A STUDY OF BONE MINERAL CONTENT PERFORMED BY THE GAMMA RAY ABSORPTION TECHNIQUE IN PROLONGED BED REST SUBJECTS MAINTAINED IN A METABOLICALLY CONTROLLED ENVIRONMENT.

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The Ultimate Application of Gamma Ray Absorption Techniques to In-flight Bone Mineral Measurements.

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Exhibit B

TERMINAL REPORT

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OBJECTIVE

To measure the bone mineral changes which occurred during and after a nine month period of bed rest in the three subjects described in the Exhibit A terminal report.

INTRODUCTION

The measurement of bone mineral is rapidly gaining a place of importance in medicine. A large proportion of previous studies have been conducted using standardized X-ray tubes with subsequent densitometry of the radiograph, Cameron and Sorenson (1) introduced a monoenergetic photon source technique in 1963 which provided many advantages. Since then, many laboratories have either reproduced their technique or have modified it. We have applied this technique to a study of the os calcis in a group of patients undergoing a detailed metabolic study of calcium balance during a nine month period of bed rest.

Most techniques measured regularly shaped bones. The os calcis is irregularly shaped therefore it was necessary for us to devise a method whereby the bed-ridden subject can have his os calcis measured repetitively over a long period of time with acceptable reproducibility of position and accuracy of measurement.

This report describes the development of the instrumentation, and the observed changes in bone mineral during and after nine months of bed rest of the three subjects described in Exhibit A of this contract.

The radiographic density data collected by Dr. Pauline Mack is the subject of a separate report to be submitted by her. The location of our scan rows was scribed on X-rays of each subject

and was given to the contract monitor to relay to Dr. Mack so that she would be able to make a direct comparison. Conclusions should be drawn by an independent group of observers about the value of each method.

APPROACHES

Several decisions had to be made:

1. Which bone should be studied ?
2. What source should be used ?
3. What type of scanning device and what type of data presentation would be appropriate for the bone to be studied ?
4. How would the patient be positioned and how could this be done repetitively so that the same position on the bone is studied each time ?
5. In what units would the data be presented ? Would there be a way in which the units could be converted into actual bone mass as gm/cc ?
6. How reproducible would scans of an os calcis be ?
7. For how long should the subjects be studied after re-ambulation to assure that a baseline had been reached and the degree of loss validated.

THE SOLUTION

1. Choice of Bone.

The choice of bone for study was based on information available from previous space flight studies. The radiographic bone densitometry studies of the early Gemini flights indicated that there was a significant loss of bone mineral in the os calcis and the

hand phalanx 5-2. The choice was therefore clearly one of the two. The available data suggested that there was a uniform mineral content along the shaft of the phalanx and this bone would therefore lend itself to scanning by conventional scanners. However, we chose to study the more difficult os calcis because the degree of activity which could be anticipated for the hands during the nine month bed rest period would not be significantly different from the pre-bed rest period. Even though the muscular activity of the lower extremity could not be completely removed the gravitational forces would be kept to a minimum. Therefore, we decided to study the left os calcis on all subjects. The choice of the left bone was dictated by the fact that Dr. Mack had already decided to study the left foot.

2. Choice of Source.

Two easily available monoenergetic sources, namely ^{125}I and ^{241}Am, were considered because mass absorption characteristics of these two energies permit a suitable differentiation between soft tissue and bone, and because of the purity of the monoenergetic beam. At the ^{125}I energy, the ratio of the linear absorption coefficients (u_{HA}/u_{H_2O}) was 18/1 and at the 60 Kev ^{241}Am energy it was 6/1. Mass absorption coefficients for water, protein and fat at 30 and 60 Kev are given in exhibit 1. It can be seen that there is less discrimination between fat, water and protein at the 60 Kev energy than at the 30 Kev energy. This suggests that the 60 Kev energy would discriminate least between any changes in the fat,

water, and protein composition of the soft tissue covering.

Originally, both an ^{241}Americium source as well as an ^{125}Iodine source were to be used for the studies. The early Americium scans failed to provide accurate measurements because of the restrictions imposed by the low count rate of the 150 mc source. This observation has been subsequently reported by others. This is not to say that ^{241}Americium is not a useful scanning agent, but rather, that for heel scanning the self-absorption and low specific activity limits the number of photons available at the source strength permitted by the AEC. A source strength of about 700 mc should be used. The hazard of using a source of this strength and Americium's predilection for bone, precludes its use in any projected in-flight experiment.

The 27.5 Kev energy of ^{125}I, on the other hand, showed an excellent ability to discriminate between hydroxyapatite and soft tissue. The ratio between the linear absorption coefficients of hydroxyapatite and water is three times that of the 60 Kev energy. This allows for excellent statistics because ample photon yield could be obtained from a 200 mc source.

The ^{125}I was first obtained from Cambridge Nuclear as a carrier free solution ranging from 10 to 100 mc/cc with about 0.66% to 5% ^{126}I contamination. This solution was passed onto

1x8 ion exchange resin beads, housed in a half-inch length of polyethylene 240 tubing, held in place between thin discs of filter paper by small sections of PE 160 tubing placed within both ends of the 240 tubing. A Luer hub needle was inserted at each end and the iodine solution was pulled through the tubing with a syringe This manuever was carried out behind one inch lead shielding as shown in exhibit 2. After 90+ % of the iodine was held on the beads, usually obtained on the second pass, the tubing was cut just above each of the filter paper discs, sealed at each end and placed in an appropriate sized hole in the center of a one inch diameter brass plug (exhibit 3.) The brass plug was sealed with a thin mylar window using Eastman 910 glue and covered with a 2 mil. thickness of tin. Leakage or contamination of the source holder was not experienced during the study. The tin filters out the 31 and 35 Kev photons yielding an almost pure 27.4 Kev beam. The brass plug was then placed in a source holder (exhibit 3.) Toward the end of the study, the iodine sources were obtained from Atomic Energy of Canada, Ltd. as a silver needle onto which the iodine had been electroplated and covered with acrylic. This needle was also placed in a polyethylene tubing which was then placed in the brass plug and sealed.

The Americium source used in our preliminary studies was obtained from NUMEC Corp., Apollo, Pennsylvania. It consisted of a 1/4" x 3/4" stainless steel capsule with an exit window thickness of .075 inch stainless steel. This was placed in the source holder about three inches from the iodine source (exhibit 4.) The source

holder was then housed in a larger lead block, 11x4x4 inches in size, fitted with a rack and pinion device that permitted either of the sources to be placed in proper alignment with the detector (exhibit 5.) The housing was fitted with interchangeable collimators (exhibit 6.) Studies conducted with varying size straight bore lead collimators showed a 3/32" x 1/2" length to be optimum.

3. SCANNING DEVICE

Early flight studies showed a variability in the degree of mineral loss in various parts of the os calcis. This made it necessary to study more than one cross section of this bone. Because this bone has an irregular shape, it was imperative to construct a device that would permit the rectilinear scanning of an adequate volume of this bone in a manner that would be reproducible anatomically. The scanning data must also be reproducible. The problem was complicated further by the need to study these subjects in the recumbent position. Rectilinear scanners of the conventional type do not lend themselves to scanning in the X-Z direction; that is, in the vertical as well as horizontal plane. Furthermore, conventional electronics have been designed either to give imaging on a photographic plate which is not linear with respect to the information supplied to the light source, or to develop a tapper pattern on a piece of paper clearly unsuited to our purposes. Color modulated scanners do not add anything to the ability to obtain a linear response. It was therefore necessary to utilize a digital system which provided not only density information but location information as well. Commerical instruments which

provided a capability for accepting such data were limited. It was necessary therefore to build an appropriate rectilinear scanner and interface it with suitable electronics which would satisfy these requirements.

The basic scanner we constructed consists of the 1) scanning head, 2) X & Y axis drives, 3) control system, and 4) data acquisition system (exhibit 7.)

In contrast to most scanning instruments which generate a horizontal raster pattern, it was designed to scan in a vertical plane by using vertical and horizontal driven members (exhibit 8.) A combination of heavy duty components and precision machinery has created a smoothly functioning system free of detectable backlash.

The main frame has a horizontal track with a bearing surface for the horizontal moving member (exhibit 9.) A Superior Electric Co. stepping motor mounted on the frame drives the horizontal member through a 5-pitch lead screw. A precision gear train provides for gear ratios from 1:1 and 6:1. A 2:1 reduction is used to drive the 10-turn potentiometer system described below.

The horizontal member is a large box that moves on roller bearings in the main frame track. The vertical member is a block which fits within the horizontal member and is counter balanced by heavy duty springs. The vertical drive motor is mounted on the horizontal member and therefore moves with it. Source and detector

assembly are attached to the vertical block.

The source assembly is rigidly mounted to the vertical block by heavy brass tubing clamped at the top and the bottom of the block thus eliminating a pendulum effect during motion (exhibit 11.) The source holder itself is attached to a bracket rigidly bolted to the other end of the brass tube.

The detector assembly is mounted in apposition to the source by a similar arrangement (exhibit 11.) In contrast, independent adjustments can be made to provide for alignment with the source: vertical, by loosening the tubing clamps on the vertical block and sliding the assembly up or down; horizontal, by a rack and pinion built into the detector housing; and rotational, by loosening a set screw which rides in a groove.

Adjustable limit switches actuated by the horizontal block determine the length of the horizontal scan line and cycle the scanner control.

Electronics include a DC power supply, stepping motor supply, and automatic controls system (exhibit 10.) To minimize noise in the signal line, low voltage DC control relays were used. The DC supply is well filtered and was built to supply the needed power for control by the limit switches. The motor power supply is capable of driving the stepping motors and was built according to the specifications of the Superior Electric Co. It provides for speeds from 1 to 200 steps per second and direction changes. This system allows for both manual

and unattended automatic scanning once the scan parameters have been set. Manual control of all scan functions permit single line or special scans. In the automatic mode control relays energized by the horizontal limit switches generate the scan raster. A time delay relay dictates the duration during which motor power is applied to the vertical motor, and, therefore the speed of the vertical motor during this interval determines the line spacing. After completion of the vertical interval, the circuitry restores power to the horizontal motor, reverses the horizontal direction, and the scanner moves in the opposite direction. Horizontal scan speed can vary from 1 to 2.75 inches per minute; the vertical interval has been set at 1/8 of an inch.

The source housing consists of a block of lead with 1 1/2" walls in which there is a rack and pinion mounted source holder which permits the alignment of either the Iodine or Americium source with the exit collimator, as was previously described. Thermo luminiscent dosimeters and film badges are placed twelve inches lateral to the detector mounting to measure both source leakage as well as scatter on a 24-hour per day basis and are read monthly. Up to the present time, there has been no evidence of radiation leak or scatter. Quarterly wipe tests are performed by the Radiation Detection Company, Mountain View, California.

The detector consists of a Harshaw SHG X-ray detector with a 2 millimeter by one inch sodium iodide thallium-activated crystal covered with a thin beryllium exit window and housed in an aluminum tube fitted with two lead discs, 5 millimeter thick and two inches in diameter.Each disc contains a 2.4 millimeter bore in its

center and is placed one and one-half inches apart (exhibit 11.) This collimation is adjusted so that the exit port from the source is in exact alignment with the detector collimator holes. As previously mentioned, though the source housing is fixed to the vertical scanning block, the detector housing has three dimensional adjustments, permitting precise alignment.

Three Methods of Data Acquisition Were Designed:

1. A simple programmed dual scaler system which permits each scaler to accumulate data from the detector alternately and to print this data on a parallel printer and on a serial punch paper tape. The programmer permits the dead time between each scaler cycle to be limited to less than 5 microseconds. This system has advantages over a digital ratemeter or a single scaler where deadtime is dictated by the print out time and is not uncommonly 500 microseconds. The scaler timing is determined by a precision timer adjustable to 0.1 seconds by means of thumb wheel switches. This system has a disadvantage: it is not able to accumulate position data whereby the bone contours can either be displayed or manually plotted (see below) however, it serves as an excellent backup system.

2. Multichannel Analyzer: Multiparameter Mode. This system permits the generation of both an X & Y index along with the data. The data can then be assigned position locations and therefore permit plotting of the bone outline or displaying it on an oscilloscope screen.

Two methods of addressing the analyzer were developed:

1) Potentiometric or voltage address.

A voltage ramp is generated by means of a precision 10-turn potentiometer mounted on the end of the X axis screw and connected to the analyzer bias supply. This voltage ramp addresses the X ADC of the analyzer with a continuously variable voltage signal from 0 to 8 volts. The location of the scanning head is accurately given to the analyzer since the voltage address is 0 at one end of the X traverse and 8 volts at the other end. Each row of the X axis was divided into 256 increments in the memory. The 5-pitch lead screw rotates 5 turns per inch or 20 turns per 4 inches, the limit set on the X travel. A precision 2:1 gear reduction train made by PIC Design Corporation reduced these 20 turns to 10 turns to accomodate a 10-turn potentiometer for the 4 inches. Simple arithmetic therefore sets each increment at 1/64 inch; i.e. 4x64 = 256 increments for 4 inches. It should be clear that the analyzer at any point in the traverse knows exactly the channel and location of the scanning head. The channel I.D. therefore allows us to locate spatially the edge of the bone and subsequently plot the contour.(exhibit 12.) This was an important consideration because repetitive scans needed to be compared. Sixteen rows of 256 channels are accumulated in the 4096 channel memory. Overlays could be made which allowed comparisons between scan rows on different days.

Since the analyzer had to search for the location of each data point, a certain amount of dead time had to be accepted. The analyzer

chosen, Nuclear Data Corporation, Model 3300, had the option of using a fixed dead time (exhibit 8.) This was one of the options which led us to choose this analyzer. By using a fixed dead time, a ramp effect between channel 0 and 256 could be avoided. Without a fixed dead time and with a count rate of 2600 counts per channel, a ramp of up to 9% is generated. When the dead time is fixed at 50 microseconds, the ramp is less than 1%. Loss of data at very high count rates can be considerable with this fixed dead time. However, at count rates of less than 2000 counts per channel, the response is linear as shown in exhibit 13. The fixed dead time of 50 microseconds was later altered by an internal modification to a value as low as 30 microseconds. This option was not completed during the study and was not used.

2) Use Of An Optical Shaft Encoder.

This mode required extensive modification of the analyzer. This method couples a shaft encoder to the end of the X axis screw. A 1024 pulse per revolution encoder was used. A six binary bit pre-scaler links the shaft encoder to the main address register. The pulse train originating within the encoder is directed to the prescaler which contains a front panel switch control that can be adjusted so that when the shaft encoder has generated a selected number of pulses, an output from the pre-scaler directs the main address scaler to increment the address. This causes: a) interruption of the scaler count, b) transfer of the scaler count into the memory register and generation of a memory cycle to store the data within the core, and c) re-start counting

for address into the next channel. This process continues until the last channel in X (256) has been incremented. At this point, an internal programmer terminates the data input to the scaler, transfers the scaler content into the memory register, generates a storage memory cycle, increments the Y address scaler and alerts the scanner to increment along the Y axis. The prescaler incorporates up/down scaling logic to compensate for any tendency of motor over travel which would cause scalloping or narrow edge channels. The control unit then initiates a command which starts the X motor on a new row. The new row data is properly addressed to allow proper geometric configuration. This is to say, the first channel on the new row will begin not at 257, but rather at 512 and count down, thereby placing the first new data point above the last data point of the row below. The circuitry allows bi-directional communication between the scanner and analyzer to avoid positional or timing errors. This system is directly interchangeable with the potentiometer system when a prescale of 80 is selected: 80/1024 of a revolution of the encoder addresses a new channel. Since 20 revolutions cover 4 inches, 20 x 1024 pulses are generated from the shaft encoder along a single row. Since 80 are needed to address each new channel, 20 x 1024/80 or 256 channels are accumulated along one row just as in the previous system. This system is capable of reducing the dead time to 30 microseconds for a 10,000 count per channel input and considerably less for lower count rates. It does have one drawback: it does not give absolute position location in space and the starting point must be appropriately chosen

to encompass the bone when beginning the first row. All subsequent rows will be accurately related to this first row, provided the analyzer and scanner are not stopped during the scan. Under these conditions this method also produces bone edge channel identification which can then be used to outline th bone as by the previous method.

The encoder system required considerable modification of the analyzer and therefore was not chosen at the beginning of this contract. Similarly, the scaler system did not provide for development of a precise bone contour for subsequent overlays and it too was not chosen at the beginning of the study. Therefore the potentiometer method of address was initially developed for this study and the other two systems were designed and developed subsequently.

The data presentation for the potentiometer system took several forms:

1) Direct print readout on a Franklin High Speed Printer, Model
2) Transfer to a digital magnetic tape system, Hewlett Packard, Model D 2020.
3) Paper tape punch on a Talley Punch, Model 420.
4) Oscilloscope display in either of two modes:
    a) Volumetric - a planar representation of the 16 rows of 256 data points

where each point on the screen is intensity-modulated by the numerical magnitude of the data at that location (exhibit 14.)

b) Isometric display - a three-dimensional display where each row is depicted in an analog fashion with each point located above the 0 baseline in proportion to its digital value. Therefore, each row resembles a chart recorder display. The rows are sequentially displayed one behind the other, giving a three dimensional effect (exhibit 15.)

5) Chart recorder tracing.

4. PATIENT POSITIONING.

Clearly, one of the most important considerations was the need to reposition accurately the subject for each of the scans. Since the study was to cover a year, any device used had to be sturdy and durable, and had to have the capability of being placed on the scanner in exactly the same location for each scan. A platform which could be adjusted by a rack and pinion was mounted on the main scanner frame. The device to hold the foot in position was placed on this platform in a position that would allow the scanner to traverse its 4-inch path with the center of the bone always at the 2-inch mark.

Several approaches were tried in developing a foot holder. The first and most important requirement was that the device be capable of serving as a water bath since scans were to be performed with the foot immersed in water to serve as a tissue equivalent. A plexiglass block molded to the foot was tried; plexiglass has a tissue equivalency very little less than water. This block was then machined to have parallel sides. Unfortunately, air bubbles and foot-air-plastic interfaces did not permit a uniform tissue equivalency and this method was discarded. The mold had to fit accurately to the foot and any spaces left had to be filled with water. Ordinary plaster was not durable enough and adjustable wooden or metal frames were clumsy to use and generally did not permit satisfactory reproducibility. The logical choice was the fabrication

of a foot mold of Duroc (a dental rock material), which, though it withstood water treatment when cured, was further protected by spraying several times with a plastic coating. Assays of water left in contact with the bloc for several days did not show any traces of solution of the material. This mold was manufactured to fit exactly into a plexiglass box with a scanning dimension of 5 inches and external dimensions of 6"x12"x5". This box with the mold inside was then filled with distilled water and the foot inserted. The plexiglass area in apposition to the part of the bone to be scanned had a thickness of 1/16 inch, while the remainder of the box was made of 1/4 and 1/2 inch stock. The detector housing to source housing distance was 6 inches and the box tissue equivalent width was 5 inches, allowing ample room on either side. A separate box and mold was made for each subject. These remained in excellent condition throughout the study. The orientation of the foot in the mold was such that the vertical plane of the mold was on an axis perpendicular to a line drawn through the waist of the os calcis. Lead ball and lead grid markers were placed on the box to permit recrientation for each scan. The ball was located 2 inches from the bottom of the os calcis, serving as a marker for selecting the starting row and thereby allowing 16 rows to be scanned downward each 1/8 inch apart. This 16 row format was used throughout the entire study. X-rays weretaken of each subject in the mold and 1/8 inch spaced-scan lines were superimposed (exhibits 16, 17, 18.)

5. DATA CALCULATION

The general format of the data generated for an average row

is shown in exhibit 19 The average count rate to the left and the right of bone, i.e. through tissue plus water, represents the I_o^* or 100% transmission. The bone edge is defined as the point which is 0.85 of the I_o^* value at each side of the bone. The data values encompassed between these two limits are then ratioed to the I_o^* value and the natural logarithm computed. To permit data averaging, the mean of 3 successive points was used for each ratio and logarithm. These triplet values, as they will be referred to, are then summed for each row and expressed as a positive number representing absorption. Therefore, the higher the number, the denser the bone. No bone would give a value of $\ln I_o^*/I_o^*$ or $\ln 1$ or 0. These sum values for each row are expressed as computer units. To permit the calculation of the data, an Olivetti Programma 101 was used with a program designed to compute the I_o^*, the 85% cut off, and to ratio the values to I_o^*, compute the ln and sum them. The Olivetti program is shown in exhibit 20. It also computed the number of channels per row which when multiplied by 3/64 inch, gave the bone width in inches. In March 1969, a computer program was written for the Mark II General Electric Computer located in Los Angeles. A data phone teletype system was installed which accepted our Tailey punch tapes. This system has been used for recent data. The program is shown in exhibit 21.

6. DATA REPRODUCIBILITY AND CALIBRATION

To assure accurate repositioning in the mold which would give us reproducible scans, each subject was studied along an ar-

bitrary scan line repetitively prior to the start of our determinations. After each scan pass, the subject removed his foot from the mold, rested for a few minutes, and then replaced his foot in the same manner as before. Five passes at the same level were made, in alternating directions. Determination of I_o*, width of bone, and relative bone mineral expressed as computer units are shown in exhibit 22. Reproducibility was within 1.5 to 2.0% at a 95% confidence level. Scans of the os calcis were performed on a normal ambulant subject on 4 successive days and then again 20 days later for 3 successive days. The computer values for 9 rows beginning at 3/8" from the bottom of the os calcis up to 1 3/8" from the bottom of the os calcis are shown in exhibit 23. The standard deviation was ± 1.17% for rows 3-11 and 1.55% for rows 4-12. The subsequent presentation of composite data for the subjects represents the sum of 9 rows, thus encompassing the central os calcis. This area will subsequently be shown to have the greatest mineral change. The first 2 rows are not used since they traverse an area where the bone width is rapidly changing and accurate reproducibility is not possible: a shift up or down of as little as 1/64 of an inch can make a considerable difference in the results. This is clearly evident in exhibits 34 to 39. Above this area, slight variations in the starting location are averaged out over the entire scan area.

Daily calibrations were made using a section of femur embedded in a plastic block (exhibit 24.) Two lead bails are embedded on either

side of the bone in a direct scan line to allow rescanning at exactly the same location each day. This bone gives a C.U.* value of 63.592 ± 1.24 (± 2 S.D.) or ± 1.9% over a period of months. Use of this standard has permitted us to make corrections when new sources with inappropriate ^{126}I contamination were received. It also permitted us to determine that an 0.05% ^{126}I contamination was the upper acceptable value.

In July of 1968, I visited Professor Friederich Heuck at the Katharinenhospital, Stuttgart, Germany who generously provided a calcium hydroxyapatite step wedge which has subsequently been used for weekly calibrations (exhibit 24.) This wedge consists of 6 steps each of 3 different hydroxyapatite concentrations; i.e. 100, 240, and 350 mg/cc. The use of this wedge as a standard, when scanned in water, provides 18 points of varying concentrations of hydroxyapatite for direct comparison with our data. The regression coefficient for a line fit by least squares has consistently been better than r = 0.9950. The best fit is shown in exhibit 25. All data presented in this report have been normalized to a wedge value of $y = 0.00227x$ where y is in computer units and x is in mg/cm^2 hydroxyapatite.

Published data relating to bone mineral content of the radius and ulna describe the ash weight mineral content per cm length of bone. Because the os calcis is an irregular bone and primarily trabecular

*C.U. = computer units. Represents $\Sigma \ln I_o^*/I$ for a row.

it was necessary to seek another method of converting our computer units to ash weight. Studies with os calcis obtained from surgical amputations have been scanned with and without the soft tissue covering. These studies have not been completed because a sufficient number of usable heels is difficult to obtain. However, one such study is described:

The bone is fixed by cross pins to a plastic box and immersed in water (exhibit 26.) Twelve scan rows are made. The bone is then scanned without soft tissue covering, dried, and embedded in a plastic resin or paraffin and cut into 6 sections of about 1/8" thickness with a diamond wheel of 0.025" thickness (exhibit 27.) Exhibit 28. illustrates the location of each scan line, the bone lost by each cut, and the location of the cut. The bone sections were ashed at 600° C for 48 hours, weighed, and analyzed for calcium and phosphorus according to the methods described in the Exhibit A Terminal Report. The calcium and phosphorus values are shown in exhibit 29. The first and last bone cut could not be used in plotting the ash weight vs. computer units. The remaining 4 points are shown in exhibits 30, 31. Because of insufficient data it is not possible to express our data in mg of ash weight change for each row scanned, at this time. However, further studies are in progress.

7.- DURATION OF STUDY

Due to the instrument design requirements our studies could not begin until the subjects had already completed 3 months of bed rest.

The subjects completed their bed rest phase in early September 1968 and it appeared reasonable to assume that pre-bed rest bone mineral values would be restored by the end of the contract time. Literature references relating to the rate of regain of mineral after periods of immobilization were limited and those available suggested that there was good probability that pre-bed rest values might never be attained. This should have made us wary about the projected period required to complete the study. As will be shown in the discussion of results, one of the subjects required 45 weeks after reambulation before he exceeded the 12 week bed rest value. Therefore, completion of this study could not be accomplished until mid-June 1969, three weeks ago. This time period could not have been foreseen at the beginning of this study and clearly exceeded the initial contract period by 6 months.

SCANNING PROCEDURE ADOPTED:

The instrumentation received the following calibration procedure:

1. Daily print out of the gamma ray spectrum to permit correction of detector or analyzer drift.
2. Daily scan of the plastic embedded bone phantom.
3. Weekly or more frequent scans of the hydroxyapatite st p wedge to assure linearity of response of the scanner to bone mineral content.

After assuring that the calibration values were within 2% of the mean obtained over several months, the mold for the subject to be scanned is filled with distilled water and the scanner location adjusted to the lead ball row (previously determined to be approximately 2" from the bottom of the os calcis as oriented in the mold). The patient is brought in on a stretcher, his left foot is placed into the mold, and the scan is begun for 16 rows, 1/8 inch apart. Using this format 12 to 15 usable rows are scanned. The row interval, though automatically advanced through 1/8 inch is checked throughout the scan by observing the vertical traverse of the scanning block in its housing. After returning the patient to the Metabolic Ward, the mold is emptied and dried.

The data stored in the analyzer memory is dumped onto digital magnetic tape for future use, punched on paper tape for direct G. E. Mark II computer input, and printed in hard copy on the Franklin printer

for calculation using the Olivetti Programma 101 computing calculator. The latter method was the only one available for the better part of the year. The data is calculated as has been described. The bone contours are observed on the oscilloscope screen and plotted according to channel ID's to permit comparison with previous scans by superimposition.

RESULTS AND DISCUSSION

The ability to scan repetitively a single line within 2% when these scans are performed on the same day, has been demonstrated (exhibit 23.) Over a period of time, changes in the soft tissue resulting from inactivity of the foot, especially related to not wearing shoes, can change the overall external anatomy of the foot. Under these circumstances the foot might slip a little lower into the mold, so that individual scan lines might not be exactly reproducible and might vary by approximately 1/32 inch. The volumetric or contour display mode of data presentation assured us of being able to superimpose row data within this limit of variation on successive scans. Because the os calcis is irregular, changes in location can result in significant differences when the lowest 2 rows of the bone are scanned since there is a rapid change in cross sectional diameter. Furthermore, the trabecular pattern is not uniform in a medulary bone such as this, with the result that values might be somewhat different when the rows do not match precisely. To overcome these problems in data presentation, bone mineral content for 9 rows in the central os calcis were summed in order to minimize small positional changes. These 9 rows comprise a total of 1 1/8 inches of bone in the vertical axis and the total bone in the horizontal axis. In this way a small shift upward for example will have the highest line include portions of an area 1/32 inch higher on the bone and the lowest line will delete 1/32 inch portion of a row previously scanned. When the bone mineral for this large segment is compared from scan to scan in a normal subject, very small variations between scans are seen (exhibit 23.)

Individual rows might show greater variation. However exhibits 31, 32, 33 illustrate that individual row value differences are not important in describing the overall change in mineral pattern as bone is lost.

In this respect, the presentation of our data had to take a different form from that presented in studies of the radius or ulna, where slight longitudinal differences in the selection of a scan row make very little difference in the results obtained. The variation of the mineral content as one scans higher on the os calcis is graphically represented in exhibits 31 to 33. These graphs illustrate very clearly the changes in mineral content as one begins at the lower cortex and moves upward through an area of less mineral content and up to areas containing more mineral. These graphs further illustrate that the changes during the bed rest as well as reambulation period are greatest in the area which originally had the least mineral content with lesser changes being observed above and below this area. We have chosen to refer to this area as the "central os calcis". The computer unit values for each row for each scan are presented in tabular form (exhibits 34 to 39.) The mineral content of the central os calcis comprising the sum of 9 rows (3/8" - 1 3/8") is given at the bottom of a row data. The percent of initial value as well as the percent of loss calculated from an arbitrary baseline (accepted as either the last value obtained or a mean of 3 successive scans if little change was occuring) is shown for each subject after 20-40 weeks of ambulation. The ultimate

values obtained after 20-40 weeks of ambulation exceeded the initial values in all cases and it is reasonable to assume that the difference between the initial value and these arbitrary baseline values could represent the amount of mineral lost during the first 12 weeks (exhibit 40.) Studies in progress in which baseline values have been obtained for several weeks prior to bed rest show similar results so that our confidence in this assumption is strengthened.

Using the central os calcis as an index of mineral loss in an area of high risk, the percent lost during the 12-36 week bed rest period of C.S. and R.R. were 44.5 and 33.3% respectively (exhibit 41.) The percent loss for G.B. during the period of 12-30 weeks of bed rest was 25.1%. The regain of mineral after ambulation was 37.9% for 30 weeks in the case of R.R., 52.8% for 21 weeks for C.S., and 27.2% in 46 weeks for G.B. Since these regain values exceeded the initial values, we believe that the mineral loss in the first 12 weeks for G.B., R.R. and C.S. were 2.1%, 4.6% and 8.3% respectively. The mineral profile for the os calcis illustrates the fact that the area with least mineral content loses the most and conversely regains mineral the most rapidly. Areas higher on the bone which lost the least mineral were the slowest to regain it and, indeed, in some areas did not completely regain all of the mineral lost during the bed rest period (exhibits 42, 43, 44.)

Considerable confusion exists regarding the degree as well as the speed with which demineralization occurs during recumbency,

immobilization, or space flight, especially where the os calcis is concerned. The reported values have ranged from a mean change of -10.23% in the Command Pilot of Gemini V (2) an eight day flight, to + 2.27% in one of the crew of Apollo VII, a 10.8 day flight (3); and from -12.35% on a 300 mg calcium per day diet to -4.96% on a 2000 mg calcium per day diet in the 14 day recumbency studies (4). Clearly, the dietary as well as activity status of the subjects in these studies differed widely. In the dietary recumbency studies of Mack (4), subjects with a dietary calcium intake comparable to our study had a bone mineral loss of from 3.5% to 7.5% in 14 days. Our subjects had 90 day mineral losses of 2.1, 4.6 and 8.3% from a baseline subsequently established 21 to 46 weeks after reambulation.

Exhibit 40. shows the greatest loss to occur in the second 3 month period. The loss is smallest in the third 3 month period when a full nine months of bed rest is maintained. Current studies appear to be duplicating this observation.

The difference in the rate of loss in varying parts of the os calcis previously reported in the space flight studies (2) were also observed in our subjects. When the os calcis is scanned in planes parallel to a line drawn through its waist, the area showing the most rapid loss was on a line about 5/8" to 1.0" from the bottom of the bone. This area was also the one which had the least relative mineral at the beginning of the study. It is interesting to note

that the subject with the least bone mineral at the beginning of the study (C.S.) lost the most and the one with the most bone mineral (G.B.) lost the least (exhibit 40.) Although changes in the soft tissue in an uneven fashion could result in changes in absorption of the gamma ray beam, all of our studies have been performed with the foot immersed in a uniform thickness water bath to minimize this effect. Furthermore, the maximum change in mineral measurement observed when a bone phantom was scanned in 100% mineral oil vs. water was only 8%, clearly not sufficient to account for our observations on the basis of changes in the fat content of the soft tissue rather than on the basis of mineral loss. The soft tissue covering was not of the thickness of the water bath used.

The rate of regain of mineral after ambulation appeared to reflect the loss pattern in a reverse fashion (exhibits 31, 32, 33.) The areas which had lost the greatest amount and at the most rapid rate were the first and most rapid to regain the mineral. The areas above the central os calcis which lost the least and at a slower rate appeared to demonstrate a minimal change early after ambulation even though rapid gains were beginning to be evident in the areas below this area. This non-uniformity in loss as well as regain, clearly points to a remodeling activity within this bone as the gravitational and muscular forces are again brought into action. The subject who had the least loss of mineral (G.B.), appeared to have a much longer time to return to the 12 week value and therefore to baseline. It

is interesting that he returned to school after the study and physical activity was less than that of the other two subjects. It took 46 weeks after ambulation before a clear increase over the initial 12 week bed rest value was observed.

The subjects returned to baseline in 18, 21 and 46 weeks respectively. This is contrary to the conclusions of Stevenson (5), who indicated that significant replacement of bone lost during immobilization may not occur. He quoted Gill as well as his own observation which were primarily observations in tubercular cases. However, he noted the loss of mineral to occur in non-tuberculous areas during immobilization which did not return to normal.

George P. Vose (8) stated that "bone mass losses have been observed in relatively short periods of time in studies of dogs under anti-gravity simulation, and in humans on varied levels of dietary nitrogen. Under conditions of such relatively rapid bone mass loss, the recovery period is often considerably slower than the depletion period". Our results, therefore, lie somewhere between these two observations since the subjects on bed rest for 36 weeks returned to baseline in 18 and 21 weeks, and the subject on bed rest for 30 weeks returned to baseline in 46 weeks, the latter subject having had the least mineral loss during bed red.

The rate of regain in the immediate post-recumbency period, that is - within hours - was not studied due to the immediate require-

ments of other studies which were given priority (Exhibit A). Future studies are required to determine whether the rapid regain of mineral as observed by Mack in the Gemini flight astronauts also applied to this ground-based simulation. One subject (G.B.) had less than 1% increase in mineral after 2 weeks of ambulation. R.R. had a 5.6% increase in 2 weeks (exhibit 40.) On the other hand, C.S. had almost a 40% increase over his lowest value obtained.

SUMMARY

1. A method of estimating the mineral content of a large volume of the os calcis by scanning with a monoenergetic photon source and observing the degree of photon absorption has been described.

2. The degree of reproducibility of scan data as well as repositioning has been found to be most satisfactory in following the bone mineral content changes in a group of subjects for 42 to 60 weeks.

3. Bone mineral loss from the central os calcis during a 30-36 week period of bed rest is substantial; 26.9, 36.7, and 53.5% in the three subjects.

4. Areas with the least bone mineral at the beginning of the study lost it more rapidly during bed rest and regained it more rapidly after reambulation than areas with greater mineral content.

5. The subject with the least total bone mineral, lost the most, and conversely, the one with the most, lost the least.

6. The os calcis, a bone repetitively subjected to full body weight stresses, can be placed at risk after return to G conditions if these substantial mineral losses occur during a prolonged period of weightlessness.

REFERENCES

1. Cameron, J.A., and Sorenson, J. Measurement of Bone Mineral in vivo: an improved method. Science 142, 230, 1963.
2. Mack, P.B., LaChance, P.A., Vose, G.P., and Vogt, F.B. Bone demineralization of foot and hand of Gemini-titan IV, V and VII astronauts during orbital flight. Am. J. Roent. 100, 503, 1967.
3. Berry, C.A. Preliminary clinical report of the medical aspects of Apollos VII and VIII. Aerospace Med. 40, 245, 1969.
4. Mack, P.B. and LaChance, P.L., Effects of recumbency and space flight on bone density. Am. J. Clin. Nutrition 20, 1194, 1967.
5. Stevenson, F.H. The osteoporosis of immobilization in recumbency. J. Bone Joint Surg. 34B, 256, 1952.
6. Rose, G.A. Immobilization osteoporosis. A study of the extent, severity and treatment with bendrofluazide. Brit. J. Surg. 53, 769, 1966
7. Birge, S.J. and Whedon, G.D. Bone. Chapter 9, Hypodynamics and hypogravics. Academic Press Inc., New York, 1967.
8. Vose, G.P. X-ray bone densitometry in short and long duration studies. Conference on Progress in Methods of Bone Mineral Measurement. National Institutes of Health, Bethesda, Maryland, February 15 - 17, 1968

Presented in part at the following society meetings:

1) Meeting of the Society of Nuclear Medicine, June 27, 1969 New Orleans, Louisiana.

Rectilinear Transmission Scanning of Irregular Bones for Estimating Mineral Content.

J. Nucl. Med. 10, 379, 1967.

2) The 53rd Annual Meeting of the American Societies for Experimental Biology, April 14, 1969, Atlantic City, New Jersey

Changes in Bone Mineral Content of the Os Calcis Induced by Prolonged Bed Rest.

Fed. Proc. 28, 374, 1969.

EXHIBITS

Figures, tables, and graphs.

1. Mass absorption coefficients for fat, water, protein and hydroxyapatite
2. Method of I-125 resin bead source production
3. Sources and source holder
4. Source holder in lead housing
5. Lead housing
6. Collimators
7. Block diagram of the scanning system
8. Scanner unit with multichannel analyzer and digital tape system, front view.
9. Scanner unit, top view.
10. Motor control unit
11. Detector, foot mold and source holder in position.
12. Bone contour map as prepared from channel I.D.'s
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15. Isometric display.
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17. R.R. X-ray of the heel in the mold.
18. C.S. X-ray of the heel in the mold.
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25. Mg/cm^2 hydroxyapatite vs. computer units.
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27. Os calcis in-vitro study. X-ray of the os calcis in scanning position, after cutting, and of each slice.
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29. Os calcis in-vitro study. Calcium and phosphorus values of the cut slices.
30. Os calcis in-vitro study.
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31. G.B. Bed rest and ambulant periods bone mineral profiles.
32. R.R. Bed rest and ambulant periods bone mineral profiles.
33. C.S. Bed rest and ambulant periods bone mineral profiles.
34. G.B. Bed rest period. Individual row values and composite central os calcis mineral content as computer units.
35. G.B. Ambulant period. Individual row values and composite central os calcis mineral content as computer units.

36. R.R. Bed rest period. Individual row values and composite central os calcis mineral content as computer units.
37. R.R. Ambulant period. Individual row values and composite central os calcis mineral content as computer units.
38. C.S. Bed rest period. Individual row values and composite central os calcis mineral content as computer units.
39. C.S. Ambulant period. Individual row values and composite central os calcis mineral content as computer units.
40. Per cent change in mineral content of the central os calcis as a function of time.
41. Mean per cent change for all subjects. Central os calcis.
42. G.B. Per cent bone mineral change by row
43. R.R. Per cent bone mineral change by row
44. C.S. Per cent bone mineral change by row

EXHIBIT 1

MASS ABSORPTION COEFFICIENTS

	30 KEV	60 KEV
PROTEIN	0.34	0.20
FAT	0.28	0.20
WATER	0.37	0.21
HYDROXYAPATITE	2.10	0.40

EXHIBIT 2

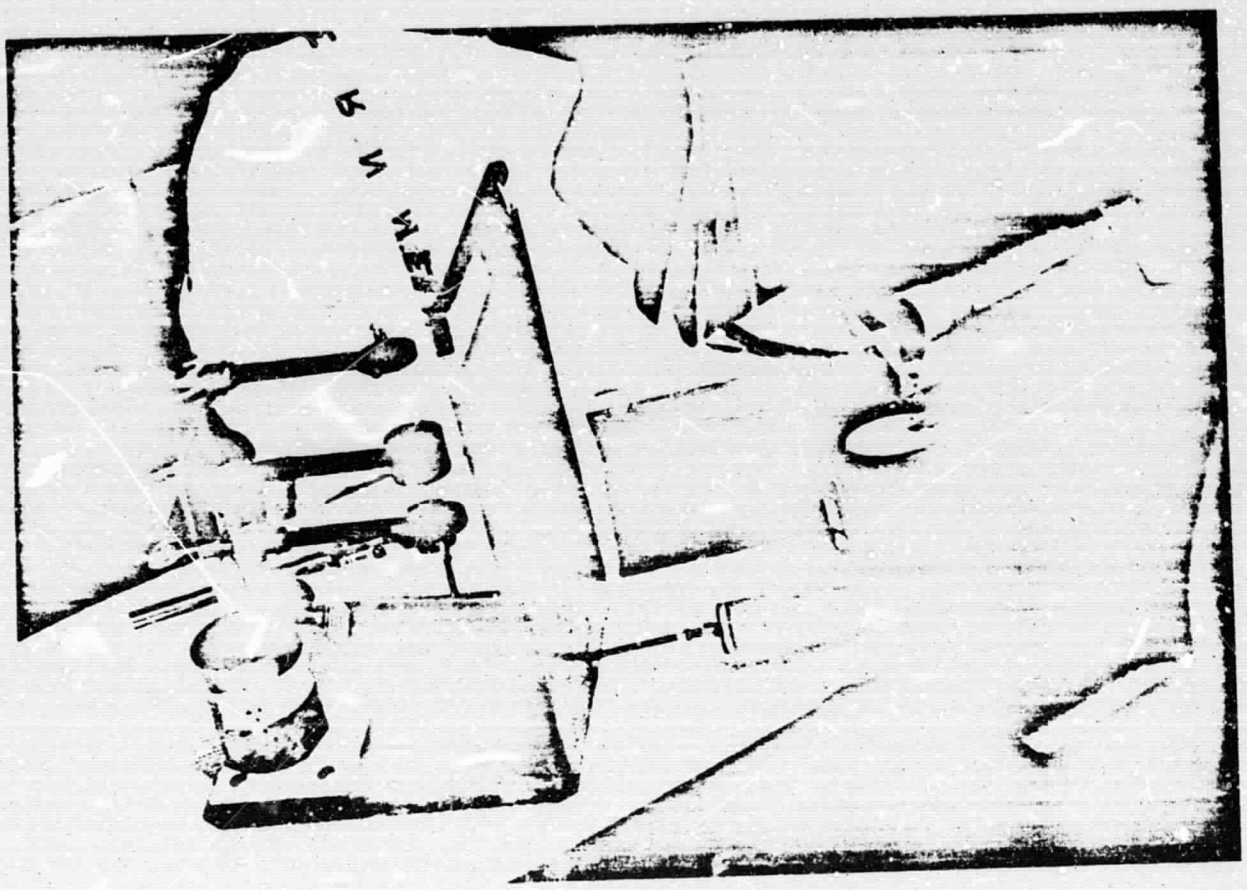
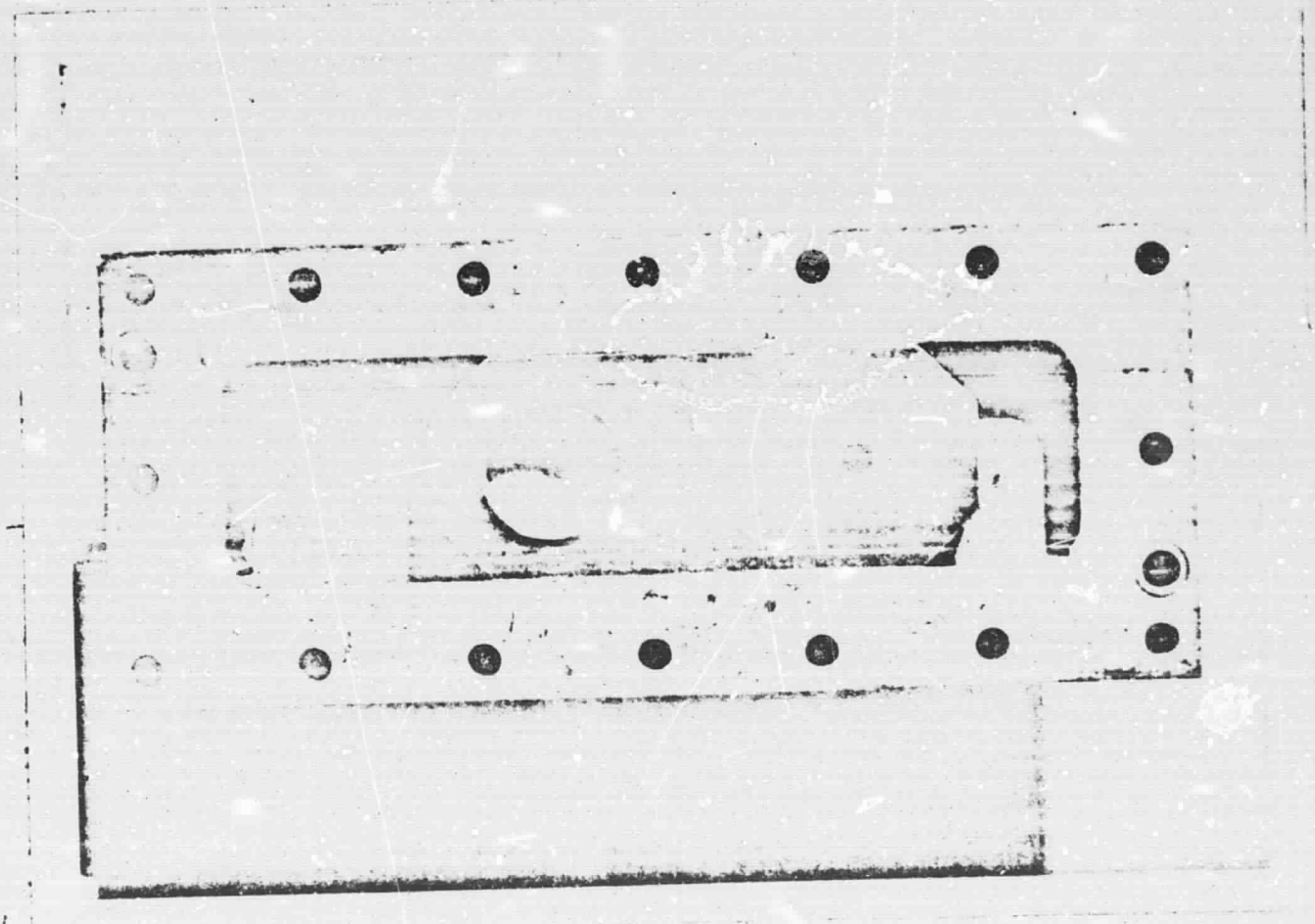


EXHIBIT 3

EXHIBIT 4



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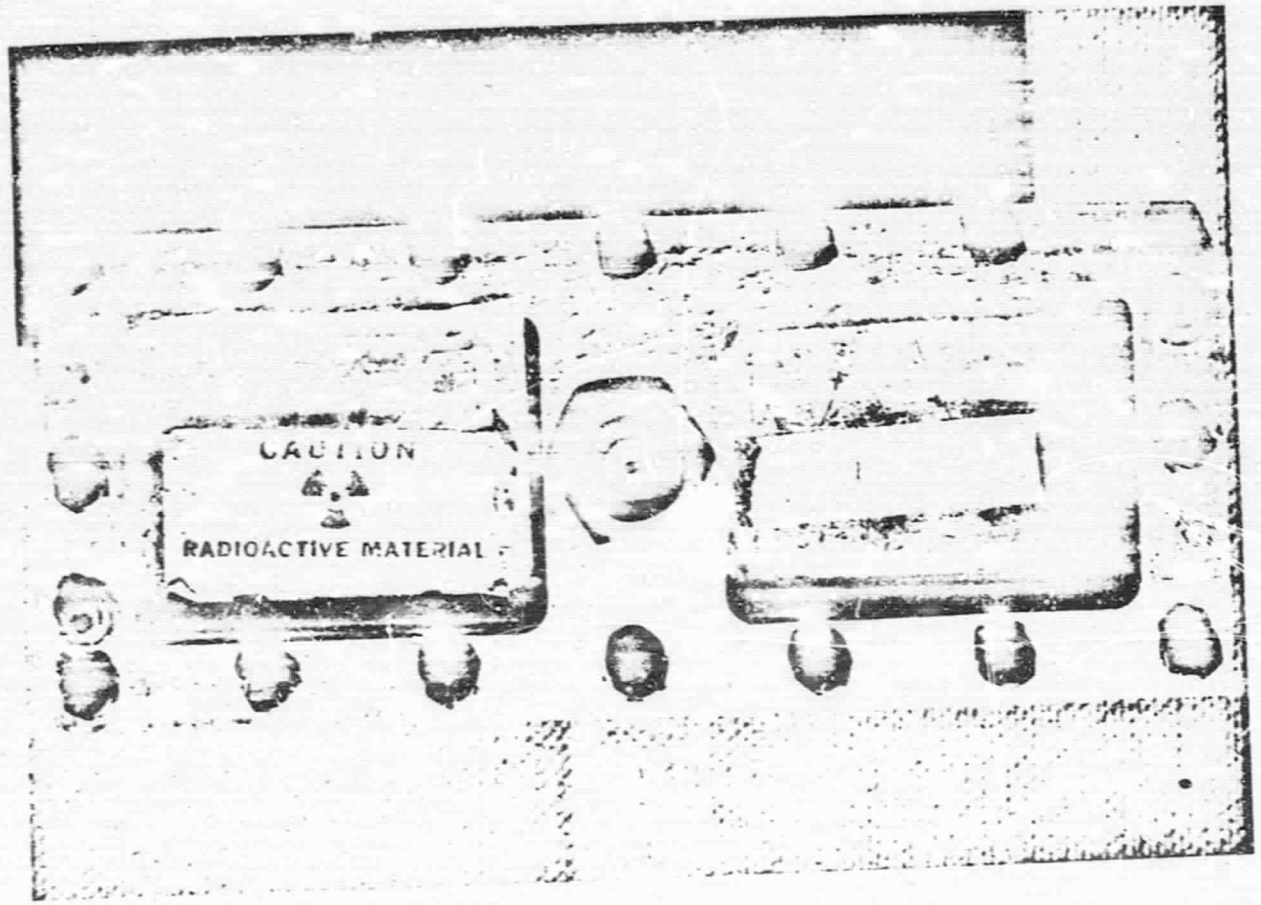

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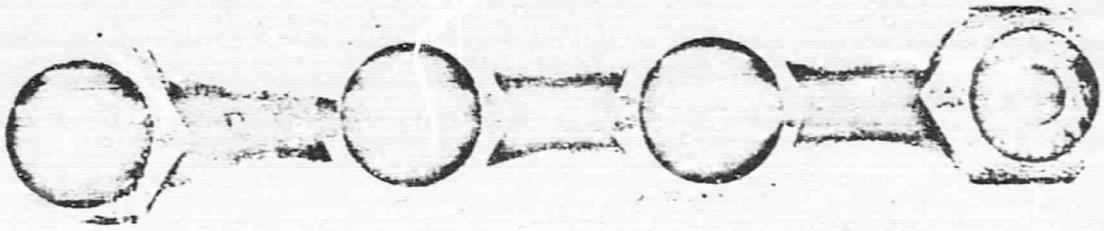

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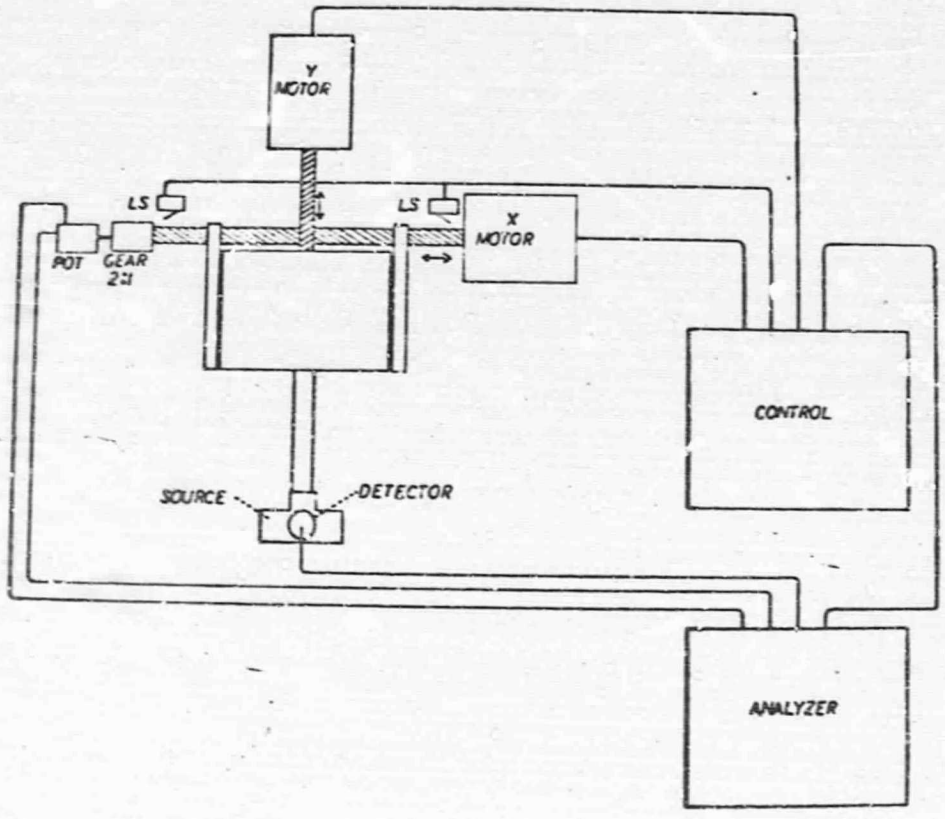
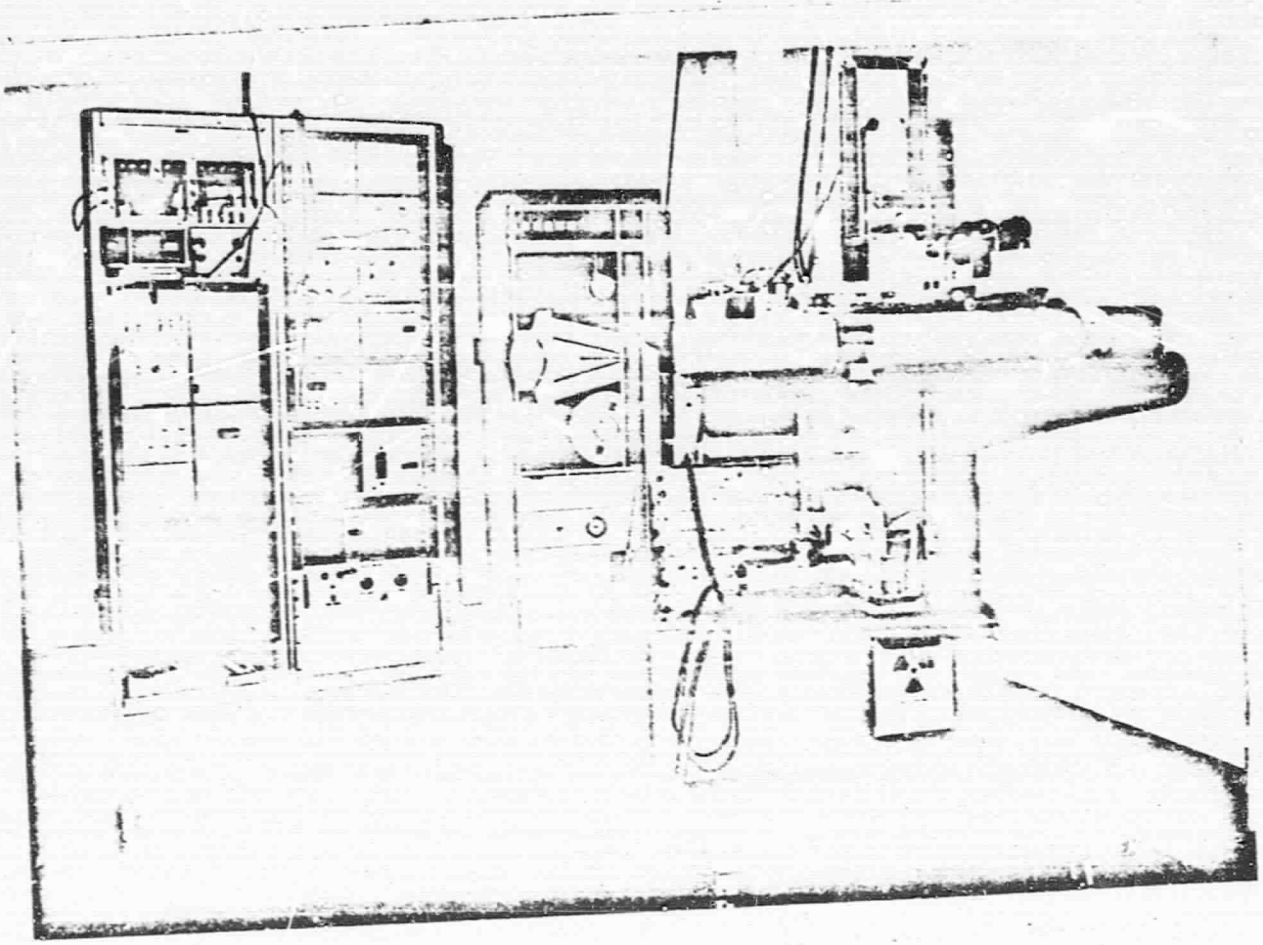

Y
MOTOR
LS
LS
X
MOTOR
POT
GEAR
2:1
CONTROL
SOURCE
DETECTOR
ANALYZER

EXHIBIT 8



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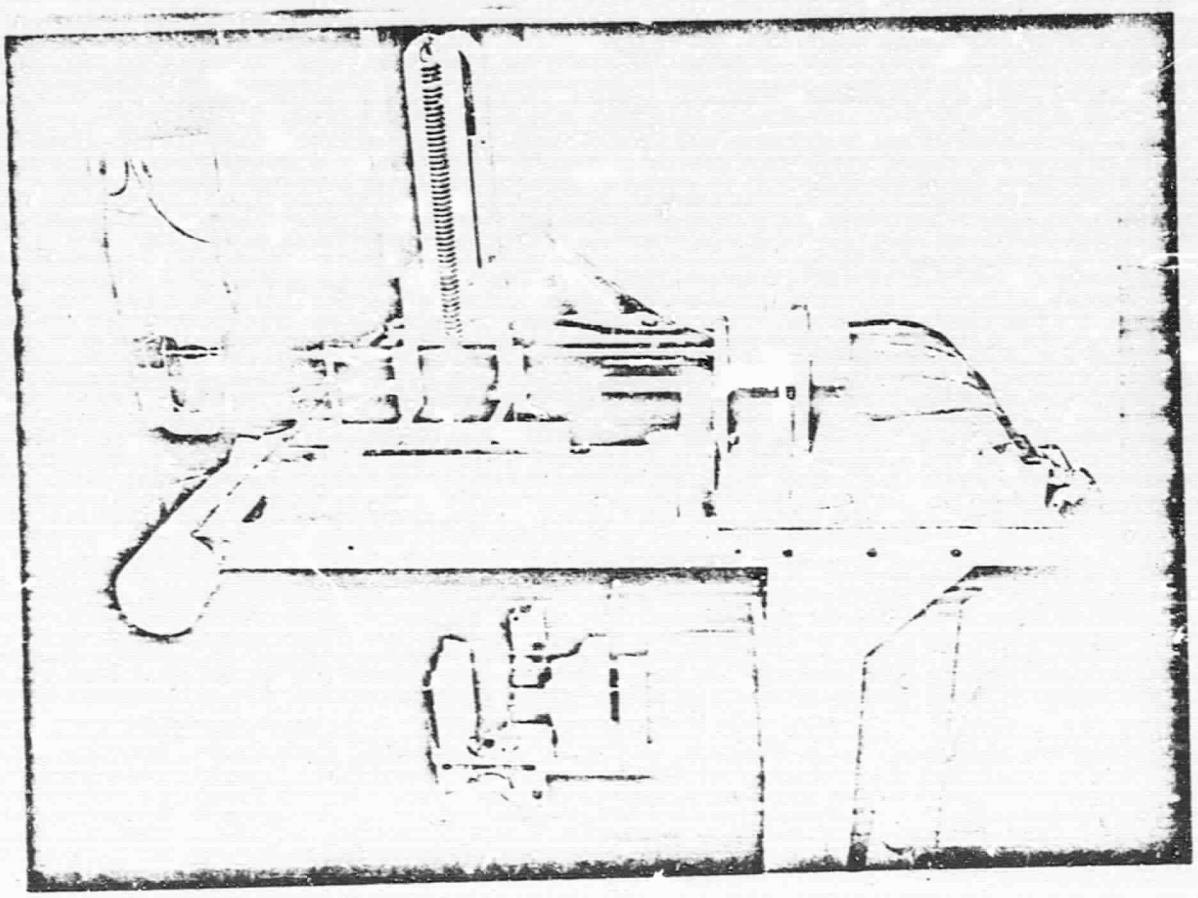
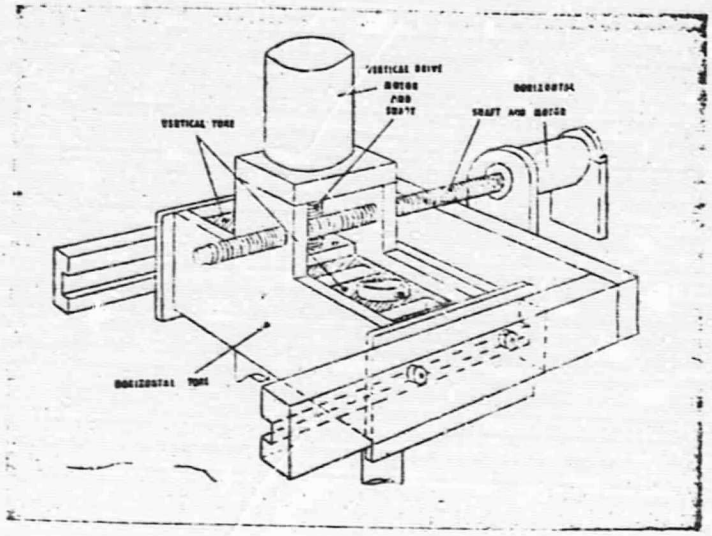


EXHIBIT 9 A



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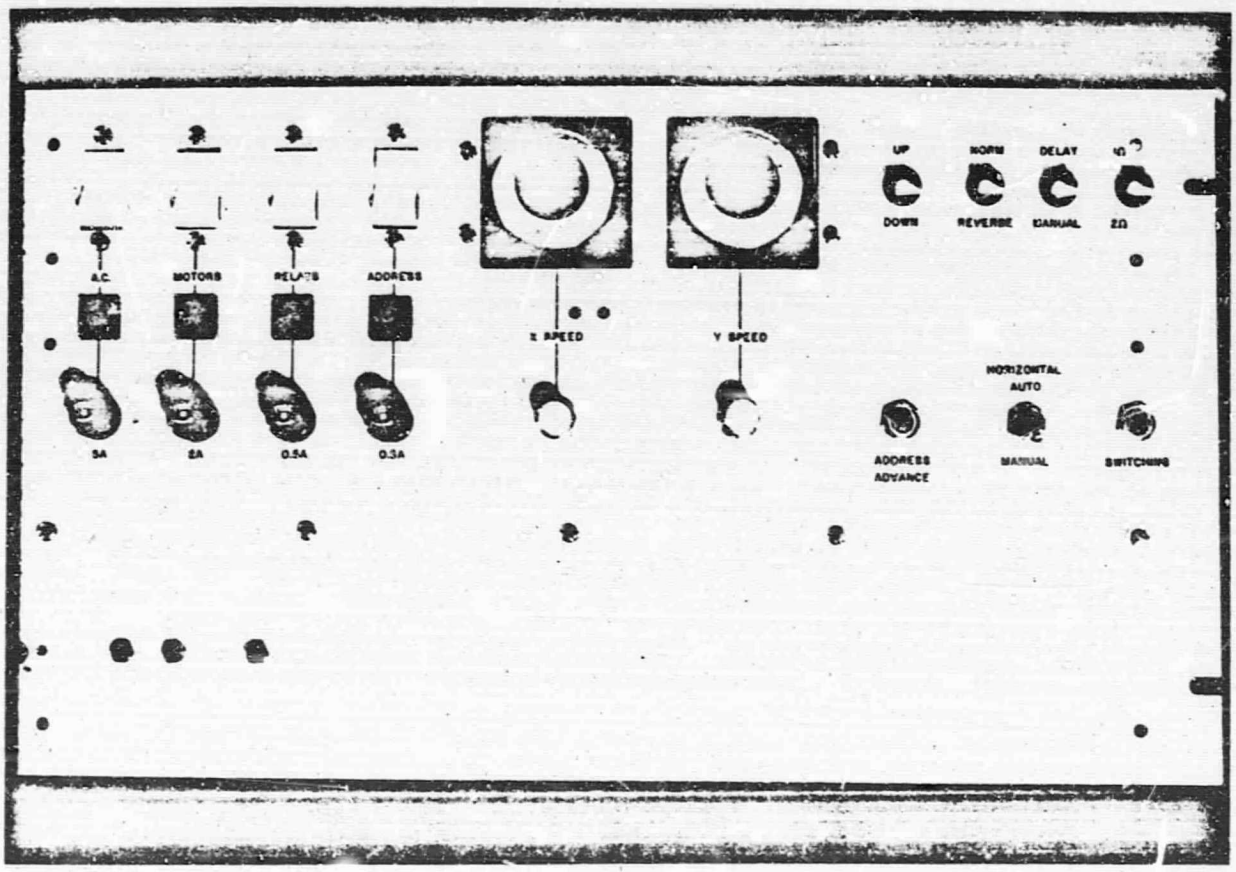
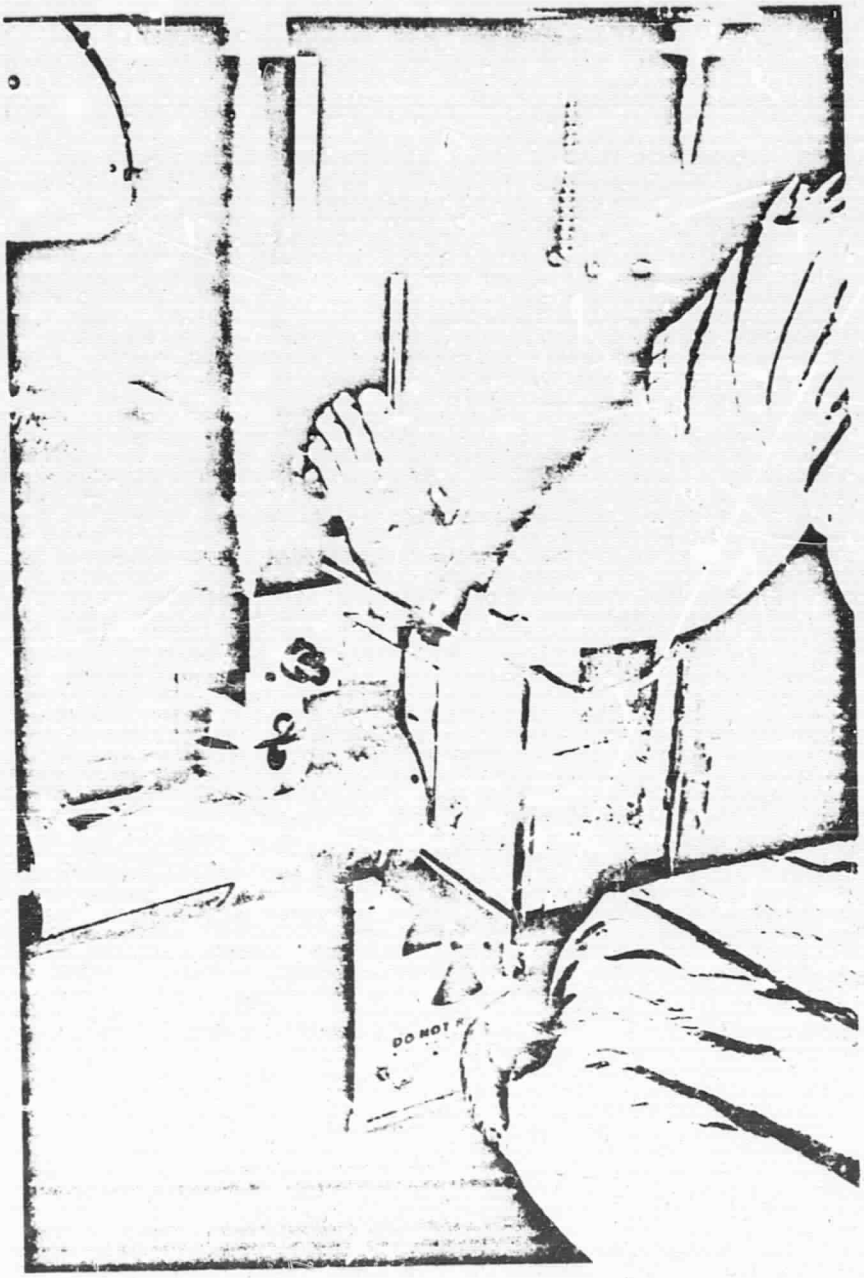

A.C.
MOTORS
RELAYS
ADDRESS
5A
2A
0.5A
0.3A
X SPEED
Y SPEED
UP
DOWN
NORM
REVERSE
DELAY
MANUAL
ADDRESS
ADVANCE
HORIZONTAL
AUTO
MANUAL
SWITCHING

EXHIBIT 11



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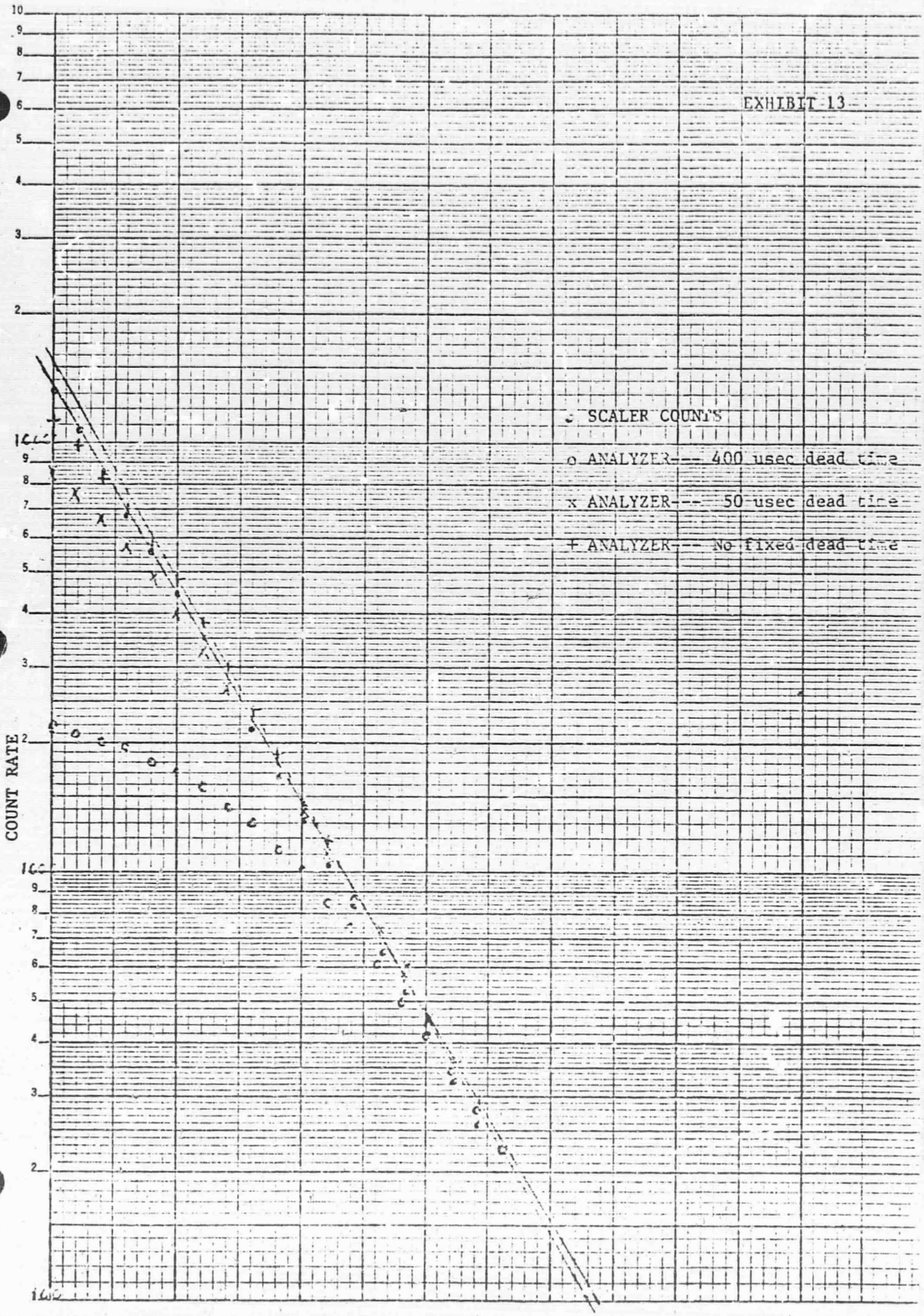
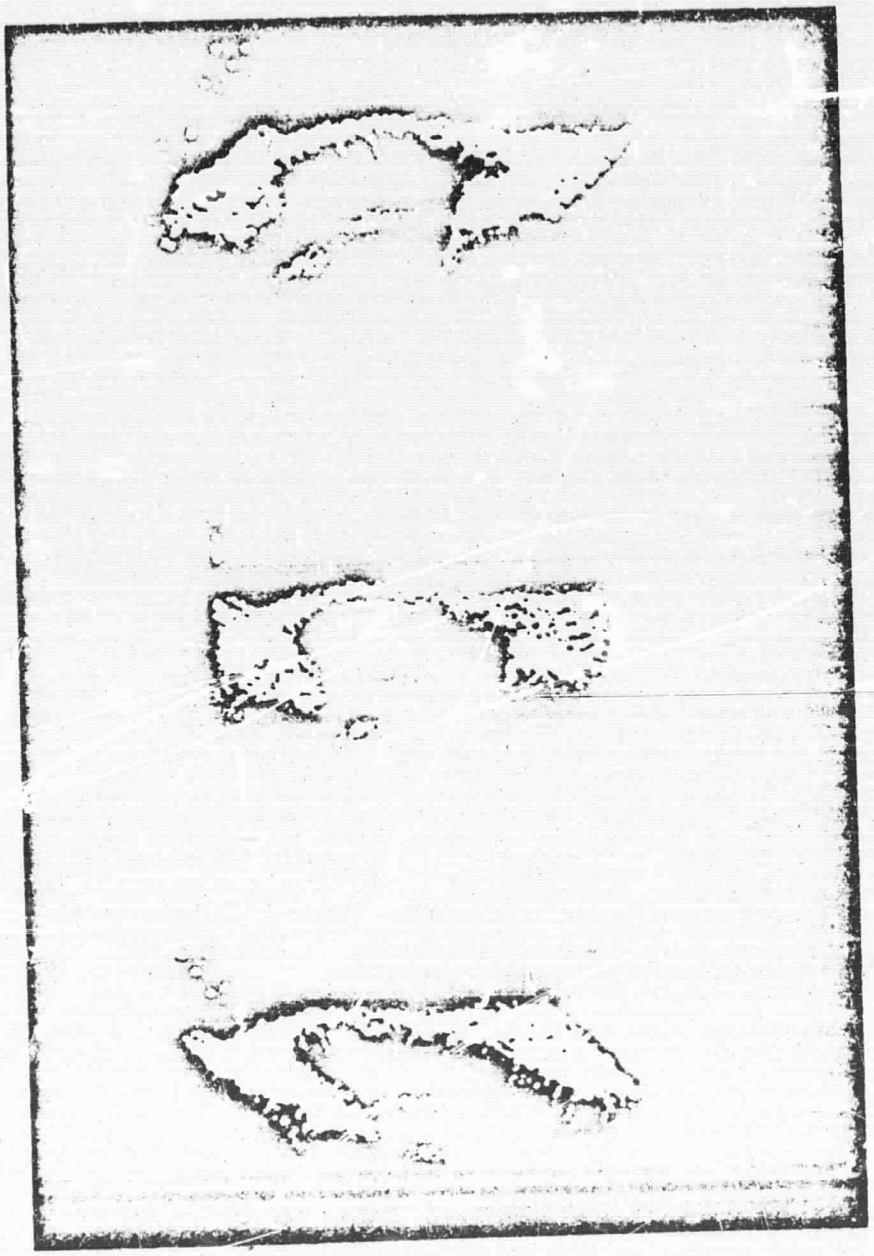


EXHIBIT 14

EXHIBIT 15



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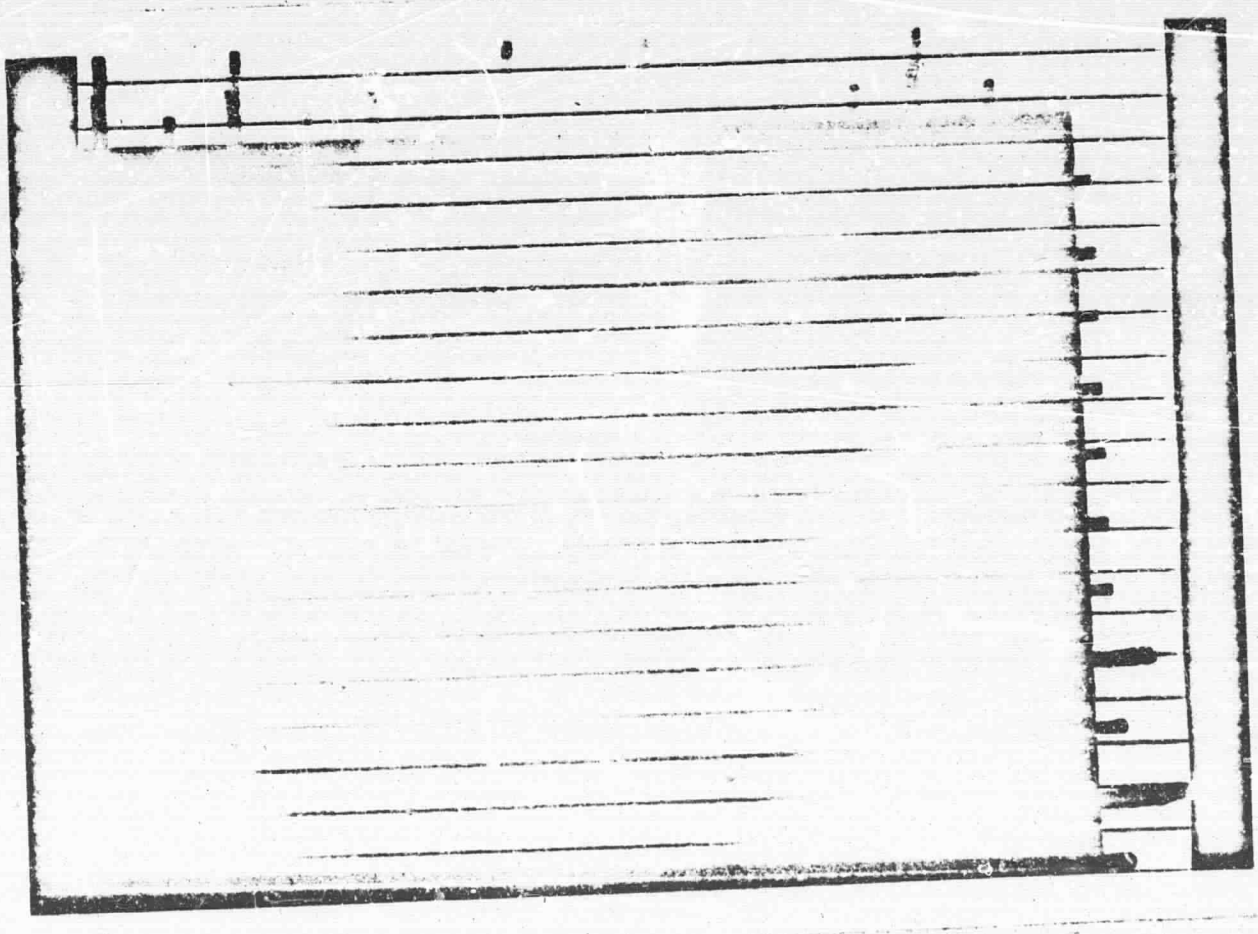

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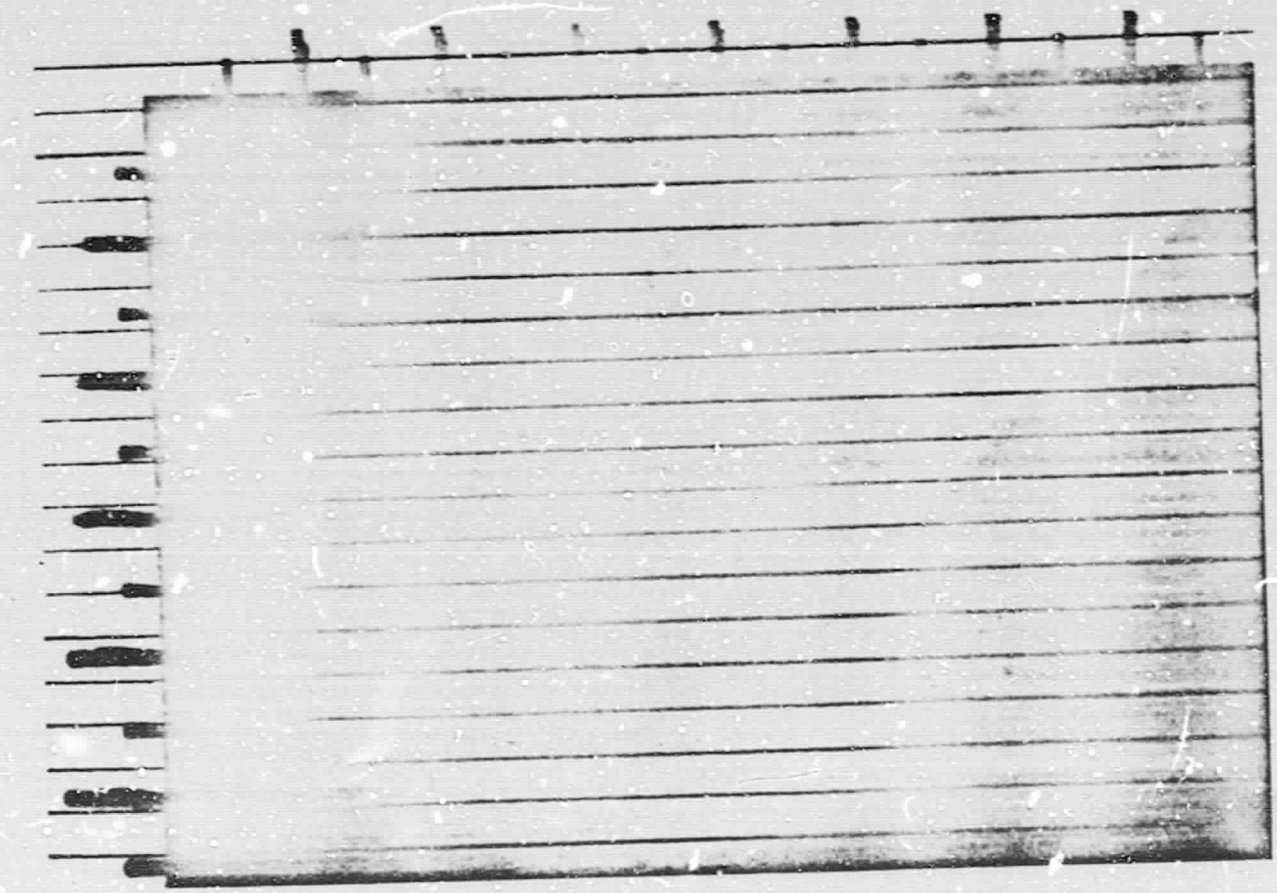
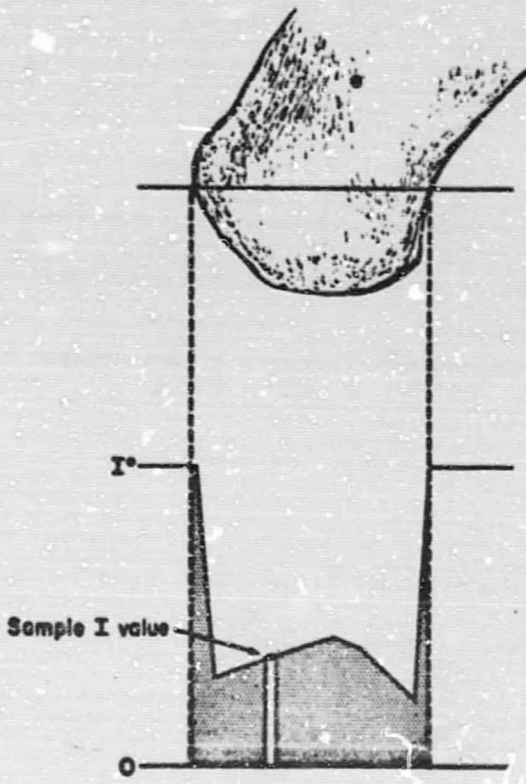


EXHIBIT 18

EXHIBIT 19



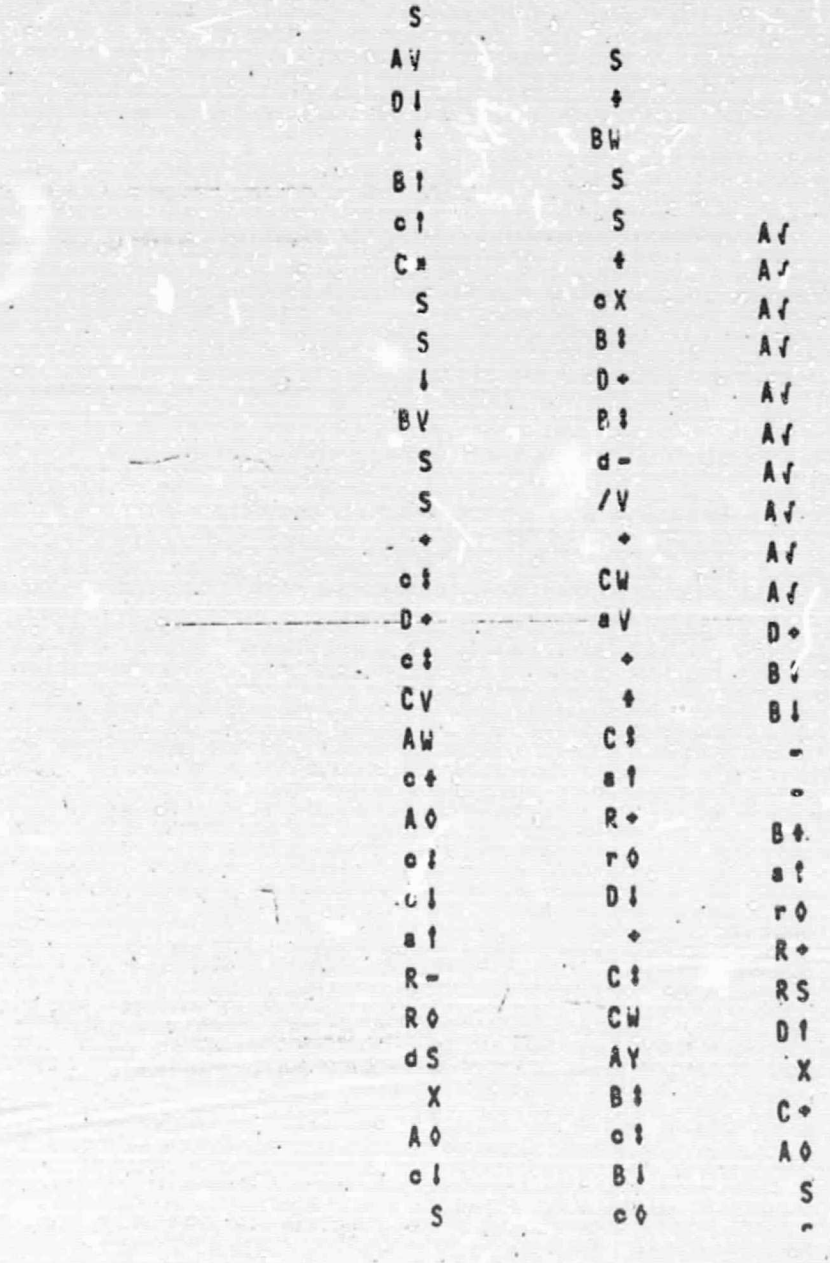
I° = 100% transmission in tissue equivalent material

I = Total counts accumulated during each 3/64" travel

Sum of ln I°/I gives value for each row

16 rows are measured

1.000000 D◊
100000000.000000 d◊



```
ØLD
ØLD FILE NAME--BØNCHN
READY
EDI LIS 100-1550
100 FILES BØNFIL
110 DIM A(261)
120 PRINT "PATIENT'S NAME";
130 INPUT B$
140 PRINT "HØW MANY RØWS DØ YØU HAVE";
150 INPUT Z
160 PRINT "WHAT IS YØUR FIRST RØW";
170 INPUT B1
180 IF Z=1 THEN 220
190 PRINT "RØWS TØ SKIP";
200 INPUT B2
210 GØ TØ 220
220 LET N=260
230 LET N1=.65
240 LET P=100
250 LET P2=.85
260 LET P1=600
270 GØ TØ 290
290 LET C=D=J=F1=F2=X=K=0
300 LET Y=B3=E1=G=M4=0
310 MAT A = ZER
320 FØR I=1 TØ N
330 READ#1,A(I)
340 NEXT I
350 IF (B2-1)>=M THEN 1490
360 IF M>0 THEN 370
370 GØ SUB 1520
380 LET W=1
390 IF W>200 THEN 580
400 FØR I=W TØ N
410 IF A(I)>P1 THEN 430
420 IF A(I)>P THEN 450
430 NEXT I
440 GØ TØ 570
450 IF A(I+5)<P THEN 760
460 LET W2=I
470 LET M1=1
480 FØR I=W2 TØ N
490 LET F=0
500 IF A(I)>P1 THEN 560
510 LET F1=F1+A(I)
520 IF A(I+1)>(.75*F1)/(M1+1) THEN 550
530 LET D=I+1
540 GØ TØ 600
550 LET M1=M1+1
560 NEXT I
570 PRINT "DATA ØUTSIDE THE P-P1 LIMIT, EDI LIST 250,270 ØR"
580 PRINT "NØ LEFT EDGE FØUND"
590 GØ TØ 1490
```

EXHIBIT 21B

```
600 IF M1>6 THEN 630
610 LET D2=D-2
620 GØ TØ 640
630 LET D2=D-5
640 LET M6=1
650 FØR J=D2 TØ W2 STEP-1
660 IF A(J)<62144 THEN 630
670 LET A(J)=A(J)-62144
680 IF A(J)<P1 THEN 700
690 GØ TØ 730
700 IF M6>=9 THEN 740
710 LET F=F+A(J)
720 LET M6=M6+1
730 NEXT J
740 LET F=F/(M6-1)
750 GØ TØ 780
760 LET W=I+5
770 GØ TØ 390
780 LET M2=W2=M3=0
790 FØR I=D+3 TØ N
800 IF A(I)/F<N1 THEN 890
810 IF A(I+5)/F<N1 THEN 890
820 IF A(I+4)/F<N1 THEN 890
830 IF A(I+3)/F<N1 THEN 890
840 IF A(I+2)/F<N1 THEN 890
850 IF A(I+1)/F<N1 THEN 890
860 LET W2=I
870 LET X=I+9
880 GØ TØ 980
890 LET M2=M2+1
900 NEXT I
910 LET M4=M4+1
920 LET W2=1
930 IF M4>1 THEN 950
940 GØ TØ 470
950 PRINT "F1=";F,N1*100;"%DRØP AT ";D;A(D),"NØ RIGHT EDGE ØR"
960 PRINT "DATA ØUTS. THE P-P1 LIMIT, EDI LIST 220&240,ØR ERRØR IN BØNFIL"
970 GØ TØ 1490  'GØ TØ NEXT SET ØF DATA
980 IF M2<12 THEN 480
990 IF M6<9 THEN 1020
1000 LET M1=7
1010 GØ TØ 1030
1020 PRINT "WARNING ØNLY";(M6-1);"F1'S"
1030 LET M7=0
1040 FØR K=X-4 TØ X+12
1050 IF A(K)<P1 THEN 1070
1060 GØ TØ 1110
1070 IF A(K)>N1*F THEN 1090
1080 GØ TØ 1140
1090 LET M7=M7+1
1100 IF M7>14 THEN 1160
1110 NEXT K
```

EXHIBIT 21C

```
1120 IF M7>0 THEN 1140
1130 GØ TØ 950
1140 IF M7>4 THEN 1160
1150 LET X=X-4
1160 LET X3=K-1
1170 LET M8=1
1180 FØR K2=X TØ X3
1190 IF A(K2)<62144 THEN 1210
1200 LET A(K2)=A(K2)-62144
1210 IF A(K2)<P1 THEN 1230
1220 GØ TØ 1260
1230 IF M8>=9 THEN 1270
1240 LET M8=M8+1
1250 LET F2=F2+A(K2)
1260 NEXT K2
1270 LET F2=F2/(M8-1)
1280 IF M8>8 THEN 1300
1290 PRINT "WARNING ØNLY";(M8-1);"F2'S"
1300 PRINT "F1=";F
1310 PRINT"F2 =";F2;
1320 LET Y=(F+F2)/2
1330 LET B3=0
1340 FØR E1=D-M1 TØ X+4
1350 IF A(E1)<62144 THEN 1370
1360 LET A(E1)=A(E1)-62144
1370 IF A(E1)/Y>P2 THEN 1410
1380 LET B9=E1-1
1390 LET G=LØG(Y/A(E1))+G
1400 LET B3=B3+1
1410 NEXT E1
1420 LET G2=G/3
1430 LET H =B3*(1/64)
1440 LET B8=B9-B3
1450 PRINT TAB(T1+1);G;
1460 LET T3=T2
1470 PRINT TAB(T3);"H ="H
1480 PRINT "Y=";Y;TAB(T1);G2;TAB(T3);"B3=";B3;B8;"-";B9
1490 LET M=M+1
1500 IF M >= Z THEN 1580
1510 GØTØ 290
1520 PRINT"------------------------------------------------------------"
1530 PRINT B$;"-";(M+B1)
1540 LET T1=20
1550 LET T2=40
1560 PRINT"I-ZERØ'S";TAB(T1);"SUM ØF LØGS";TAB(T2);"BØNE WIDTH"
1570 RETURN
1580 END

READY
```

EXHIBIT 22

REPETITIVE SCAN ---- FOOT REPOSITIONED

I_0	CHANNELS	LN I_0 / I
536.8	81 - 198	40.6185
541.2	81 - 201	40.0808
547.0	82 - 199	40.5655
536.1	83 - 199	40.3008
530.9	82 - 198	40.0942
	MEAN ± 2 S.D.	40.3319 ± 0.5066

REPETITIVE SCANS ON A NORMAL AMBULANT SUBJECT

Date		6/10	6/11	6/12	6/13	7/1	7/2	7/3
				Ln I_o^*/I (channel width)				
Row	1	24.02 (58)	24.88 (62)	22.75 (58)	25.77 (60)	25.27 (57)	26.06 (59)	14.19 (44)
	2	40.88 (84)	39.95 (89)	39.95 (79)	40.12 (79)	36.01 (73)	38.24 (83)	35.20 (77)
	3	43.71 (102)	44.29 (97)	42.86 (97)	44.62 (103)	41.02 (93)	39.45 (100)	43.07 (90)
	4	35.33 (107)	38.54 (106)	36.70 (105)	34.50 (115)	35.50 (107)	32.84 (109)	38.07 (100)
	5	30.86 (111)	34.01 (112)	33.01 (112)	32.90 (111)	30.59 (109)	30.31 (109)	33.51 (110)
	6	27.34 (107)	29.11 (107)	26.05 (101)	28.36 (100)	27.93 (105)	29.71 (107)	27.48 (105)
	7	27.35 (100)	26.77 (100)	28.12 (101)	27.38 (101)	28.75 (98)	30.81 (106)	27.48 (102)
	8	30.47 (104)	29.44 (100)	32.21 (102)	30.92 (99)	33.35 (101)	33.47 (99)	27.31 (97)
	9	32.52 (101)	31.86 (100)	33.10 (102)	32.76 (101)	32.74 (99)	31.97 (103)	32.38 (100)
	10	33.75 (102)	32.45 (100)	34.53 (101)	34.75 (100)	34.78 (102)	32.52 (100)	32.10 (100)
	11	35.12 (102)	34.06 (100)	36.15 (101)	37.68 (103)	37.13 (102)	34.45 (101)	34.25 (100)
	12	37.25 (104)	38.03 (101)	39.40 (105)	39.82 (106)	40.20 (107)	37.70 (106)	36.87 (104)
	13.	39.77 (107)	38.52 (104)	40.22 (108)	39.83 (105)	41.42 (108)	38.49 (109)	39.61 (106)
	14	40.80 (111)	40.45 (107)	41.06 (112)		36.73 (109)	38.69 (112)	40.21 (111)
	15	43.71 (116)	39.77 (113)					40.50 (113)
Total of Row 3-11		296.48	300.53	302.73	303.87	301.79	295.53	295.75
Total of Row 4-12		289.99	294.27	299.27	299.07	294.20	293.78	289.58

Mean ± S.D. Row 3-11 . 299.53 ± 1.17% Row 4-12 295.28 ± 1.55%

EXHIBIT 23

EXHIBIT 24

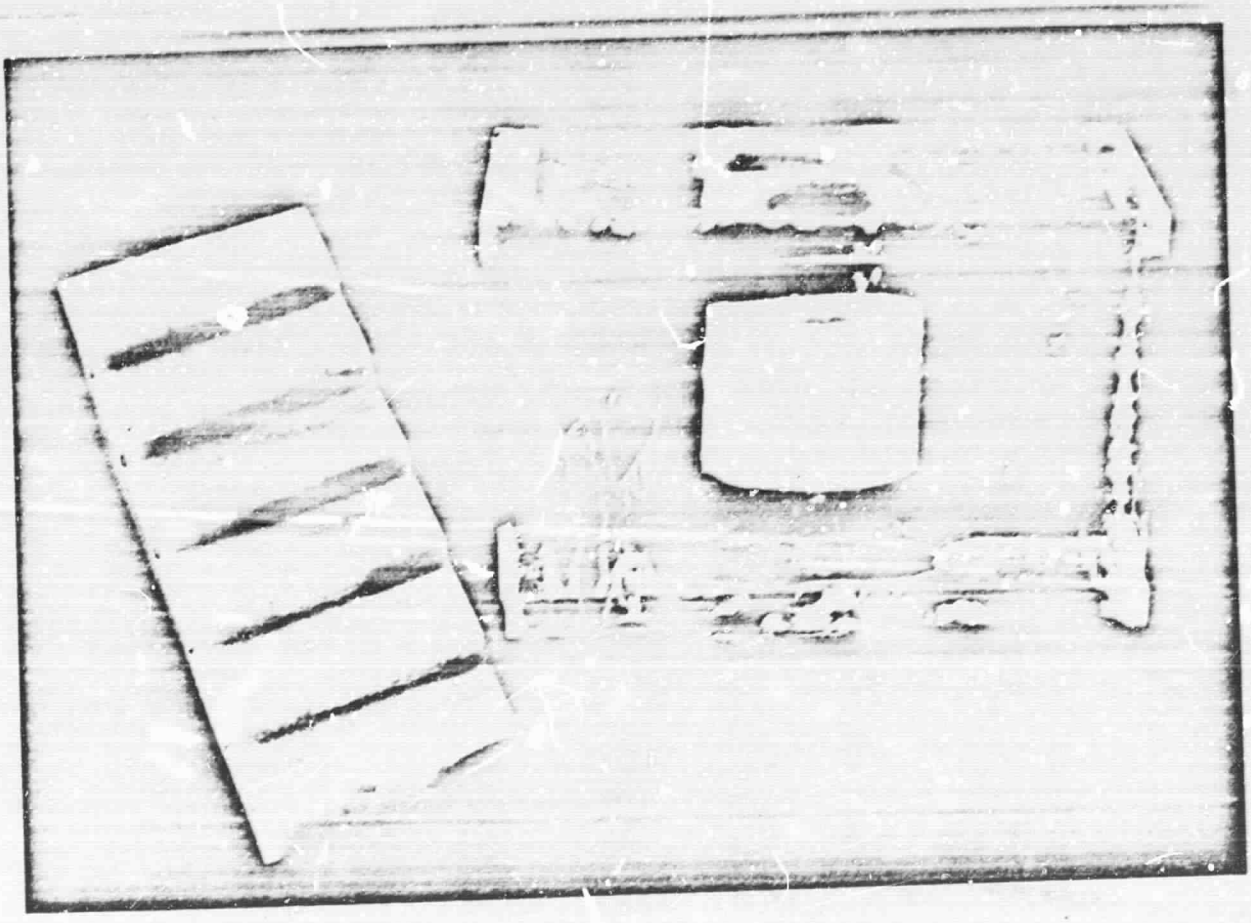


EXHIBIT 25

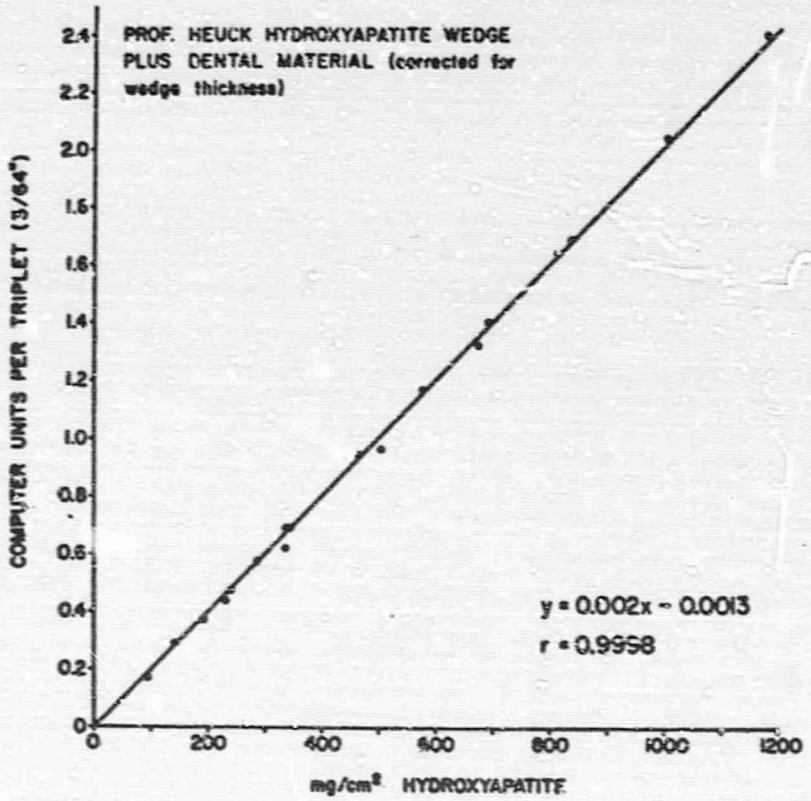

PROF. HEUCK HYDROXYAPATITE WEDGE
PLUS DENTAL MATERIAL (corrected for
wedge thickness)
COMPUTER UNITS PER TRIPLET (3/64")
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
0
200
400
600
800
1000
1200
mg/cm² HYDROXYAPATITE
y = 0.002x - 0.0013
r = 0.9998

EXHIBIT 26

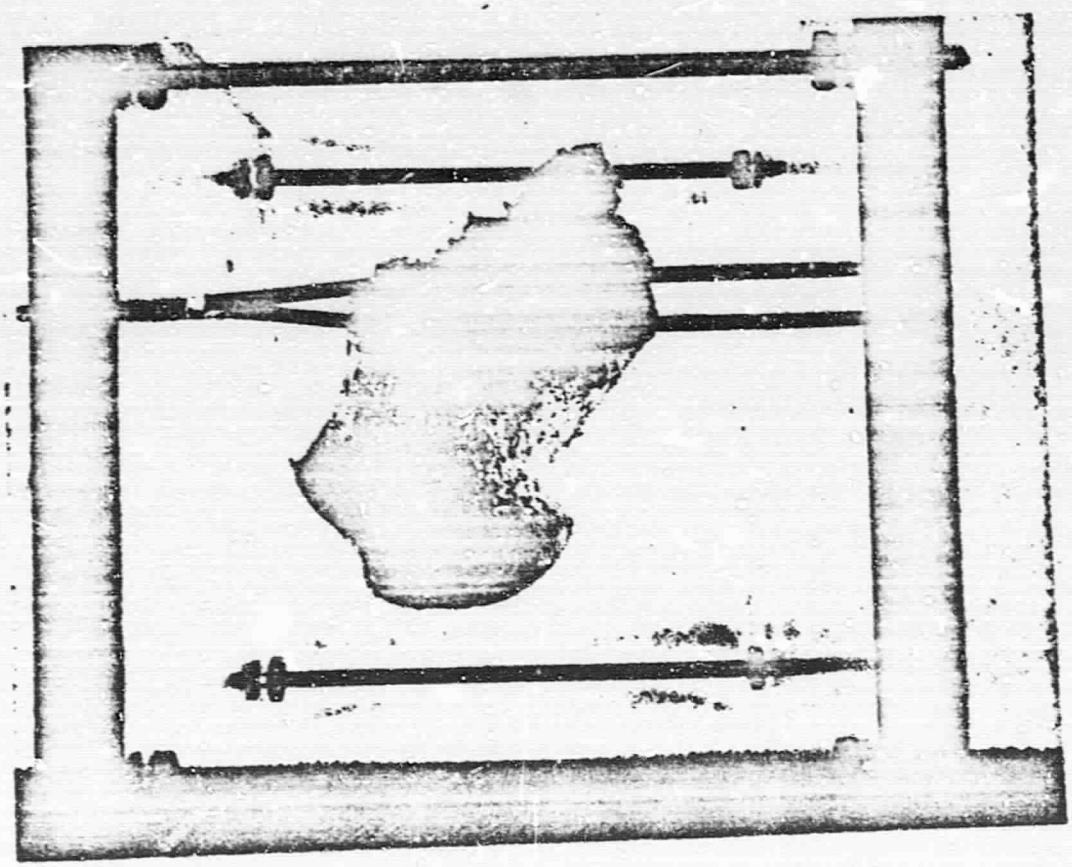


EXHIBIT 27

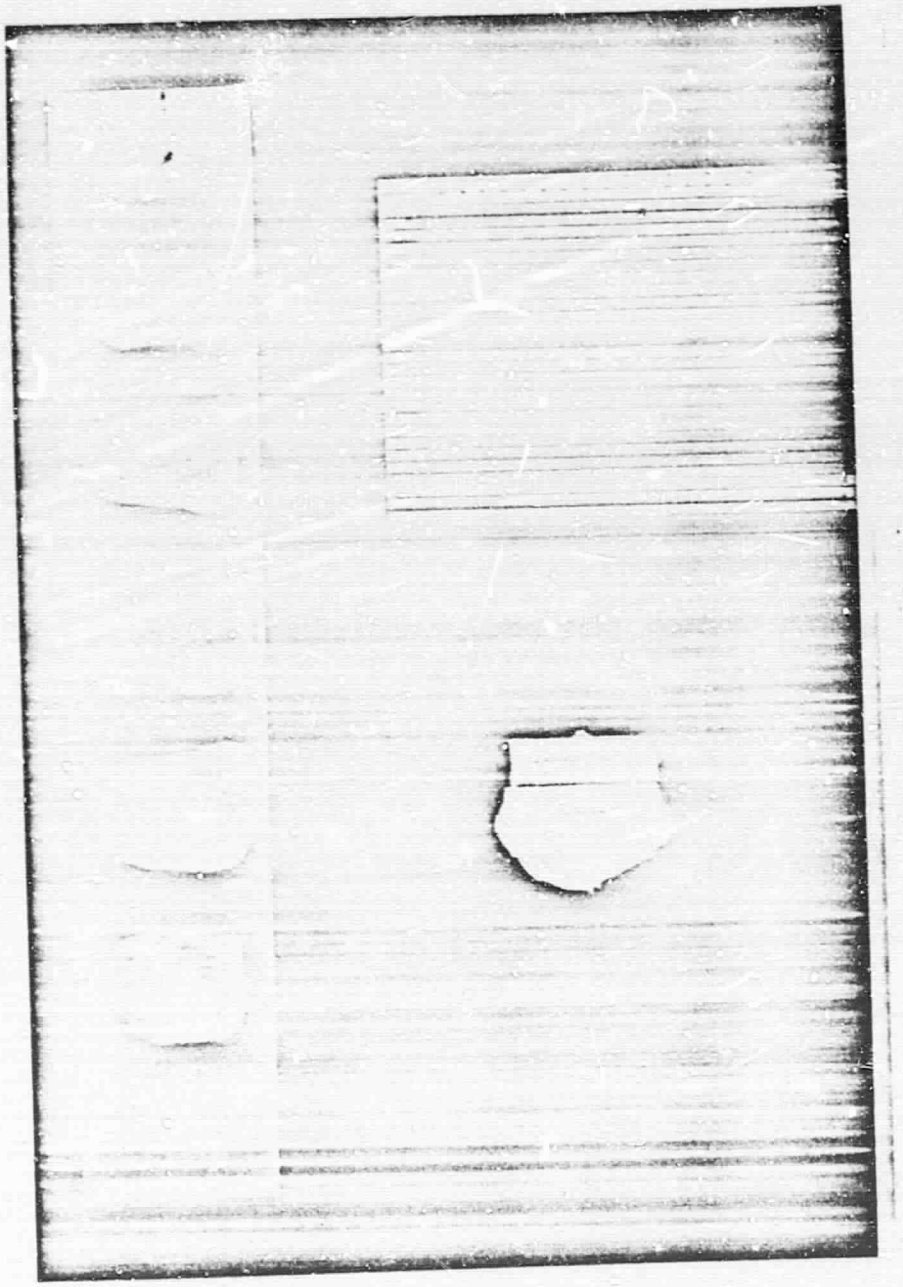


EXHIBIT 28

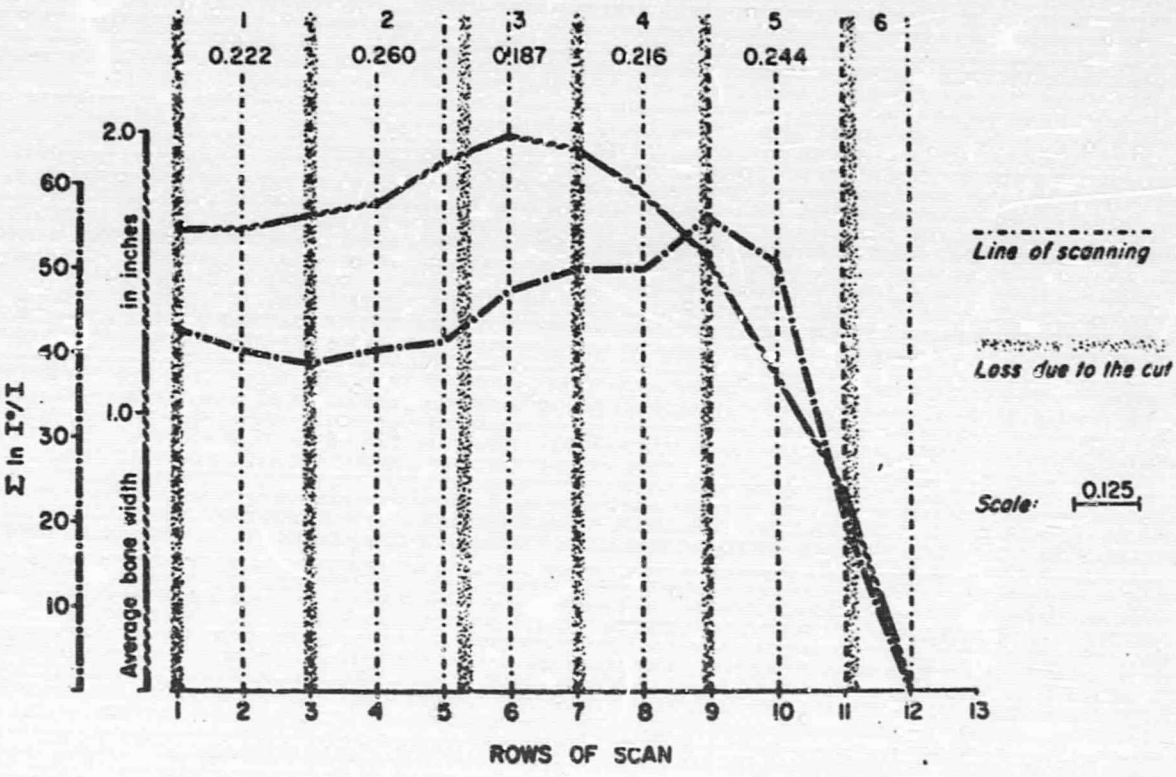

1
0.222
2
0.260
3
0.187
4
0.216
5
0.244
6
2.0
1.0
Average bone width in inches
60
50
40
30
20
10
Σ ln I°/I
1 2 3 4 5 6 7 8 9 10 11 12 13
ROWS OF SCAN
Line of scanning
Loss due to the cut
Scale: 0.125

EXHIBIT 29

Calcium and Phosphorus Assay

Sample	Total ash	mg Ca/ gm.	mg PO_4/ gm.	Ca / PO_4
1	1.6205	385.1	520.0	1.75
2	1.7322	372.8	517.1	1.70
3	1.9806	375.3	524.8	1.69
4	1.6537	378.9	533.3	1.68
5	2.0953	376.1	523.7	1.70
6	2.2393	373.6	522.4	1.69

EXHIBIT 30 A

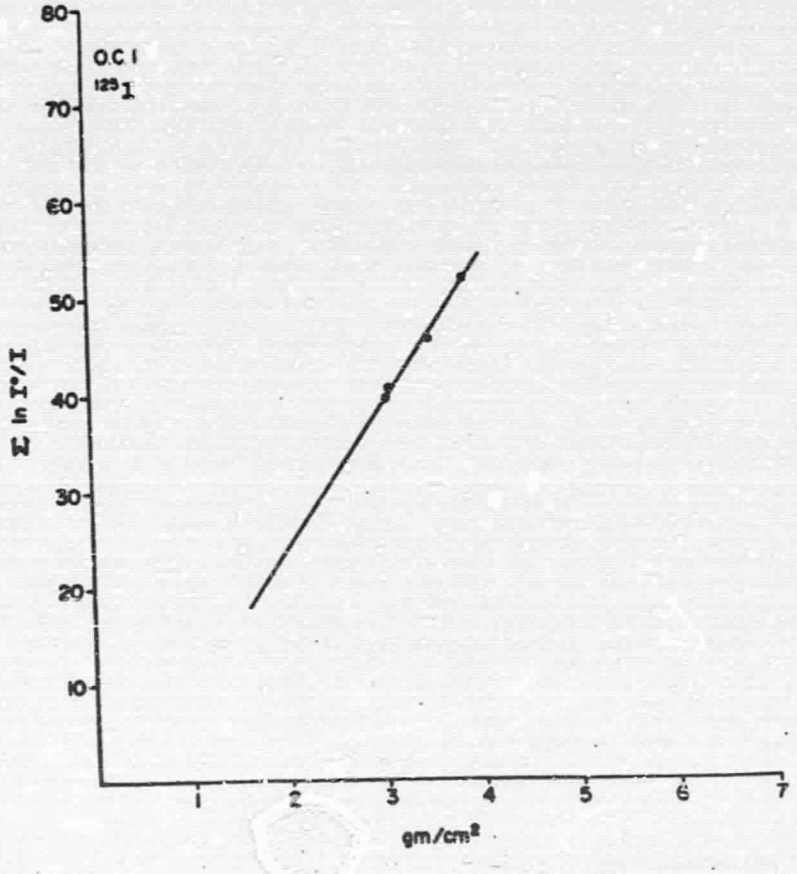


EXHIBIT 30 B

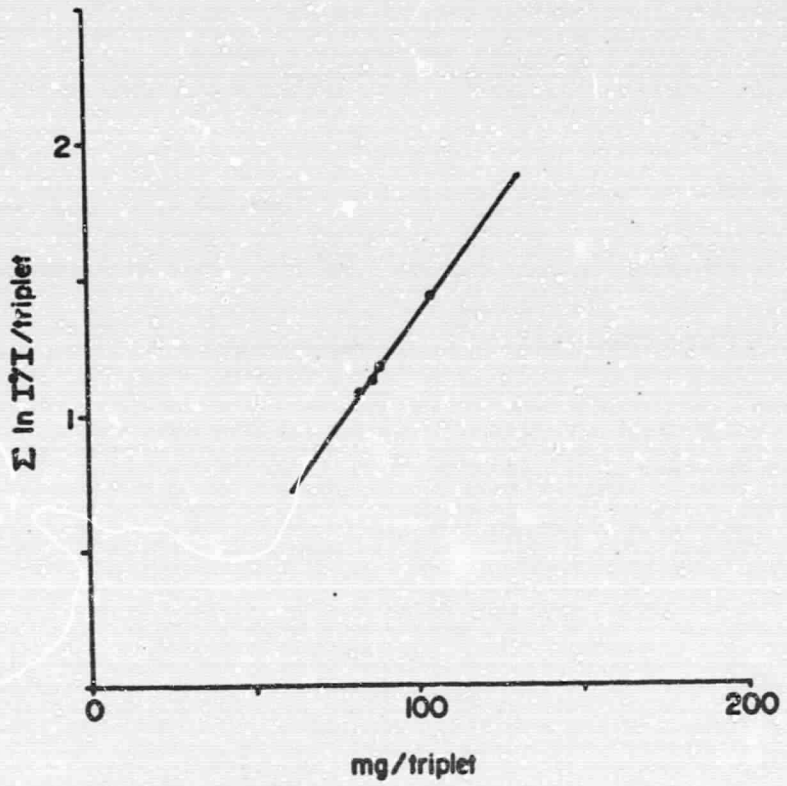
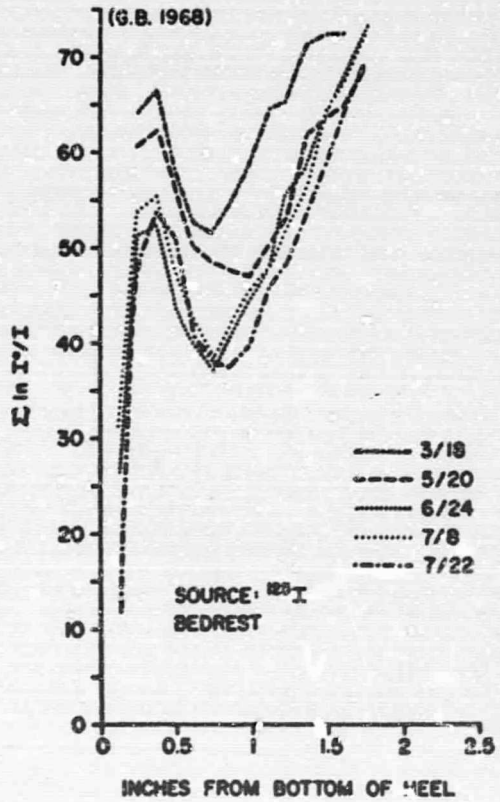


EXHIBIT 31


(G.B. 1968)
Σ ln I'/I
70
60
50
40
30
20
10
0
0
0.5
1
1.5
2
2.5
INCHES FROM BOTTOM OF HEEL
3/18
5/20
6/24
7/8
7/22
SOURCE: 125I
BEDREST

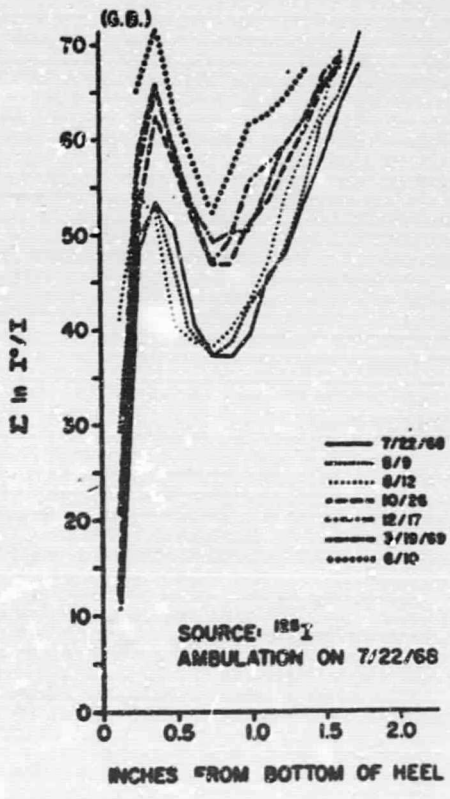
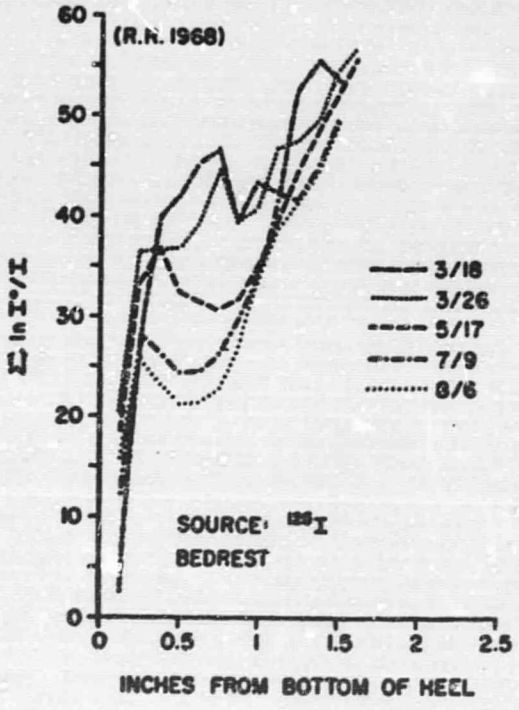

(G.B.)
Σ ln I'/I
70
60
50
40
30
20
10
0
0
0.5
1.0
1.5
2.0
INCHES FROM BOTTOM OF HEEL
7/22/68
8/9
8/12
10/26
12/17
3/19/69
6/10
SOURCE: 125I
AMBULATION ON 7/22/68

EXHIBIT 32


(R.R. 1968)
Σ ln I°/I
60
50
40
30
20
10
0
0
0.5
1
1.5
2
2.5
INCHES FROM BOTTOM OF HEEL
3/18
3/26
5/17
7/9
8/6
SOURCE: 125I
BEDREST

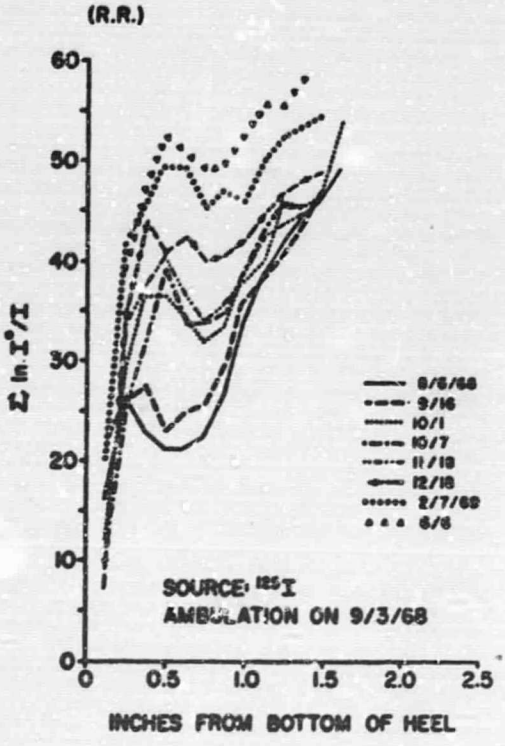
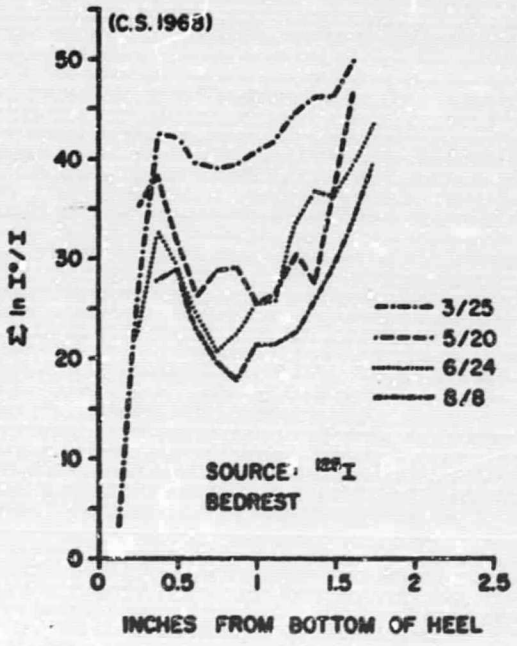
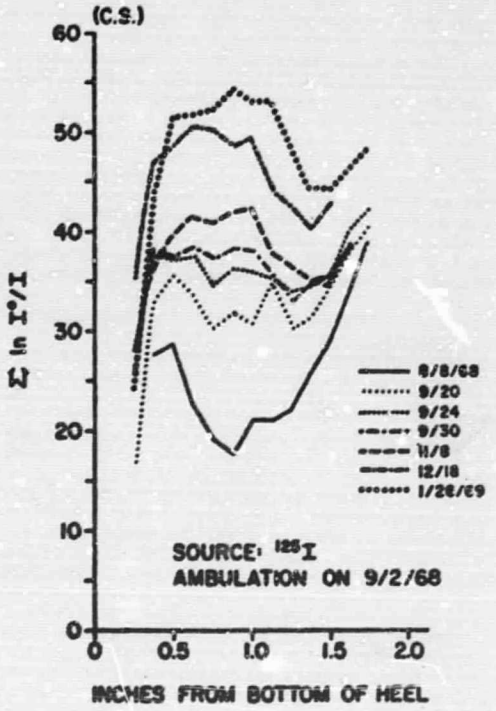

(R.R.)
Σ ln I°/I
60
50
40
30
20
10
0
0
0.5
1.0
1.5
2.0
2.5
INCHES FROM BOTTOM OF HEEL
9/16
10/1
10/7
11/18
12/18
2/7/69
8/6
SOURCE: 125I
AMBULATION ON 9/3/68

EXHIBIT 33


(C.S. 1968)
50
40
30
20
10
0
0
0.5
1
1.5
2
2.5
INCHES FROM BOTTOM OF HEEL
3/25
5/20
6/24
8/8
SOURCE: 125I
BEDREST


(C.S.)
60
50
40
30
20
10
0
0
0.5
1.0
1.5
2.0
INCHES FROM BOTTOM OF HEEL
8/8/68
9/20
9/24
9/30
11/8
12/18
1/26/69
SOURCE: 125I
AMBULATION ON 9/2/68

G.B.

BED REST PERIOD

Individual row values expressed as computer units *.

Weeks	12	21	26	28	30
Row 1/8"			26.7	31.1	11.2
1/4"	64.1	60.7	51.2	53.7	48.1
3/8"	66.5	62.1	52.4	55.6	53.4
1/2"	58.2	56.1	43.8	47.3	50.7
5/8"	52.8	50.2	39.9	42.3	40.5
3/4"	51.5	48.2	37.0	38.4	37.5
7/8"	54.4	47.3	40.9	42.1	37.2
1 "	58.7	47.0	44.7	45.8	39.8
1 1/8"	64.3	50.1	48.0	48.2	45.6
1 1/4"	65.2	53.0	55.4	52.0	48.3
1 3/8"	70.3	62.0	58.0	56.4	53.5
1 1/2"	72.8	63.6	63.4	64.7	58.5
1 5/8"	72.8	64.9	68.0	67.7	64.0
1 3/4"		68.4	77.3	73.2	68.2

CENTRAL OS CALCIS

Σ 3/8"- 1 3/8"	541.9	476.0	420.1	428.1	406.5
% initial value	100.0	87.6	77.3	78.8	74.8
% below projected baseline	- 2.1	-14.4	-24.4	-23.0	-26.9

* Computer units = $\Sigma \text{ Ln } I_o^*/I$

EXHIBIT 35

G.B.

AMBULANT PERIOD

Individual row values expressed as computer units *.

Weeks		2	3	13	21	34	46
Row	1/8"	29.2	41.0	12.4	10.9	20.6	
	1/4"	49.5	54.0	52.4	56.5	57.1	65.0
	3/8"	52.8	51.6	62.5	65.8	66.0	71.1
	1/2"	46.0	40.3	57.3	58.8	59.0	62.3
	5/8"	39.3	38.8	51.3	51.5	52.7	57.6
	3/4"	37.2	38.2	46.8	47.0	49.4	52.0
	7/8"	38.7	40.1	46.9	49.7	50.3	56.4
	1 "	42.8	43.1	51.3	55.7	50.5	61.3
	1 1/8"	45.4	47.3	53.9	57.4	55.5	62.9
	1 1/4"	49.2	54.2	57.6	59.6	59.9	64.8
	1 3/8"	56.1	59.1	61.3	62.2	62.4	67.2
	1 1/2"	62.6	63.9	65.7	67.2	65.4	
	1 5/8"	65.1	69.9	67.8	68.5	68.5	
	1 3/4"	71.4					
CENTRAL OS CALCIS							
	Σ 3/8" - 1 3/8"	407.5	412.7	488.9	507.7	510.7	556.1
	% initial value	75.2	76.2	90.2	93.7	94.2	102.6
	% below projected baseline	-26.7	-25.7	-12.0	- 8.7	- 8.1	0

* Computer Units = $\Sigma \ln I_O^*/I$

EXHIBIT 36

R.R.

BED REST PERIOD

Individual row values expressed as computer units *.

Weeks		12	13	20	24	28	31	32
Row	1/8"	2.3	20.6	18.9	20.9	12.4	20.5	16.1
	1/4"	27.6	36.1	33.5	31.3	27.9	29.3	25.8
	3/8"	39.9	36.7	36.8	29.4	26.3	23.9	23.0
	1/2"	42.0	36.9	32.4	27.9	24.3	22.2	21.1
	5/8"	45.1	39.1	31.7	24.7	24.6	20.4	21.1
	3/4"	46.2	44.5	30.7	27.0	26.3	22.5	22.5
	7/8"	39.6	39.1	31.8	26.1	29.3	28.1	26.9
	1 "	43.3	40.7	35.0	32.6	34.5	33.2	33.5
	1 1/8"	41.9	46.8	39.6	33.8	42.6	40.5	38.9
	1 1/4"	52.8	47.3	44.8	42.0	41.6	43.2	41.8
	1 3/8"	55.3	49.4	48.8	42.2	44.9	46.8	44.5
	1 1/2"	53.3	54.4	52.0	46.2	49.6	47.4	46.1
	1 5/8"		56.4	55.3	46.1		47.9	49.3
	1 3/4"		54.3	56.3				

CENTRAL OS CALCIS

Σ 3/8"- 1 3/8"	406.1	380.5	331.6	285.7	294.4	280.8	273.3
% initial value	100.0	93.6	81.6	70.3	72.4	69.1	67.2
% below projected baseline	- 4.6	-11.5	-22.9	-33.5	-31.5	-34.7	-36.7

* Computer Units = $\Sigma \ln I_o^*/I$

EXHIBIT 37

R.R.

AMBULANT PERIOD

Individual row values expressed as computer units *.

Weeks		2	3	4	5	11	15	18	22	30	39
Row	1/8"	22.3	24.5	11.9	11.1	15.5	7.5	3.1	19.4	29.3	15.6
	1/4"	25.8	33.7	29.7	26.4	33.4	35.0	30.0	41.7	45.5	39.0
	3/8"	27.1	33.1	36.3	31.9	37.2	43.8	47.4	45.3	44.9	47.2
	1/2"	23.0	30.0	36.5	39.0	40.1	41.8	47.5	49.5	43.1	52.2
	5/8"	24.9	30.0	34.4	33.7	36.3	42.2	49.1	49.1	45.2	48.7
	3/4"	25.8	29.5	31.9	33.7	33.5	40.0	46.4	45.1	45.7	48.9
	7/8"	29.6	33.2	33.5	34.6	35.3	40.4	43.3	47.0	47.5	49.5
	1 "	36.0	39.7	37.9	38.8	37.9	41.9	44.8	46.0	50.5	52.7
	1 1/8"	38.3	43.5	39.9	43.1	42.6	44.6	46.7	49.9	51.4	55.7
	1 1/4"	40.7	47.0	46.0	45.6	43.5	46.8	50.3	52.4	51.9	55.3
	1 3/8"	43.4	48.1	45.3	45.2	44.2	48.0	49.2	48.2	53.3	58.1
	1 1/2"	47.0	52.2	46.8		44.5	48.6		54.2		
	1 5/8"			54.0		47.5					

CENTRAL OS CALCIS

Σ 3/8"- 1 3/8"	288.8	334.1	341.7	345.6	350.6	389.5	424.7	432.5	433.5	468.3
% initial value	71.0	82.2	84.1	85.0	86.2	95.8	104.5	106.4	106.6	115.2
% below projected baseline	-32.8	-22.3	-20.5	-19.6	-18.5	- 9.4	- 1.2	+ 0.5	+0.7	+ 8.8

* Computer Units $= \Sigma \ln I_o^*/I$

EXHIBIT 38

C.S.

BED REST PERIOD

Individual row values expressed as computer units *.

Weeks		14	20	27	28	32
Row	1/8"	26.3	35.1	22.3	15.3	11.3
	1/4"	42.6	38.4	31.8	31.7	27.9
	3/8"	42.1	31.8	29.2	29.7	29.4
	1/2"	39.8	26.2	24.0	26.8	22.8
	5/8"	39.0	28.8	20.8	21.0	19.6
	3/4"	39.2	29.1	22.4	21.3	17.9
	7/8"	40.8	25.6	25.5	23.0	21.3
	1 "	41.6	26.8	25.9	27.0	21.4
	1 1/8"	44.1	30.5	33.3	25.5	22.7
	1 1/4"	46.0	27.3	36.9	29.3	26.0
	1 3/8"	46.1	37.1	36.5	32.5	29.7
	1 1/2"	49.7	46.4	39.7	34.6	34.0
	1 5/8"			43.3	40.6	39.1

CENTRAL OS CALCIS

Σ 3/8"- 1 3/8"	378.7	263.2	254.5	236.1	210.8
% initial value	100.0	69.5	67.2	62.3	55.7
% below projected baseline	- 8.3	-41.9	-43.8	-47.9	-53.5

* Computer Units = $\Sigma \ln I_o^*/I$

EXHIBIT 39

C.S.

AMBULANT PERIOD

Individual row values expressed as computer units *.

Weeks		2	3	4	9	15	21
Row	1/8"	16.8	28.3	25.9	28.1	35.4	24.1
	1/4"	33.3	37.7	38.0	36.7	47.0	44.3
	3/8"	35.9	37.0	37.1	39.2	48.8	51.4
	1/2"	33.8	37.6	38.8	41.5	50.4	51.9
	5/8"	30.1	34.7	37.2	40.9	50.2	52.6
	3/4"	31.9	36.4	38.4	42.0	48.7	54.2
	7/8"	30.8	36.0	38.0	42.2	49.6	52.9
	1	35.0	35.6	35.3	38.0	44.0	53.2
	1 1/8"	30.0	34.0	33.0	36.6	42.7	48.7
	1 1/4"	31.5	34.4	34.3	35.3	40.2	44.6
	1 3/8"	34.5	35.6	34.5	35.6	43.0	44.1
	1 1/2"	38.2	40.3	38.4	38.1		46.3
	1 5/8"	40.6	42.3				48.4
	CENTRAL OS CALCIS						
Σ 3/8"- 1 3/8"		293.5	321.3	326.6	351.3	417.6	453.6
% initial value		77.5	84.8	86.2	92.7	110.2	119.8
% below projected baseline		-35.2	-29.1	-27.9	-22.5	-7.9	0

* Computer Units = $\Sigma \ln I_o^*/I$

EXHIBIT 40

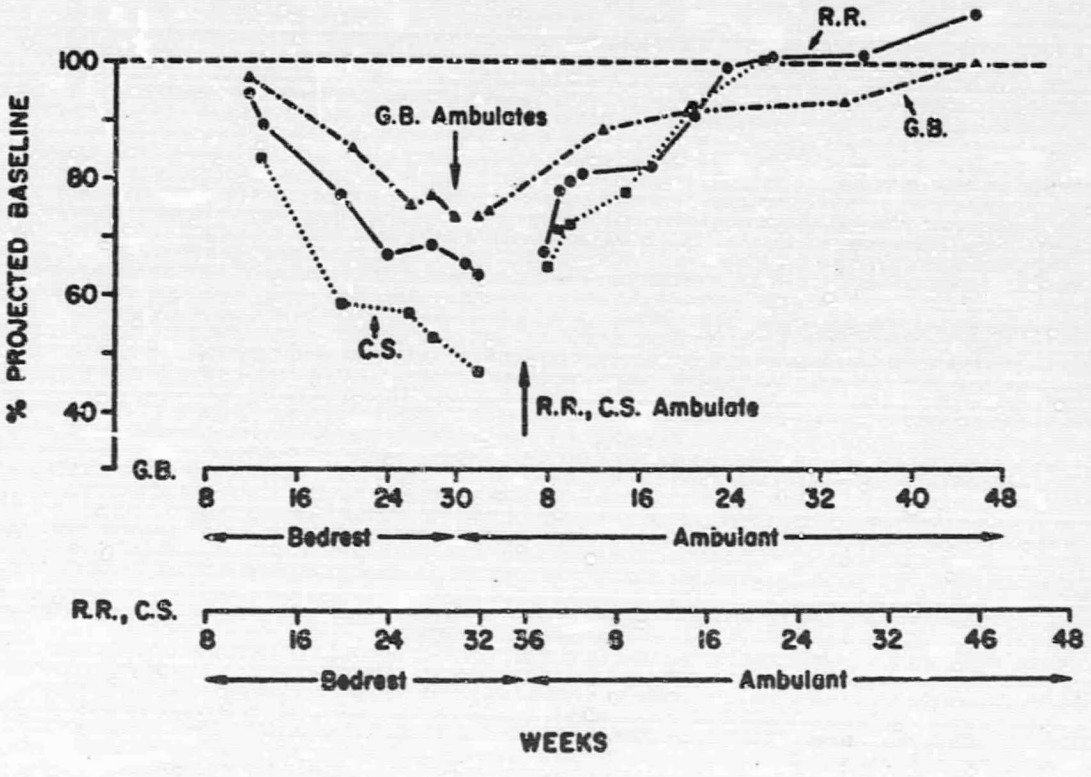

R.R.
100
G.B. Ambulates
G.B.
80
60
C.S.
40
% PROJECTED BASELINE
R.R., C.S. Ambulate
G.B.
8 16 24 30 8 16 24 32 40 48
Bedrest
Ambulant
R.R., C.S.
8 16 24 32 36 8 16 24 32 46 48
Bedrest
Ambulant
WEEKS

EXHIBIT 41

MEAN % CHANGE FOR ALL SUBJECTS *

Bed Rest		Ambulation	
G.B. 12 to 30 wk.	-25.1 %	0 to 46 wk.	+27.2 %
R.R. 12 to 32 wk.	-33.3 %	0 to 30 wk.	+37.9 %
C.S. 14 to 32 wk.	-44.5 %	0 to 21 wk.	+52.8 %

* For 9 rows 3/8" to 1-3/8" inclusive.

EXHIBIT 42

G.B.

% OF BONE MINERAL CHANGE BY ROW

	Bed Rest Period 12th wk. to 30th wk.*	Ambulation Period 0 to 46th week**
3/8"	-19.6%	+ 24.9%
1/2"	-12.9	+ 18.7
5/8"	-23.3	+ 29.7
3/4"	-27.2	+ 27.9
7/8"	-31.7	+ 34.1
1"	-32.2	+ 35.6
1 1/8"	-29.1	+ 27.6
1 1/4"	-26.0	+ 25.5
1 3/8"	-23.9	+ 20.4
Mean	-25.1%	+ 52.8%

Δ = 2.1 %

* % of 12 th week of bed rest.

** % of 46th week of ambulation.

EXHIBIT 43

R.R.

% OF BONE MINERAL CHANGE BY ROW

	Bed Rest Period	Ambulation Period
	12th to 32nd week*.	0 to 30th week.**
3/8"	- 42.4%	+ 48.7
1/2"	- 50.0	+ 51.0
5/8"	- 53.3	+ 53.3
3/4"	- 51.3	+ 50.7
7/8"	- 32.1	+ 43.3
1"	- 22.7	+ 33.6
1 1/8"	- 7.2	+ 24.3
1 1/4"	- 20.9	+ 19.4
1 3/8"	- 19.6	+ 16.5
Mean	- 33.3%	+ 37.9%

Δ = 4.6%

* % of 12th week of bed rest.

** % of 30th week of ambulation.

EXHIBIT 44

C.S.

% OF BONE MINERAL CHANGE BY ROW

	Bed Rest Period 14th wk. to 32nd wk.*	Ambulation Period 0 to 21st week**
3/8"	-30.1%	+ 42.8%
1/2"	-42.7	+ 56.0
5/8"	-49.7	+ 62.7
3/4"	-54.3	+ 66.9
7/8"	-47.7	+ 59.7
1"	-48.5	+ 59.7
1 1/8"	-48.5	+ 53.3
1 1/4"	-43.4	+ 41.7
1 3/8"	-35.5	+ 32.6
Mean	-44.5%	+ 52.8%

Δ = 8.3%

*% of 14th week of bed rest

**% of 21st week of ambulation