Short Term Tissue Distribution of Several Radionuclides Useful in Bone Scanning.* (31668)

NORMAN S. MACDONALD, LINDA BRADEN, ELLIN JAMES, AND ROBERT A. WOOD
(Introduced by G. V. Taplin)
Laboratory of Nuclear Medicine and Radiation Biology, Department of Biophysics-Nuclear Medicine; and Department of Radiology, UCLA School of Medicine, Los Angeles

Availability of gamma emitting radioactive isotopes of calcium and strontium (^{47}Ca and ^{85}Sr) in recent years has led to their increasing usage for delineating tumors and other lesions of the human skeleton by "bone-scanning" with scintillation detectors(1-3). However, the physical properties of these nuclides are not ideal. Photons from the former are too energetic for convenient collimation, whereas the latter isotope has a 65-day half-life which is unnecessarily long for scanning purposes. Therefore, other radionuclides of short half-lives and suitable energies are undergoing clinical trials at several laboratories. ^{87m}Sr and ^{18}F show promise for bone scanning(4-6) and ^{99m}Tc in several chemical forms has proven a versatile agent for brain and thyroid scanning(7,8). Its value for bone scanning is unproven. Much is known of the metabolism and mechanisms of skeletal uptake of strontium(9,10) and fluoride ion(11-13). However, there is need for detailed information of the distribution of these materials in the various tissues which are in the "field of view" of the scintillation detector during the scanning operation following intravenous injection. These tissues consist mainly of skin, muscle, bone and marrow, but it is the accumulation of isotope in bone which is of prime concern. Excessive activity in other tissues obscures the results. The purpose of the work reported here was to determine the relative distribution of ^{47}Ca, ^{85}Sr, ^{18}F and ^{99m}Tc in tissues of the leg at various intervals shortly after administration, and to compare their rates of appearance in the dog leg. Bromine-82 was included in the comparison because it enters the extracellular fluid space but is not trapped by bone(14).

Methods. Adult female Dutch rabbits were given, by ear vein, a single injection of a mixture of two radionuclides in physiological saline.† Group I consisting of 4 animals received 25 μc each of ^{47}Ca and ^{85}Sr and were sacrificed with sodium pentobarbital either 4 or 60 minutes later. Group II, 9 animals receiving 25 μc of ^{85}Sr and 100 μc of ^{99m}Tc as pertechnetate ion, were sacrificed after intervals of 5, 15 and 60 minutes. Group III, 8 rabbits, 25 μc ^{85}Sr plus 50 μc ^{82}Br were killed after intervals of 10, 15, 30 and 60 minutes; and Group IV, 4 rabbits, given 25 μc ^{85}Sr plus 100 μc of ^{18}F, after 5, 10, 60 and 180 minutes. In all cases the left lower leg was removed and all tissues between the knee joint and a point two-thirds of the leg length distal to the knee were separated into skin, muscle, white connective tissue, bone and marrow. Tissues from the proximal third of the leg were grouped separately from those comprising the middle third, or shaft of the leg. After weighing, each separate tissue collection was packed into a sufficient number of screw-capped vials so that

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† Calcium-47 was obtained from Abbott Laboratories as the chloride in sterile 0.9%-NaCl solution. Carrier Ca in each administered dose was 0.1 mg. Strontium-85 and ^{82}Br were supplied by Iso-Serve Inc., the former as the chloride in sterile 0.9%-NaCl with 0.04 mg carrier Sr per dose; the latter as NaBr which was diluted with 0.9%-NaCl. Carrier Br was 0.2 mg per dose. Technetium-99 m was eluted with NaCl solution as carrier-free pertechnetate ion from an ion exchange column containing the parent nuclide, ^{99}Mo, as supplied in "generator" form by Nuclear Consultants Corp. Fluorine-18 was prepared from Li_2CO_3 enriched in Li^6 (obtained from Oak Ridge National Laboratory) by irradiation in the nuclear reactor of the UCLA Engineering Dept. After processing, the product was carrier free ^{18}F as fluoride ion in 0.9%-NaCl solution at pH 7. The nuclear reactions involved are: ^{6}Li(n, α) T followed by ^{16}O(T, n) ^{18}F. The physical half-lives are: ^{47}Ca-4.7 day; ^{85}Sr-65 day; ^{87m}Sr-2.8 hr; ^{99m}Tc-6.0 hr; ^{82}Br-35 hr; ^{18}F-1.8 hr.

TABLE I. Comparison of Distribution of ^{47}Ca and ^{85}Sr in Tissues of the Rabbit Lower Leg.

Tissue	4 min		60 min		60 min		60 min	
	^{47}Ca	^{85}Sr	^{47}Ca	^{85}Sr	^{47}Ca	^{85}Sr	^{47}Ca	^{85}Sr
Proximal section								
Skin	16.4	16.7	11.2	10.6	8.5	8.7	7.8	8.1
Connective tissue	1.8	1.7	2.2	2.0	2.5	2.6	2.9	3.0
Muscle	40.2	37.8	22.6	21.5	14.8	13.5	6.7	5.8
Marrow	1.2	1.1	.6	.2	.2	.2	.03	.02
Bone	15.7	15.4	26.2	24.6	52.3	52.7	60.0	60.8
Mid section								
Skin	8.1	8.5	10.2	9.7	4.0	4.2	3.3	3.3
Connective tissue	4.4	4.3	2.2	2.1	2.9	2.9	1.7	1.5
Muscle	5.3	7.3	15.2	15.3	2.4	1.9	5.5	5.0
Marrow	.4	.4	.5	.3	.1	.1	.1	.1
Bone	6.6	6.9	9.3	13.4	12.3	13.2	11.8	12.4

Mixture of ^{47}Ca and ^{85}Sr given intravenously; animals sacrificed at 4 min or 60 min later. Group I.

Each value represents the percentage of total activity in lower leg.

each vial contained approximately 5 ml of tissue in order to equalize counting geometries. Collections of marrow, white connective tissue and terminal blood drawn by heart puncture were mixed with water to make up 5 ml volumes in the counting vials. Dose aliquots were prepared in similar fashion. Radioassays were carried out with a 3-inch well-type NaI scintillation crystal equipped with two channels for pulse height selection so that both nuclides in each pair could be determined simultaneously. Appropriate corrections were made for backgrounds, radioactive decay, and interchannel Compton scatter contributions. Since ^{18}F and ^{85}Sr have identical gamma spectra (0.51 Mev photons) their radioassay was achieved by counting the samples before and after decay of the ^{18}F component. Interference by low energy gammas from the ^{47}Sc daughter of ^{47}Ca decay was avoided by appropriate pulse height discrimination.

Radioisotope osteograms of the uptake of the nuclides in the knee area were obtained in dogs. This technique measures the rates of accumulation of radionuclide with a collimated probe-type scintillation detector placed next to the skin over the area of interest(15). Each dog was immobilized by sodium pentobarbital anesthesia, the external probe set in place and approximately 50 μc of one of the nuclides given intravenously. The observed counts for each minute were recorded by a pulse height analyzer and printing scaler for the ensuing 45 minutes. The window of the analyzer was then raised to bias out the pulses of the first nuclide, a second nuclide emitting higher energy photons was injected and the sequential counting was resumed. In this way the accumulations of each isotope of the following pairs were compared: ^{85}Sr-^{47}Ca; $^{99m}TcO4$-^{85}Sr; ^{85}Sr-^{82}Br and ^{85}Sr-^{18}F. In the last pair, the dose of ^{18}F was about 10 times that of the ^{85}Sr and the pulse height analyzer setting was not altered, because the gammas arising from annihilation of the positrons emitted by the ^{18}F are of the same energy as the gammas accompanying the disintegrations of ^{85}Sr. All counting data were corrected for background and radioactive decay back to time of injection.

Results and discussion. Table I shows that the ^{85}Sr activity was distributed among the soft tissues and bone of the leg in almost exactly the same proportions as was ^{47}Ca. It is also evident that the activity measured by an external detector such as a scanner placed over the leg would not be coming solely from bone. Skin and muscle contained considerable activity even after one hour. As time passed the ^{47}Ca and ^{85}Sr content of muscle declined and that of bone increased. Similar changes have been deduced from observations in humans(16). Both tracers were found in greater concentrations (counts/min/g) in the upper section of the tibia than in the more distal portion, probably because the accessibility of exchangeable bone mineral is

TABLE II. Distribution of Radioactivity in Lower Leg of the Rabbit.

		Group II						Group III				Group IV	
Interval (min)	Tissue	Sr	Tc	Sr	Tc	Sr	Tc	Sr	Br	Sr	Br	Sr	F
4–10	Bone	35	18	25	17	34	17	36	15	36	19	32	42
	Muscle	41	54	41	49	40	54	28	35	37	44	25	24
	Skin	14	18	20	21	19	20	23	30	19	25	36	28
10–15	Bone	40	19	41	20	46	23	36	13	43	15	70	76
	Muscle	31	46	32	56	31	56	29	39	27	42	14	12
	Skin	21	26	19	20	19	20	22	28	18	24	12	8
30	Bone							42	12	45	12		
	Muscle							26	36	25	38		
	Skin							19	28	19	30		
60	Bone	55	20	55	15	44	13	55	15	59	14	87	83
	Muscle	21	41	23	37	28	66	23	40	21	41	6	8
	Skin	17	28	15	12	19	14	13	27	13	29	5	5
180	Bone											97	92
	Muscle											1	4
	Skin											1	2

Group II consisted of 9 animals each receiving 25 μc of ^{85}Sr and 100 μc of ^{99m}TcO4 and sacrificed after intervals of 5, 15 and 60 min. Group III, 8 rabbits each, received 25 μc ^{85}Sr plus 50 μc ^{82}Br and were killed after intervals of 10, 15, 30 and 60 min. The 4 rabbits in Group IV each received 25 μc ^{85}Sr plus 100 μc of ^{18}F and were killed after intervals of 5, 10, 60 and 180 min.

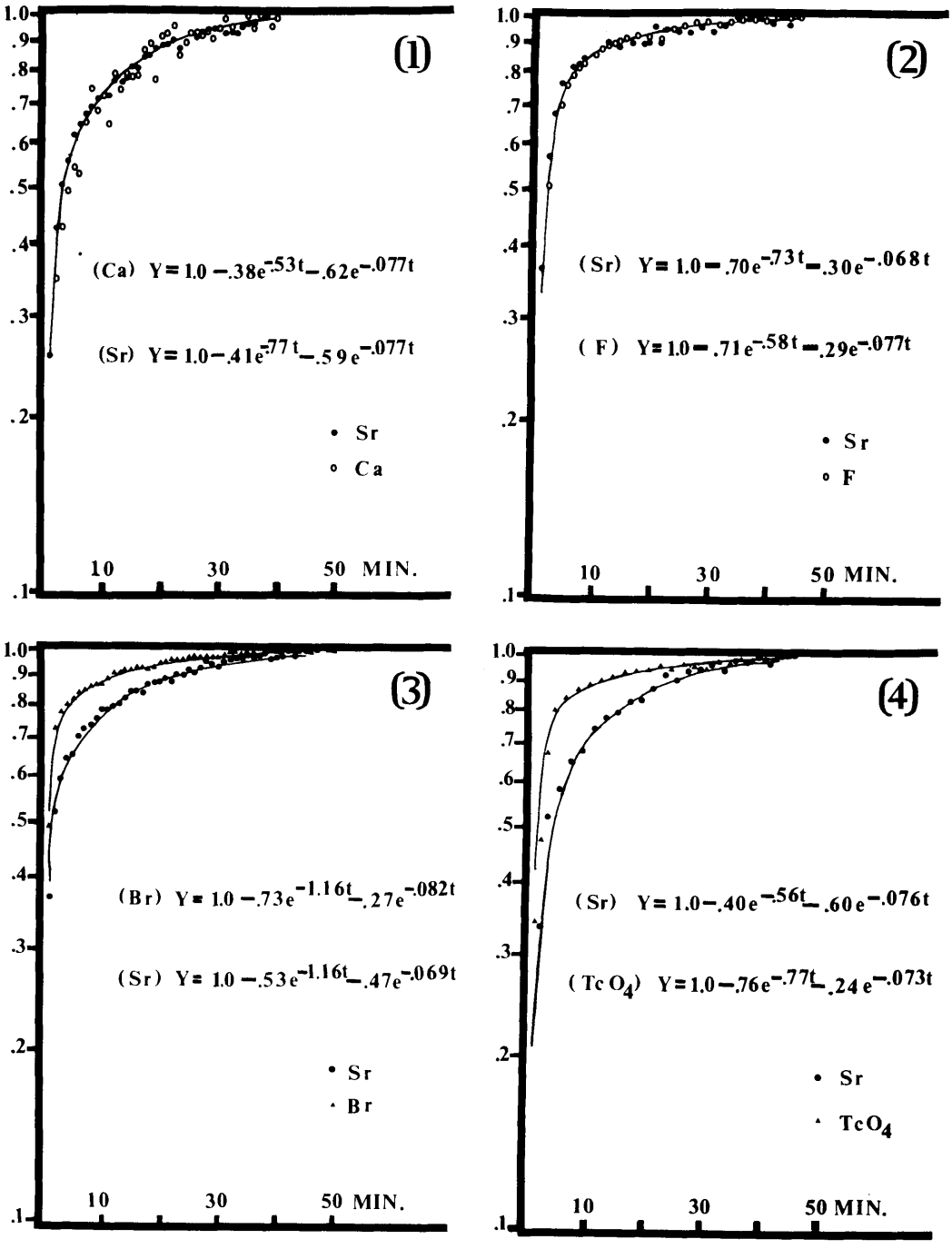
Each numerical value represents the percentage of total activity for the nuclide in the lower leg. The remainder of the activity necessary to total 100% in each case was in marrow and white connective tissue. Each set of 6 values is from one animal. Rabbits in Group IV were somewhat younger than the others which probably accounts for the higher uptake of ^{85}Sr in bone tissue at the later time intervals.

greater in the spongiosa of the metaphyseal region than in the compacta of the shaft.

Table II presents similar data for the other isotopes. The distribution of ^{18}F was almost identical with that of ^{85}Sr and therefore, by inference, with ^{47}Ca distribution. Here again the fraction of total leg activity of ^{85}Sr and ^{18}F found in muscle decreased with time while the fraction in bone increased. The absolute activity in bone, in terms of counts/min/g, also increased with time. Thus, to minimize interference by "non-bone" radioactivity, scanning with ^{18}F or ^{87m}Sr should be postponed until 1-2 hours after injection, even at the expense of loss of total activity due to physical decay of these short-lived nuclides.

Technetium-99m in the form of pertechnetate ion did not parallel ^{85}Sr in tissue distribution. Although considerable ^{99m}Tc was found in the bone samples, this was overshadowed by the larger amount in muscle. The proportions found in skin were similar for both ^{85}Sr and pertechnetate. Pertechnetate distribution in the leg was remarkably like that of ^{82}Br when both were compared to Sr. The data suggest that pertechnetate ion may behave like bromide ion which enters and remains temporarily within the extra cellular fluid (ECF) space in the leg but does not become sequestered by bone mineral. For example, the fraction of the total leg activity found in the bone component was 2-4 times higher for ^{85}Sr than for ^{82}Br and ^{99m}TcO4. Conversely, the amounts of ^{85}Sr in the muscle component were only ½ to ¾ of the amounts of ^{82}Br and ^{99m}TcO4 in the same muscle. Sr:F ratios were essentially 1:1 for bone and muscle.

The osteograms in Fig. 1 demonstrate that accumulation of radioactive isotope in the knee area, as seen by an external detector, follows the same kinetic pattern in the cases of ^{47}Ca, ^{85}Sr and ^{18}F. On the other hand, the ^{99m}TcO4 and ^{82}Br patterns differ from ^{85}Sr. Thus, by 5 minutes post injection both ^{99m}TcO4 and ^{82}Br had reached more than 80% of their 45-minute value, whereas the true bone seekers ^{47}Ca, ^{85}Sr and ^{18}F had attained only 60-65% of their 45-minute level of activity. Compartmental analysis of these and a number of similar curves in dogs and humans and attempts to assign physiological meanings to the mathematical terms will be



FIGS. 1-4. Rates of appearance of the radiotracers in left knee areas of 4 dogs, as seen with an external, collimated scintillation detector. Abscissa (t) of each graph represents time elapsed from injection. Ordinates represent the observed counts per minute normalized to the counting rate of 45 min post injection and plotted on a logarithmic scale. $Y = (c/m)_t \div (c/m)_{45}$. Triangles and dots represent observed values. Smooth lines represent the curves fitted to these points and the equations express these fitted curves in exponential form.

presented later. The curves show clearly that ^{18}F behaves like calcium and strontium during the early time period (1-2 hours) when most scanning for bone lesions or abnormalities would be performed with this nuclide, but that pertechnetate and bromide ions do not.

Summary. ^{18}F and ^{85}Sr administered intravenously in rabbits and dogs yield the same information as ^{47}Ca, insofar as early distribution and kinetics of tissue uptake affect "bone scanning" with external gamma scintillation detectors. Uptake by bone spongiosa exceeds that by compacta. Muscle and skin contain large fractions of the total activity during the first hour, but muscle content drops rapidly. ^{99m}Tc as pertechnetate ion does not behave like the bone seeker, but closely parallels bromide ion, ^{82}Br, in its early distribution throughout leg tissues.

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The Nature of Antibody in Swine Naturally Infected with Japanese Encephalitis Virus. (31669)

JIRO KONNO, KOKI ENDO, AND NAKAO ISHIDA (Introduced by T. Francis, Jr.)
Department of Bacteriology, Tohoku University School of Medicine, Sendai, Japan

The cyclic outbreak of Japanese encephalitis (J.E.) among man and pigs in Miyagi Prefecture was outlined previously(1). One of the main observations of that work was that the outbreak of J.E. among pigs was 3 weeks ahead of the one among people, suggesting that a serological survey of pigs through the summer season might be used for the purpose of prediction of J.E. outbreak in the human population. For that purpose, it would be most important to find out the first occurence of infection among pigs. However, several serum specimens taken at an early phase of the epidemic season, *i.e.*, June and July, showed high hemagglutination inhibition (HI) titers and it was difficult to decide whether these were due to antibodies elicited by infection the previous year or the current year.

The present study was undertaken in 1965 to determine if recent infection of pigs with J.E. virus could be diagnosed by the susceptibility of specific antibodies to 2-mercaptoethanol (2-ME).

Materials and methods. Collection of swine sera: As a rule, 50 serum specimens were collected weekly at a slaughterhouse in Miyagi Prefecture. All sera were obtained