Radioactive Calcium Tracer Studies in Bone Grafts * †

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INTRODUCTION

During the last half century, the fate of bone grafts has been studied by many techniques; yet no general agreement exists at present concerning the sequence of events which follows the insertion of a bone graft. The availability of radioactive isotopes now makes possible tracer experiments which can show the distribution of some elements of the grafted tissues. The present study was undertaken in order to determine what information radioactive calcium (Ca^{45}) tracer techniques could add to our knowledge of the behavior of bone grafts. In particular, we have tried to determine what happens to calcium in and around a graft area. Does grafted calcium diffuse into adjacent bone or contribute to nearby callus formation, or is the grafted calcium removed by circulating blood as the graft area undergoes repair? Does the calcium supply to the graft area come from adjacent bone, or does it come from circulating blood?

In order to answer the first question we implanted homogenous-bone grafts containing Ca^{45} into dogs; in order to answer the second question we implanted non-radioactive homogenous-bone grafts into dogs which had been injected with Ca^{45}. Samples of blood were taken at intervals up to the time of sacrifice and were analyzed for specific activity. When the dogs had been sacrificed, sections from the graft, the callus, and the nearby bone were analyzed for stable calcium (Ca^{40}) and for radioactive calcium (Ca^{45}). Adjoining sections were examined histologically. Adjacent sections were autoradiographed, and the films were analyzed quantitatively for localized values of specific activity by means of a microdensitometer.

All of the quantitative data are given in terms of specific activity, that is, in microcuries of Ca^{45} per gram of Ca^{40}. Ca^{45} activities obtained densitometrically have been corrected for decay from the time of injection, a half-period of 163 days being used. Ca^{45} activities obtained by counting with Geiger-Müller counter have been corrected by means of Ca^{45} standards. Since the amount of injected Ca^{45} differed somewhat for different dogs, all the data were normalized to an injection of 0.1 millicurie per kilogram of body weight. The use of *specific activity* instead of the *total activity* of Ca^{45} made it possible to compare the activity values for blood with those for bone, which helped to indicate the source of deposited calcium and the time of calcification. The normalization made it possible to compare the specific activity results for different dogs.

MATERIALS AND METHODS

The animals used were adult, healthy, mongrel dogs, maintained in cages under standard laboratory conditions with additional precautions taken in order to minimize radioactive contamination. The dogs were fed a mixture of fresh frozen horse meat, bran, and water as one meal in the morning. The average daily intake of calcium was about 1.7 grams a day. Water was given *ad libitum*.

The Ca^{45} solutions for injection were prepared by diluting the Ca^{45} supplied by the Atomic Energy Commission (specific activity ranging from 0.6 to 4.2 millicuries per milligram of calcium) with distilled water

* Supported in part by a grant from the Playtex Park Research Institute and by Contract AT(30-1)-952 with the United States Atomic Energy Commission.

† Read at the Annual Meeting of the Orthopaedic Research Society, Chicago, Illinois, January 28, 1956.

and by adjusting the pH to 4 to 5 with dilute hydrochloric acid and sodium hydroxide. The standardization * of the Ca^{45} (maximum energy = 0.254 million electron volts; half-life = 163 days ± 1 day **) was carried out with a 4 π beta proportional counter. The standard deviation in the uncertainty of the absolute activity was ± 5 per cent.

Injections of Ca^{45} were performed by clean venipuncture; and leakage or loss of activity was minimized by multiple washings-through of blood, by delayed withdrawal of the needle, and by prolonged pressure on the puncture site after withdrawal. The adequacy in administering the dose was determined by measuring the residual Ca^{45} in the needle and syringe. The residual activity was found to average about 3 per cent and no corrections were applied for this small loss. A new syringe and needle were used for each injection and for each venipuncture for sampling. The dose levels of Ca^{45} for different dogs varied from 0.06 to 0.24 millicurie per kilogram of body weight.

The grafting procedures are summarized in Table I. Four dogs were used as donors, three of them having received Ca^{45} and the fourth having received no Ca^{45}. Bones from each of these donors were removed, separated from soft tissues under sterile conditions, and kept refrigerated at −20 degrees centigrade until used. Seven dogs served as recipients and received inlay grafts, approximately three to eight centimeters long by 0.5 to one centimeter wide, into the diaphyses of the long bones. The cortical grafts, the measurements and weight of which had been recorded, were either fitted into slots in the cortex or inserted in the medullary cavity. Weighed cancellous grafts were packed as granules into troughs formed in the cortex. The grafts were held in place when necessary by periosteal sutures.

A complete postmortem examination was performed on each animal, and many specimens of bone from different portions of the skeleton were assayed. Each grafted area was excised in full, and the graft segment was divided into three parts. One of these was fixed in formalin for histological studies, another was analyzed for specific activity, and the third was prepared for autoradiography.

Multiple histological preparations of each graft site were made by use of celloidin embedding and hematoxylin and eosin staining techniques. The amount of persisting grafted bone was estimated as follows: when cortical implants had been used, a fragment could be found which, because of its position and devitalized appearance, represented that part of the graft persisting intact. The indistinct border of the area of callus could be recognized by the differing patterns of trabeculation. On each slide the areas of callus and implant were circumscribed in ink. An ocular grid was used to make an approximation of the proportions of new bone and residual implant present. Each square of the grid (100 micra by 100 micra) which contained bone in more than half of its area was recorded as containing callus or implant depending upon which type of bone predominated. A summation of the results on all squares in the demarcated area yielded an estimate of the relative amounts of implanted and new bone.

For determination of specific activity, the graft site was divided into three parts as accurately as could be done with a scalpel under a hand lens. These three parts were called respectively the implant, the callus, and the bed, the latter being that portion of recipient cortex opposite the graft. Each of these specimens was known to contain variable amounts of materials from adjoining areas as demonstrated by histological examination of their counterparts. They represented regions of sampling and were not thought of as pure samples of the designated material.

Ca^{40} analyses of serum samples were carried out in triplicate by use of a modification of the method of Salomon, Gebrio, and Smith. The proteins of serum aliquot-samples were removed by precipitation with trichloracetic acid. Ca^{40}, in the protein-free serum, was precipitated directly as the oxalate at pH 4.5. After standing overnight, the precipitate was centrifuged and washed several times with dilute, freshly prepared ammonium hydroxide. After the final centrifugation the precipitate was dissolved in perchloric acid ($HClO_4$), and the oxalate ion was titrated with cerium $^{++++}$, nitroferrin being used as indicator. The serum aliquots were chosen to contain 0.02 to 0.07 milligram Ca^{40}. Conductivity water was used for all solutions. Routine checks of the procedure were maintained by titrating the cerium $^{++++}$ with standard oxalate ion, by blank tests for the indicator, and by a standard calcium solution to monitor the procedure as a whole.

For Ca^{40} analyses, the bone samples were prepared by dissecting the soft tissues from the bone. The cleaned bone was oven dried and was dry ashed in porcelain crucibles in a muffle furnace at 550 degrees centigrade for forty-eight hours. The Ca^{40} analyses were carried out in duplicate on aliquots of the dissolved dry-ash residue by means of either the procedure just described or the standard potassium permanganate titration procedure [1], depending on the calcium content of the sample.

Ca^{45} analyses of serum were carried out with the same samples as those used for the Ca^{40} analysis; Ca^{45} analyses of bone were performed on aliquots of the original dry-ashed solutions. The samples were prepared for beta-ray counting by precipitating the calcium as the oxalate. Prior to precipitation, sufficient carrier calcium was added to make each sample contain four milligrams of calcium. After precipitation, the solution was poured through a two-section glass filter, and the calcium oxalate was collected on a disk of filter paper.

* This absolute standardization is necessary only for purposes of dosimetry with regard to radiation effects. The other aspects of this study merely require a self-consistent relative standard.

** This value is a recent measurement obtained in this laboratory (J. B. W.) and is in agreement with the value obtained by C. F. G. Delaney and J. H. J. Poole, Phys. Rev., **89**: 529, 1953.

TABLE I

SUMMARY OF EXPERIMENTAL SCHEDULE

Dog No.	Procedures	Millicuries of Ca^{45} Injected or Implanted per Kilogram of Body Weight	Type of Graft	Location	Sacrificed at	Remarks
101	Injection	0.242			11 days	
104	Injection	0.242			26 days	
119	Injection	0.059			14 days	
102	Radioactive graft	0.00056	Cortical (intermedullary)	9 ribs, femora	5 days	Thoracic infection, died
103	Radioactive graft	0.00071	Cortical (intermedullary)	Humeri, femora, tibiae	10 days	Humeral fracture, died
106	Radioactive graft	0.0026	Cortical	Femora, tibiae	90 days	
107	Radioactive graft	0.0030	Cancellous	Femora, tibia	94 days	
105	Injection followed by non-radioactive graft	0.127	Cortical	Femur, tibiae	102 days	11-day interval between injection and graft
108	Injection during non-radioactive graft	0.187	Cortical	Femora, tibiae	93 days	Simultaneous graft and injection
109	Non-radioactive graft followed by injection	0.165	Cortical	Femora, tibiae	89 days	14-day interval between graft and injection

The filter paper was glued on a copper planchet, and the Ca^{45} content was determined by counting on automatic apparatus equipped with thin end-window Geiger-Müller counters. The Ca^{45} preparation procedure was monitored by the routine preparation of Ca^{45} standards. The counters were monitored with electroplated radioactive cobalt (Co^{60}) standards [4] and with a continuous record of gamma-ray background. Duplicate samples were counted, each for three cycles, a total of 3×4096 counts being accumulated.

In the specific-activity analyses, the recoveries of Ca^{40} and Ca^{45} were 98 ± 2 per cent. Standard deviations in the measurement of individual samples of Ca^{40}, Ca^{45}, and Ca^{45}/Ca^{40} were determined on large numbers of replicate samples and found to be 1 to 2 per cent, 2 to 3 per cent, and 2 to 4 per cent, respectively.

For *autoradiography* the bone was dehydrated through alcohols and xylol, and was embedded in bioplastic. The embedded bone was sectioned with a thin circular saw and faced on a lathe according to the method of Marshall, White, and Cohen. Exposures of a week to several months were made on Eastman Autoradiographic No-Screen plates. The plates were developed under carefully controlled conditions, and each development batch was calibrated with a Ca^{45} plaster-of-Paris radiator. This radiator, consisting of several areas of graded activities as described by Dudley and Dobyns, contained quantities of Ca^{45} standardized within ± 5 per cent by 4π beta counting. The blackening of the autoradiographs was measured with a microdensitometer. The microdensitometer consisted of a standard densitometer (Macbeth-Ansco Colar Densitometer, Model 12), the photocell head of which was adapted to fit a standard photomicrographic camera. The aperture for the densitometer was located at the optical axis of the microscope in the plane normally occupied by the camera film. With a microscope magnification of 63 ×, this aperture corresponded to a circular aperture 80 microns in diameter on the autoradiograph located on the object plane of the microscope. All other apertures of the microscope were reduced as much as possible in order to minimize the amount of stray light entering the aperture of the densitometer.

The autoradiographic density data were reduced to terms of specific activity as follows. By use of different exposure times with the Ca^{45} radiator, the density of the film was measured as a function of relative exposure. From this observed relation, the densitometer data were corrected for slight non-linearity. The relation between corrected density (less film background) and specific activity can be expressed as follows:

1. $D = a\ r\ k\ F$

where

D = measured net density corrected for non-linearity;

a = specific activity of bone in microcuries per gram of calcium referred to the time of injection;

r = grams of calcium per gram of bone as embedded = 0.25;

(the value $r = 0.25$ was used for all the autoradiograph data; however, subsequent measurements gave 0.250 and 0.235 gram of calcium per gram of embedded bone);

k = film density produced per microcurie per day per gram of embedded bone;

f = exposure time in days corrected for the decay of Ca^{45} (half-life = 163 days).

A similar relation applies to the plaster-of-Paris radiator:

2. $D^* = a^* r^* k^* F^*$

where

D^* = measured net density of the radiator spot;

$a^* r^*$ = Ca^{45} activity per gram radiator (measured by a 4 π counting);

k^* = film density per microcurie per day per gram radiator;

F^* = exposure time used for the radiator.

Since the same beta-ray emitter (Ca^{45}) is used in both the bone sample and the radiator, k will equal k^* if the curve of absorption of beta rays in relation to superficial density (milligram per square centimeter) is the same for embedded bone as it is for plaster of Paris. The respective densities of bone and plaster of Paris are not important so long as the diameter of the active area is large compared with the range of beta rays. Since plaster of Paris has an atomic composition comparable to that of bone, the assumption that $k = k^*$ should be excellent, and we may solve for a, the specific activity in a small region of bone from the above expressions:

3. $$a = \frac{D F^* a^* r^*}{D^* F r}$$

This relation between the specific activity in bone and the density of the autoradiograph film applies properly to thick, uniformly distributed sources. The maximum range of Ca^{45} beta rays is about sixty milligrams per square centimeter (300 micra in cortical bone with observed density two grams per cubic centimeter). Since all bone sections that were autoradiographed were at least 300 micra thick, the thick-source requirement was fulfilled. The resolution of the method (thick source in contact with an autoradiographic plate) was measured by embedding a thread ten micra in diameter drawn from a viscous sugar solution containing dissolved radioactive calcium chloride, and by exposing a film to a cross section of the thread. The resulting exposure spot, measured with the eighty-micra densitometer aperture, showed a characteristic density profile with a width at half maximum density of about 120 micra. The specific activity derived from a microdensitometer reading therefore represents the effective average specific activity whose distribution is uniform in a cylinder with a diameter of about 120 micra. The autoradiographic values for effective specific activity reported in the tables have been calculated as if the activity were in the above distribution and therefore these values underestimate the actual specific activity in proportion to the non-uniformity of its distribution.

The error (standard deviation) in the densitometric specific activity, a, is a proper summation of the errors in the variables of Equation 3. The errors in the exposure factors F and F^* arise from the uncertainty in the half-period of Ca^{45}, applied to the time between injection and the conclusion of exposure. With an error of one day in the Ca^{45} half-period, the maximum errors in F and F^* are calculated to be 3 per cent and 1 per cent, respectively. The quantity $a^* r^*$ has an error of 5.5 per cent and is primarily due to the error in the standardization of Ca^{45} with the 4 π counter. The quantity r^* has been determined independently and is 0.240 $\pm$ 0.002 gram of calcium per gram of radiator, this determination falling between the two corresponding measured values for bone. The error in the quantity r is approximately 3 per cent. Therefore, the combined error or standard deviation in the quantity $F^* a^* r^*/Fr$ is approximately 7 per cent and is nearly constant for all the densitometric measurements.

Measurements with the eighty-micra circular aperture result in a standard deviation of approximately 5.5 per cent for density values between 0.6 and 2.5. This error is primarily due to the number of grains per unit area in the film. Since the error due to subtracting film background density is relatively small at these density values, the error in net density, D and D^*, is about 5.5 per cent, although theoretically it is expected to decrease slowly as the density increases to higher values. Thus, the error in the quantity a is 10 to 11 per cent for values of net density equal to or greater than about 0.4 when background density is about 0.2 to 0.25, and is the minimum error for any value of a. As the density decreases below about 0.6, the errors in D and D^* begin to increase rapidly due to two additional factors: the significant fluctuations arising from the number of beta rays per unit area interacting with the film, and the error due to the increasing relative importance of film background density in total density measurements. The error in the observed calibration curve for the response of the film to exposure is a third factor, small when compared with the other errors at total density values greater than about 0.3, but increasingly significant as the density values decrease below about 0.3. Therefore, as the net density values D and D^* decrease to 0.4, 0.2, and 0.1, the error in the quantity a increases to approximately 20 per cent, 45 per cent, and 70 per cent, respectively. At some of the lowest densities which have been measured, the error or standard deviation approaches 100 per cent.

No fading was detected over the two-month period of the long exposure.

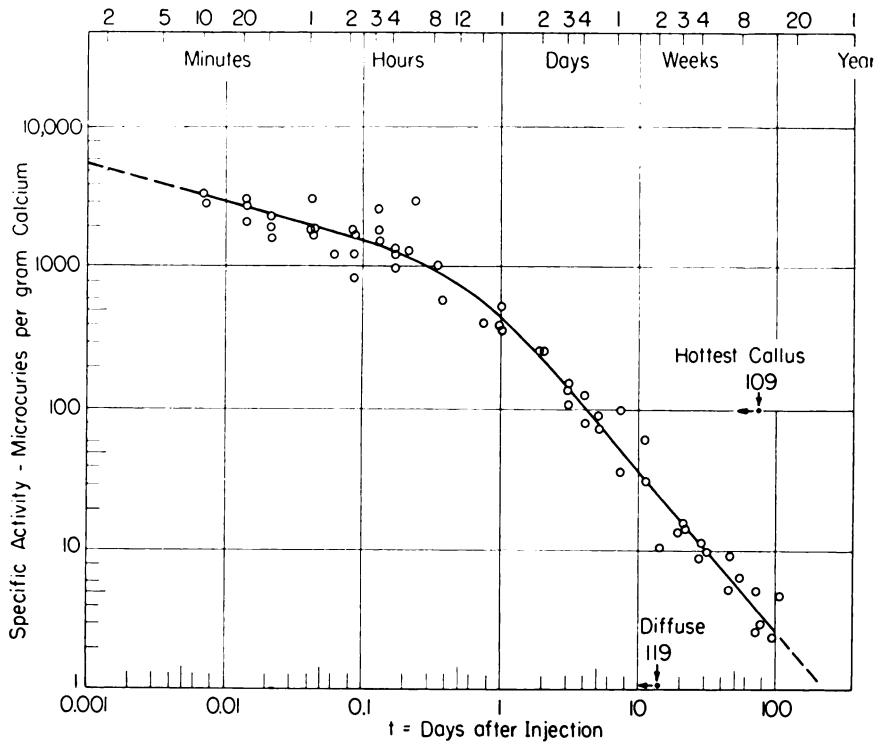

CHART I

Serum specific activity of dogs injected with Ca^{45}. A log-log plot is presented for convenience. The specific activity decreases monotonically over the entire observation period. The dotted sections at the two extremities of the curve are extrapolations. The dogs represented in the figure are not identified since there was no significant difference between the animals. All points are normalized to an injected dose of 0.1 millicurie per kilogram of body weight. The specific activity of the most active callus (dog 109, left tibia) and a characteristic diffuse value (dog 119, right femur) are included for comparison.

RESULTS

The specific activity of the blood in microcuries per gram of calcium is plotted as a function of time after injection in Chart I. Different dogs showed no significant variation from the curve, so measurements on all injected dogs were plotted together, after normalization to an injected dose of 0.1 millicurie per kilogram of body weight. The dashed curves represent extrapolations from the solid curve at its extremities.

A summary of the measurements of specific activity in bone is given in Table II under three headings: (a) donor dogs injected with Ca^{45}, (b) dogs receiving radioactive grafts, and (c) dogs receiving non-radioactive grafts and injections of Ca^{45}. The densitometer data for bone outside the graft areas are given in three columns: maximum hot spot, diffuse, and periosteal or endosteal maximum. The utility of this separation can be seen by reference to Figures 2 and 3. The distribution of activity in cross sections of the shafts of the long bones consists in a general diffuse component upon which are superimposed a few intense hot spots and often a ring of increased activity in or near the endosteum or periosteum. The diffuse activity, which contains the largest fraction of the total activity in the shaft, has a relatively constant specific activity of about one microcurie per gram of calcium for all the dogs when normalized to an injected dose of one millicurie per kilogram of body weight, whether they lived twelve days or 100 days after the injection. Because of the normalization of the data to one dose, this constancy means that the specific activity of the diffuse component is proportional to the injected dose. The contrast between the relatively constant values for the diffuse component (Table II) and the decreasing serum specific

activity (Chart I) is to be emphasized even though the error in the measurement of the diffuse component may be as much as a factor of two.

The hot spots correspond anatomically to individual Haversian systems. The obvious variation in specific activity of the hot spots (Table II) and in their numbers and anatomi-

TABLE II

II - A. S.A. of Specimens from Radioactive Dogs without Grafts

	Autoradiograph Data						Counter Data		
Dog	Maximum Hotspot	Diffuse	Periosteal Maximum	Maximum Implant	Average Implant	Maximum Callus	Bed	Implant	Callus
101	21	0.6	—				0.8		
104	18	1.0	3.4				2.1		
	12	1.0	2.3				2.0		
	21	1.4	3.3				4.0		
119	38	1.1	4.2				—		
Average Value	20	1.0	3				2		

II - B. S.A. of Specimens from Non-radioactive Dogs with Radioactive Grafts

103	<0.2	<0.2	<0.2	32	—	<0.2	<0.01	—	—
	<0.5	<0.5	<0.5	24	0.9	<0.5	<0.01	—	—
106	<0.5	<0.5	<0.5	23	1.6	<0.5	0.021	1.7	0.1
	—	—	—				0.013‡		
107	<0.5	<0.5	<0.5	10	2.3	<0.5	0.046	0.7	0.4
	—	—	—				0.020‡		

II - C. S.A. of Specimens from Radioactive Dogs with Non-Radioactive Grafts

105	25	1.0	—	—	—	9	1.5	0.7	0.7
108	5	0.6	81	7	5*		1.9	8.5	8
	24	1.9	9.5	15	—	—	2.9	8.5	8
	14	0.9	11	17	8*		1.7	9.1	6
109	21	1.0	39	36	9	110	41†	57	55
	17	0.6	12	—	—	—	5.5	56	86

(*See page 567*)

cal distribution (Figs. 1 and 2) required an arbitrary threshold of density for defining a hot spot. For the purposes of this experiment, the threshold was set at double that of the diffuse component, provided the characteristic density profile on cross diameters on the hot spot was present. This procedure avoided confusion of film artefacts with hot spots. The maximum hot spot in the cross sections of long bones was found to average twenty microcuries per gram of calcium as measured with the 120-micron resolution and normalized to an injected dose of 0.1 millicurie per kilogram of body weight. With higher resolution, the maximum hot-spot value would probably be higher.

The average specific activity, determined by beta-ray counting, of a segment of cortical bone in the graft bed, that is, the cortical segment opposite the area of graft insertion, is about twenty microcuries per gram of calcium. This value compares favorably with the autoradiographic data since the bed value should be somewhat higher than the diffuse value because it contains some hot spots and periosteal or endosteal activity.

The specific activity data for bones obtained from dogs which received radioactive grafts are shown in Table II, *B*. The autoradiographic data for the graft areas are given in three columns: maximum implant (representing the most active regions in the implant area), average implant (representing the average of the entire implant area), and maximum callus (representing the most active region of callus). These regions were identified from their relative positions on the autoradiograph and the corresponding section. Because all the data for these dogs have been normalized to the injected dose of their donor dogs, the average values of specific activity shown in Table II, *A* represent the values expected to be found for the unabsorbed portions of the implants in Table II, *B:* "maximum implant" in Table II, *B* corresponds closely to "maximum hot spot" in Table II, *A;* "average implant" in Table II, *B* corresponds to "diffuse" in Table II, *A;* the counter value for "implant" in Table II, *B* corresponds closely to "bed" in Table II, *A*. Estimates of the total amount of activity, as distinguished from the specific activity, implanted into dogs 103, 106, and 107 are given in Table I.

The important result apparent in Table II, *B* of the autoradiographic data is that, within the limit of measurement, no activity was detectable outside the area of the implant. The upper limits for the specific activity near a hot graft are set by the background of the film, by the limited exposure times, and by the activity of the particular samples, which had decayed several half-periods before autoradiographs were made.

The counter data are more sensitive than the autoradiographic data and show de-

In Table II are shown the specific activities (microcuries per gram of calcium) of normal and grafted bone areas obtained by autoradiographic and counter measurements.

All values are normalized to an injected dose of 0.1 millicurie per kilogram of body weight. A dash indicates that data for the item were not obtained. A blank indicates that the column does not apply to the particular sample. A less-than sign (<) represents a practical limit of sensitivity in the measurement of the value and indicates that the value is less than that recorded or, alternatively, that the value cannot be larger than that recorded. The standard deviations for the autoradiographic values are given by the following error code:

The standard deviations for the counter values are all <10 percent and are not specified individually. Errors due to biological sampling are specifically noted. See section on autoradiographic method in text for the physical limitations on the meaning of autoradiographic S. A.

‡ These values are for tail vertebrae to represent samples distant from any grafted area in dogs 106 and 107.

* These values represent areas where no differentiation between callus and implant could be made grossly. Compare Figs. 5 and 6.

† This value is probably inaccurate due to inclusion of callus in sampling.

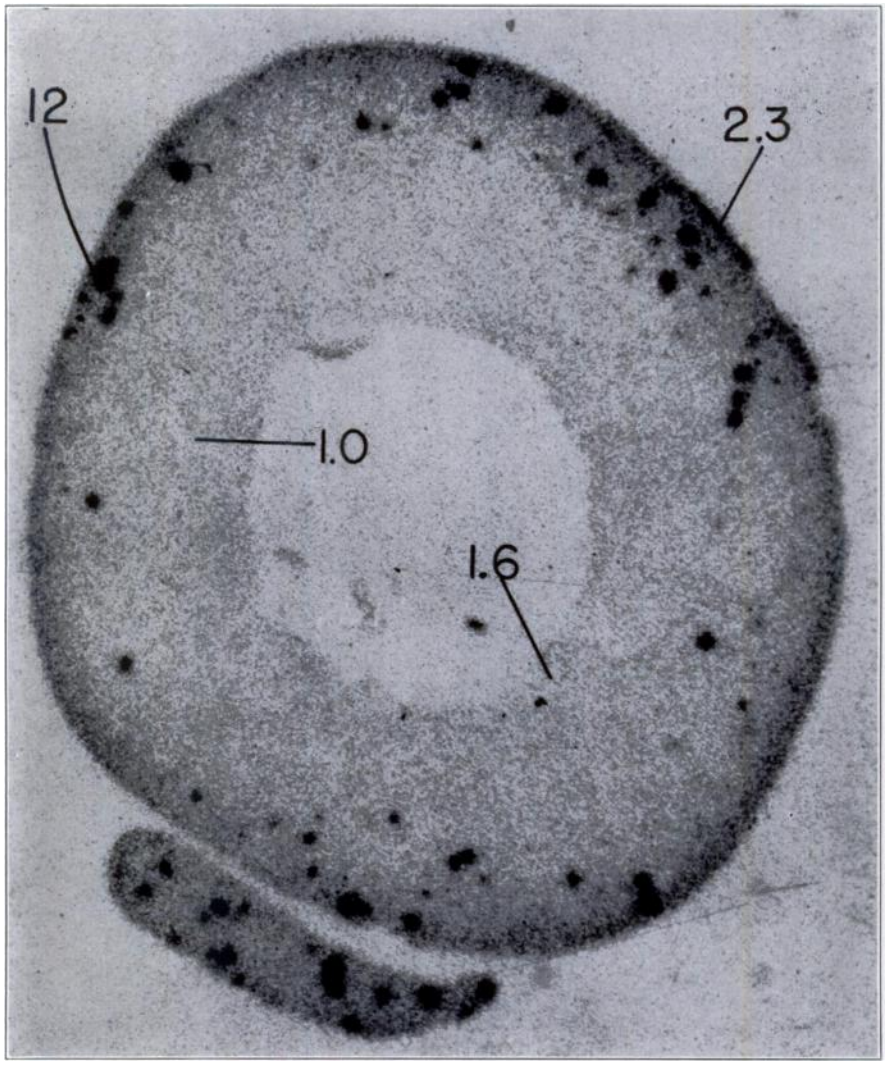

FIG. 1

Autoradiograph (× 15) of cross section of left tibia of dog 104, a donor injected with Ca^{45} twenty-six days before sacrifice. The hot spots are irregularly distributed and seem to predominate in the subperiosteal compacta especially under the external circumferential lamellae. The maximum hot-spot specific activity was twelve microcuries per gram of calcium for the large hot spot situated at about 10 o'clock. The periosteal maximum (at 2 o'clock) was 2.3 and the endosteal (5 o'clock) was 1.6. The diffuse value was 1.0 microcurie per gram of calcium and was constant within a factor of 2 throughout the section. All data are normalized to an injected dose of 0.1 millicurie per kilogram of body weight.

tectable specific activity in the bed of dogs 106 and 107, that is, in the cortical bone near the hot-graft area. From the data of Table II, *A* the maximum hot spot would be expected to have about ten times the specific activity of the bed. Therefore, one would not expect to observe hot spots in autoradiographs of recipient bone from dogs in which radioactive grafts were implanted unless the lower limit of detectability on the autoradiographs was 0.1 instead of 0.5 microcurie per gram of calcium. The specific activity of the bed in dogs 106 and 107 may be compared with the values for tail vertebrae in the same dogs. The specific activity of the blood at the time of sacrifice was 0.33 microcurie per gram of calcium for dog 106, and 0.26 microcurie per gram of calcium for dog 107. Blood measurements for other times were not made. It is evident that some of the activity from the hot grafts has been redeposited throughout the body by way of the circulating blood. Whether

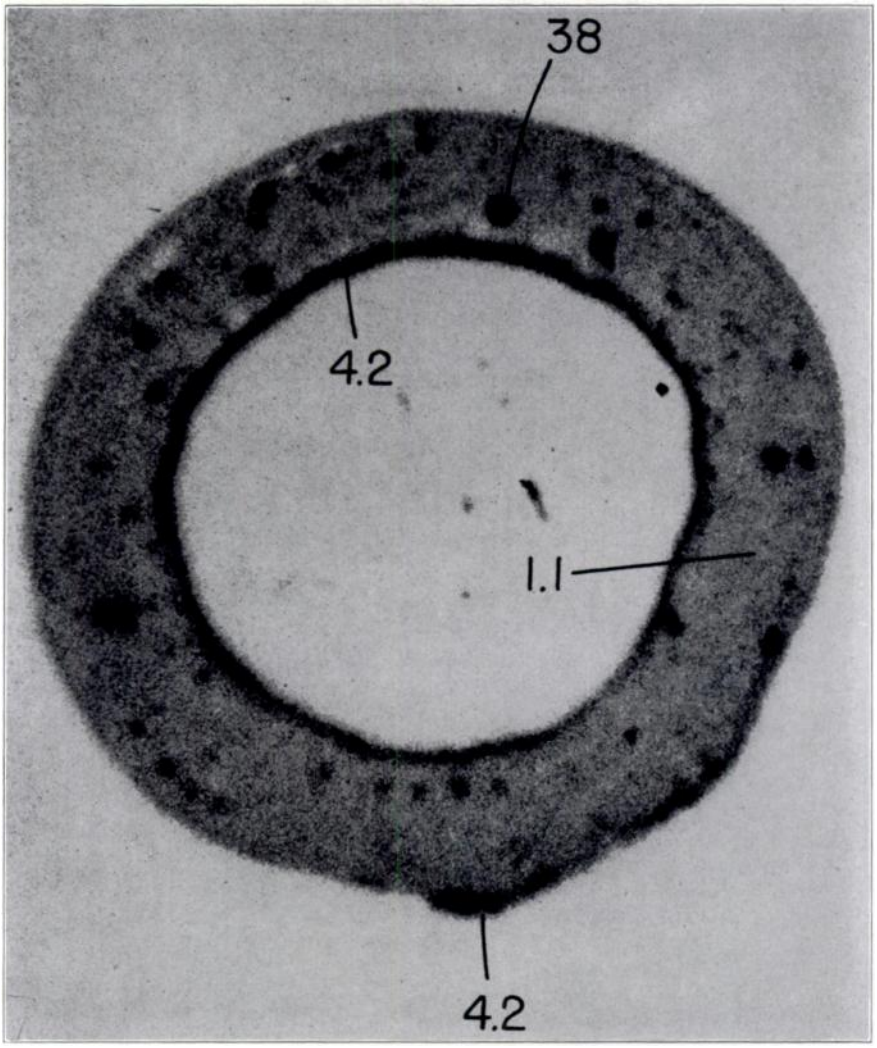

FIG. 2

Autoradiograph (× 15) of cross section of right femur of donor dog 119, injected with Ca^{45} fourteen days before sacrifice. The hot spots are more irregularly distributed than those of Fig. 1. The endosteal area shows a high activity over most of its circumference. The maximum hot-spot specific activity was thirty-eight microcuries per gram of calcium for the hot spot at 12 o'clock. The endosteal maximum measured at 9 to 12 o'clock and the periosteal maximum at 6 o'clock were 4.2. The diffuse activity was 1.1 microcuries per gram of calcium and was constant within a factor of 2. All data are normalized to an injected dose of 0.1 millicurie per kilogram of body weight.

the counter bed values for these two dogs indicate a significantly higher deposition of active calcium near the graft than in the body as a whole is doubtful. Additional and unpublished data from this laboratory have shown that in adult dogs the specific activity of adjacent small volumes in the diaphyses may vary by as much as a factor of three, that the average skeletal specific activity is slightly higher than the diaphyseal specific activity and that the tail vertebrae may have specific activity two to three times higher than the skeleton. From these data one may at least say that the specific activity near the hot grafts is comparable with that in the rest of the skeleton. Considering how small a quantity of bone immediately surrounds a graft, compared with the total skeleton, we may conclude that very little, if any, of the graft calcium is preferentially utilized near the graft.

The autoradiographs used to obtain the data for Table II are exemplified by Figures 3-A and 3-B which show a photograph and corresponding autoradiograph of a section of

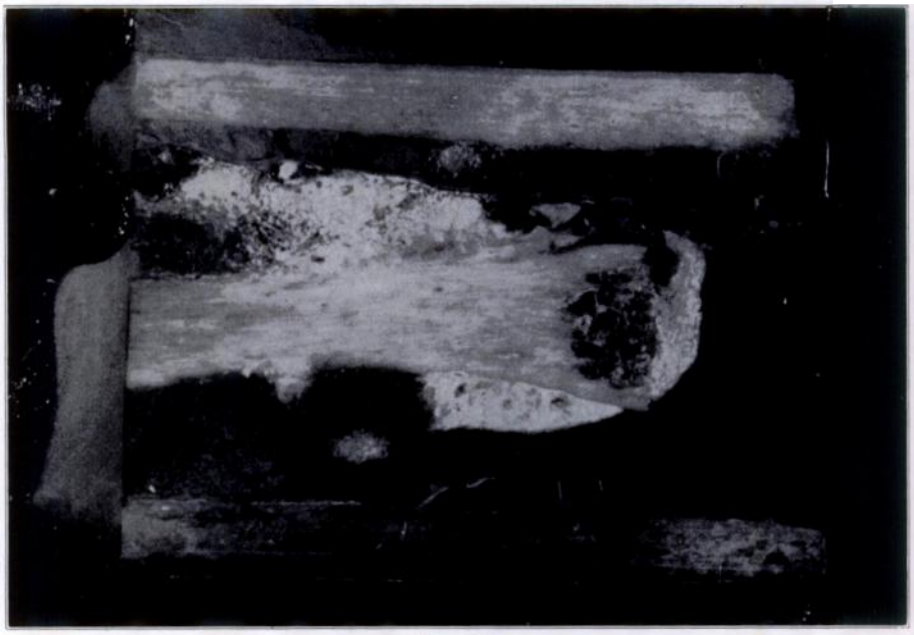

FIG. 3-A

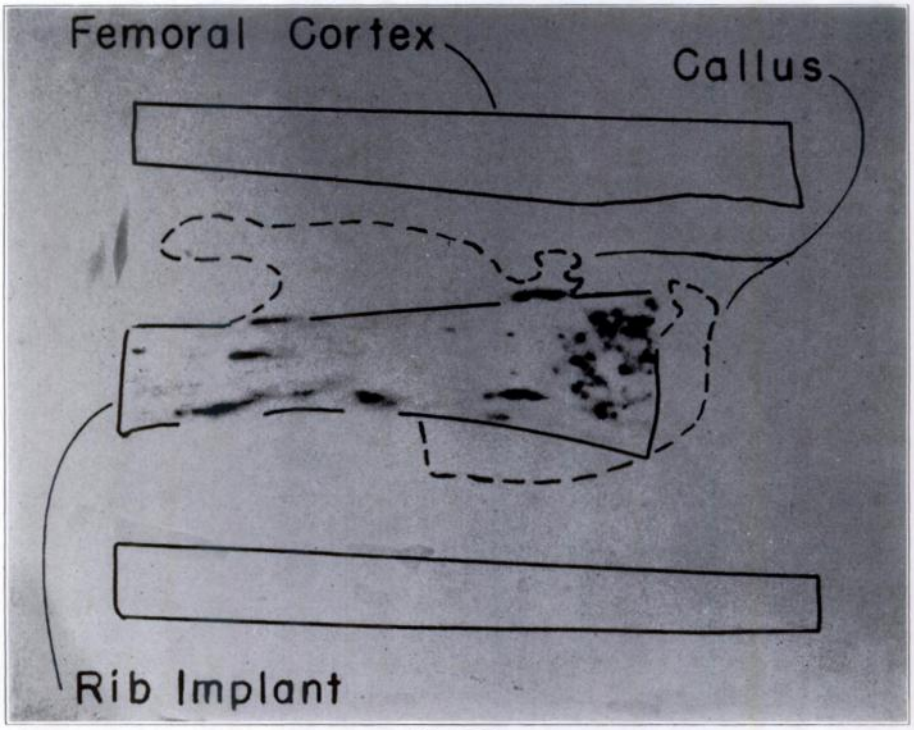

FIG. 3-B

Figs. 3-A and 3-B: Photograph and corresponding autoradiograph (× 5) of longitudinal section of radioactive graft of rib segment from dog 101 implanted into left femur of dog 103, ten days after grafting. The cancellous structure of the right-hand portion of the rib is well shown and this provides the lacework pattern of the activity in the autoradiograph. The rib cortex has a few active areas and in general is much less active than the cancellous portion. Callus in moderate amount is present particularly on the right end and both sides of the implant. India ink outlines of the implanted rib and femoral cortex (solid line) and of major portions of callus (dashed line) have been superimposed on the autoradiograph. Neither callus nor femoral cortex shows detectable activity.

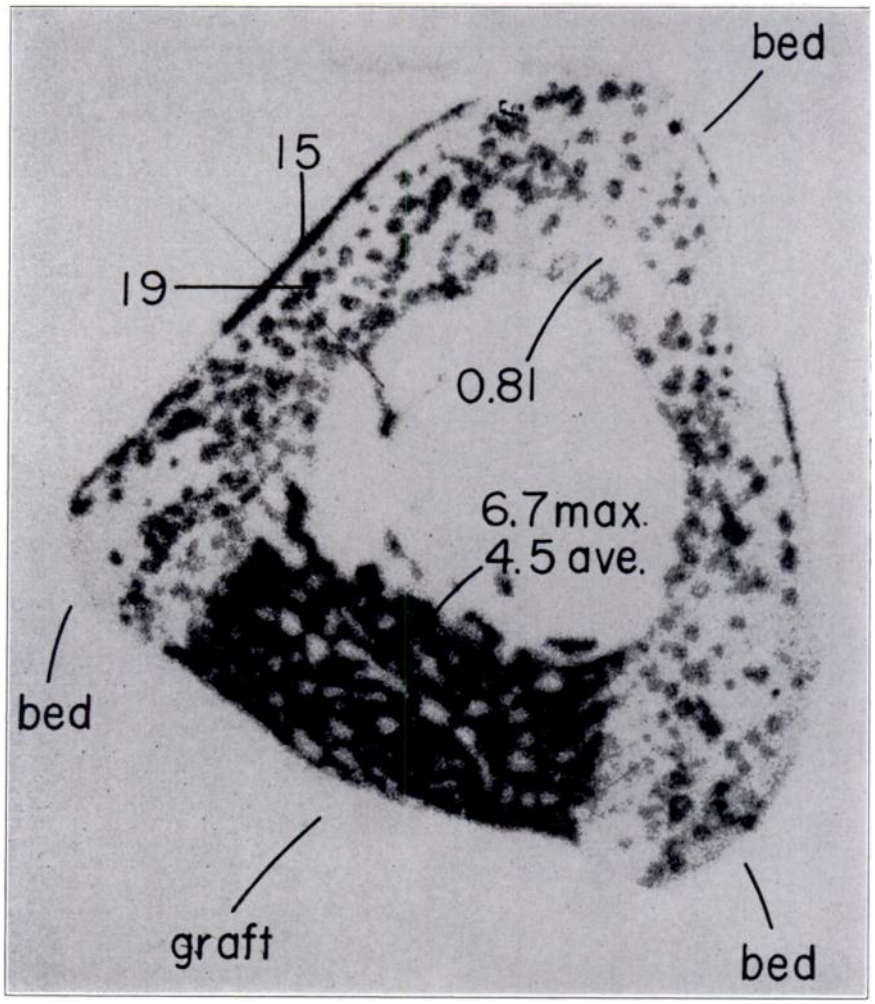

FIG. 4

Autoradiograph (× 15) of a cross section of a graft and its bed from dog 108, which was implanted and simultaneously injected with Ca^{45} ninety-three days before sacrifice. The maximum graft specific activity measured at the central portion of the graft near the endosteum was 6.7 microcuries per gram of calcium, while the average specific activity was 4.5. The periosteal portion at 10 o'clock measured 15. The maximum hot spot in the same region was 19. The diffuse activity was 0.81 microcurie per gram of calcium. The two bands of lesser density flanking the graft contain devitalized bone of the host, as demonstrated histologically. These devitalized areas, burned by the rotary saw, have taken up less activity than the diffuse component elsewhere in the section. All data are normalized to an injected dose of 0.1 millicurie per kilogram of body weight.

rib from dog 101 implanted in the medullary cavity of the left femur of dog 103. There is a sharp boundary between the activity of the implanted rib and its surrounding host bone. A considerable quantity of callus can be seen, some of it in intimate contact with the active implant. Yet none of the callus shows detectable activity. If more than one-tenth of the calcium in the callus had been obtained from the implant, the callus activity could have been detected on the autoradiograph. The counter callus values for dogs 106 and 107 are not zero, but they do not necessarily indicate a slight callus activity because, as mentioned in the section dealing with methods, it was difficult to obtain a counter sample of callus without contamination with implant material.

The specific activity data for the injected dogs which received non-radioactive grafts are shown in Table II, *C*. The values for maximum hot spot and for diffuse activity are not significantly lower than the values in Table II, *A* for the donor dogs, despite the fact that

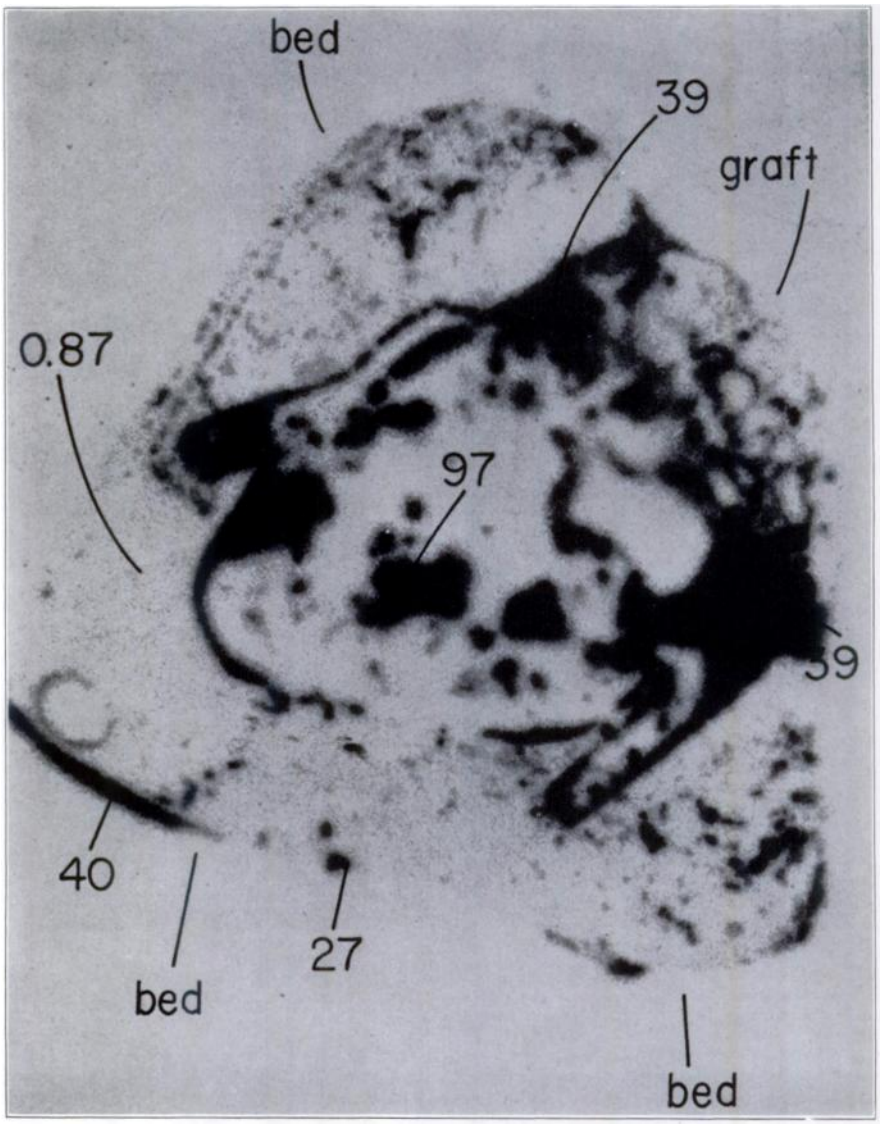

FIG. 5

Autoradiograph (× 15) of a cross section of a graft and its bed from dog 109, which was grafted, injected two weeks later with Ca^{45}, and then sacrificed eighty-nine days later. The maximum graft specific activity measured thirty-nine microcuries per gram of calcium, while that of the callus in the center of the medullary cavity measured ninety-seven. The average specific activity of the entire graft area was 9.1. The periosteal maximum (8 o'clock) was forty. The maximum hot spot (6 o'clock) measured twenty-seven. The diffuse value was 0.87 microcurie per gram of calcium. Two saw cuts have penetrated into the cortex opposite the graft and show, as in Figure 5, specific activity adjacent to the devitalized host bone lower than the diffuse. All data are normalized to an injected dose of 0.1 millicurie per kilogram of body weight.

most of the hot spot and diffuse values in Table II, *C* were measured only a few millimeters from a graft area.

Figure 4 shows a cross-section autoradiograph of the left tibia of dog 108, which was grafted and injected simultaneously. Hot spots and diffuse activity are seen throughout the cortical recipient area. The cancellous-graft area at the lower left shows a high concentration of activity within the graft. The implanted bone, however, originally contained no activity, and therefore, the activity found in the graft at the time of sacrifice is due to the process of reconstruction during the ninetey-three days which elapsed between grafting

and sacrifice. Histological examination showed that the activity may be attributed to callus in the interstices of the implant. By histological assay only about 8 per cent of the implant area represents residual graft. There are two zones of lesser specific activity at the side of the callus area which contain recipient bone devitalized by burning by the rotary saw.

Figure 5 is an autoradiograph of the left tibia of dog 109, which was injected two weeks after grafting. The implant area, at the upper right, contains much less activity than that in Figure 4. The histological assay showed that the implant area contains about 65 per cent residual implant. The two saw cuts containing callus reach into the cortex opposite the graft, and next to each is an inactive cortical zone devitalized by the saw. The callus shown in Figure 5 has the highest specific activity measured in this experiment: about 100 microcuries per gram of calcium, the counter and autoradiograph measurements agreeing fairly well. The activity of the callus, as shown in Figures 4 and 5, is characterized by the absence of hot spots and is distributed rather diffusely and in a continuous pattern as compared with the activity distribution of cortical bone in these and other cross sections.

The data of Table II, *C* show that the callus laid down either in or around the implant has a specific activity which depends strongly upon the time of injection relative to the time of grafting. Dog 105 was injected eleven days before grafting; dog 108 was injected on the day of grafting; and dog 109 was injected fourteen days after grafting. The specific activity of the callus of dog 109 is by far the highest, reaching 100 times the diffuse activity. It is evident that this activity did not come from the cortical bone near the graft area since that region shows approximately the same specific activity it would have if there were no graft nearby. There is a significant increase in the periosteal activity opposite the graft areas because of subperiosteal deposition of new bone. A comparison of Table II, *A* with *C* with respect to "maximum hot spot", "diffuse", and "bed" specific activity reveals the corresponding values to be very similar, and therefore, suggests that despite multiple grafting procedures on the dogs listed in Table II, *C*, no apparent perturbation of the distribution of injected Ca^{45} has occurred in these animals.

DISCUSSION

In discussing these data as they pertain to bone-grafting, three points must be emphasized:

1. A single element, calcium, is being traced in the grafting procedures. Since many other elements compose the bony substance of the host, of the graft, and of the callus, it should not be assumed that the other elements are similar to calcium in their mode of redistribution; each may follow a separate course.

2. Our results apply to homogenous grafts of refrigerated bone in dogs; other grafting methods may give entirely different data.

3. The tracing of an element may not portray the entire effect of the grafting procedure; whether these grafts stimulated, retarded, or had no influence on the healing process at the operative site is too speculative for discussion here.

The data do demonstrate that under the conditions of the experiments, the calcium of the grafts entered the blood and was distributed in a manner indistinguishable from that of calcium entering the blood from other sources. No evidence can be drawn from the data for local preferential transfer of calcium to the callus from grafts or from bone stores adjacent to the grafted area. On the contrary, all of the data may be used to support the hypothesis that the calcium in callus is entirely derived from the serum.

If callus is indeed derived entirely from the serum, then it should be possible to predict callus specific activity by calculation from the known blood curve (Chart I) and from knowledge of the time interval after grafting during which callus calcifies. Variations in the timing of callus formation occur, but let us assume for the moment that these variations are not large. The data from dog 109 can be used to indicate a reasonable arbitrary

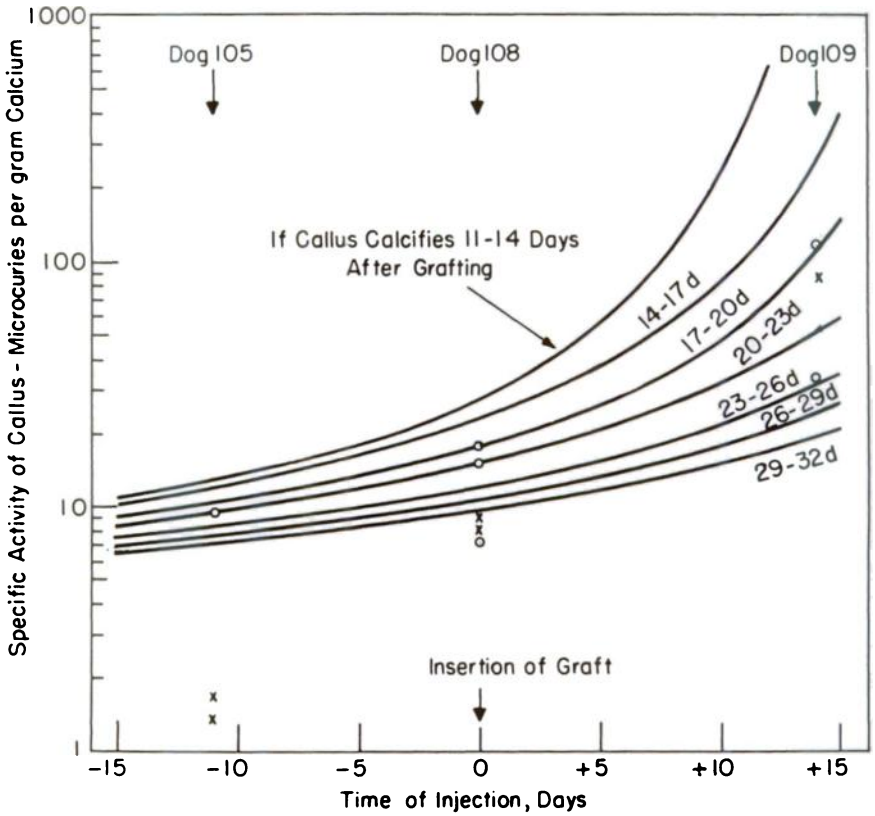

CHART II

Comparison between observed and predicted specific activity of callus as related to the injection-graft interval. Each group of points (circles for autoradiographic data and crosses for counter data) represents experimental callus specific activity obtained from each dog according to its injection-graft interval indicated on the abscissa. The solid lines are a family of curves of the calculated average specific activity of serum extending from fifteen days before to fifteen days after grafting, and occurring during the assumed callus-formation periods designated on the curves. These calculated values represent the predicted specific activity of callus, assuming that it calcifies entirely from serum calcium during the designated three-day intervals and that the rate of calcium deposition in the callus is uniform during these three days. The data for the curves are obtained from the serum specific activity curve of Chart 1, with the time scale of Chart 1 appropriately adjusted to the injection-graft interval. For example, assuming that most of the callus calcified in the three-day interval seventeen to twenty days after grafting, the calcium in the callus of dog 105 (injected eleven days prior to grafting) would have been derived from serum with an average specific activity of about ten microcuries per gram of calcium, which was the average level twenty-eight to thirty days after injection on Chart 1. All data are normalized to an injected dose of 0.1 millicurie per kilogram of body weight.

interval during which it may be assumed for purposes of the calculation that all calcium deposition occurs. In this dog, injected fourteen days after grafting, the high specific activity of callus indicates that most of the calcium in the callus was deposited in the third week after grafting. At less than two weeks, the blood contained no activity, and at more than three weeks after grafting the specific activity of the blood had dropped below the level at which the most active callus could possibly have been formed. If a three-day interval is chosen (for example, fourteen to seventeen days after grafting) as a reasonable period during which the greatest amount of calcium is deposited in callus, and if it is assumed that the rate of calcium deposition is uniform during these three days, the average specific activity of the blood during this period will be the predicted specific activity of callus for injection time fourteen days after grafting. A similar calculation made for all injection times extending from fifteen days before grafting to fifteen days after grafting has been made and is represented in one of the curves of Chart II. The other curves represent

similar calculations based on the assumptions that most of the calcium callus is deposited in the designated three-day periods after grafting. The observed maximum specific activity of callus in implants for dogs 105, 108, and 109, are also plotted on this figure according to the time of injection relative to grafting. It is interesting that the maximum specific activity of callus in or around the implant corresponds predominantly to the average specific activity of the blood for a three-day period between seventeen and twenty-six days after grafting, whether the time of injection was eleven days before, the same day as, or fourteen days after the grafting procedure.

The maximum observed callus values obtained from the autoradiographs should be the most reliable in this comparison because most of the possible errors of measurement, impurity of counter callus samples, resorption of callus during the life of the dog, and low autoradiographic resolution would tend to reduce the specific-activity values. If intervals longer than three days (for example, six or fourteen days) are chosen over which to calculate the average blood specific activity, the curves which fall close to the 17–20, 20–23, and 23–26 day curves are those for 16–22 days or 14–28 days. Comparison of blood and maximum callus specific activity does not yield a very sensitive method of timing callus calcification, but despite this lack of sensitivity, it provides a quantitative check that callus calcium does indeed come from the circulating blood. For if any reasonable period is chosen for callus calcification at about two to three weeks after grafting, then the average specific activity of the blood during that period falls very close to the maximum callus values for dogs 105, 108, and 109.

In studies of the deposition of radioactive isotopes in bone, the question often arises whether high local concentrations of radioactivity are fixed in the same region of bone from the time they are deposited until the time they are recorded on autoradiographs. The term "hot-spot migration" is often used to describe a possible relocation of radioactive deposits without dilution in the whole volume of circulating blood, that is, deposition at a specific activity greater than that of the blood at the time of deposition. In the results in Table II, *B*, no evidence of hot-spot migration from radioactive grafts has been observed. This result, however, may not apply to a normal situation since it refers essentially to the implantation of a foreign body. The observations of Table II, *C* may represent a more nearly normal situation from the point of view of migration. The formation of active callus presents a demand for calcium on the body to which nearby areas of recipient bone have not responded preferentially, since the hot spot and diffuse specific activity are approximately as high as those in injected dogs with no grafts.

In a fracture incurred many days after the injection of an animal with radium, Aub and his associates found by counting with a Geiger-Müller counter that there was a higher specific activity in the callus than in cortical bone, and interpreted this as evidence of hot-spot migration. The explanation of these results and those of the present experiment, which are analogous, does not require the hypothesis of hot-spot migration. All that is necessary is that callus be formed with the same specific activity as that of the serum during the relatively short period of deposition, at a time when the serum specific activity is higher than the average specific activity of cortical bone. These are the conditions observed in the present experiment. If in one of the dogs of this experiment, a callus had formed at any time up to 100 days after injection with Ca^{45}, the specific activity of callus would be expected to be higher than the average specific activity of cortical bone. However, at some later date the specific activity of serum may be expected to decline to values below the average specific activity of cortical bone and the specific activity of callus formed then would be lower than that of cortical bone.

The following additional points while not germane to the subject of graft tracer experiments, are evident at once from the data given, and merit mention from the point of view of radiation effects to bone by radioactive materials fixed in bone. Dudley and Dobyns measured the radiation-dosage distribution of Ca^{45} deposited in bone using a tech-

nique similar to that described in this paper, but with much lower resolution. Their findings indicated an average ratio between the specific activity of the most active areas in the epiphysis and the least active areas in the cortex of dog bone of approximately 10 to 1. The maximum ratio they observed was 14 to 1. The data on the injected dogs (Table II, *A*) provide a useful comparison with their data. From the present data the ratio occurring within cortical bone averages 20 to 1, but ranges from 12 to 1 to 35 to 1. Because of the normalization of the data to a single dose level, the values for the diffuse component are shown as constants, but it should be realized that the absolute levels of specific activity in all components are therefore proportional to the injected dose. As a result of the higher resolution of the present method, the ratio between hot spot specific activity to diffuse component specific activity is higher, even within cortical bone, than that observed by Dudley and Dobyns, and this ratio may easily be higher than the maximum given above, when resolution is further improved. The specific activity of different hot spots ranges in value from that of the diffuse component to the maximum values reported in Table II, *A* although the quantitative aspects of this variation are not presented in this communication. These findings are of obvious importance in any consideration of optimal or minimal dosage for biological damage due to radiation, whether it be to the whole skeleton or to any localized portion thereof.

SUMMARY

1. Homogenous-bone grafts in dogs were studied by implantation of radioactive bone from donor dogs previously injected with Ca^{45} and by implantation of non-radioactive bone into hosts which were injected with Ca^{45} prior to, during, or after the grafting procedure. Determinations of serum and bone specific activities were made and compared.

2. A procedure for quantitative autoradiography was developed and applied to the analysis of cross sections of grafted and non-grafted areas.

3. The activity in cross sections of the shafts of the long bones consisted principally of a general diffuse component, the specific activity of which was relatively constant over the 100-day period of study and was proportional to the administered dose. Upon this diffuse component were superimposed a few intense hot spots and often a ring of increased activity in or near the endosteum or periosteum. The hot spots corresponded to individual Haversian systems, and their specific activities ranged up to thirty-five times that of the diffuse component as determined with an autoradiographic resolution of 120 micra.

4. The distribution of Ca^{45} from radioactive grafts were systemic and no preferential transfer of Ca^{45} to callus or nearby bone was detected.

5. In dogs which received non-radioactive grafts and injections of Ca^{45}, the specific activity of the callus was the highest observed anywhere in bone, up to 100 times that of the diffuse component of the host's cortical bone and was strongly dependent on the relation between the time of injection and the time of grafting.

6. The time of calcification of callus occurred predominantly at about the third week after grafting.

7. Relocation of localized deposits of activity without dilution in the whole volume of circulating blood (hot-spot migration) to or from graft areas was not detected in this experiment.

8. The data of this experiment provided two points which are considered pertinent and applicable to the problem of radiation hazard evaluation with respect to radioactive materials fixed in bone. These are the proportionality between the administered dose and the specific activity of the diffuse component and the large ratio between the specific activity of hot spots and that of the diffuse component.

NOTE: We would like to thank Prof. Robley D. Evans and Mr. Joel B. Bulkley of Massachusetts Institute of Technology, and Dr. William Green of the Children's Medical Center for their support and interest in this work. We are also indebted to Miss Marie Helmick, Mrs. Dorothy Kuchta, and Miss Virginia White for the innumerable analyses and measurements and for the development of many details of technique.

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A Comparative Study of Xeroroentgenography and Routine Roentgenography in the Recording of Roentgen Images of Bone Specimens*

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In 1940 and 1944, Carlson obtained patents for a photographic process based on photoelectric rather than photochemical principles. It was called xerography because of the dry nature of the process. Technical details of its roentgenographic counterpart, xeroroentgenography, have been described by McMaster and Wenk, Roach and Hilleboe, and Oliphant. In xeroroentgenography an electrostatic image of the size, shape, and radio density of the interposed object is produced on a xeroplate, which is a metallic plate coated with a semiconductor such as selenium. This image is made visible by placing finely divided powder granules on the charged pattern of the xeroplate. Permanent records are best made by photographic techniques.

Roach and Hilleboe, and Oliphant noted a slight exaggeration of detail in the xeroroentgenogram as compared with that in the routine roentgenogram. Roach and Hilleboe showed that the strength of the electrostatic field was considerably greater at the edge of the charged area than in the center. This was manifest by an increase of powder along

* Presented at the Annual Meeting of the Orthopaedic Research Society, Chicago, Illinois, January 28, 1956.