:	0.130	0.0764	0.0542	0.0495	0.0416	0.0366	0.0299	0.0267

Estimated Value

TABLE IV

Comparison of Net Calcium Accretion and Change in ^{85}Sr Retention
in Three Groups of Bones of Swine Injected at 6 Months of Age

	% Increase in <u>Calcium/Bone*</u>	% Decrease in <u>% Injected Dose of ^{85}Sr*</u>
Group I	128	61
Group II	132	41
Group III	47	70

*Pig 8 (killed at 12 months of age) compared to Pig 1 (killed at 6 months of age).

Group I = Skull and Mandible

Group II = Scapula, Humerus, Radius-Ulna, Femur, Tibia, Fibula,
7th Thoracic Rib

Group III = 1st Cervical Vert., 2nd Cervical Vert., 7th Thoracic Vert.,
4th Lumbar Vert.

TABLE V
 Net Calcium Accretion and ^{85}Sr Retention in One Young (0-50 day) Pig
 (Data from Anderson, 1968)

<u>Bones</u>	<u>Net Calcium accretion as % of original</u>	<u>Percent of injected dose of ^{85}Sr retained on day 50 P.I.</u>
Group I	83.7	39.5
Group II	84.5	20.9
Group III	90.5	11.0

TABLE VI

Arbitrarily Assigned Rates of Accretion and Resorption Relative to Age
in Young (0-50 days) and Mature (6-12 months) Swine.

	0-50 Days		6-12 Months	
	<u>Accretion</u>	<u>Resorption</u>	<u>Accretion</u>	<u>Resorption</u>
Group I	5*	3	10	7
Group II	6	4	7	3
Group III	10	7	5	4

*Scale where: 5 = Average Rate; 0 = Zero Rate; and 10 = Accelerated Rate

Summary of Estimated Accretion and Resorption Rates
in Two Age Groups of Swine

Age: 0-50 days

Group I average accretion with decreased resorption
Group II average accretion with slightly decreased resorption
Group III accelerated accretion and average resorption

Age: 6-12 months

Group I accelerated accretion with average resorption
Group II average accretion with decreased resorption
Group III average accretion with slightly decreased resorption

LEGEND

Fig. 1. Plot of the specific activity (% of injected dose per gram of calcium) of selected bones as a function of time in days of pigs killed after intravenous injection of strontium 85.

Dose (mg/kg)

Time (hr)