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**RADIONUCLIDE BONE IMAGING  
IN THE EVALUATION OF OSSEOUS  
ALLOGRAFT SYSTEMS**

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**Bethesda, Maryland**

Research was conducted according to the principles enunciated in the "Guide for Laboratory Animal Facilities and Care," prepared by the National Academy of Sciences - National Research Council.

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## ABSTRACT

Evaluation of the progress of osteogenic activity in mandibular bone grafts in dogs by a noninvasive, nondestructive radionuclide method is feasible. The method provides a meaningful sequential interpretation of osseous repair more sensitive than conventional radiography. It is presumed that accumulating hydroxyapatite is being labelled by the imaging agent technetium diphosphonate. The osseous allograft systems studied were comparable to or exceeded autografts in their repair activity in mandibular discontinuity defects as judged by radionuclide imaging. A lyophilized mandibular allograft segment augmented with autologous cancellous marrow was more active than autograft controls at 3 and 6 weeks and was the most active system studied. Allograft segments augmented with lyophilized crushed cortical allogeneic bone particles were equal to controls at 3 weeks and more active than controls at 6 weeks. Lyophilized crushed cortical allogeneic bone particles retained in a Millipore filter while not clinically stable at 6 weeks did show osteogenic activity equal to control autografts at this interval.

## I. INTRODUCTION

One of the major disadvantages of autologous rib or iliac bone grafts is the necessity for a donor site operation which imparts surgical morbidity that is often greater than at the recipient site. Availability of simply prepared allogeneic bank material which is comparable to autologous tissue in its reparative capacity could circumvent this disadvantage and simplify the bone graft reconstruction of osseous defects in the oral and maxillofacial area.

Sequential evaluation of osseous repair is imprecise as it is largely dependent on radiography which, as a result of numerous technical and biological variables, does not provide a consistently accurate measure of bone healing. End point analyses used in evaluating the progress of osteogenic activity in animal experiments provide valuable information, but these techniques are both invasive and destructive and necessitate termination of studies. The study of skeletal tissues by radionuclide methods has provided valuable information particularly in such clinical problems as the detection of osseous tumor metastases. Positive radionuclide scans have been shown to precede positive radiographic findings thus providing a more sensitive measure of osseous changes.<sup>5, 9-11, 13</sup> Stevenson et al. and Bright et al. evaluated cancellous and cortical grafts to ulnar defects in an animal model by a radionuclide imaging method and found that radioactive technetium provided sensitive and reliable information about the rate and location of graft incorporation.<sup>3, 14</sup>

This paper presents the results of a comparison of selected osseous allograft systems for mandibular reconstruction by means of radiography, radionuclide bone imaging and visual plus manual end point analyses.

## II. RADIONUCLIDE BONE IMAGING

The radioisotopes which emit gamma rays are applicable for bone scanning or imaging as gamma rays will penetrate tissue and can be measured by an external monitor. The radioisotope technetium-99m is a gamma emitter and in the radiopharmaceutical form in which it is prepared is known to be a bone seeking agent.<sup>16</sup> An aliquot of this agent is administered intravenously and after an appropriate interval, when the agent is known to be localized in the skeleton, the area to be studied is positioned in front of a gamma ray monitor called a gamma camera.<sup>2</sup>

The gamma camera which is a stationary solid scintillation counter has two major components, the imaging head and the data integration and display console (Figure 1). The camera head includes a collimator, a sodium iodide crystal (NaI(Tl)) and an array of photomultiplier (PM) tubes. The reception head of each PM tube is covered by a photocathode (Figure 2).

The collimator improves resolution of the image that is being produced by selecting out unwanted rays, i.e., scattered radiations. The crystal absorbs energy from the collimated gamma rays and converts this energy into flashes of fluorescent light or scintillations. The points of incidence of the gamma rays on the crystal coincide with the location of the radioisotope within the bone. The scintillations emitted from the crystal are detected by photocathodes in the photomultiplier tubes with each tube producing an electrical signal proportional to the light intensity at its location (Figure 2).

The signals are then transmitted to the console for processing and recording. Finally, each representative scintillation event transmitted to the console is displayed on an oscilloscope (Figure 1) as a bright point of light in the same relative position as it occurred. The integration of these bright points of light forms a useful image. The events may also be digitally recorded on tape for later replay or transmitted to a computer (Nuclear Data Med-II) where they may be displayed on the computer's display oscilloscope.

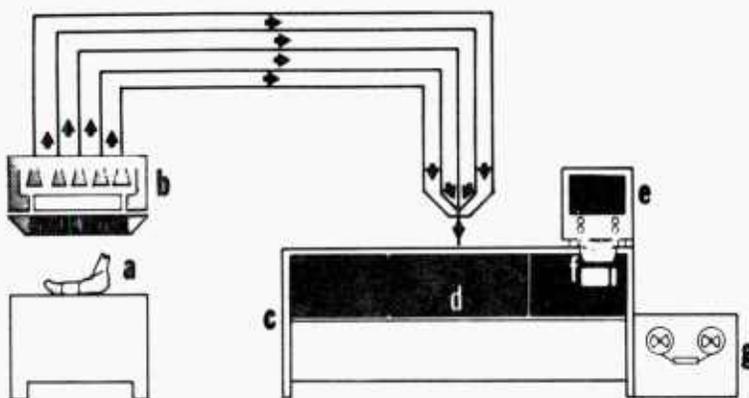


Figure 1. Diagrammatic illustration of the gamma camera evaluation system. (a) skeletal part being imaged, (b) imaging head, (c) camera console, (d) camera controls, (e) oscilloscope screen, (f) recording photographic camera, (g) digital tape recorder.

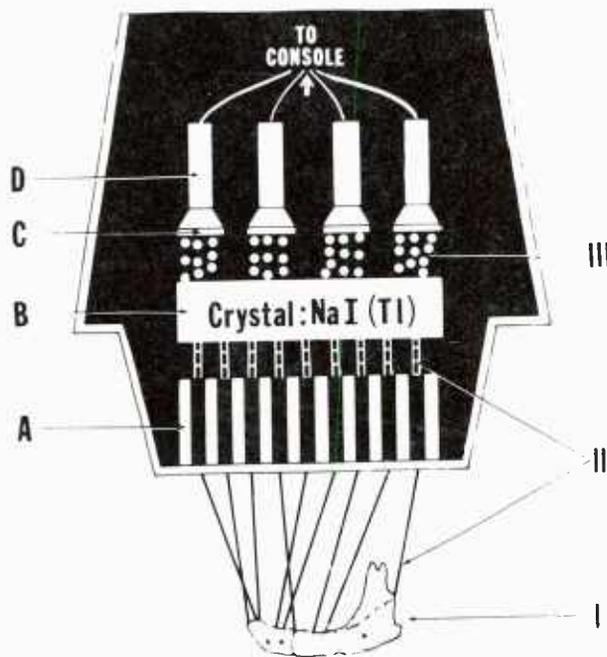


Figure 2. The major components of a gamma camera imaging head are diagrammatically illustrated. (A) high resolution collimator, (B) sodium iodide crystal, (C) photocathode, (D) photomultiplier (PM) tubes. The labelled bone (I) emits gamma rays (II) which are collimated prior to striking the crystal. Scintillations (III) produced by the crystal excite the photocathodes which emit electrical pulses that are transmitted to the console after amplification by the PM tubes.

Image differences are identified by visual interpretation. Areas of isotope uptake reflect osteogenic activity and are seen as "hot spots" while areas of decreased or absent uptake, reflecting diminished osteogenic activity, are seen as darker voids (Figure 3A). To verify visual interpretation, an image profile can be developed by the computer from the digitized data. Any desired slice or section of the bone image can be selected for analysis by simply adjusting appropriate controls associated with a display screen that is integrated with the computer. All of the image data along the selected slice are displayed as a count versus distance histogram or image profile on

the computer display oscilloscope thus graphically confirming activity within the bone (Figure 3B). In order to develop data from a greater cross-sectional area of the bone, an accumulated image profile can also be produced in which all of the image data

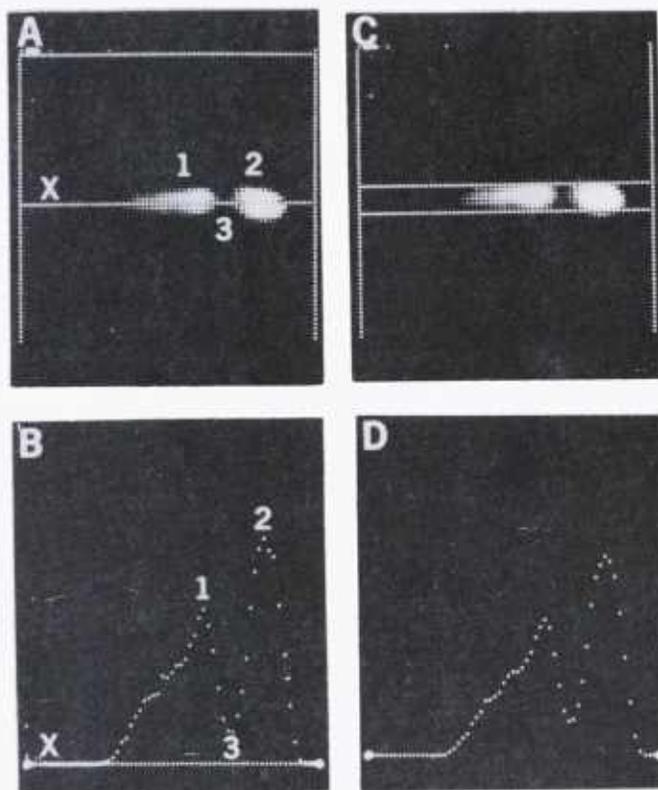


Figure 3. (A) Radionuclide image of a right dog mandible shortly after placement of a bone graft. (1) and (2) are areas of labelled bone referred to as "hot spots". The dark void at (3) represents the graft area where there is little osteogenic activity at this interval. The x-axis (horizontal white line) through the bone image represents the selected slice from which a count versus distance histogram or image profile is developed. (B) Image profile of image shown in A. (1) and (2) represent the "hot spot" areas of bone which have been labelled while (3) is the graft area which is minimally active. (C) Image of bone from computer display screen on which the upper and lower x-axis lines (horizontal white lines) have been positioned to outline boundaries of the area from which data are to be developed for an accumulated image profile. (D) Accumulated image profile developed from the image in (C).

between two slices can be displayed as a profile (Figure 3C and D). Figure 4 illustrates sequential changes occurring in images and image profiles of a dog mandible treated with an osteogenically active bone graft.

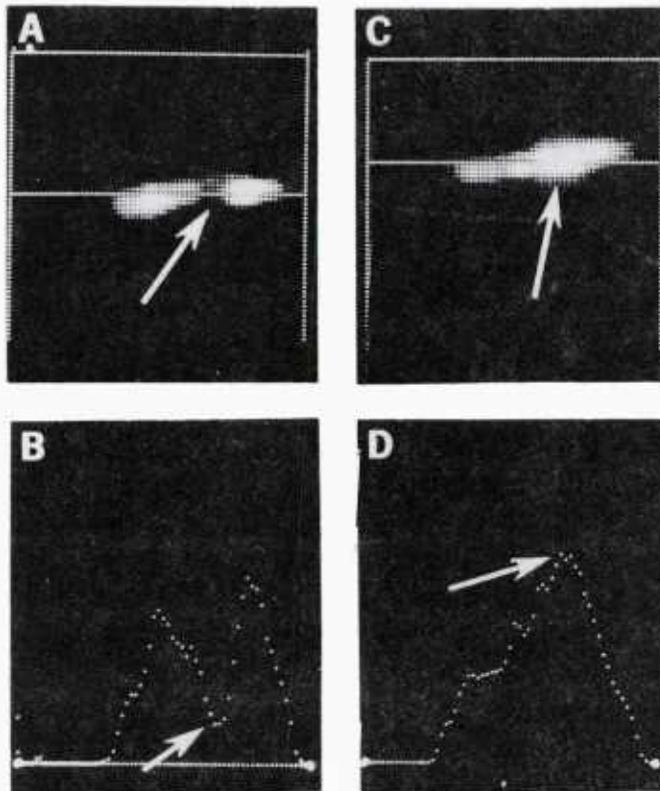


Figure 4. An image (A) and image profile (B) are seen in a dog mandible 1 week after placement of an autogenous bone graft (arrows). (C) At 6 weeks the graft has become osteogenically active and the area where the graft is located in the image (arrow) has assumed the appearance of a "hot spot". (D) In the image profile the graft area is seen as a peak of activity.

### III. MATERIALS AND METHODS

Twenty-four beagle dogs were partially dedentated and standardized 2-cm mandibular defects were created bilaterally subsequent to application of Sherman plates. Postoperative diet consisted of liquid, then soft ration. The animals

were divided into three experimental groups of eight. The animals in Group I received lyophilized mandibular allografts augmented with autologous cancellous marrow. Group II animals received lyophilized mandibular allografts augmented with lyophilized crushed cortical allogeneic bone particles. Group III animals received a graft system consisting of lyophilized crushed cortical allogeneic bone particles retained in a Millipore filter with a pore size of 45  $\mu\text{m}$ . Contralateral autograft segments acted as controls. Crushed cortical allogeneic bone was prepared from sterile femur bone by crushing in a stainless steel instrument and sieving to produce particles of less than 4 mm in diameter. Submento-occlusal radiographs and technetium diphosphonate bone images and image profiles were obtained at weekly intervals up to time of euthanasia for evaluation of osteogenic activity in the grafts. Half of the animals were euthanatized at 3 weeks and half at 6 weeks. Specimens were examined postmortem to qualitatively evaluate the state of repair and graft stability.

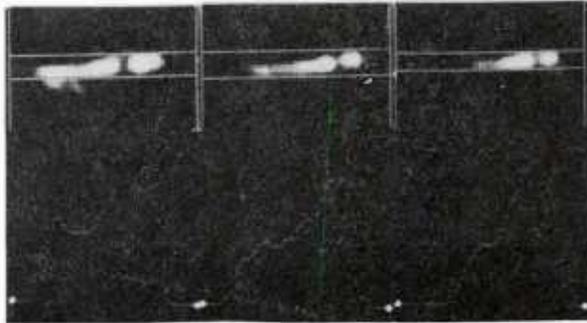
#### IV. RESULTS

Group I. Lyophilized mandibular allograft augmented with autologous cancellous marrow.

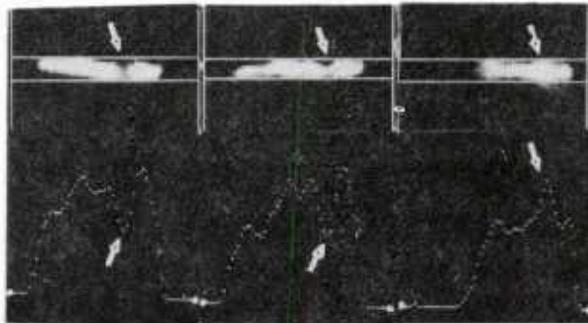
Three weeks. Bone images and profiles demonstrated greater osteogenic activity within the experimental grafts in five of eight animals while the radiographs were unremarkable. In the four animals euthanatized at 3 weeks, clinical stability of the grafts was comparable. Figure 5 illustrates the images and profiles for control and experimental grafts in a Group I animal at 1, 2 and 3 weeks. At 3 weeks there was increased activity in the experimental allograft confirmed by both image and image profile.

GROUP I ANIMAL: THREE WEEKS

CONTROL GRAFT



EXPERIMENTAL GRAFT



1 WEEK

2 WEEKS

3 WEEKS

Figure 5. Image and image profiles for grafts from a 3-week, Group I animal. In the experimental graft area, depicted by arrows, greater activity is noted at 3 weeks.

Six weeks. Imaging data exhibited significantly more activity in the experimental allograft sites in three of four animals. With the exception of an unusual morphologic observation in one animal, the radiographs were unremarkable; clinical stability was comparable at euthanasia. Images and image profiles of a control autograft at 1, 2, 4 and 6 weeks are seen in the top of Figure 6. A slight increase in osteogenic activity is observed at 6 weeks.

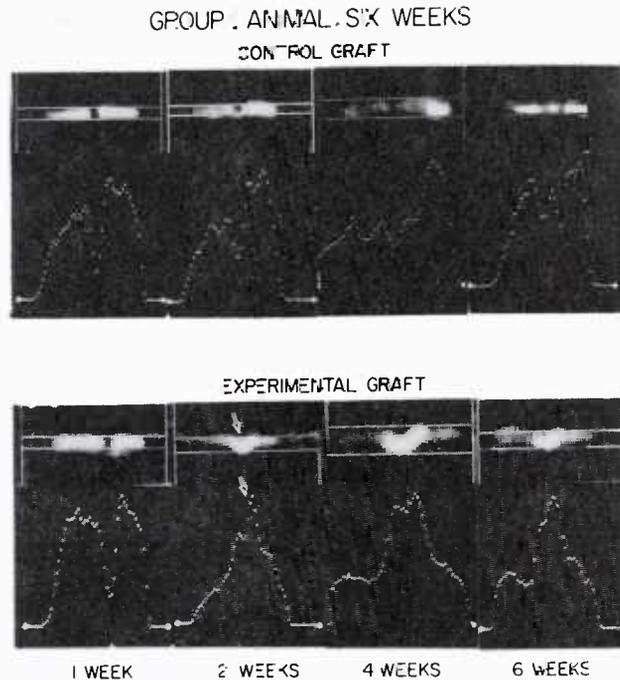


Figure 6. Image and image profiles for grafts from a 6-week, Group I animal. Greater activity is seen in the experimental graft beginning at 2 weeks (arrow) and continuing through 6 weeks. Note image confirmation of the unusual graft morphology seen in Figure 7.

In Figure 7 the unusual bowing configuration which developed during healing in one of the experimental grafts is seen in superior view at time of euthanasia. The distinctive morphology of this highly active allograft composite was not confirmed radiographically until 4 weeks. However, the images and image profiles displayed marked osteogenic activity, as well as the distinctive morphology, as early as 2 weeks as seen in the bottom of Figure 6.

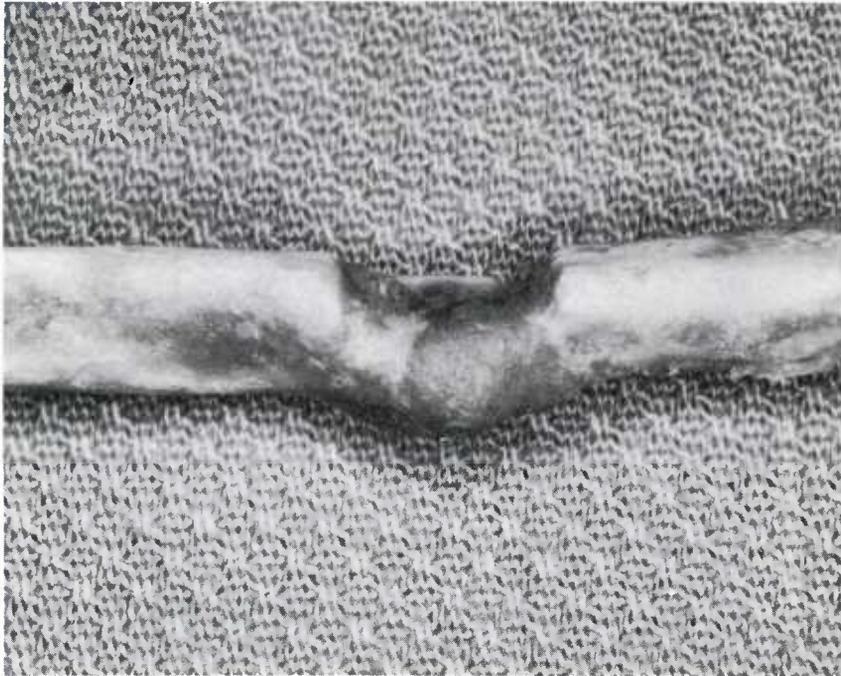


Figure 7. A superior postmortem view of an experimental graft from a 6-week, Group I animal illustrating the unusual bowing configuration which developed as the graft healed.

Group II. Lyophilized mandibular allograft augmented with lyophilized crushed cortical allogeneic bone particles.

Three weeks. Six of eight animals exhibited equal control and allograft activity as evidenced by imaging data. Radiographic examination revealed no observable differences in the progress of healing. Postmortem evaluation of the four animals euthanatized at 21 days showed mobility at all graft sites. The images and image profiles from a 3-week animal illustrate equal 1- and 3-week activity in both autograft and allograft (Figure 8).

Six weeks. Two of three experimental grafts showed greater activity on radionuclide evaluation. In the fourth animal it was not possible to determine the

## GROUP II ANIMAL : THREE WEEKS

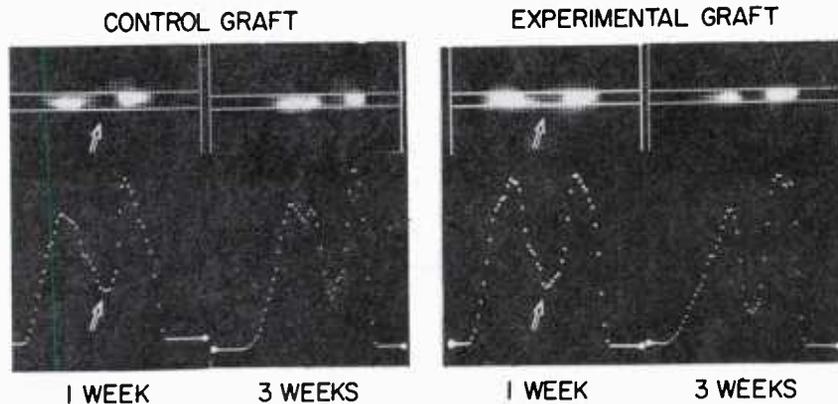


Figure 8. Image and image profiles from a 3-week, Group II animal. Graft areas are designated by arrows. Activity is equal in autograft and allograft.

progress of osteogenic activity in either graft beyond 4 weeks because of inability to accurately localize the graft sites on images and image profiles. At euthanasia stability was comparable in controls and experimentals. The radiographs did not provide any meaningful information regarding the progress of healing. Radiographs of an animal in this group are seen at 4 and 6 weeks in Figure 9. The medial and superior appearance of an experimental graft at euthanasia is seen in Figure 10. Corresponding images and image profiles from a 6-week animal revealed little change in activity in either graft until 6 weeks when an increase was noted in both the control and experimental grafts (Figure 11).

Group III. Lyophilized crushed cortical allogeneic bone particles retained in a Millipore filter.

Three weeks. Five of eight animals exhibited equal osteogenic activity in control and experimental graft sites as determined by the radionuclide data.



Figure 9. Radiographic appearance of grafts from a 6-week, Group II animal at 4 weeks (left) and 6 weeks (right). Other than the basic difference in graft appearance, there is no demonstrable sequential variation in the grafts.

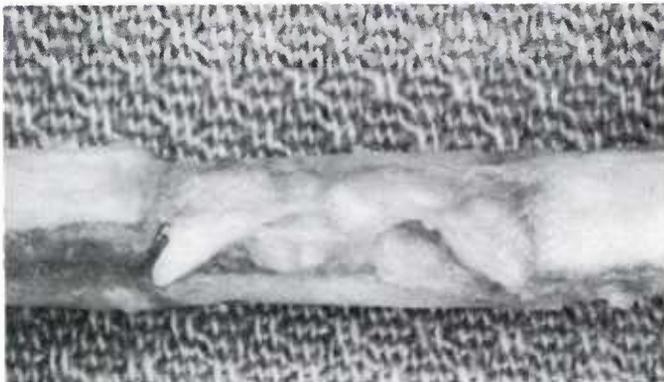


Figure 10.  
(Top) Medial postmortem view of an experimental graft from a 6-week, Group II animal.  
(Bottom) Superior view of graft.

Radiographically there was no demonstrable variation between the grafts. Experimental graft sites in the four animals euthanized at 3 weeks were very mobile or showed nonunion.

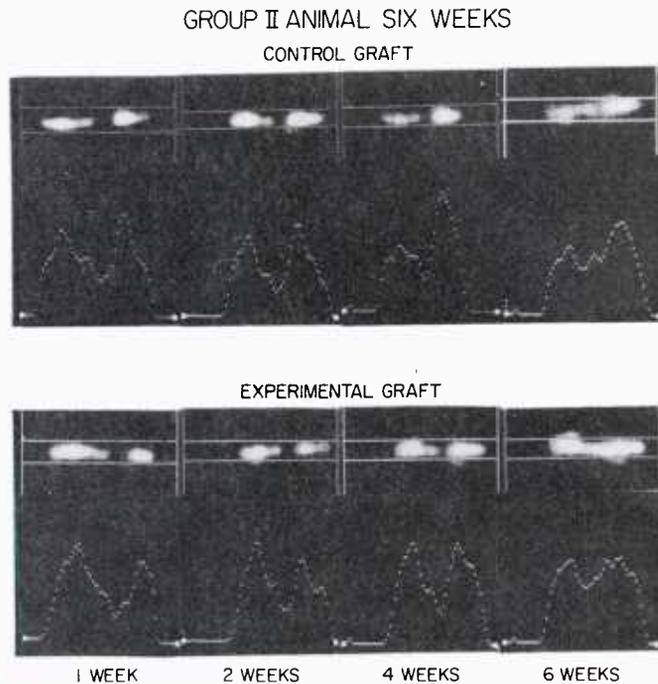


Figure 11. Images and image profiles from a 6-week, Group II animal. Increase in activity in both autograft and allograft is observed at 6 weeks.

Six weeks. One of the 6-week animals developed a wound infection at the experimental graft site and the data were excluded. In the remaining three animals, two of three had equal activity in both grafts as determined by evaluation of the imaging data. In the third animal the radiographs and images suggested that the control was exceeding the experimental; the image profiles, which are less qualitative, showed equal activity. Control grafts were more stable at euthanasia. In Figure 12 the

experimental lyophilized crushed cortical allograft in a Millipore filter is seen at time of euthanasia, with the filter in place and with the filter removed. Images and image profiles at 1, 4 and 6 weeks depict equal activity in the control autograft and the experimental allograft system (Figure 13).

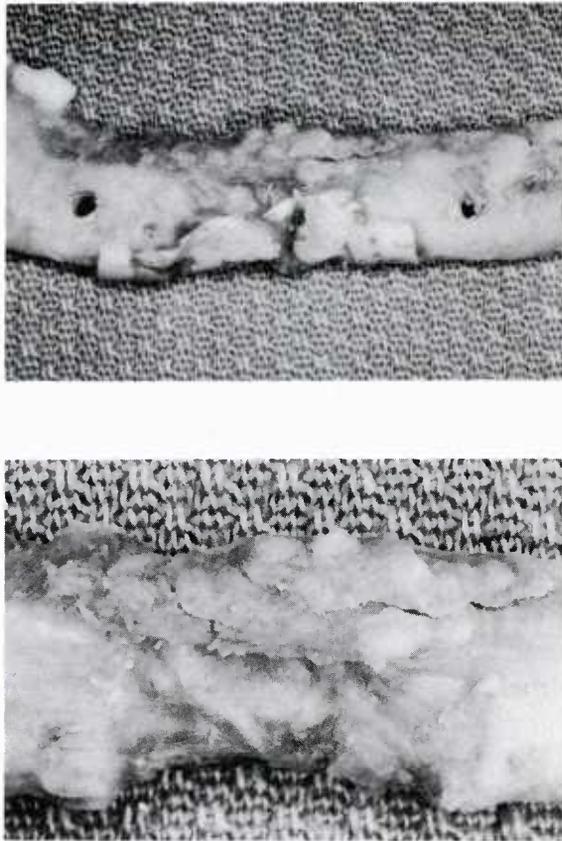
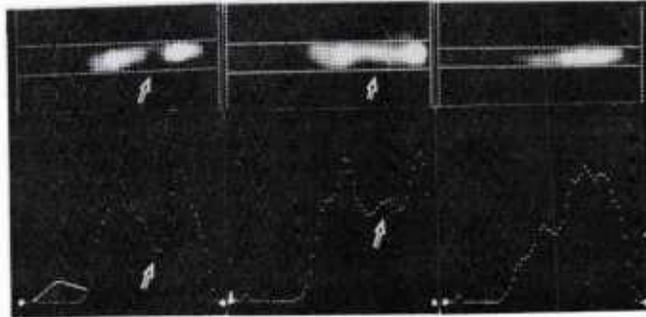


Figure 12. Postmortem appearance of an experimental graft from a 6-week, Group III animal with the Millipore filter in place (top) and with filter removed (bottom).

GROUP III ANIMAL : SIX WEEKS  
CONTROL GRAFT



EXPERIMENTAL GRAFT

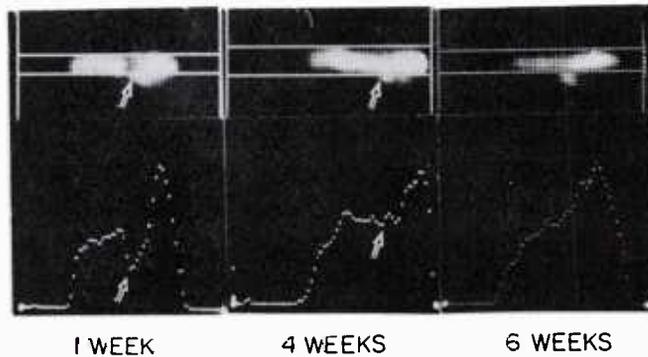


Figure 13. Images and image profiles from a 6-week, Group III animal. The graft areas (arrows) show increasing activity at 4 weeks in autograft and allograft.

V. DISCUSSION

Fluorine-18 and strontium-87m were the most frequently used of the early bone scanning and imaging agents, but in spite of their undisputed value these agents have disadvantages and limitations.<sup>5,9-11</sup> Technetium-99m has almost ideal properties for imaging. It has an easily collimated 140-keV gamma emission, with the absence of biologically hazardous beta decay; a physical half-life of 6 hours; low radiation dose; easy availability and relatively low cost.<sup>13</sup> The low radiation dose

permits safe sequential imaging. Technetium-99m has been used to scan and image almost every major organ system of the body, but only with the recent development of phosphate labeling was it possible to use this radionuclide effectively to scan and image the skeleton.<sup>4, 15-17</sup>

Through the work of Russell, Fleisch, Francis and others<sup>1, 6-8, 12, 16</sup> it has been shown that various phosphate compounds chemisorb onto the surface of developing hydroxyapatite crystals in vitro and in vivo. Of these phosphate compounds the diphosphonates possess physical and chemical properties which suggest an advantage over other agents for skeletal imaging. The diphosphonates are close structural analogues of pyrophosphate and polyphosphates, which have been studied extensively in relation to bone mineralization, but unlike these compounds they are stable to chemical and enzymatic hydrolysis. Thus when diphosphonates are introduced into an in vitro or in vivo system they more consistently attach to active bone surfaces as they are not subject to rapid degradation.

The radiopharmaceutical agent used in this study, technetium-99m diphosphate, has recently been studied by Tofe and Francis.<sup>16</sup> Their data showed that in an in vitro system disodium etidronate when complexed with technetium and stannous tin binds to synthetically developed hydroxyapatite thus offering further evidence that technetium diphosphate as a bone imaging agent localizes in areas of osteogenic activity.

Nuclear medicine departments equipped with gamma camera imaging equipment are located in numerous medical centers and community health facilities. Imaging of maxillofacial structures has only recently been investigated and while both research

and clinical techniques need to be refined the availability of professional expertise and appropriate equipment suggests that these methods will gain wider acceptance in the future for evaluation of a variety of osseous repair phenomena.

The study of bone grafts in an animal model system does not provide the final answer for the solution of clinical problems. However, the results of this study, which are based on a newly introduced sequential evaluation parameter, strongly suggest that bone allograft systems compare very favorably to autografts in their repair capacity. The objective of ongoing studies in our laboratories will be to further refine imaging methods to produce more precise resolution and localization. Additionally, longer imaging studies will be conducted in conjunction with an attempt to sequentially document mineral accretion and dissolution through other noninvasive radioisotope methods.

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