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ANALYSIS OF BODY CALICIUM

(REGIONAL CHANGES IN BODY CALCIUM BY IN VIVO NEUTRON ACTIVATION ANALYSIS)

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INTRODUCTION

A major medical concern for future long duration space flights and even repeated short missions is the alterations in bone metabolism which are expected to occur. The effect of space flight on urine and fecal calcium loss was documented during the three lone-term Skylab flights.

Within one day following insertion of Skylab crewmembers into the weightlessness of orbital flight, the quantity of calcium appearing in the urine began to increase. Within ten days the positive calcium balances which prevailed preflight were abolished and the body as a whole began to lose calcium. The loss was slow at first, amounting to about 50mg per day at 10 days, and then increased dramatically to almost 300mg per day by the 84th day of flight. Examination of the data reveals that at the end of 84 days in flight, the average Skylab crewmember had lost approximately 25g of calcium from his overall pool of the element. From this it is evident that approximately 2.5% of total body calcium was depleted in this period of time.

Similar results but of lesser magnitude have been documented in bedrested subjects. The cause for this loss and its ultimate extent is not known. The bones from which this loss is occuring are also not known. It is probable that certain bones will lose calcium more rapidly than others. For this reason it is important to have techniques which complement balance measurements; techniques which can accurately and nonivasively determine regional calcium loss. Neutron activation analysis is one such technique.

Neutron activation has several advantages when compared to the other non-invasive techniques for skeletal analysis. It is much more precise than standard radiographic procedures which require Ca losses on the order of 30% to be detected. Gamma-ray absorpitiometry is a precise technique but it is normally limited to a few smaller regions of bone (commonly the os calcis or the mid-shaft of the radius) which may be neither typical of the skeleton nor a region of particular interest. When

compared to total body neutron activation, partial body activation has the advantage of technical ease, and therefore lower cost. It also allows the skeletal regions where large changes are expected to be selectively measured.

The objective of this work was to 1) investigate various designs for regional neutron activation analysis; 2) based on the results of the various methods available construct a factilty which could be used for both animal and human research purposes; and 3) support various research projects outlined by NASA investigating calcium loss and counter measures related to space flight.

FEASIBILITY AND DESIGN

After it was determined that we wished to measure Ca in vivo by neutron activation the next task was to examine its feasibility. The first part of this examination was a search of the literature. Two major design decisions were required at an early stage, 1) the type of neutron source and 2) the type of detector system.

The activation reactions that we were interested in using in this project were capture of neutrons of thermal ($\frac{1}{40}$ eV) energy, particularly Ca-48 (n,γ) Ca-49. In order to obtain reasonably uniform irradiation throughout a sample more than a few cm thick it is necessary to use higher energy neutrons and have them moderate to thermal energies within the sample. As the neutron energy increases, the uniformity of thermal flux increases but so does the radiation exposure per unit thermal flux. When selecting a source for in-vivo activation there is a trade off between these two factors.

Among the neutron sources that have been used for in-vivo activation are nuclear reactors (external ports) and cyclotrons. These were ruled out without serious consideration due to the great expense of either. The sources that were considered were neutron generators, α -Be sources and Cf-252.

Neutron generagors are low energy linear accelerators that create neutrons by the reaction ^{2}H (^{3}H, ^{4}He)n. The reaction is exothermic (16MeV) so that the deuterium ions need only be accelerated sufficiently to overcome the coulomb barrier. The accelerator is only a few feet in length and the entire device could be contained in a room of normal size. The neutrons produced are monoenergetic with an energy of 14MeV. The α-Be sources produce neutrons from the reaction $\alpha(^{9}Be, ^{12}C)n$. A variety of radioactive isotopes have been used as the source of α particles, among the most common are Ra-226, Pu-238, and Am-241. The neutron energy spectrum is typically fairly flat with a mean energy of about 5MeV and a maximum of about 10 MeV. The detailed structure of the spectrum will depend on the particular

α emmitting isotope used.

Californium (Cf-252) produces neutrons by spontaneous fission. The neutrons have a typical fission spectrum distribution ($N(E)=E^{\frac{1}{2}}e^{-E/1.3}$) with a mean energy of 2.3MeV. The half life of C^{f}-252 is 2.6 years.

Each of these possible sources had advantages and disadvantages, but the one significant disadvantage of Cf-252 (its relatively short half life requiring periodic replacement) was out-weighed by its several advantages. Compared to a neutron generator the output is highly stable (when decay corrected) and there is very little maintenance required. For the source strength we needed, an α-Be source would have been rather large and expensive. The large size would have made handling and collimating the source more difficult than for a Cf-252 source. The energy of the Cf neutrons was adequate to provide uniform irradiation through the samples in which we were interested (human extremities, and small animals) and gave more thermal neutrons per unit radiation exposure than the other possibilites.

The decision on the basic detector type for measuring the activation was much simpler than for the source type. The requirements of high efficiency and adequate energy resolution have caused virtually everyone measuring in-vivo activation to select NaI (Tl) detectors.

These detectors consist of a NaI (Tl) crystal in which the γ-rays energy is converted into light. The crystal is viewed by one or more photomultiplier tubes in which the light is converted to an electrical pulse and amplified. The height of the pulse is proportional to the energy deposited in the crystal and hence to the energy of the γ-ray being detected. Using a pulse height analyzer one can obtain a spectrum of the γ-rays hitting the crystal and hence the activities of each of the elements present.

The specific detector set up we finally chose consisted of two detectors (11.5" diameter x 4" thick) vertically opposed with an adjustable separation. The lower

detector is stationary. The steel plate containing the upper detector is moved by hydralic jacks and positioned on blocks attached to the support frame. By summing the counts from the two detectors the counting efficiency is relatively uniform along a line parallel to the crystal axis.

The design of the neutron irradiator and storage cask was done in cooperation with Don Garret of Gamma Industries whom we contracted for the construction and installation. The facility was to be placed in a shielded (5ft concrete) room in the basement of the Fondren-Brown building of Methodist Hospital. The entrance to the room was a narrow doorway and baffled hallway. This limited the size of any of the pieces. A Cf-252 source emits both neutrons and γ-rays so a shield must be designed to stop both. The optimum shield materials for γ-rays are dense materials of high atomic number, (e.g. lead). The neutrons have quite different requirements. The typical history of a neutron emitted from a source is to lose energy by elastic collisions with atomic nuclei (from 1MeV to .025eV) to thermal energies. The thermal neutron is then captured by an atomic nucleus typically emitting γ-rays totalling several MeV. (The energy emitted is characteristic of the capturing nucleus). The amount of energy that can be transferred in an elastic collision is a maximum for particles of the same mass and falls off very rapidly (as the square of the mass ratio) as the masses become dissimilar. Hydrogen is by far the best element for slowing down fast neutrons so materials with high hydrogen contents such as water, paraffin, or various plastics are normally used. To minimize the secondary γ-rays a substance which has a high capture cross section for thermal neutrons and that emits only a small amount of energy as γ-rays may be dispersed throughout the principal shield material. The final shield consisted of an inner portion of lead (3" thick) surrounded by boron loaded water extended polyester. water extended polyester is a plastic like substance made from 50% water and 50% polyester resin. The boron, boric acid, was dissolved in the water before mixing with the resin so it was uniformly dispersed throughout the shield.

Preliminary to the receipt of our irradiation system and our Cf-252 source, feasibility and design studies were performed with a small Cf source. These studies were done at M.D. Anderson Hospital with a source borrowed from them. The aim of these studies was to determine how the various geometrical factors would affect the radiation dose and thermal neutron flux in a sample. Among the factors studied were 1) collimator size and shape; 2) premoderator area and thickness; and 3) backscatters.

Two techniques were used for making measurements of the thermal neutron flux, 1) Thermoluminescent dosimeters (TDL); 2) activation foils. Thermoluminescent dosimetry is based on the fact that when certain cyrstals are irradiated the deposited energy can excite some electrons to a metastable band. When the crystal is heated these electrons can de-excite emitting light. By measuring the amount of this light (by summing the current from a photomultiplier tube viewing the crystal) one can deduce the amount of radiation to which the crystal was exposed. One of the crystals often used as a TLD is LiF. Lithium has two naturally occuring isotopes with very different interactions with thermal neutrons; Li-6 (7% abundance) and Li-7 (93% abundance). Li-7 has a very low cross section for thermal neutrons (0.037 barns for neutron capture). Li-6 has a high cross section (953 barns) for the reaction Li-6 (n, α) H-3. The energy from the α particle and H-3 nuclei is deposited in the crystal in a very short range. Crystals of LiF made from isotopically separated Li are commercially available. Since ^{6}LiF will interact with both the γ-rays and neutrons in a radiation field while the ^{7}LiF will interact with only the γ-rays by using a pair of cyrstals both components can be measured.

The second technique for measuring the thermal flux used the activation of Mn by neutron capture. When Mn-55 captures a neutron it changes to Mn-56 which has a half life of 2.58 hours. The decay of Mn-56 results in a 847 keV γ-ray (99% of the time) which may be measured to determine the amount of activity present.

Manganese was a convenient element to use for the flux measurements because of its single naturally occuring isotope (Mn-55), a high capture cross section, (13.3 barns) and convenient half life (2.58 hours). The half life was long enough that several samples could be measured after a single irradiation yet short enough that they could be reused in a couple of days with no correction for the residual activity. The samples routinely used consisted of small (about 1" square) plastic packages containing MnO2 (typically 0.05 g) spread over a area of about ½" square. Each packet was numbered and the exact amount of MnO2 weighed at the original filling.

A paper was written describing our feasibility studies in greater detail and has been included in this report as Appendix A.

NEUTRON ACTIVATION ANALYSIS FACILITY

The activation analysis laboratory consists of two rooms located in the basement of the Fondren-Brown building of Methodist Hospital. The room containing the Cf source has five foot thick concrete walls to provide radiation shielding. The adjacent room is used for counting the activated samples.

The irradiator was designed and built to our specifications by Gamma Industries of Baton Rouge, La.. Its construction and operation are described in detail in the instruction manual (Appendix B) and will only be outlined here. The Cf-252 source was obtained on loan from the Savannah River Laboratory (Department of Energy, formerly E.R.D.A., formerly A.E.C.). The original source of 3 mg was obtained in 1975 and was replaced in 1980 (the original source had decayed to under 1 mg). The source is contained in a cylindrical steel capsule (.37" diameter x 1.48"). This capsule can be moved between the storage position in the irradiator and the irradiation port. This movement is controlled from the adjacent room by a manually operated flexible cable.

The main position of the shield is constructed of blocks of boron loaded water extended polyester (WEP). Water extended polyester is a substance of approximately half water and half polyester resin that hardens to a consistency similar to plastic. The boron loading is used to reduce the radiation from gamma rays following thermal neutron capture. Boron has a capture cross section of 756 barns and a .477 MeV γ-ray per capture compared to hydrogen (the other major contributor in the shield) of 0.33 barns and a 2.22 MeV γ-ray. When the source is in the storage position there is a minimum of 3" of Pb and 21" of WEP between it and the point of closest approach. The maximum exposure at the shield surface was 33 $\frac{\text{mrem}}{\text{hr}}$ (detailed safety survey in Appendix D, Table 1).

The interlock system prevents entrance to the room when the source is not in the storage position. It also prevents movement of the source from the storage

position unless the door to the source room is locked, all the safety plugs are in and the control panel is unlocked. The locks on the source room door can be overriden from inside the source room (as an emergency safety provision). A geiger tube is positioned at the ceiling over the irradiation port. This is used to trigger a flashing red light in each room when the source is in the irradiation position.

The source is moved between the storage position and the irradiation position in a ½" diameter steel tube. The bottom portion of the irradiation port is a 10" cubical opening. Above this there is a 8" by 12" opening 10 3/4" high. When the source is in the irradiation position it is in the center of the bottom portion, 15 3/4 " (40cm) from the top face of the port. Normally a backscatterer (8" x 8" x 4" thick Pb) is beneath the source to increase the upward flux of fast neutrons.

The size of the irradiation port permits considerable flexibility in the choice of irradiation conditions. The collimator opening may be used as it stands, it may be stopped down, or an insert may be used. The irradiation conditions may also be modified by the choice of premoderators (between source and sample) and backscatterers (above sample).

Three collimator inserts were constructed of Plexiglas. The first was a tapered collimator (Appendix C, Figure 3(a)) with a 4" x 8" rectangular opening. The sides were constructed so that any particle emitted from the source toward the opening would be unobstructed while other particles would pass through as much material as possible. This collimator provided a narrowly defined beam of neutrons with an energy spectrum close to an unmoderated Cf spectrum.

The second collimator was used primarily in activating small animals (rats or small guinea pigs). It was a rectangular collimator with a 4" x 8" opeining (appendix C, Figure 3(b)). The sides were constructed of 2" Plexiglas. Mounted in one end of the collimator was a small 6r.p.m. motor and a gear. The small animal holder

consisted of a Plexiglas tube with teflon bearings on the ends and a non-metalic gear. The holder fit in the collimator with the bearings riding in slots at the ends of the collimator and the gears driven by a gear attached to collimator motor. Since the animal remained in the holder both during irradiation and counting it was critical that the holder not contribute to the spectrum. This meant that not only could metals not be used, but adhesives with high Cl content could be a problem (and was until identified in one holder).

The counting set up consists of two large (11.5" diameter x 4" NaI (TI)) detectors. The detectors are vertically opposed with a variable separation. The lower detector is stationary. The upper detector may be moved by hydralic jacks and may be reproducibly positioned on blocks attached to the detector support frame. Each detector is viewed by three photomultiplier tubes (5" diameters) which are wired together and fed into the preamplifier (Ortec, 113). From the preamplifier the signal goes to a stabilized amplifier (Harshaw, Na23). The amplifier automatically adjusts its gain to maintain a constant pulse height for a stablilization peak (Cs-137, 661keV). Normally a stabilization peak is chosen above the energy region of interest but this was not possible because of the high energy in which we were interested (e.g., 3084 keV for the Ca-49 line). From the amplifier the pulse went to a mixer/router (Norther Scientific, NS-459) and then to a pulse height analyzer (Norther Scientific, NS-710A). Signals from the upper detector were routed into the first 512 channels of the analyzer and those from the lower detector into the second 512 channels. To measure the live time of the system a pulser (60 Hz) was fed into the preamplifier of the lower detector and was of amplitude to fall in an analyzer channel above the spectrum.

To measure the irradiation time and wait time (between end of irradiation and start of counting) a clock panel was constructed. At the start of the irradiation the three clocks on the panel started, switched on by a signal from the geiger tube over the irradiation port. At the end of the irradiation the first clock

stopped, controlled by the gieger tube. The second clock was turned off by a switch that started the pulse height analyzer. The third clock was an independent duplicate of the second clock.

CALCULATION OF ELEMENTAL CONCENTRATION

The technique of neutron activation analysis is a means of measuring the amount of an element present in a sample. The sample is exposed to a flux of neutrons and the induced radioactivitiy is then measured. Assuming the radioactivity measured results from a single reaction process its intensity will be proportional to the amount of the target element in the sample. In neutron activation this process is usually the capture of a thermal neutron (one whose kinetic energy is about 1/40 ev) by the nucleus of an isotope of the element of interest. The decay of the resulting isotope is then measured. It can easily be seen that the sensitivity of the technique is dependent on the properties of element of interest, specifically the isotopic distribution, neutron capture cross sections, and the decay characteristics. These properties can vary by many orders of magnitude resulting in some elements being unmeasurable (e.g. carbon, oxygen) while others can be measured with great sensitivity (e.g. calcium, sodium, chlorine).

The amount of an element present in a sample is given by the formula below:

$$N = \frac{C}{\varepsilon(BR)(IA)\left(\int \sigma(En)\,\phi(Fn)\,dEn\right)\left(1-e^{-\lambda ti}\right)e^{-\lambda tw}\left(1-e^{-\lambda tc}\right)\frac{lt}{ct}}$$

Where C is the number of counts detected

ε is the detection efficiency

BR is the branching ratio for the gamma ray being counted

IA is the isotopic abundance

En is the neutron kinetic energy

$\sigma(En)$ is the neutron capture cross section, as a function of energy

$\phi(En)$ is the neutron flux, as a function of energy

λ is the decay constant of the isotope being detected

ti is the irradiation time

tw is the wait time (from end of irradiation to start of counting)

tc is the counting time

$\frac{lt}{ct}$ is the fraction of time the detection system time is

Several of the factors in the above equation are physical constants and depend only on the reaction being used (BR, IA and $\sigma(En)$). Other factors depend on the particular irradiation and counting setup being used but will be constant from one sample to another (ϵ, $\emptyset(En)$). There is actually a long term variation in $\emptyset(En)$ due to the decay of the Cf-252 source ($t_{\frac{1}{2}}$ = 2.6y) which must be accounted for.

In principle the various components in this factor could be obtained separately either from published sources (the neutron capture cross section as a function of energy and the branching ratio) or measured for our own setup (the neutron flux spectrum and the counting efficiency). In practice since both the flux spectrum and detector efficiency vary from point to point in the sample the technique would be both tedious and subject to a number of errors. By constructing and measuring a phantom of known composition approximating the geometry of the sample to be measured the factor $\epsilon(BR) \int \sigma(En)\ \emptyset(En)\ d\ En$ can be obtained without separately measuring each component. The neutron flux is directly proportional to the activity of the Cf source so in comparing measurements on different days the source decay ($t_{\frac{1}{2}}$ = 2.6y) must be accounted for. The procedure we have found most convenient is to decay correct all measurements to the same arbitrary date.

The activity of the sample is measured with NaI (TI) crystals and a multichannel analyzer. The spectrum obtained is the sum of the spectra from each of the elements activated plus the background.

It is necessary to obtain separate measures of the activities of each of the elements of interest. There are several possible methods to obtain this information, most of which would require a computer to strip the spectrum into its components. Because of the characteristics of our samples we were able to use a simpler

procedure adaptable to a hand calculator. Since we were dealing with biological samples (patient or experimental animals) the elements present and their approximate concentrations were known prior to the measurement. The major elements contributing were normally Na, Cl, and Ca with much smaller contributions from Mn and K. Regions of interest in the pulse height spectrum were selected over the principal peaks for each of the elements of interest. These peaks were Na-24 (1368, 2754, and 1368 + 2754), Cl-38 (1643, 2167, and 1643 + 2167), Ca-49 (3084), K-42 (1524) and Mn-56 (847). For each of the elements of interest a phantom was made containing this element with approximately the same distribution as was expected in the sample. By irradiating and counting this phantom a reference spectrum was obtained. The sample spectrum consists of the sum of the background plus the spectrum of each of the elements activated. Each spectrum (both sample and reference spectra) can be divided into regions of interest under the principal peaks of each of the elements in the sample. The number of counts in region N_i of the sample spectrum is equal to $B_i + \sum_j A_j F_{ji}$ where A_j is the number of counts from the element j and F_{ji} is the fraction of A_j in region i. The coefficients F_{ji} are determined from the reference spectra. If one region of interest is chosen for each element there will be a set of simultaneous equations which may be solved for the activities. An alternative procedure is to reduce the number of equations to the number of unknowns. This can be done by adding together the equations for the several peaks of a given element. These equations can then be solved for the spectral amplitudes of each of the contributing elements. For a given set of irradiation and counting conditions this amplitude will be directly proportional to the amount of the element present and to a normalization factor dependant on the decay constant of the element and on the times of irradiation, decay (before starting to count), and counting. The normalization factor is of the form $(1-e^{-\lambda t_i}) e^{-\lambda t_w} (1-e^{-\lambda t_c})$ where λ is the decay constant, t_i the irradiation time, t_w the waiting time, (between end of irradiation and start of counting) and t_c the counting time.

PHANTOM STUDIES

Phantoms were used for two basic purposes in our activation studies; to provide an absolute normalization for the measurements and to provide a check on the long term srability of the system. In those cases where absolute measurements were of interest (all cases except the renal failure patients) it was necessary to make phantoms that were reasonable approximations of the sample to be tested. Reasonable approximation had to be thought of in terms of its interaction with neutrons (moderation and capture), its composition of elements which could be activated and its absorption of decay gamma rays. Hydrogen is by far the most effective element in moderating the energy of neutrons so the sample and phantom had to have comparable amounts. The elements which make the major contribution to the activation spectrum, Ca, Na, and Cl had to be present in known amounts, comparable to the sample for absolute normalization.

Some phantoms were made to determine the shape of the spectrum for a particular element. In these cases the requirements were slightly different, only that the distribution of the element be similar to the sample of interest and that there be no other significant contributors to the activation spectrum. These phantoms could not in general be used for absolute normalizations since high concentrations of an element with a high capture cross section depress the neutron flux.

Carbon and oxygen have very low capture cross sections and do not contribute to the activation spectra so their concentrations can be chosen for convenience. Nitrogen does have an appreciable cross section (for the reaction ^{14}N (n,p) ^{14}C) but does not contribute to the activation spectrum. The nitrogen concentration in the phantom would have been importanly only at high levels that would have caused a flux depression. In a few of the phantoms Mn and K were included in physiologic concentrations. These elements gave only small contributions to the spectra and these contributions were chiefly at energies below those used for the Ca,

Na, and Cl measurements. The inclusion of Mn and K in the phantoms (or in the analysis procedure) did not have a significant effect on the measurements of the other elements. There was not adequate data in the sample spectra to permit useful measurements of either Mn or K. This was partially because our procedures were normally optimised for Ca ($t_{\frac{1}{2}}$ = 8.7 min) and not for Mn ($t_{\frac{1}{2}}$ = 2.6 h) or K ($t_{\frac{1}{2}}$ =12.4h). Other elements were sometimes included in the phantoms but only after checking the capture cross section and activation characteristics.

Physically the activation phantoms we used were of three basic types. 1) A mixture of powdered chemicals, to simulate ash or excised bone. 2) A solution of chemicals, to simulate whole body activation of rats or guinea pigs. 3) paraffin phantoms containing bones or packets of chemicals to simulate the partial body activations of monkeys or of patients.

The simplest phantoms were those that simulated ashes (of rats or guinea pigs). The ashes were irradiated and counted in small plastic (Nalgene) bottles. The phantoms consisted of measured quantities of chemicals (e.g., $CaCO_3$, $NaNO_3$) of volume similar to the ashes.

The phantoms for the live rats and guinea pigs consisted of Nalgene bottles containing aqueous solutions containing the elements of interest. A typical phantom was the one used to simulate a guinea pig of about 500g. It was a ½ liter plastic bottle filled with deionized water plus the chemicals shown in Table 1. This choice of chemicals provided a phantom with no precipitates, no extaneous activation spectra, and no elements (not present in living guinea pigs) with significant absorption cross sections. Similar phantoms containing only one element of interest were used to obtain the reference spectra. The third basic type of phantom was used for samples that could not be well approximated by simple geometric shapes such as bottles. These phantoms consisted of a central core, (either bones or chemical packets) covered with paraffin. Phantoms of this type were made for human hands and feet and for monkeys. The foot phantom was the bones of a human foot

and leg which we encased in paraffin. In the other phantoms sealed plastic packets containing known amounts of the elements of interest were encased in paraffin. Since our primary interest was in measuring Ca and most of the Ca in the body is in the bones the relative weight and geometry of the packets simulates the bones. For the hand phantoms a glove was used as a mold, a packet of chemicals was placed in each finger and one in the palm. The glove was then filled with molten paraffin. Since the glove contained Cl (polyvinylchloride) it was removed after the paraffin hardened. The monkey phantoms were made in several pieces to simulate the trunk, limbs, and tail. These pieces were placed in the monkey holder in a geometry similar to that of the monkeys.

The long term reproducibility of measurements of several of the activation phantoms is shown in Tables 2, 3, 4, and 5. Each of the phantom measurements duplicated as closely as possible the measuring conditions of the sample being simulated.

TABLE 1

GUINEA PIG PHANTOM

The chemicals shown were disolved in ½ liter of distilled water. This solution, in a plastic bottle, was used to simulate a guinea pig both for calibration and for system stability. Similar phantoms of different size were used in various rat and guinea pig studies.

CHEMICAL	AMOUNT	ELEMENTS	AMOUNT
$MnSO_4 \cdot H_2O$	.0199 g	Mn	.00647 g
NaCl	1.3290 g	Cl	.8061 g
$NaNO_3$	.9154 g	Na	.7705 g
$CaNO_3 \cdot 4H_2O$	38.1990 g	Ca	6.438 g
KNO_3	6.1518 g	K	2.377 g

TABLE 2

HAND PHANTOM MEASUREMENTS WITH INSERT

A plexiglass collimator with a 4" x 8" opening was in the irradiation port. The Ca value is given in arbitrary units. The uncertainty in each measurement due tc counting statistics is typically 600 (2.2%) for one standard deviation.

DATE	Ca
10/3/77	27957
10/17/77	27826
10/26/77	26768
11/22/77	27260
12/1/77	27286
12/8/77	27733
12/8/77	27050
1/19/78	26201
1/19/78	27302
1/31/78	27040
2/7/78	27070
2/7/78	26058
3/29/78	26707
4/10/78	27715
4/19/78	27047
5/9/78	26004
5/10/78	26220
5/11/78	27522
5/26/78	26736
7/12/78	26114
8/30/78	28232
8/31/78	27670
10/10/78	27294
10/11/78	27803
11/14/78	27499
11/15/78	26870
11/27/78	27459
11/30/78	26996
12/5/78	27913
12/6/78	27132

DATE	Ca
1/19/79	26914
1/22/79	28078
1/26/79	27454
2/6/79	28229
4/24/79	26969
4/25/79	28287
5/2/79	27474
5/7/79	27924
5/16/79	29135
5/17/79	28923
5/17/79	28654
5/18/79	28421
7/2/81	26872
7/11/79	29125
7/12/79	28109
9/6/79	26480
1/3/80	27329
1/9/80	24666
1/9/80	26356
1/21/80	26830
1/23/80	26086
1/23/80	26695
2/20/81	27585
3/12/81	28254

TABLE 3

HAND PHANTOM MEASUREMENTS, NO INSERT

There was no insert in the 8" x 12" irradiation port. The Ca value is given in arbitrary units. The uncertainty in each measurement due to counting statistics is typically 600 (2.2%) for one standard deviation.

DATE	Ca	DATE	Ca
3/20/79	26789	2/18/80	25585
3/21/79	27588	2/19/80	25015
3/21/79	25891	2/27/80	27255
4/12/79	26782	11/26/80	26742
4/12/79	27789	11/26/80	26613
4/17/79	26074	11/28/80	26964
4/18/79	25840		
5/7/79	26373	1/12/81	27001
6/5/79	26094	2/16/81	26710
7/23/79	27056	2/18/81	26883
11/8/79	27017	3/5/81	26445
11/14/79	27270	3/6/81	26609
11/27/79	25745	3/20/81	26911
		4/3/81	26842
1/3/80	27329	4/17/81	26603
1/9/80	24928	4/20/81	26405
1/9/80	26916	4/27/81	26303
1/24/80	25985	6/12/81	25407

TABLE 4

FOOT AND LEG PHANTOMS

The Ca value is given in arbitrary units. The uncertainty due to counting statistics is typically 1500 (1.4%) for one standard deviation.

DATE	Ca
10/4/77	104974
11/22/77	108282
11/23/77	106843
12/1/77	104067
12/7/77	104722
12/15/77	104129
1/19/78	108326
1/31/78	105961
2/7/78	106513
3/29/78	108006
3/29/78	106796
3/30/78	103731
4/5/78	105994
4/6/78	105552
4/10/78	109021
4/10/78	111316
4/10/78	108082
4/11/78	107136
4/19/78	103856
5/26/78	108733
5/30/78	110248
7/12/78	107660
7/13/78	106894
7/13/78	106891
7/14/78	104876
7/17/78	104815
7/18/78	108501
7/19/78	109339
8/25/78	109027
9/11/78	103148
9/12/78	104808
9/14/78	110901
9/27/78	105923
9/27/78	107519
10/18/78	109450
10/24/78	107707
10/24/78	107072
11/13/78	111130
11/15/78	107072
11/16/78	109508
11/17/78	107127
11/29/78	107025
12/5/78	104827
12/15/78	109456
1/25/79	108913
4/12/79	108511
5/18/79	105908
5/29/79	107470
6/5/79	109162
6/11/79	109723
6/12/79	111170
7/3/79	107166
7/13/79	108547
9/5/79	111641
9/25/79	107201
11/14/79	103628
11/21/79	107423
1/2/80	105721
1/3/80	103818
1/10 80	105273
1/23/80	101565
1/24/80	102941
11/26/80	110123
11/26/80	109240
2/11/81	109355
2/12/81	107489
3/5/81	107372

TABLE 5

RAT PHANTOM MEASUREMENTS

The Ca value is in arbitrary units. The uncertainty in each measurement due to counting statistics was typically 600 (1.5%). The phantom contained 2.65 g Ca.

DATE	Ca
9/17/76	39445
9/20/76	39718
9/20/76	39148
9/21/76	38427
9/22/76	39324
9/23/76	38767
10/11/76	39455
10/12/76	39532
10/13/76	38644
11/5/76	39301
11/8/76	39175
11/9/76	39764
11/29/76	39972
11/30/76	39675
12/1/76	38649
12/30/76	38921
12/30/76	38781
1/3/77	38918
1/4/77	39293
1/5/77	39406
1/22/77	39261
1/25/77	38428
1/26/77	39930
1/27/77	37784
2/11/77	39156
2/14/77	39003
2/15/77	39592
3/7/77	39484
3/8/77	38847
3/9/77	38301
3/28/77	39866
3/29/77	40025
3/30/77	39017
5/2/77	39269
5/3/77	39160
5/4/77	38832
6/21/77	39990
6/22/77	38344
6/23/77	38884
6/27/77	39930
6/28/77	40300
6/29/77	39342
7/12/77	39301
7/13/77	39195
7/14/77	40410
7/25/77	39848
7/26/77	39933
7/28/77	38967
8/8/77	38691
8/9/77	39016
8/10/77	39865
8/29/77	39757
8/30/77	38881
8/31/77	40509
9/1/77	39852
9/19/77	39802
9/20/77	39704
9/21/77	39664
10/18/77	39933
10/19/77	39388
11/28/77	40926
11/29/77	39342
1/17/78	40038
1/18/78	38699
1/25/78	38438
1/26/78	39215
2/13/78	37700
2/14/78	39042
3/6/78	39413
3/7/78	39279
4/17/78	38844
4/18/78	38994
6/5/78	39593
6/6/78	38520
7/6/78	38266
7/7/78	39352
7/26/78	39545
8/14/78	39281
8/15/78	39920
8/21/78	39727
8/22/78	39063
5/12/80	39746
5/13/80	41505
5/13/80	40817
5/14/80	40682
5/27/80	40859
5/28/80	41142
6/10/80	40226
6/11/80	39433
6/24/80	41931
6/25/80	40690

FAST NEUTRON DOSIMETERY

Three techniques were used for measurements of fast neutrons in our studies; a BF_3 counter, paired ionization chambers, and indium activation foils. The BF_3 counter was a commercial instrument (Eberline Instrument Corporation, Model PNC-4) based on the nuclear reaction $^{10}B(n,\alpha)\ ^{7}Li$. This is a thermal neutron capture porcess. The detector has an outer shield of cadmium (to absorb thermal neutrons) followed by a layer of parafin (to thermalize the fast neutrons) to adapt it to measuring fast neutrons. This instrument was used mostly for radiation safety measurements. It was not well suited for precise measurements.

The paired ionization chambers we used were of the personnel dosimeter type (Don L. Collins and Associates) with a maximum reading of 200 mrad. One chamber was nominally insensitive to neutrons and the other equally sensitive to neutrons and γ-rays. This was not precisely true and the actual formulas used for obtaining the neutron and gamma ray doses were:

$$D_n = A(R_{n+g} - R_g)$$

$$D_g = A(R_g - BR_{n+g})$$

where D_n is the neutron dose

D_g is the gamma ray dose

R_{n+g} is the reading of the neutron-gamma chamber

R_g is the reading of the gamma only chamber

A and B are experimental constants.

The constants A and B were experimentally measured to be 1.751 and 0.325 respectively. The determinations were made by taking readings of the radiaton field of a Cf-252 source (5.4μg) immersed in a bath of tissue equivalent liquid. Using published values of the dose distribution (Colvett et al. Phys. Med. Biol. 17 (1972) 356) the calibration constants were determined.

The third technique we used for making fast neutron measurements was threshold activation foils. This method was based on the inelastic neutron scattering reaction In-115 (n,n') In-115m. The meta-stable state of In-115 is at 335 keV

and has a half life of 4.5 hours. The decay of In-115m is by emission of 335 keV gamma ray with a branching ratio of .50. The intensity of this gamma ray was measured in NaI well detector to determine the amount of In-115m produced. The cross section for this reaction is energy dependent so the activity produced is proportional to $\int\sigma(En)\ \emptyset\ (En)\ dEn$ where $\sigma(En)$ is the cross section, $\emptyset$ (En) is the neutron flux, and En is the neutron energy. Throughout our work we assumed that the flux had the spectral distribution of a Cf-252 source. This allows the use of the mean cross section (averaged over the energy distribution). This assumption would be precisely true only in a vacuum but it seemed a reasonable approximation even in conditions of considerable scatter. This would not be true of a thermal or epithermal reaction but the literature indicates that the shape of the fast neutron spectra is not easily altered.

The technique for these measurements was to use small plastic packets containing a known amount of In_2O_3. The amount of In per packet ranged from .05g to 3.7g, depending on the desired sensitivity. Indium has a very high thermal capture cross section so to lessen the activity of the irradiated packets they were covered with Cd during irradiaton. Even with Cd covers the dominant activity in the irradiated samples was normally In-116m (from capture of epi-thermal neutrons penetrating the Cd). The half life of In-116m is 54 minutes compared with the 4.5 hours of In-115m. It was possible to allow the In-116m to decay to low levels before the In-115m activity was measured.

RENAL FAILURE PATIENTS

The pathophysiologic derangements which result in uremic bone disease include acidosis, intestinal malabsorption of minerals, alterations in Vitamin D metabolism and secondary hyperparathyroidism. Histologic examination of uremic bone has identified osteitis fibrosa, osteomalacia, osteosclerosis, and osteoporosis. The multiple factors operative in uremia together with the varied histologic pattern of uremic bone diesase suggest that the uremic population is heterogenous with respect to the altered mineral metabolism. Further, no one factor emerges as primarily responsible for the pathology of uremic bone disease.

In order to trace the course or progression of uremic bone disease or the effectiveness of various therapeutic interventions, a sensitive and accurate measure of skeletal calcium is required. Partial body neutron activation was selected because it offered advantages over the more common techniques.

Standard radiologic techniques are not adequate for detecting changes of less than 30%. Bone biopsys are invasive, only semi-quantitative and sample only a small portion of the skeleton. Gamma-ray absorptiometry has been widely used because of its high precision. Its chief disadvantage is that only small sections of certain areas of the skeleton are measured and these areas (most often midshaft of the radius) are often not where the first signs of bone loss would be expected. Neutron activation may be used for measuring Ca in-vivo either in the whole body or in selected regions. Total body activation has the advantage of giving an absolute measure of Ca in the body with high precision (2 to 4%). Partial body activation has the advantages over total body activation that it is technically simpler, exposes only selected regions to radiation, and allows regions where the greatest change is expected to be selectively measured.

PATIENT MEASUREMENT PROCEDURE

Before the patient arrived the apparatus was set up for the first measurement to be performed (either the left hand or foot). This consisted of arranging the irradiation geometry (premoderators, collimators, and holders), the counting geometry (detector separation, holders, shielding) and electronic settings (baselines, regions of interest, count time). As a final check that the system was ready a phantom was irradiated and counted. These measurements served to check both that the system was properly working for an individual patient and that the system was stable over a period of years.

When The patient arrived for his/her first measurement the procedure was explained and the patient was asked to read and sign a consent form (Appendix D). No patient was measured without a signed consent form (either by the patient or legal guardian). The standard procedure was to make 3 separate Ca measurements (each hand and the left foot) for each patient. Occasionally it was necessary to omit one measurement due to the inability to position the hand or foot correctly (eg. due to a highly swollen hand).

When measuring the hand the patient was taken into the source room and seated with his/her arm extending horizontally. The hand was positioned on a Plexiglas plate over the collimator opening. The hand was slid forward until it touched two pins (½" dowels) extending between the fingers. This allowed the irradiation position to be reproduced for subsequent measurements. A backscatterer (2 inch thich Plexiglas) was placed over the hand. This served both to increase the neutron flux uniformity in the hand and to provide additional radiation shielding to the rest of the patient. The operator then left the source room. From the panel in the counting room the source was brought out for 6 minutes irradiation. During this time the patient was monitored by means of a closed circuit television and in communication by means of an

intercom. After the irradiation the patient was escorted from the source room to the counting room. The patient's hand was then placed between the detectors on a positioning holder similar to that used for irradiation. The activity in the hand was counted for 15 minutes. The other hand was then measured in the same way. The procedure for measuring the foot was slightly different. For the irradiation the patient was placed on a specially constructed stretcher with his/her left foot positioned on a Plexiglas holder at the end of the stretcher. The foot was lightly strapped in position on the holder. The holder extended over the collimator opening. A 2" thick Plexiglas backscatterer was placed over the foot. After the 3 minute irradiation the stretcher with the patient in place was wheeled to the counting room. The foot holder end of the stretcher was placed between the detectors and the activity in the foot counted for 15 minutes. The total time for measuring the 3 sites was about an hour and 15 minutes. On the first visit an extra 15 minutes were required for the explanation of the procedure and reading the consent form. For most patients there seemed to be no discomfort in the procedure, for a few sitting or lying in a fixed position for 15 minutes was a strain. The radiation dose to each hand was approximately 1.4 Rem and to the foot 0.7 Rem. This can be compared to the 75 Rem/year which is the maximum permitted dose to the extremities of an occupational exposed person.

We have measured the Ca of 54 renal faiulure patients. Of these, 25 have been measured multiple times, the results for these patients are shown in table 6. The other 29 patients were not remeasured for a variety of reasons; a few are not yet due, some moved, some changed physicians, some died and some simply did not wish to be retested. These patients have been undergoing a variety of dialysis procedures, haemodialysis, peritoneal dialysis, and continuous ambulatory peritoneal dialysis. Some of them have been receiving drug therapy, ($CaCo_3$, Calcitriol) to counter the loss of Ca. There was no evidence of bone loss in the group as a whole, the simple average of all the changes measured was slightly positive. Two

of the patients showed increases which may have been due to normal growth (CM and MN), they were 16 and 17 years old respectively at the time of the first measurement.

TABLE 6

PATIENT	DATE OF 1st MEASUREMENT	MONTHS LATER	LEFT FOOT	LEFT HAND	RIGHT HAND
J.B.	8/78	9	5 ± 2	4 ± 3	2 ± 3
T.C.	10/77	6	5 ± 2	-3 ± 2	1 ± 2
		12	2 ± 2	-6 ± 3	2 ± 3
		20	8 ± 2	0 ± 3	4 ± 3
M.E.C.	9/78	8	-4 ± 3	-3 ± 3	0 ± 3
D.C.	11/78	6	-8 ± 4	4 ± 4	16 ± 4
	2/81	27	-8 ± 3	3 ± 3	13 ± 4
J.D.	12/78	9	2 ± 2	6 ± 3	3 ± 3
S.D.	9/78	8	-1 ± 3	0 ± 3	-2 ± 3
E.L.D.	12/77	7	3 ± 2	7 ± 3	-7 ± 3
		13	5 ± 2	-3 ± 3	1 ± 3
V.G.	11/78	6	-4 ± 3	4 ± 5	15 ± 5
W. J.	10/77	6	-8 ± 2	-1 ± 3	-7 ± 3
		14	-2 ± 3	4 ± 3	-4 ± 3
C.J.	8/78	12	---	1 ± 4	2 ± 4
A.J.	5/79	6	-4 ± 3	-7 ± 4	0 ± 4
D.K.	11/78	14	-2 ± 2	1 ± 3	-3 ± 3
		27	-5 ± 2	4 ± 2	1 ± 2
A.G.L.	11/78	8	-1 ± 3	3 ± 4	2 ± 4
C.M.	12/77	7	-3 ± 2	-10 ± 3	-6 ± 3
		13	-3 ± 2	-2 ± 3	-8 ± 3
Ch. Mo.	11/78	8	7 ± 3	6 ± 4	9 ± 4
M.N.	10/77	7	10 ± 3	8 ± 4	9 ± 4
		15	2 ± 3	3 ± 4	24 ± 5
T.A.P.	9/77	7	4 ± 2	1 ± 2	0 ± 2
W.R.	4.78	7	3 ± 2	3 ± 3	7 ± 3
		21	-1 ± 3	-10 ± 3	-----
H.S.	9/78	7	(-10 ± 3) Problem	6 ± 3	-5 ± 3
R.G.S.	12/77	8	4 ± 2	0 ± 3	2 ± 3
		14	5 ± 2	2 ± 3	2 ± 3
C.T.	4/79	7	4 ± 3	1 ± 3	-1 ± 3

PATIENT	DATE OF 1st MEASUREMENT	MONTHS LATER	LEFT FOOT	LEFT HAND	RIGHT HAND
G.T.	5/[illegible]	9	-3 ± 3	____	-19 ± 3
I.M.T.	4/78	7	11 ± 3	2 ± 4	-1 ± 4
B.J.W.	1/78	9	4 ± 3	-7 ± 3	-3 ± 3
		15	2 ± 3	-4 ± 3	-4 ± 3
		24	5 ± 3	-1 ± 4	2 ± 4
M.W.	7/78	9	-3 ± 3	-4 ± 3	7 ± 3

RAT STUDIES

Prolonged human bedrest studies have been used frequently to simulate the changes of spaceflight as it affects the cardiovascular and skeletal systems. For studying countermeasures against calcium loss a major difficulty with this technique is the high cost per subject and the technical problems of multifaceted metabolic balance studies. The problem is compounded by the very low loss rate from the skeleton and the apparent inhomogeneity of this loss. Because of the expense of human bedrest, it seems reasonable to develop an animal model to study countermeasures as a screening device before testing in humans.

A number of animals have been used as models for the study of calcium metabolism including the rat and to a lesser extent the dog, cat, rabbit, chicken and the monkey. Studies with laboratory rats have shown that increased bone calcium was associated with increased muscle mass in the exercised animals and that the relationship between muscle mass and bone calcium remained constant. Studies using normal and bipedal rats have demonstrated that increased muscle mass was followed by increased femur density and breaking strength of the weight bearing limbs. Disuse of a limb leads to atrophy of both muscle and bone.

The Russian data from Cosmos-783 has shown that zero-g rats demonstrate decreased bone growth and a decrease in bone breaking strength. Inhibited bone growth was the major finding when the tetracycline growth lines of Russian flight rats were compared with control animals. Rats flown on Cosmos-605 have shown microscopic bone changes similar to those seen in the autopsies of the three cosmonauts who died during reentry.

We have chosen the laboratory rat as our experimental model for several reasons. First, there is extensive background literature on all phases of its bone metabolism. The histology including growth, remodeling and repair of cancellous and cortical bone is nearly identical to that of the human. Second, the rat can be trained and when given the opportunity will voluntarily excercise.

When exercise opportunities are limited it voluntarily assumes a sedate inactive existence without the need for physical restraints. We have the opportunity then of simulating the human bedrest situation where healthy conditioned individuals are abruptly put in a situation of "voluntary" inactivity. This model may be closer to the conditions encountered during bedrest than are other osteoporosis producing models such as forced immobilization, water immersion, or endocrine manipulation. A third reason for choosing the laboratory rat is that the Russians have made extensive use of this animal. Their flight data suggest that the rat is suitable for studying skeletal changes induced by spaceflight. Lastly, techniques not tolerable in humans can be employed which are capable of measuring the small changes expected after the short duration shuttle flights. Shuttle missions might then become useful for testing countermeasures found to be effective during animal experiments conducted under one-g conditions.

Figure 1 shows the mean calcium for 16 rats as a function of the age of the rat. The experimental (exercise) and control (non-exercise) calcium data for the three groups of rats are shown in Figures 2, 3, and 4. Each study was divided into three time periods, pre-exercise, exercise and post exercise. The animals in group A were fed ad lib throughout all phases. Even so, the forced exercise period caused a small weight loss in the exercise period. The rat to be measured was placed in a cylindrical plastic holder (2½" i.d. x 6"). The holder was placed in the collimator insert with its axis 8" above the source. The holder was rotated at 6 r.p.m.. The normal irradiation and counting times were 900 seconds and 1000 seconds respectively.

For each time period the mean total body calcium vs days was fit by linerar regression and is shown by the solid lines in each of the figures. It should be noted that the pre-exercise data for groups A and C were obtained during the rapidly growing phase and therefore the slopes are considerably greater than group B during the pre-exercise period. All rat groups began an exercise regime after the rapid growth

phase. For group A, exercise was started at 118 days of age, group B 226 days, and group C 187 days.

Shown in Table 1 are the slopes and 95% confidence limits of the least squares fit for the mean mg of calcium vs days for the three time periods. In order to account for calcium change not due to exercise, the control group's slope is subtracted from the experimental to calculate the net rate of change. Subtracting the exercise net rate from the post exercise net rate gives the overall change in rate resulting from this change in activity, i.e. changing from exercise state to non-exercise. For group A this gives a value of 1.6. In group B the value is 4.9; however, the control value post exercise consisted of only 3 data points and therefore has a larger statistical uncertainty. If for the non-exercised rats, the exercise and post exercise data periods are considered one period and the combined least squares are determined, one obtains an overall slope of 1.4. When this value is used for both the exercise and post exercise periods, the calcium change becomes +1.6 and -1.2 respectively for an overall Δ of 2.8. This is very close to the value in group C , 3.1, which had better statistics in the post exercise period. In every case the experimental rats had a significant decrease in slope (95% confidence) after cessation of exercise while no significant change was seen in the control animals. In group A (forced exercise) no significant difference was seen between experimental and control animals during the exercise period while voluntary exercise in group B and C resulted in a significant increase over control. This is thought to be due to the factors discussed in the rationale for using voluntary exercise.

TABLE 7

CHANGE IN TOTAL BODY CALCIUM WITH EXERCISE

The slope and the 95% confidence level of the least square fit of the mean calcium vs days is given for experimental and control rats of each group. Rats in group A were given a regimen of forced exercise, and groups B and C were given one of voluntary exercise. It should be noted that the pre-exercise data for group A and C were obtained during the animal's rapid growth phase and therefore the slopes are considerably greater than during the remainder of the experiment. The difference between experimental and control rats gives the net rate of calcium gain (+) or loss (-). The animals in group A were the youngest and group B the oldest.

Mg Calcium/Day

	N	Pre Exercise	Exercise	Post Exercise
			Group A	
Experimental	4	15.2 ± 2.8	3.7 ± 1.3	0.9 ± 1.7
Control	5	16.1 ± 3.7	3.9 ± 1.3	2.7 ± 1.5
Net Rate		-0.9	-0.2	-1.8
			Group B	
Experimental	5	2.1 ± 2.8	3.0 ± 0.1	0.2 ± 0.7
Control	6	2.1 ± 2.6	1.2 ± 2.6	3.2 ± 3.0
Net Rate		0	+1.9	-3.0
			Group C	
Experimental	7	10.2 ± 2.0	5.5 ± 0.5	2.0 ± 1.6
Control	7	10.2 ± 2.4	3.8 ± 0.1	3.5 ± 1.5
Net Rate		0	+1.6	-1.5

CEBUS MONKEY STUDIES

It is well known that a complication of high level corticosteroid treatment in man is osteoporosis. In spaceflight increased urinary cortisol in conjunction with calcium loss has been observed. This led us to look for an animal model to study the effects of cortisol administration on calcium loss. An opportunity was presented to collaborate with Texas A & M and to use eight members of their cebus monkey colony in this study.

Cebus monkeys are small South American monkeys whose average weight is approximately 2 kg. Two body regions were measured in this experiment. One was the lower body including the pelvis, legs and tail, the other was the mid-body primarily the spine. The study extended over a 5 month period with each region remeasured at approximately two week intervals. During the entire period the monkeys were fed a diet of known and constant composition.

The monkeys were divided into two groups of 2 males and 2 females each. One group was kept as a control throughout the experiment. Following a five week control period the experimental group was given high doses of corticosteriods over a 16 week period. For the first 10 weeks of the experimental period the drug administered was cortisone, for the initial 6 weeks at 10mg/kg body weight/day and then at 15mg/kg body weight/day. For the final 6 weeks the drug administered was cortisol, at a dosage of 35mg/kg body weight/day. This change was made because the anticipated calcium loss was not occurring and we were concerned that cortisol might not be physiologically equivalent in cebus monkeys.

To make the calcium measurements the monkeys were anesthetized by an intramuscular injection of ketamine. They were then strapped in a holder constructed of acrylic and paraffin in which they were irradiated and counted. The positioning for irradiation was determined by aligning the pelvis in a constant position relative to the collimator. The conditions for irradiation and counting of the spine were similar.

The irradiation time was 10 minutes and the counting time 17 minutes. The radiation exposure was 650 mrad neutrons to the lower body and 450 mrad neutrons to the spine. The lower body and spinal measurements were made on successive days. During the control period each region of each monkey was measured 3 times. As an indication of the precision of the technique the percent standard deviation was calculated for each group of three measurements. For the lower body measurements the mean of these standard deviations was 2.8% and for the spine it was 2.5%. The standard deviation expected from counting statistics alone are typically 1.5% for the lower body and 2.5% for the spine.

To determine the calcium change during the experiment a line was fitted to a plot of the measured values versus time. From the slope of this line the percent change during the experimental period was calculated. These changes are given in Table 8. The experimental period was 16 weeks except for 2 monkeys (E4 and C3) where it was 12 weeks. Monkey C3 died from heat stroke following air conditioning failure during transport. Monkey E4 died from unknown causes. Upon necropsy the adrenals were atrophied, presumably due to the corticosteroid administration. The only indications of change in the lower body region are the 11% and 8% losses in experimental monkeys E1 and E3. There is no indication of loss in the spinal region.

The clear and substantial calcium loss we had anticipated in the corticosteroid treated monkeys did not occur. Our expectations of large calcium losses were based partially on the work of Jaffe et al. with rabbits. Over a 9 week period they administered about 4mg/kg body weight/day of cortisone. They noted evidence of generalized osteoporosis both histologically and roentrographically by the 5th week. In our experiment no substantial calcium loss was seen in spite of dose rates starting at 2½ times and going to almost 10 times that of Jaffe et al.. The explanation for this probably lies in the adreno-cortico function of cebus monkeys and possibly new world monkeys in general.

Yamamoto et al. have compared the plasma corticosteroids of new world marmosets to macaque monkeys and found the levels over 5 times as great in the marmosets. Another small new world monkey (the squirrel monkey) has been studied by Brown et al. and been found to have very high plasma cortisol levels and a turnover rate of 30mg per day. These studies in addition to our own work have led us to conclude that the cebus monkey is not a good model for studying the effects of corticosteroids on calcium balance in man.

TABLE 8

REGIONAL CALCIUM CHANGE IN CEBUS MONKEYS

The experimental monkeys received daily corticosteriod injections. The experimental period was 4 months except for monkeys E4 and C3 where it was 3 months.

GROUP	MONKEY	LOWER BODY % CHANGE	SPINAL REGION % CHANGE
Experimental	E1	-11	1
Experimental	E2	2	4
Experimental	E3	-8	-1
Experimental	E4	-4	0
Control	C1	-4	3
Control	C2	-2	-1
Control	C3	-3	4
Control	C4	4	5

GUINEA PIG STUDIES

These studies were conducted in collaboration with the Departments of Physiology and Pediatrics of Baylor College of Medicine. The initial phase of the study was aimed at evaluating the accuracy of in-vivo neutron activation compared with chemical analysis for Ca, Cl and Na. This part of our studies is reported on in a paper (Appendix F) which has been accepted for publication in the American Journal of Physiology. The material included in this paper will only be sketched over in this section along with a few of the technical details omitted from the paper. The second phase of this experiment was a study of the effect of varying levels of dietary Ca in lactating guinea pigs. Both the mothers and babies were measured.

Technically the guinea pig measurements were very similar to the rat measurements described previously. The two significant differences in technique were caused by the greater range in size of the animals and a desire to obtain accurate measurements for the Cl and Na rather than just Ca. The guinea pigs ranged in size from 90g for one of the nursing babies to over 900g for one of the lactating mothers. This large range in size compared with the 100 to 400g rats required a new holder insert at the small end and a new collimator insert and holder for the larger animals. Appropriate phantoms were constructed to calibrate the system. For most of our studies the primary interest has been in measuring Ca the irradiation and counting times are normally chosen comparable to the half life of Ca-49 ($t_{\frac{1}{2}}$ = 8.7 min). In the initial phase of this experiment accurate measurements of the longer lived isotopes of Cl and Na (Cl-38, $t_{\frac{1}{2}}$ = 37 min and Na-24, $t_{\frac{1}{2}}$ = 15 h) were also desired. The irradiation time was still chosen as 15 minutes (a little less thant 2 half lives of Ca-49) but rather than a single 1000 second count, 5 successive 1000 second counts were made. The amount of each of the elements Ca, Cl, and Na was determined in each of the 5 counts. A weighted average of these 5 determinations per element was taken as the value of the measurement. The weighting factor was determined by

the statistical uncertainty for each count. The Ca measurement was largely determined by the first count while for Na each count contributed approximately equally.

The results from the initial phase of the study were that there was no statistically significant difference in the elemental concentration determined by chemical analysis and by neutron activation for Na, Ca and Cl. The variations were higher than would be expected strictly from counting statistics and higher than in phantom studies but still adequate to provide useful information. The chief advantage of neutron activation over chemical analysis is that it simplifies experimental design. Since an animal may be measured in-vivo it is not necessary to have a cross sectional design with the animals sacrificed and measured at various times.

The second phase of guinea pig work was studying the Ca changes in mothers and babies as a function of diet. The question was whether inadequate Ca in the diet would cause Ca depletion of the mother or reduce accretion of the infant. Unfortunately due to difficulties with the animals we are unable to provide a firm answer to the question. Within a few weeks of their arrival at the vivarium the animals would get sick and die. This seemed to be caused by an infectious agent present in the vivarium. This caused our study to be suspended with only very preliminary results. Of the six mother-baby pairs, 4 were on diets with normal Ca and 2 on diets with no Ca. In no case did any mother show a significant loss of Ca. Each of the infants whose mothers were on normal Ca diets gained Ca (from 23% to 109%). Neither of the infants whose mothers were on Ca free diets gained Ca. This last statement is based on very sparse data, 4 measurements on one infant and only 2 on the other. The illness of the mothers may have been the cause of the lack of Ca accretion. Most of the Ca accretion of the infants whose mothers were on normal diets occurred before the last few measurements. These problems make conclusions impossible but the fact no evidence was seen of the mother depleting her Ca for the infant is interesting and hopefully can be pursued in the future.

FEASIBILITY OF NITROGEN MEASUREMENT

Of the elemental contituents of animals one of the most interesting to measure is nitrogen. Proteins are nitrogenous compounds which are the principal contituents of cell protoplasm. A measure of the nitrogen in an animal would give a measure of the protein present.

Nitrogen is not an element readily adaptable to measurement by neutron activation but because of its physiological importance several laboratories have worked on the problem. Two major approaches have been used. The first makes use of the fast neutron reaction ^{14}N (n,2n) ^{13}N. The ^{13}N decays with a 10 minute half life by positron emission. The decay may be detected by the 511 keV positron annihilation γ-rays. There are a number of possible interferences with this technique either from positrons from other sources (e.g., pair production by high energy γ-rays) or from ^{13}N created by ^{16}O (p,α) ^{13}N. Because of the high energy threshold of the ^{14}N(n,2n) ^{13}N reaction (11 MeV) a neutron source other than Cf is required for its use.

The neutron technique which has generated the most interest for nitrogen measurement is prompt γ-ray analysis. When a thermal neutron is captured by ^{14}N an excited state of ^{15}N is formed which promptly (10^{-16} second) decays to the ground state. About 15% of these captures result in a 10.8 MeV γ-ray. The very high energy of the γ-ray allows it to be distinguished from the very high backgrounds. The high detector backgrounds are a great difficulty with this technique. The counting must be simultaneous with the neutron capture so the γ-ray detector must be viewing the sample during the irradiation. During irradiation a number of things occur in addition to thermal capture of neutrons by nitrogen in the sample, that can cause pulses in the detector. Among the sources of background are γ-rays from the source, γ-rays from neutron capture by elements other than nitrogen (whether in the sample, the shielding or the detector) and fast neutrons scattering in the detector.

Our interest in measuring nitrogen was primarily focused on small animals. This meant that the problems were different than those of the groups that have made in-vivo measurements in humans. Since there is much less nitrogen in a small animal either the neutron flux or detection efficiency must be higher to give adequate signal strength. The neutron source in our facility is much stronger than those which have been used for measurements in humans which led us to hope that small animals could be measured. In a neutron activation technique one can completely compensate for a decrease in the amount of a sample to be measured by an increase in the neutron flux. In a prompt γ-ray technique this is not always possible. The problem arises from the fact that in the prompt γ-ray technique the detector must be present during irradiation. The conditions that gave high fluxes in the sample (and thus an adequate signal) also resulted in unacceptable count rates in the detector. Various methods were attempted to overcome this problem but none were sufficiently promising to expand the studies beyond feasibility testing. The detector used for the feasibility studies was a 5" diameter x 4" NaI crystal. A thermal neutron shield (of paraffin and boric acid) was constructed to fit over the detector both to lessen the background from neutron capture in the detector during a measurement and to reduce the amount of activity induced in the detector. Since the difficulties with the technique stemmed from the high count rates our efforts were aimed at lowering the rates (while preserving the signal) and at increasing the systems ability to handle high rates. The efforts at lowering the count rates in the detector were basically limited to shielding the detector and to reducing the neutron flux by stopping down the irradiation port. A third possibility, backing the detector away from the sample, decreased the signal faster than the background. The chief recognizable γ rays in the background were 477 keV from neutron capture in boron and 2.2 MeV from capture in hydrogen. The 477 keV γ-ray could be managed either with lead shielding or by designing boron out of the system. The hydrogen line is a greater problem since it would originate from any

irradiator storage cask system. Shielding the detector is also difficult since the attenuation coefficient of lead for 2.2 MeV is lower than for 10.8 MeV (the γ-ray from capture in nitrogen). For most of the feasibility tests the detector was shielded not only on the sides away from the sample (2-4 " of Pb) but also from the front (typically ¼" of Pb). Our electronic efforts consisted basically of clipping the detector pulse (typically to 100 ns) and biasing out the low energy pulses before they reached the pulse height analyzer. This increased the rate tolerance but not sufficiently.

There were a number of obvious steps that could have been taken to improve the set up in our feasibility tests. Among them were 1)buying a detector with a photomultiplier tube and base chosen for high count rate capability; 2) designing and constructing a detector shield rather than relying on Pb bricks; 3) using a smaller neutron source closer to the sample, to reduce the background while maintaining the signal. None of these steps were cheap or easy and did not seem sufficiently promising to warrant the effort and expense.

CURRENT PROJECTS

I. Anti-estrogen Effects on Osteoporosis in Ovarectomised Rats

This project has recently been begun in collaboration with Dr.'s L. Misra and P. Beal of the Department of Physiology of Baylor College of Medicine. This pilot project is aimed at testing the hypothesis that the anti-estrogen, clomiphene, can protect against post-ovarectomy osteoporosis in mature breeder female rats. Thirty rats have been divided into 3 groups of 10 rats each. The first group receives a weekly injection of 1mg of clomiphene in 0.25 ml of vegetable oil. The second group has been ovarectomised and receives a weekly injection containing only 0.25 ml of vegetable oil. The third group are normal control rats, not ovarectomised, and given no injections. The Ca in each rat was measured initially and again at 2 months. The preliminary results indicate increased gains for the rats receiving clomiphene. None of the groups lost Ca. It is planned to continue the test at least 2 more months.

II. Renal Failure Patients

The studies of renal failure patients as described in a previous section are continuing on new and old patients.

III. Normal Rats

In collaboration with Steven Altchuler of NASA the Ca in a group of 5 normal rats is being measured periodically. The aim is to provide a normal growth curve to provide an aid in the design of future experiments. During the rapid growth phase measurements were made at intervals of 2 weeks. As the rats have matured the intervals between measurements have been lengthened.

LIST OF FIGURES

FIGURE 1

Age Vs Calcium

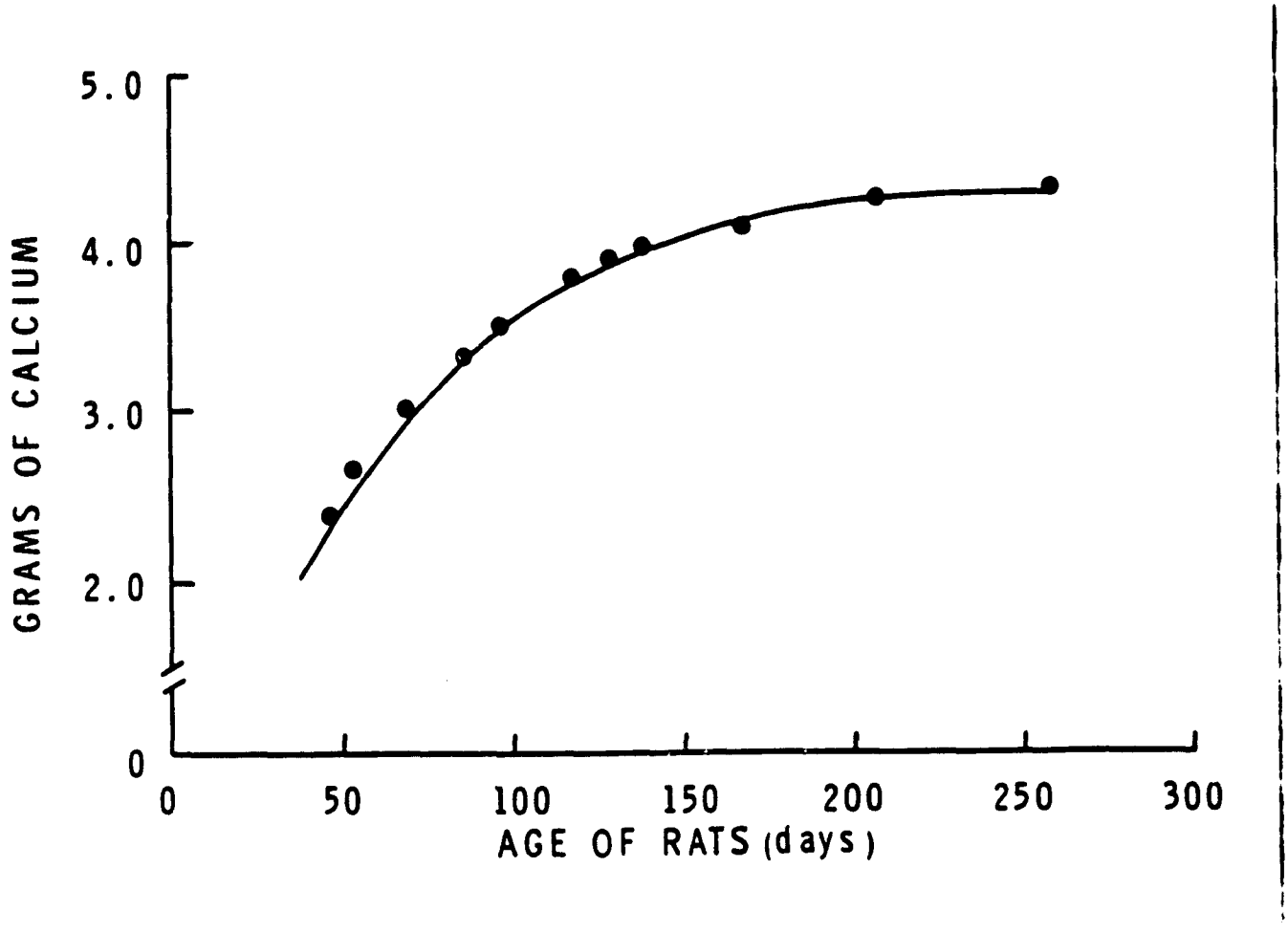
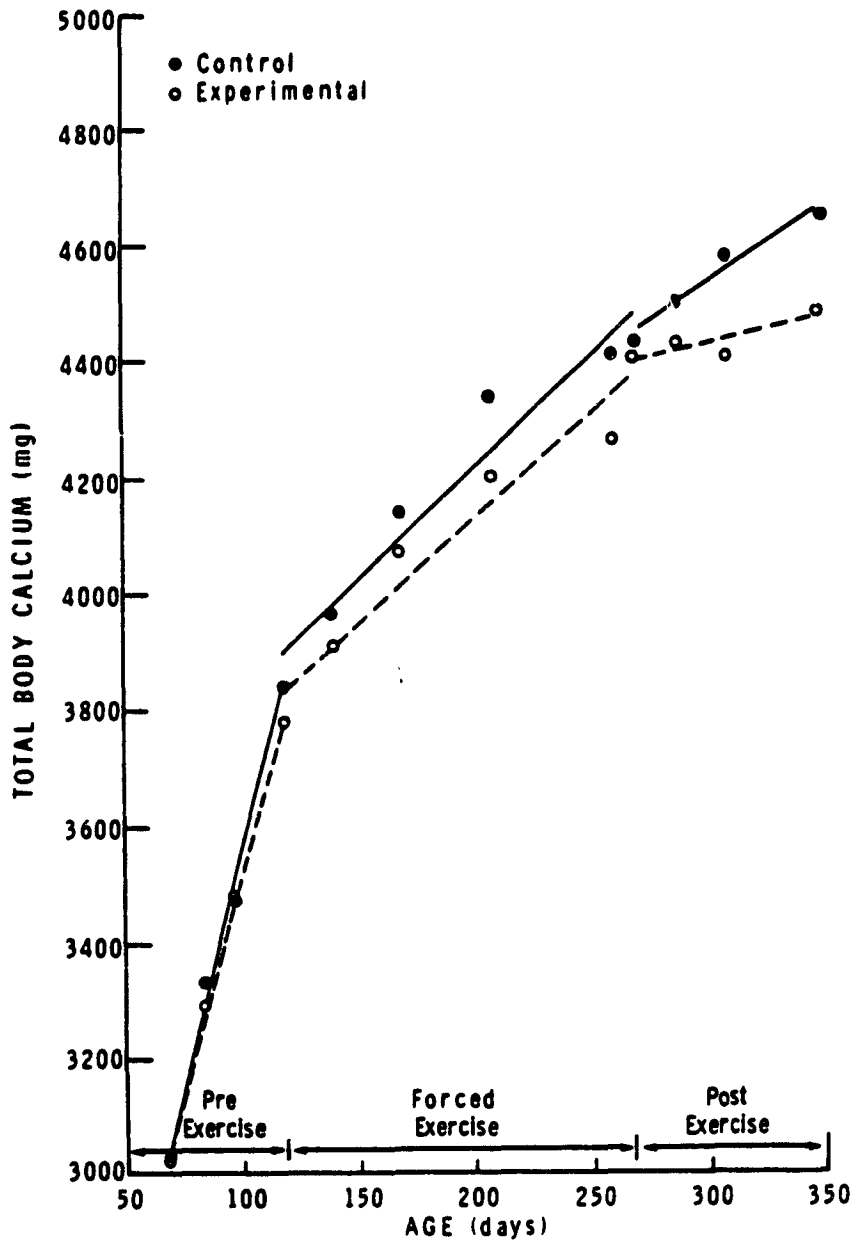


FIGURE 2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4800
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3200
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TOTAL BODY CALCIUM (mg)
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o Experimental
Pre Exercise
Forced Exercise
Post Exercise
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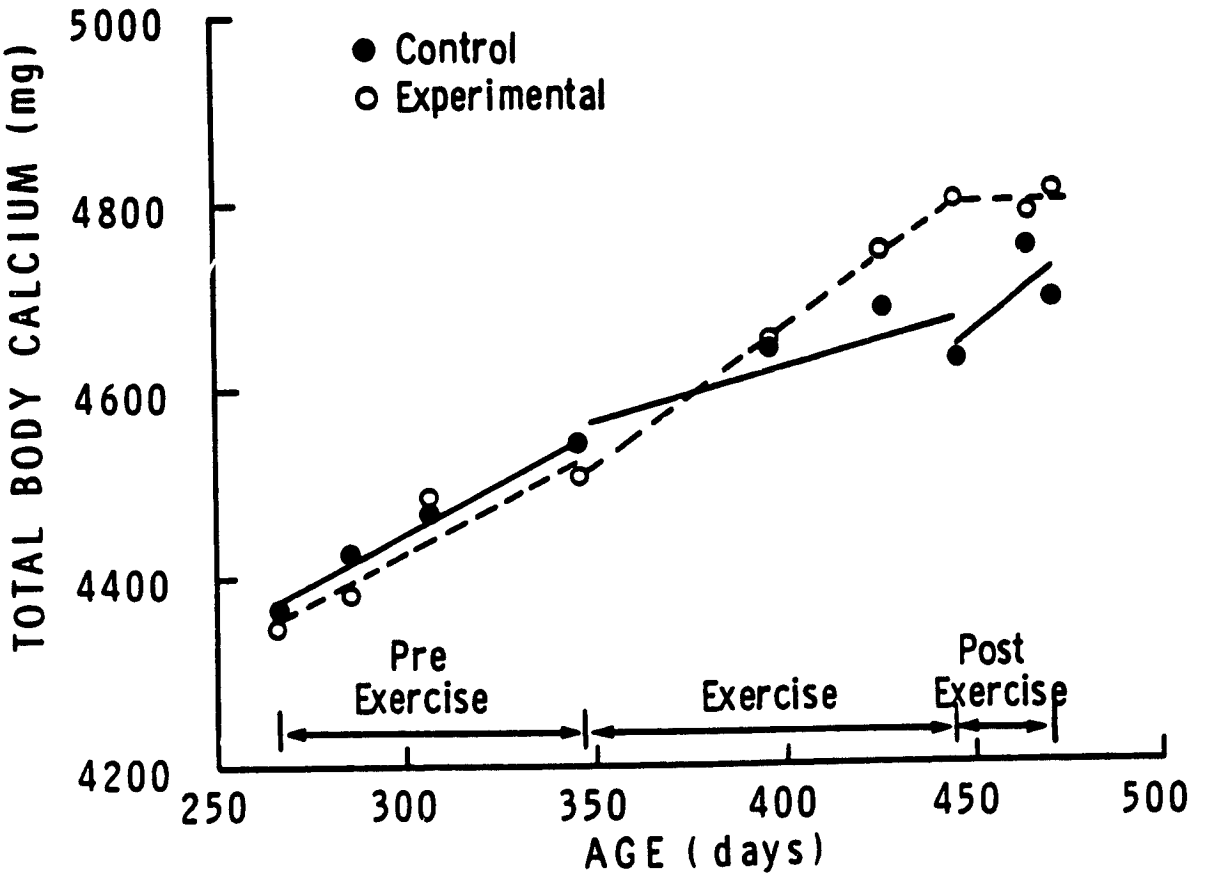
150
200
250
300
350
AGE (days)

FIGURE 3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4400
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Experimental
Pre Exercise
Exercise
Post Exercise
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400
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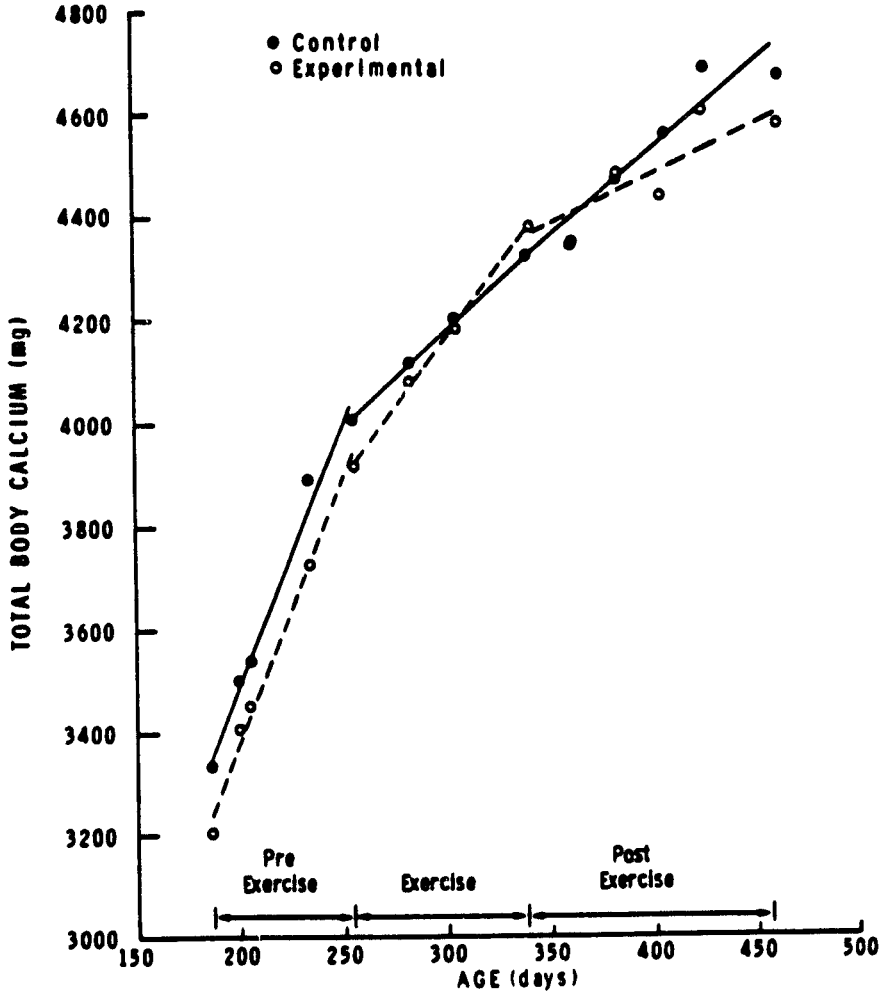
500
AGE (days)

FIGURE 4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Experimental
TOTAL BODY CALCIUM (mg)
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4200
4000
3800
3600
3400
3200
3000
Pre Exercise
Exercise
Post Exercise
150
200
250
300
350
400
450
500
AGE (days)

APPENDIX A

Feasibility study: *In vivo* neutron activation for regional measurement of calcium using Californium 252*

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The feasibility of using a collimated ^{252}Cf neutron source to measure regional changes in skeletal calcium was tested because *in vivo* regional activation of diseased bone should offer advantages over the more widely reported total-body calcium measuring techniques. Regional activation allows examination of discrete regions where the greatest changes in calcium content occur. Additionally, a simpler radiation facility is required for regional studies. Using a 5.5-μg ^{252}Cf source, thermal neutron flux and absorbed dose were measured in a tissue-equivalent phantom. Detection efficiency of ^{49}Ca γ rays for conditions simulating regional activation were measured using a 29-cm-diameter $\times$ 10-cm-thickness sodium iodide detector. These *in vitro* measurements indicate that a collimated ^{252}Cf source can be used for regional neutron activation of the lower spine and legs. Preliminary calculations indicate that a 1–3-mg source provides adequate count rates for statistical accuracy with a bone marrow dosage acceptable for human patients and normal subjects.

I. INTRODUCTION

Osseous calcium loss occurs in many diseases and as a result of prolonged bedrest or spaceflight. Traditionally, osteoporosis has been evaluated in semiquantitative fashion from roentgenograms of the skeleton. This has not been satisfactory because early changes are difficult to detect.[1] To quantitate the small changes in bone calcium associated with early disease, more sensitive techniques for measuring bone calcium are required. *In vivo* neutron activation of whole-body calcium has been shown to be accurate and sensitive.[2–4] However, it is probable that certain bones lose calcium more rapidly than others and, in some diseases, calcium loss may be occurring in one bone, e.g., a single immobilized extremity, while calcium accretion is occurring in others. In these situations, regional measurements of calcium may have an advantage over a whole-body method. If the regional measurements were confined to areas of active bone remodeling, earlier biological changes would be observable than if the overall mean change in total-body calcium were to be determined.[5] The primary purpose of this study was to investigate the feasibility of measuring calcium *in vivo* in the lower spine. The lower spine was chosen because the lumbar vertebrae can be expected to show the earliest and most pronounced change in calcium content during common pathological states.[6,7]

A method for measuring phosphorus and calcium content of bone has been described using small, uncollimated $^{241}AmBe$ or ^{252}Cf neutron sources placed on both sides of the hand and ankle.[8,9] A similar method has been described for measuring calcium content in the hands after the subject grips a $^{241}AmBe$ source contained in a plastic moderator.[4] Partial-body calcium has been measured by locating PuBe sources above and below the trunk of recumbent subjects. This irradiates 33%–60% of the total-subject dose[10] More recently an unfiltered reactor spectr has been used to activate the calcium in phantoms of t spine and appendages.[11] A precision of about 3% was o tained for doses of 50 mrad. However, wide variation thermal flux occurs through the spine as a result of the l neutron energy.

Except for the nuclear reactor method, regional activati techniques have used uncollimated neutron sources plac close to the skin. A collimated source with a greater sourc to-skin distance (SSD) has theoretical advantages, especial if bilateral irradiation is not practical as is true for t lumbar spine. A larger SSD with collimation (1) has bett uniformity of thermal flux by minimizing inverse squa variations and (2) more shielding can be placed between t source and areas not being irradiated. A disadvantage is th larger sources and more extensive shielding are required.

Of the several radioactive neutron sources available, on ^{252}Cf is available in sufficient strength for SSD greater tha a few centimeters and yet is contained within a reasonab small volume. Large-volume sources are less satisfactor since the source volume increases the penumbra of t radiation field. The required source size will depend on t counting accuracy desired, detector sensitivity, mass calcium activated, absorbed dose levels, collimator geometr source-to-skin distance (SSD), and the mean thermal neu tron flux per microgram of ^{252}Cf. To determine the require source size and absorbed dose, the thermal flux and absorbe dose distribution of ^{252}Cf were measured in a tissue-equiv lent phantom for two different collimator geometries, tw SSDs, and two different collimator wall materials. Althoug this work was undertaken to investigate the feasibility measuring calcium in the lumbar spine, the data indicat that a collimated source of ^{252}Cf can be used for oth

II. METHOD

A 5.5-μg ^{252}Cf source (type SR-Cf-100) with a yield of 1.3×10^{7} neutrons/sec was used. Measurements of thermal flux and the absorbed dose from fast neutrons and γ rays were performed in a 30×50-cm acrylic phantom filled with tissue-equivalent liquid to a depth of 20 cm. In all cases the phantom was placed directly on top of the collimator opening.

Two rectangular collimators were investigated, 10×20 and 20×30 cm. The collimators had parallel, vertical walls. The source was placed in the center of the collimator opening. Source-to-phantom distances were 50 and 41 cm. The collimator consisted of water-extended polyester (WEP) impregnated with 1% boron carbide. Provision was made for removing the inner 5 cm of the collimator to permit insertion of 5-cm Pb bricks.

Thermal neutron flux was measured using water-filled plastic vials (0.6-cm diameter × 3 cm) containing 400-mg MnO_2. Activation was determined by measuring the 0.85-MeV γ ray from the decay of ^{56}Mn to ^{56}Fe. A flux-depression correction of 11% was used. No correction was made for epithermal absorption, which could cause an error of about 4%.

To measure the fast-neutron dose, a pair of pocket ionization chambers (Don L. Collins and Associates) was used, one equally sensitive to neutron and gamma and the other less sensitive to neutrons. Fast-neutron dose was calculated as follows: $R_{N,\gamma}=A(D_\gamma+D_N)$ and $R_\gamma=A(D_\gamma+BD_N)$, where $R_{N,\gamma}$ is the reading of the neutron- plus gamma-sensitive chamber, R_γ is the reading of the chamber which is less sensitive to neutrons, D_γ is the gamma dose, D_N is the neutron dose, and A and B are instrumental constants. The constants A and B for the chambers were determined with the source and chambers in a tissue-equivalent phantom, using the standard neutron and gamma dose distributions from Colvett *et al.*[12] From seven measurements at three points between 4.2 and 6.5 cm, the constants were determined to be $A=0.848\pm0.028$ and $B=0.325\pm0.050$ (mean ±s.d.). Based on the errors in the determination of the constants A and B and in reading the dosimeters, the error in the fast-neutron dose is estimated to be less than ±20%. As a check of these constants, readings were taken in air at 13.5 cm. The ratio of the measured-to-expected neutron dose was 1.13. A quality factor of 10 was used for the fast-neutron component to obtain dose equivalent in rems.

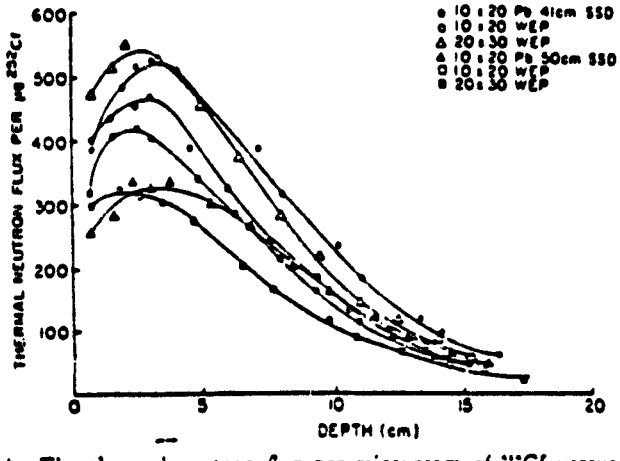

FIG. 1. The thermal neutron flux per microgram of ^{252}Cf versus the depth in the phantom for several collimator geometries.

TABLE I. Expected mean thermal flux per microgram through the spine.

Collimator (cm)	SSD (cm)	Mean flux (n/cm² sec μg)	Percentage of the mean low value	high value
10×20 Pb	50	265	83	115
10×20 WEP		242	64	120
20×30 WEP		308	66	123
10×20 Pb	41	409	80	116
10×20 WEP		349	63	120
20×30 WEP		411	68	120

TLD 700 LiF, calibrated against a ^{137}Cs source, was used as a secondary check on the gamma dose measured with the ionization chambers. At a depth of 6.5 cm in a tissue-equivalent phantom, the TLD gamma dose was 5.8 mrad/h while the ionization chambers gave a value of 5.2 mrad/h.

A 29-cm-diameter×10-cm-thick NaI(Tl) detector (Bicron Corp.) was used; its efficiency for counting ^{49}Ca in the lower spine was determined. The most intense γ-ray following neutron activation of ^{48}Ca is 3.10 MeV. To determine the detector sensitivity at this energy, the 2.60-MeV γ ray of a standard ^{56}Co source was used. A correction was made for the 0.85-MeV γ ray which occurs in cascade with the 2.60-MeV γ ray. To correct for the decrease in efficiency between 2.60 and 3.10 MeV, the measured intensities were multiplied by 0.92. The detector efficiency was determined at a distance of 7.5 cm from the crystal face over the area of interest (7.5×20 cm) approximating the area of the lower spine. A counting window from 2.97 to 3.23 MeV was assumed for calculating efficiency.

III. RESULTS

Figure 1 is a plot of thermal flux density per microgram of ^{252}Cf as a function of depth along the central axis in the tissue-equivalent phantom. The curves were made at 50- and 41-cm source-to-phantom distances. Two collimators with WEP walls and one Pb-walled collimator are shown. The mean thermal flux density was calculated from these curves for a hypothetical 7-cm-thick spine, assuming 0.8-cm thickness of tissue-equivalent material between the spine and source. Table I gives the mean flux and the maximum and minimum percentages of the mean per microgram of ^{252}Cf. This shows that the collimator with Pb walls gives a higher mean flux density and less variation with depth than the equivalent-sized WEP collimator. The larger collimator, 20×30-cm WEP, had the highest mean flux.

Figure 2 is a plot of thermal flux density ϕ_{th} as a function of the lateral displacement from the central axis, normalized to the central-axis value. The thermal flux density over the collimator edge falls to about 70% of the central-axis value

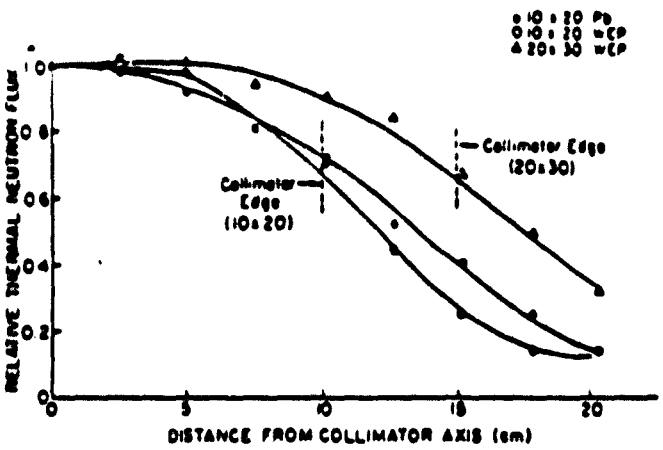

FIG. 2. The thermal neutron flux normalized to the central value versus the distance from the collimator axis at a depth of 6.5 cm in the phantom.

One of the limits of the precision of *in vivo* activation analysis is the dose which one is willing to deliver to the subject. Therefore, the ratio of ϕ_{th} to absorbed dose is an appropriate figure of merit. Table II gives the mean ϕ_{th} (Table I) divided by the absorbed dose for different depths and collimators. The results at 50 cm SSD were not significantly different from those at 41 cm SSD. The most striking thing in this table is the higher figure of merit for the 20×30-cm collimator at 1-cm depth. However, three times the area is being exposed in this case. Because of the uncertainties in the absorbed dose measurements previously discussed (see Sec. II), the difference between the WEP- and Pb-walled 10×20-cm collimators may not be significant.

The detector efficiency for 3.1-MeV gammas was determined assuming a lower spine geometry of 7.5×20 cm at an effective distance of 7.5 cm from the face of the 29×10-cm detector. Under these conditions, an overall photopeak (2.97–3.23 MeV) efficiency of 5.8% can be expected. The background count rate of the unshielded detector is 25 counts/min under the peak.

Using the data from Tables I and II and the detector efficiency and making assumptions of the amount of Ca, source size, source distance, transfer time, and counting time, the required irradiation time (and therefore absorbed dose) can be calculated as a function of the counting error.

In Table III the irradiation time and absorbed dose were calculated as a function of counting error for the following conditions: 25-g Ca target, a 3-mg ^{252}Cf source at 41 cm SSD in a Pb-walled 10×20-cm collimator, a 1-min transfer time, and a 20-min counting time. The absorbed dose given in Table III is the mean total-body bone marrow dose; it was assumed that the lower spine contains 10% of the active bone marrow, located at an effective depth of 4 cm from the skin surface (see Appendix for sample calculation). The dose to the irradiated bone marrow would be ten times the mean dose and the skin dose about 15 times the values given in Table III. The dose to the male gonads is less than 10% of the central-axis surface dose, or about equal to the values given in Table III. To obtain a statistical coefficient of variation of 1.5% using a 1-mg source, the absorbed dose given in Table III would be increased by 30%. Therefore, a 1–3-mg source would be adequate.

If areas other than the spine are to be studied, bilateral irradiation might be advantageous over unilateral irradiation. The uniformity of thermal flux density can be obtained by summing the curves given in Fig. 1 at various depths in the phantom. Figure 3 gives the resultant curves using the 10×20-cm Pb collimator for total phantom thicknesses of 14, 16, and 18 cm. The values are normalized to the central value. A flux uniformity of ±2% is obtained over 8 cm (center of 16-cm curve) and of ±6% over 12 cm (center of 18-cm curve). This would indicate that ^{252}Cf can produce acceptable thermal flux uniformity in the appendages as well as the spine by employing bilateral irradiation.

TABLE II. Expected mean thermal flux density through spine divided by absorbed dose as a function of depth in phantom.*

Depth (cm)	Mean thermal flux/absorbed dose, collimator type		
	10×20 Pb	10×20 WEP	20×30 WEP
1	12	14	18
4	22	28	29
8.5	76	74	73

* SSD = 41 cm

TABLE III. Relationship between total mean marrow dose and coefficient of variation.*

CV (%)	Irradiation time required (min)	Mean bone marrow dose (mrem)
1	6.1	570
1.5	2.5	235
2.5	0.96	90
4.0	0.45	42
6.0	0.25	23

* See text for conditions and assumptions.

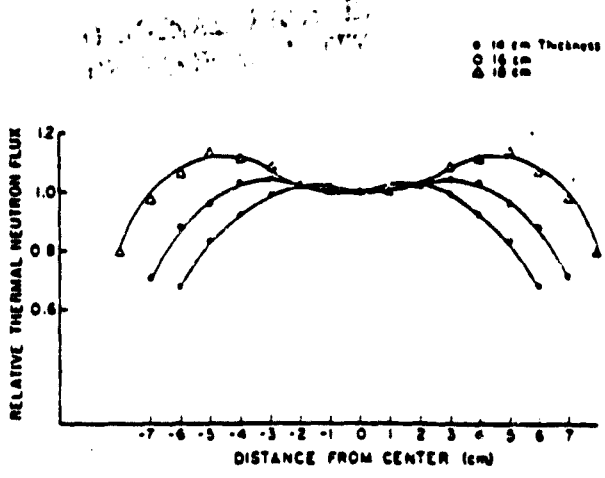

FIG. 3. The thermal neutron flux as a function of depth for bilateral irradiation of phantoms of various thicknesses, normalized to th

IV. DISCUSSION

In deciding which approach we wished to take, i.e., whole-body versus regional measurements, a number of factors were considered. Both techniques can measure relative changes in calcium while whole-body techniques are best suited for determining absolute values. The basic requirements for such quantitative determinations are that the ϕ_{th} and counting efficiency must be uniform over the body and invariant to changes in body size among individuals. Several techniques have been reported in the literature in order to achieve these two requirements.[4,13–19] The reported accuracies vary from ±4% to ±5.2%. The normal values of calcium vary with sex, age, and body build so that, for a given measurement of calcium to be meaningful, an estimate must be made of the normal or expected calcium content.[20,21] High precision has been achieved with whole-body measurements, e.g., Nelp[17] can detect a 3% change in whole-body calcium, which represents 30 g of calcium.

In disease, if calcium is not lost uniformly from all bones, then regional measurements would provide a more sensitive technique. For example, in generalized osteoporosis the spine shows early and rapid changes in calcium.[4] Similar differences are probably present in other conditions which cause loss of skeletal calcium.

Regional activation systems have a major advantage over whole-body systems in that a high counting efficiency is more easily attained. This is important since the total neutron flux required and therefore the absorbed dose to the subject is inversely proportional to the detector efficiency. Most whole-body counters are of the scanning type, where the subject is moved with respect to the detectors at some predetermined rate.[4,18] The major disadvantage of these detectors is low efficiency due to the short time near any given region. For 1% counting statistics the whole-body dose is approximately 1 rem.[4] It is possible to make whole-body detectors in which the whole subject is counted simultaneously. The Brookhaven 54-detector monitor is one of the most sensitive of this type.[13] The whole-body dose from this system is approximately 0.3 rem to achieve 1% counting statistics. A whole-body detector of this efficiency is prohibitively expensive for most laboratories. Even the Brookhaven detector has a lower efficiency than can be attained for a small region with a single detector. The Brookhaven counter has an efficiency of 6.9% for a ^{54}Mn (0.835 MeV) source in air in the center of the counter, but only a 3.0% efficiency for a source uniformly distributed in a subject.[13] With our single 29×10-cm detector, we found an average efficiency of 10.7% over an area chosen to simulate the lower spine (7.5×20-cm rectangle, 7.5 cm from the detector). This advantage results primarily from the fact that the detector can be placed closer to the region of interest. A single detector placed near the irradiated region will have a less-uniform detection efficiency over the region than would a whole-body counter. This nonuniformity will make a comparison between subjects difficult but should not be a problem in comparing serial measurements of an individual as long as care is taken to reproduce the conditions of irradiation and counting.

With a decision to use regional activation rather than whole-body activation, the next question is what source type is best. The choice is necessarily a trade-off between several factors such as cost, size, thermal neutron uniformity, and absorbed dose. As the neutron energy is increased, the depth uniformity of the thermal flux is improved but the radiation dose to the spine is increased also and the dose at depths greater than the spine increases rapidly. For example, the dose equivalents per incident fluence at 15 cm in a cylindrical phantom for 2.5-, 5-, and 14-MeV neutrons are 106.6, 274.2, and 379.7 (10^{-10} rem neutron^{-1} cm^2), respectively.[22] The cost of ^{239}PuBe or ^{241}AmBe sources having a yield of 10^9 n/sec is about $60,000. A similar ^{252}Cf source would be about $4,500 at current prices. Even though the ^{252}Cf source would have to be replaced every 3–5 yr, all other things being equal, the lower capitalization cost of ^{252}Cf would tend to favor this source over PuBe or AmBe sources. A neutron generator costs about $25,000 with 14-MeV neutron yields of 10^{11} n/sec. Low-voltage generators can produce 2–4-MeV neutrons but with yields of only 10^9 n/sec. Output variability is a disadvantage in the use of generators. A reactor is prohibitively expensive unless one is already on site and available.

For regional activation with a collimated beam, a point source is desirable in order to reduce the penumbra. For the high neutron yields required, only ^{252}Cf, neutron generators and reactors can qualify. For instance, a 10^9 n/sec source of PuBe would represent a volume of approximately 400 cm^3.

As a feasibility study, our objective was to measure the 3-dimensional distribution of thermal flux and absorbed dose in a tissue-equivalent phantom using ^{252}Cf as the neutron source. The limited strength of the ^{252}Cf source available gave too low a fast-neutron flux at working distances to obtain an accurate measurement of the fast-neutron component. However, since the dosimeters were calibrated against a known ^{252}Cf source in air and in tissue-equivalent liquid, the measured values are probably within ±20% of the true values.

The effect of several geometries on thermal flux and absorbed dose were investigated. Two source-to-surface distances, two collimator wall materials, and two collimator sizes were investigated. For measuring relative changes in calcium, acceptable uniformity of thermal flux was obtained with either 41- or 50-cm source-to-phantom distances. Small changes in the thickness of soft tissue in between the source and the spine produce minor changes the mean thermal flux. For example, it was calculated that, if the thickness of tissue in front of the spine was increased by an additional 0.5 cm, the calculated mean flux for the 10×20-cm collimator at 50 cm was reduced approximately 1%. The mean thermal flux through a distance equivalent to an adult lower spine was 44% higher for the 41-cm distance than for the 50-cm distance, although when expressed as flux per unit dose rate, the two are equivalent. The Pb-walled collimator resulted in better uniformity, a higher thermal flux, and higher absorbed dose as compared to WEP walls. The 20×30-cm collimator increased the thermal flux

density by 23% while decreasing thermal flux depth uniformity and increasing integral absorbed dose by a factor of about 3.

For measurements of the spine, bilateral irradiation adds little to the flux uniformity while increasing integral absorbed dose substantially. For the extremities, such as the tip of the femur, bilateral irradiation is preferable. Employing bilateral irradiation in the configuration described will result in less than ±6% variation from the mean when the total tissue thickness is less than 12 cm. In this feasibility study, all measurements have been made in tissue-equivalent liquid. It is anticipated that the interposition of bone will disturb the neutron flux since the hydrogen density of bone is different from that of soft tissue. However, the ratio of neutron flux divided by the absorbed dose rate should not be altered appreciably.

APPENDIX: SAMPLE CALCULATION OF COUNTING COEFFICIENT OF VARIATION VERSUS ABSORBED DOSE

Assuming the background is known with high accuracy, the coefficient of variation for counting a sample is given by the formula, $CV = \sqrt{(C+B)}/C$, where C is the number of counts in the peak and B is the background. For a CV of 0.015, C is equal to 4900 counts. C is related to the irradiation time by the following formula.

$$C = \frac{fWN_0\phi\sigma(1-e^{-\lambda t_i})(BR)\epsilon e^{-\lambda t_m}(1-e^{-\lambda t_c})}{A},$$

where

C = total counts = 4900,
f = isotopic abundance of ^{48}Ca = 0.00185,
W = weight of calcium = 25 g,
N_0 = Avogadro's number = 6.02×10^{23},
ϕ = thermal flux = 1.23×10^{6} n/cm² sec,
σ = cross section = 1.1×10^{-24} cm²,
λ = decay constant of ^{49}Ca = 0.0788 min.$^{-1}$,
A = atomic weight of calcium = 40.1,
BR = branching ratio for the 3.10-MeV γ = 0.9,
ϵ = detector efficiency = 5.8%,
t_m = time between end of irradiation and start of counting = 1 min,
t_c = counting time = 20 min,
t_i = irradiation time.

The above equation is solved for t_i, which for a CV of 1.5% is 2.53 min. To obtain absorbed dose, the irradiation time is multiplied by the absorbed dose rate. The absorbed dose rate is obtained by dividing the flux, 1.23×10^{6}, by the appropriate value in Table II, 22, to obtain 55.9 rem/h or 0.93 rem/min. Multiplying 0.93 by 2.53 min gives 2.35 rem. Since approximately 1/10 the bone marrow is irradiated, the mean marrow dose is 0.235 rem.

* This work was supported by NASA Contract No. NAS 9-13737.

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APPENDIX B

A. INTRODUCTION

The Gamma Industries Model 100 Biomedical Irradiator was designed to provide collimated neutron beams for the in vivo measurement of calcium depletion in humans and animals. Specifically the systems utilizes a moderated fission-neutron beam emitted by the ^{252}Cf isotope. The neutron beam is collimated and is employed to excite the $^{48}Ca(n.\ )^{49}Ca$ reaction.

B. MODULAR CONSTRUCTION

The irradiator design utilizes the modular concept, making it possible to easily move or modify the system. Basically, four modules comprise the irradiator.

1) Source storage module and associated BWEP shielding.
2) Irradiator with moderator cavity and BWEP shielding.
3) Control module
4) Interlock system

Neutron shielding is achieved by the use of stacked BWEP blocks which are staggared in such a way that no neutron streaming is possible when the source is in the stored position. Primary fission gamma rays are attenuated by a lead shield.

The BWEP shieldblocks which measure 4"H X 8"W X 16"L are fabricated according to the following formula per block:

H_2O	50 v/o	1.5 Gallons
WEP	50 v/o	1.5 Gallons
Boric Acid	6 w/o	1.5 Pounds
Hydrogen Peroxide		50 mL - 85 mL
Sodium Hydroxide		1 ounce or until neutral

The BWEP and water mixture temperature ranged between 68° and 70° in this particular instance.

To prevent long-term water leaching, each block was allowed to dry for several days, then coated with Steelcoat, an epoxy-based enamel.

C. CONTROL PANEL MODULE

The control panel module consists of the following controls and indicators:

1) The main power switch controls the line voltage to all interlock circuits. The line is doubly fused.
2) The key-operated interlock release switch is employed to release all interlock systems in the case of an emergency.
3) The personnel interlock plugs are pulled, making the system inoperative, whenever personnel enter the irradiation area. These must be replaced before the system can be made operative.
4) The key-operated lock is used to release the upper locking ball on the cable drive.
5) The cable drive is mounted to the right of the control panel, making it possible to manually control the source position.
6) The three control lamps indicate the source position, i.e.
    a) Green = Safe storage
    b) Amber-Red = Source in transit
    c) Red = Source exposed

D. OPERATING INSTRUCTIONS

1) Assume the source to be in the stored position at "O".

2) The Teleflex cable locking ball preventing source movement is released by unlocking the key-operated lockbox "A", mounted on the source storage module.

3) The door to the irradiation area is shut and the lock "R" is secured. This activates microswitch "I", activating solenoid lock "J", releasing the lower locking ball "P" on the Teleflex drive cable.

4) The key operated lock "K" is unlocked releasing the upper locking ball "P". The source is now free to move.

5) The source is cranked to the exposed position in the center of the moderator cavity at "E".

6) Once the source leaves the stored position at "O", the green light on the control panel is extinguished and the amber and red lights illuminated. Both lamps will remain illuminated as long as the source is in a transient condition.

7) When the source leaves the stored position, the microswitch "B" activates the solenoid lock "H" preventing access from the outside of the irradiation room.

8) In an emergency, exit from the irradiation room may be achieved by disengaging the solenoid lock "H" with the emergency exit switch "N", and manually opening the lock "R".

9) As the source is cranked into the exposed position at "E", the return upper cable locking ball Q trips the microswitch

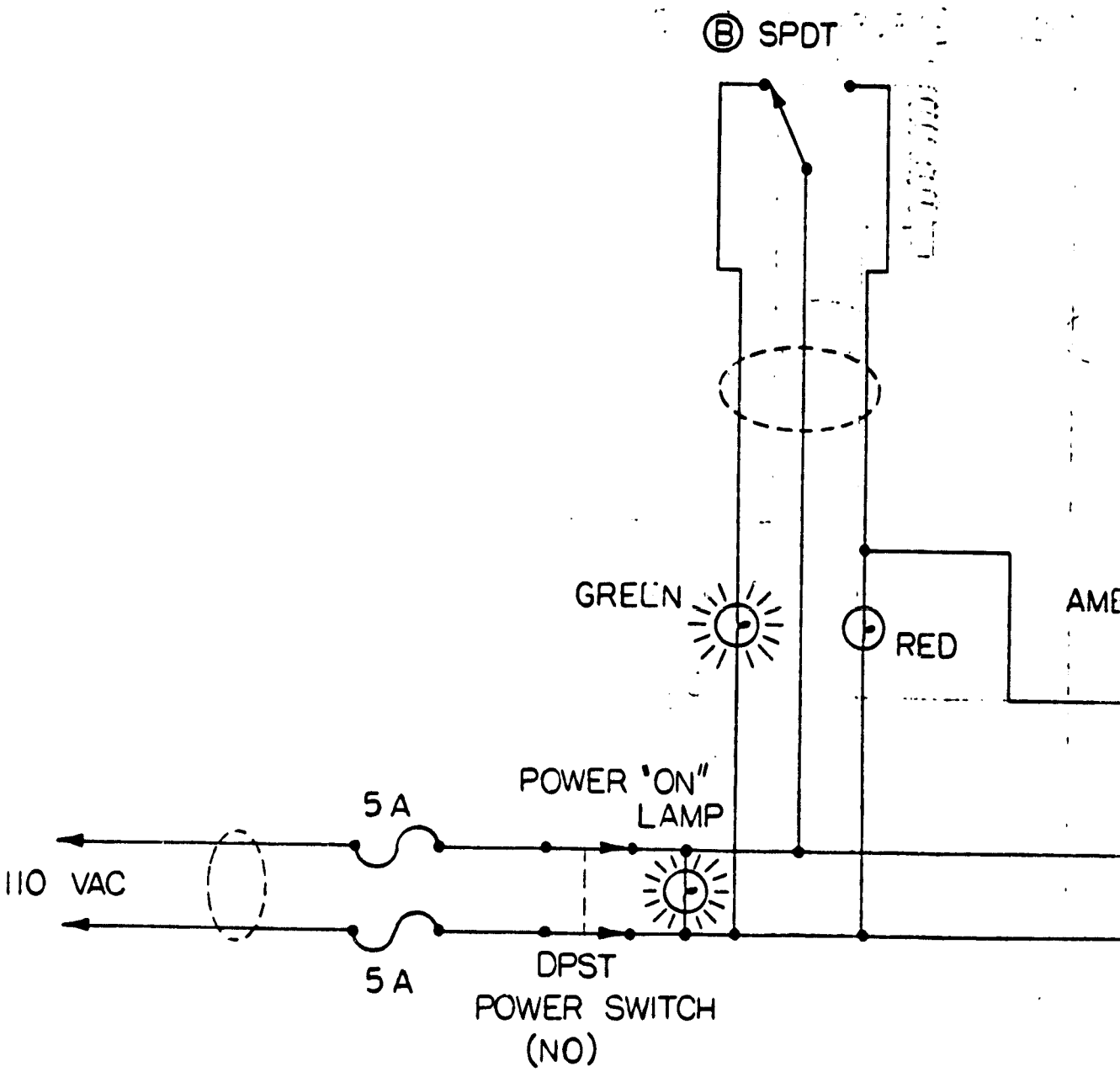
"F", causing the amber light to blink as the source position "E" is approched. A second lower locking ball Q disengages the amber light when the source reaches the final position "E", leaving only the red lamp illuminated.

WARNING: ONCE THE AMBER BLINK SIGNAL HAS BEEN OBSERVED, THE SPEED OF CRANKING SHOULD BE LOWERED TO REDUCE SHOCK TO THE END OF THE SOURCE TUBE.

E. COMPONENT LIST

ITEM		LOCATION
A)	Main Lockbox	Irradiation Area
B)	Interlock Microswitch	Irradiation Area
C)	BWEP Neutron Shielding	Irradiation Area
D)	Lead Primary Gamma Shield	Irradiation Area
E)	Exposed Source Position	Moderator Cavity
F)	Source Position Indicator Lamp Microswitch	Control Room
G)	Mechanical Insert Locks	Control Room
H)	Solenoid Door Lock	Control Room
I)	Door Lock Operated Micro-switch	Control Room
J)	Solenoid Lock-Lower Drive Cable Locking Ball	Control Room
K)	Key Operated Lock-Upper Drive Cable Locking Ball	Control Room

L)	Teleflex Drive Cable	Control Room/Irradiation Area
M)	Teleflex Return Cable	Control Room
N)	Emergency Exit Switch	Irradiation Area
O)	Source Stored Position	Irradiation Area
P)	Upper & Lower Drive Cable Locking Balls	Control Room
Q)	Upper & Lower Return Cable Indicator Lamp Actuators	Control Room
R)	Mechanical Lock	Control Room



NC = NORM

NA = NORM

NO = NORM

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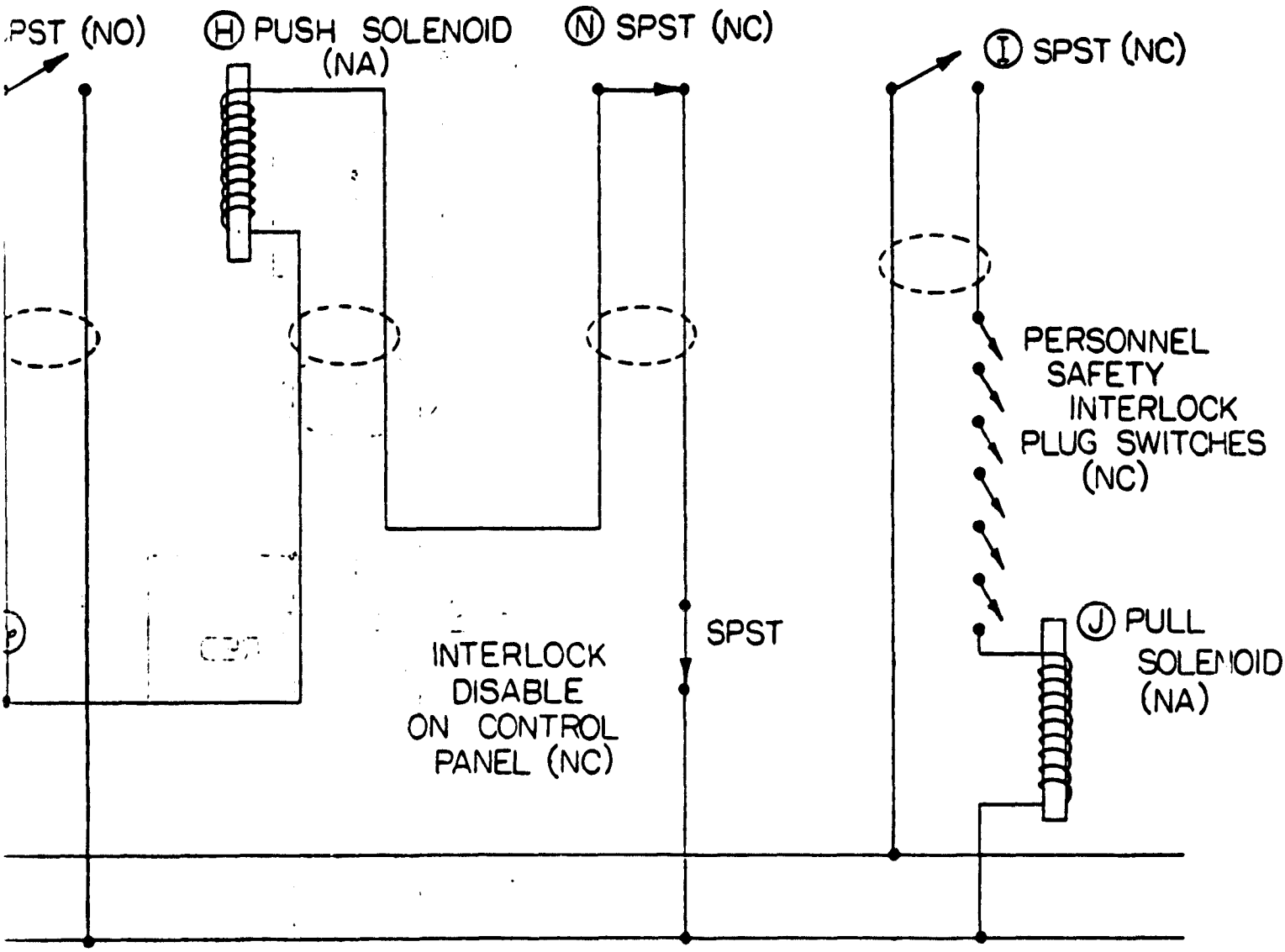

_Y CLOSED

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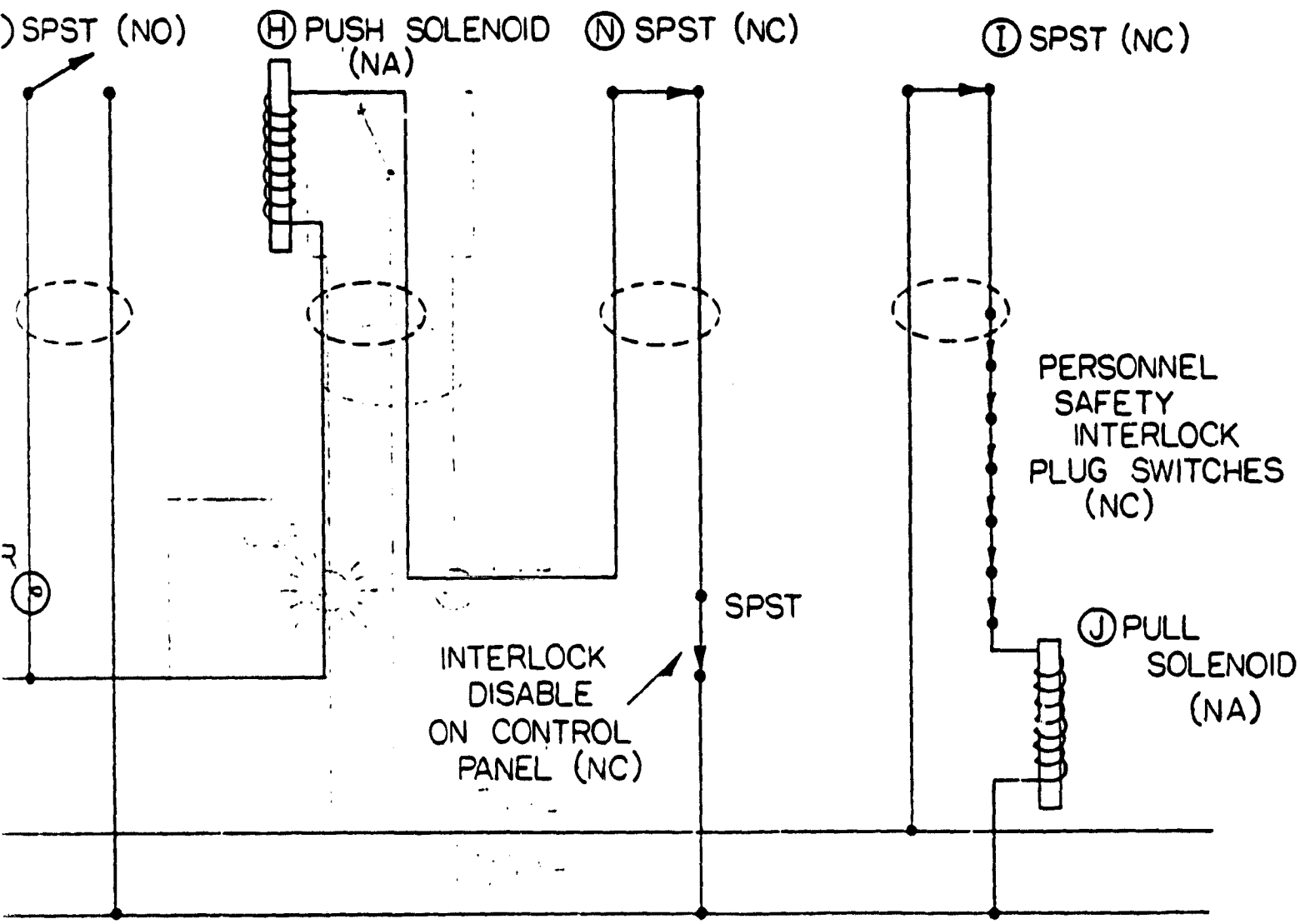
NUCLEAR SYSTEMS INC., B.R., LA.		
SCALE: NONE	APPROVED BY:	DRAWN BY WDL
DATE: 1-22-76		REVISED
SOURCE SECURED MODE		
BIOMEDICAL IRRADIATOR MODEL 100		DRAWING NUMBER 417-21



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NUCLEAR SYSTEMS INC., B.R., LA.		
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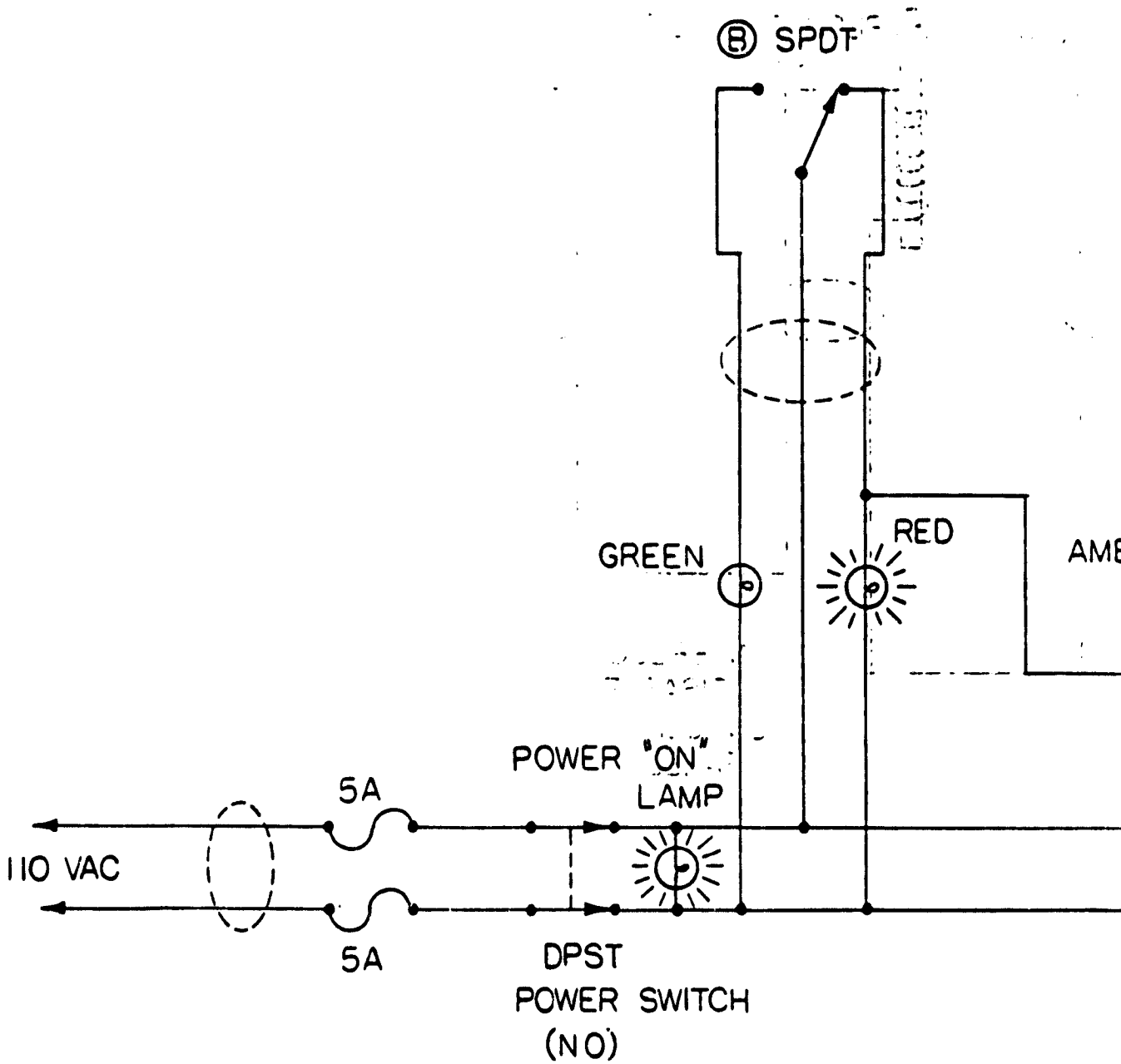
DATE: 1-22-76		REVISED
SOURCE EXPOSED MODE		
BIOMEDICAL IRRADIATOR MODEL 100		DRAWING NUMBER 417-22



NC= NORMAL

NA= NORMAL

NO= NORMAL

APPENDIX C

PHYS. MED. BIOL., 1979, Vol. 24, No. 1, 181–187. Printed in Great Britain

Technical Note

Facility for Regional *in vivo* Neutron Activation Analysis of Skeletal Calcium

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1. Introduction

Activation analysis is a recognised analytical tool. Only recently has this technique been employed for elemental analysis in human subjects and laboratory animals. The approach is similar to *in vitro* activation analysis methods except that radiation dose to the subject or animal and flux uniformity throughout non-uniform tissues become important. A number of elements can be measured accurately and safely (Boddy, Dennis and Lawson 1969, Cohn and Dombrowski 1971, Boddy and Glaros 1973, Dombrowski, Wallach, Shukla and Cohn 1973).

Two basic approaches (whole body or regional techniques) have been used for *in vivo* neutron activation analysis of the skeleton. In the whole body techniques the entire body is irradiated with fast neutrons which are slowed to thermal energies by the body tissue and a portion of them are captured by the reaction $^{48}Ca\ (n,\gamma)\ ^{49}Ca$. The beta decay of ^{49}Ca ($t_{\frac{1}{2}} = 8{\cdot}7$ min) is followed by the emission of a 3·1 MeV gamma ray in 92% of the decays. This gamma ray activity is measured with a whole body counter. There are several good descriptions of whole body radiation facilities in the literature (Cohn, Dombrowski and Fairchild 1970, Nelp, Palmer, Murano, Pailthorp, Hinn, Rich, Williams, Rudd and Denney 1970). In the regional or partial body techniques selected areas are irradiated and the induced ^{49}Ca is measured. Regional techniques are most useful when the calcium content of localised areas is undergoing rapid change or when one area of the skeleton is losing calcium while another area is gaining calcium. Regional systems using small uncollimated PuBe, AmBe and ^{252}Cf sources placed close to the irradiation site have been described (Catto, McIntosh and Macleod, 1973, McNeill, Thomas, Sturtridge and Harrison 1973, Boddy, Robertson and Glaros 1974). This paper describes our facility which was designed and built to measure regional changes in skeletal calcium of human subjects or animals and to measure whole body calcium in small animals. This facility employs a collimated beam

0031-9155/79/010181+07 $01.00

of neutrons obtained from a 3 mg ^{252}Cf source on loan from the Energy Research and Development Agency. The neutrons produced by ^{252}Cf have a fission spectrum with a mean energy of 2·3 MeV.

2. Description

2.1. *Neutron irradiation facility*

Fig. 1 is a diagrammatic view of the storage shield and irradiator (built and installed by Nuclear Systems, Inc. of Baton Rouge, Louisiana) with a subject positioned for a calcium measurement of the foot and ankle. Within the storage shield the source is surrounded by 7·5 cm of lead to shield the ^{252}Cf

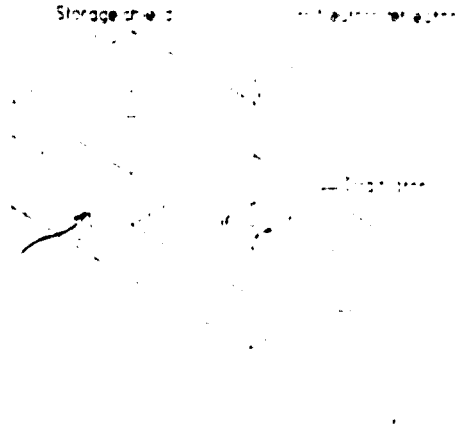

Fig. 1. ^{252}Cf irradiator and storage shield. A subject is shown in position for activation of the foot.

fission gamma rays. Surrounding the lead are blocks of borated (1·0%) water extended polyester (WEP) which provide a minimum shielding thickness of 60 cm. The 10 cm × 20 cm × 40 cm blocks are staggered to minimise streaming. Shown in table 1 are the exposure readings around the shield with the 3 mg ^{252}Cf source in the store position. The neutron exposures were measured with a BF_3 counter calibrated with a ^{252}Cf source and the gamma ray exposures were measured with an ionisation chamber. A quality factor of 10 was assumed for fast neutrons. These values are sufficiently low to allow long work periods in the room containing the shielded source.

Table 1. Storage shield survey

	Exposure readings (mrem h^{-1})		
	Neutron	Gamma rays	Total
Max. surface (side)	17	16	33
Avg. surface (side)	3	6	9
Max. 3 ft. (side)	3	2	5
Max. surface (top)	1	5	6
Avg. surface (top)	< 1	2	< 3
Irradiator well	< 1	< 1	< 1

Source movement from the storage shield to the irradiator is achieved from a control panel located in an adjacent room. The source is connected to a flexible drive cable which in turn is contained within a stainless steel tube. The source is hand cranked between the storage and irradiation positions with a travel time of 1–2 s for the 142 cm distance. Safety devices include door interlocks, source position indicator lights, radiation-actuated lights, audible alarm when the source is out of the storage position and finally the source crank cannot be rotated without first unlocking it with a key.

The exit port of the irradiator is 20 cm × 36 cm. By means of collimator inserts variations in source to tissue distances and collimator geometries are possible to optimise thermal flux, flux uniformity and absorbed dose. For example, fig. 2 shows the irradiator in cross-section arranged for irradiating the

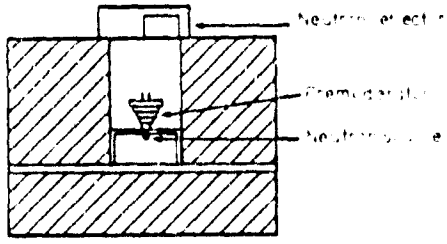

Fig. 2. Cross-sectional view of the exit port of the irradiator showing the premoderator and backscatterer used for activation of the foot.

foot. The amount of premoderator can be varied in 2·5 cm increments. With this arrangement nearly all neutrons reaching the target tissue have been scattered one or more times from the collimator, the premoderator or the plastic neutron reflector enhancing thermal neutron uniformity and decreasing radiation absorbed dose. A similar arrangement is used for irradiating the hand. Fig. 3(*a*) is an insert used for obtaining a well defined beam of unmoderated neutrons. This insert, machined from two 10 cm blocks of plexiglas, has an exit opening of 10 cm × 20 cm and is tapered toward the source. The distance from source to exit opening is 40 cm. Fig. 3(*b*) is a collimator insert

Fig. 3(*a*). Tapered collimator insert. (*b*) Collimator insert used for irradiation of rats.

used for irradiating laboratory rats. This insert consists of 5 cm thick plexiglas rectangular walls and a removable cylinder used to hold the rat. The cylinder is rotated (6 rpm) during irradiation by means of a motor mounted in the wall of the insert. A plexiglas block is placed above the cylinder during irradiation to increase backscattered neutrons.

2.2. *Counting facility*

The counting facility located in a room adjacent to the irradiation room includes two opposing 29 cm diameter by 10 cm thick NaI (Tl) detectors. The distance between the two detectors can be varied by means of hydraulic jacks from 8 to 67 cm. Because of the high gamma ray energy (3·1 MeV) of ^{49}Ca, no detector shielding is required. In our facility the background under the calcium peak is approximately 25 cpm for each unshielded detector. The pulse height spectra are recorded with a 1024 channel analyser. The counts under the calcium peak are corrected for the contribution to the count from ^{24}Na and ^{38}Cl.

3. System performance

Shown in table 2 are the results of calcium determinations in four laboratory rats by neutron activation and by atomic absorption. For the atomic absorption measurements, the carcasses are ashed in a muffle furnace. The ashes are dissolved in HCl which is neutralised and brought to known volume. The atomic absorption measurements were performed blindly by a separate

Table 2. Calcium analysis of four laboratory rats

Rat	IVNAA (g ± % SD)	Atomic absorption (g)	Δ (%)
A	3·87 ± 1·3	3·9	+ 0·8
B	4·00 ± 1·1	4·2	+ 4·8
C	4·00 ± 2·5	4·1	+ 2·4
D	4·08 ± 1·6	4·1	+ 0·5

reference laboratory. The neutron activation results are given as the mean and per cent standard deviation of four total body irradiations (12 min irradiation, 33 min counting) on successive days. The normalisation is done by carefully duplicating the geometry of each irradiation and by making appropriate mathematical corrections for irradiation time, the time between the end of irradiation and the start of counting, and for the source decay between irradiations. The radiation exposure, measured with paired ionisation chambers, was 3 rad of neutrons and 3 rad of gamma rays for each irradiation. There were approximately 16 000 net counts in the calcium peak. These results indicate a precision from 1·1 to 2·5% with a mean of ± 1·6%. The quantity of calcium in each rat was calculated by comparing its count with a water-filled phantom containing appropriate amounts of Na, Cl and Ca. Compared to atomic absorption, activation analysis underestimated calcium content from 0·5 to 4·8%. If the mean value of + 2·1% were used as a correction factor, the variability would be − 1·6 to + 2·7%.

In preparation for the regional analysis of human subjects, a plexiglas phantom of a human foot was used to study positional variation of the thermal neutron flux. Shown in table 3 is the flux distribution when this phantom was

Table 3. Thermal flux in foot phantom

Depth (cm)	Flux (10^5 n cm^{-2} s^{-1})		
	Heel	Instep	Toes
0·6	5·7	6·0	4·8
1·9	6·3	6·7	5·2
3·2	5·5	6·3	5·0
4·4	3·9	5·1	4·4

unilaterally irradiated with a premoderated beam. The mean thermal flux was $(5{\cdot}4 \pm 0{\cdot}7)10^5$ n cm^{-2} s^{-1}. The flux is adequately uniform for serial measurements of a subject's foot. If one wishes to make absolute calcium measurements greater uniformity would be desirable, but probably at the expense of increased radiation exposure or decreased counting statistics.

Another phantom of a foot was constructed by embedding the bones of a human foot and leg in paraffin. Successive activations (3 min irradiation, 20 min counting) gave a coefficient of variation of 1·7% with 1·0% counting statistics. The foot of an adult human volunteer (A.D.L.) was irradiated three times under the same conditions and the coefficient of variation was 1·9%. The skin surface dose for each irradiation was 66 mrad of neutrons and 94 mrad of gamma rays. At a position corresponding to the head of the femur, 90 cm from the exit port and perpendicular to the beam axis, exposure values were 0·3 mrad of neutrons and 2·6 mrad of gamma rays.

A human hand phantom was constructed of paraffin and 23 g of $CaCO_3$ to simulate bone. Because there is less calcium in the hand than in the foot, a longer exposure time is needed for accurate measurement. However, because of the decreased thickness of the hand, this can be partially compensated for by increasing the thickness of the premoderator. The result is that the dose to the surface of the hand phantom was very similar to that of the foot phantom, 65 mrad neutrons and 105 mrad gamma rays.

4. Discussion

The greatest potential clinical utility of activation analysis is measurement of skeletal calcium in diseases characterised by osteoporosis. Of the cases of osteoporosis, post-menopausal osteoposoris is the most widespread affecting approximately 25% of white U.S. females by the sixtieth year (Heany 1971). Less commonly skeletal calcium loss occurs because of gastrointestinal, endocrine and renal disorders or from vitamin deficiency. There is also excessive calcium loss in patients bedrested for prolonged periods of time and in astronauts as a result of weightlessness (Donaldson, Hulley, Vogel, Hattner, Bayers and McMillan 1970, Whedon, Lutwak, Rambaut, Whittle, Smith, Reid, Leach, Stadler and Sanford 1974, Rambaut, Smith, Mack and Vogel 1975). Our interest

in activation analysis occurred because these studies indicated that in spaceflight and bedrest calcium was not lost uniformly from all bones. A regional activation analysis system appeared to be ideal for studying regional calcium changes resulting from bedrest, spaceflight and various bone-wasting diseases. If bone loss in these conditions is inhomogeneous, regional measurements might prove to be more useful than whole body measurements. A method of regional activation of the spine, a body region of great medical interest, has been presented by Al-Hiti, Thomas, Al-Tikrity, Ettinger, Fremlin and Dabek (1976). Several considerations led to the beginning of our human studies by measuring the feet and hands. Technically studies of the extremities are easier because reproducible positioning is simpler, more efficient counting and therefore lower radiation exposure is possible, and a more uniform thermal flux can be achieved. Activation of the extremities also avoids exposing sensitive organs such as bone marrow to radiation. It is hoped that enough useful information can be obtained from studies of the extremities to escape the increased difficulties and risk of studying the spine.

A number of fast neutron sources have been used for the measurement of calcium, such as reactors, cyclotrons, linear accelerators and isotopic sources such as PuBe and more recently ^{252}Cf (Cohn *et al.* 1970, Nelp *et al.* 1970, Cohn, Shukla, Dombrowski and Fairchild 1972, Catto *et al.* 1973, Boddy *et al.* 1974, Boddy, Glaros and Robertson 1975, Harrison, McNeill, Meema, Fenton, Oreopoulos and Sturtridge 1975, Al-Hiti *et al.* 1976). The choice of a neutron source is governed in part by available equipment, space, funds and by the required neutron energy (Cohn, Fairchild and Shukla 1973). The high cost of nuclear reactors and cyclotrons will normally exclude consideration of them by investigators who do not have access to existing equipment. Designing a facility for regional, rather than whole body, analysis allows the use of an isotopic source such as ^{252}Cf with a relatively low average neutron energy (2·3 MeV). Isotopic sources have the advantages of compactness, high reliability and constant neutron output.

This facility has been in operation for approximately one and one-half years. To gain experience with the system it has been used to investigate the laboratory rat as a model to study the effects of spaceflight on skeletal bone calcium and to determine the effects of various hormonal interventions on regional skeletal calcium loss in monkeys. Only minor, easily solved problems have occurred with the equipment during these studies. Approval has just been obtained to begin an investigation of regional skeletal calcium changes in patients with various bone-wasting diseases particularly those undergoing renal dialysis.

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APPENDIX D

INFORMED CONSENT

Measurement of Regional Calcium Changes in Patients Undergoing Renal Dialysis

I agree to participate in a study to measure bone calcium changes which may result from kidney failure.

I understand that the calcium content of both my hands and wrist and my left foot and ankle will be measured 3 to 4 times over a one year period of time. Each determination will require approximately one hour.

I agree to the following:

1) to be seated in a shielded room and have my hand positioned over an instrument which will irradiate my hand

2) remain as positioned while the technician leaves the room

3) to be in contact with the technician and to be monitored by closed circuit TV when the technician is not in the room

4) not move my hand while the instrument is turned on for 3 to 6 minutes

5) move to an adjoining room where my hand will be placed on an instrument which will measure the radioactivity produced in my hand and wrist

6) not to move my hand and wrist during the measurement (10-15 minutes)

7) repeat steps 1-6 for the opposite hand

8) return to the shielded room and be positioned on a stretcher with my left foot over the irradiator

9) remain as positioned while the technician leaves the room

10) not move my left foot while the instrument is turned on for 1 to 4 minutes

11) move to the adjoining room where my left foot will be placed on the instrument which measures the radioactivity produced in my foot and ankle

12) not move my foot and ankle during the measurement (10-15 minutes)

I understand that:

1) There may be some slight discomfort because of the lack of movement of my arms and legs during the irradiation and measurement periods.

2) The radiation exposure resulting from this procedure is considered within safe limits.

3) I understand that in the event of physical injury resulting from the research procedures described to me that there will be no financial compensation or free medical treatment offered by Baylor College of Medicine or The Methodist Hospital.

4) I may benefit from this procedure by permitting a more accurate evaluation of bone changes which may result from kidney failure. I realize however, that this procedure is investigational and may not benefit me.

5) That my name will not be revealed in any published paper or report.

6) During the study I will be under the care of Dr. Wadi Suki or his associates.

7) If I have any questions concerning the study, Dr. Suki or Dr. LeBlanc will be glad to elaborate on the purposes and procedures.

8) I may withdraw from the study at any time.

Date:_____

_____
Legal Signature

Witness:_____

APPENDIX E

PARTIAL BODY ACTIVATION ANALYSIS USING A CALIFORNIUM -252 SOURCE

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ABSTRACT

Partial body activation of calcium has been performed in experimental animals (cebus monkeys) and human patients using a neutron source (1.5 mg ^{252}Cf) placed 40cm from the skin surface. The collimator, premoderator and backscatterer are designed to optimize the results from the specific region activated. Following activation, the irradiated area is positioned between two 29cm diameter x 10cm thick NaI detectors for counting and quantitation.

Four cebus monkeys were administered corticosteroids daily over a 4 month period. The skeletal calcium of these monkeys along with 4 control monkeys was measured 11 times in each of two body regions, the lower body including the pelvis and legs and the spinal or mid-body region. There was evidence of a slight loss in the lower body of two of the four experimental monkeys while no change was seen in the other two or in the controls. No change was seen in the spinal region of the experimental or control animals.

The long term calcium change in the feet and hands of renal dialysis patients are being studied. The mean per cent difference for 14 measurements (4 feet and 10 hands) repeated at a one week interval was -1.2 with a standard deviation of 3.4. The preliminary results from 4 patients monitored over a 6 month period show indications of slight calcium loss in one patient, a strong indication of gain in another and no change in the other two.

Keywords: Calcium, Monkeys, Corticosteroids, Neutron Activation, Renal Dialysis

I. Introduction

It is well known that a complication of high level corticosteroid treatment in man is osteoporosis (Birkenhager et al. 1967, Adams and Jowsey 1967). In spaceflight increased urinary cortisol in conjunction with calcium loss has been observed (Leach 1974; Whedon et al. 1974; Donaldson et al. 1970). This led us to look for an animal model to study the effects of cortisol administration on calcium loss. An opportunity was presented to collaborate with Texas A. & M. and to use eight members of their cebus monkey colony in this study. Because calcium loss is one of the possible complications in long term renal dialysis, we are beginning studies of the long term calcium changes in the hands and feet of renal dialysis patients. Hopefully this monitoring will provide the clinician with a rapid and accurate assessment of his attempts to prevent calcium loss.

II. Methods

A. Facility

The neutron source in our laboratory is 1.5mg of ^{252}Cf. The neutrons from californium have a typical fission spectrum with a mean energy of 2.3 MeV. Our irradiation facility is located in a room 3½ x 3½ meters in size with 1½ meter concrete walls. The storage cask and irradiator are constructed of borated water extended polyester blocks. The source is moved by cable between the storage position and a 20 x 30cm port in the irradiator section. Various collimators and premoderators can be placed in this port to optimize the beam for the animal or human body

region to be activated. The distance from the source to the port face is 40cm. Backscatterers are placed around the region of the body being activated both to increase the neutron flux uniformity and to provide some radiation shielding for the subject. Patients are monitored by closed circuit television and are in voice communication during irradiation.

The counting facility located adjacent to the source room consists of two large, 29cm diameter x 10cm thickness, NaI crystals which are separately connected to a multichannel analyzer. The detectors are vertically opposed with an adjustable separation. Because of the high energy of the gamma ray following ^{49}Ca decay, 3.1 MeV, the detector background is low and only minimal shielding is employed. Gain shifts in the detectors are minimized by using stabilized amplifiers and a reference source.

B. Cebus Monkeys

Cebus monkeys are a small South American monkey whose average weight is approximately 2 kg. Two body regions were measured in this experiment. One was the lower body including the pelvis, legs and tail, the other was the mid-body primarily the spine. The study extended over a 5 month period with each region remeasured at approximately two week intervals. During the entire period the monkeys were fed a diet of known and constant composition.

The monkeys were divided into two groups of 2 males and 2 females each. One group was kept as controls throughout the experiment.

Following a five week control period the experimental group was given high doses of corticosteroids over a 16 week period. The dosage regime is given in Table 1. After the 15th week the drug was changed from cortisone to cortisol. This change was made because the anticipated calcium loss was not occurring and we were concerned that cortisone and cortisol might not be physiologically equivalent in cebus monkeys.

To make the calcium measurements the monkeys were anesthesized by an intramuscular injection of ketamine. They were then strapped in a holder constructed of acrylic and parafin in which they were irradiated and counted. The positioning for irradiation was determined by aligning the pelvis in a constant position relative to the collimator. The conditions for irradiation and counting of the spine were very similar. The irradiation time was 10 minutes and the counting time 17 minutes. The radiation exposure was 650 mrad neutrons to the lower body and 450 mrad neutrons to the spine. The lower body and spinal measurements were made on successive days.

C. Renal Dialysis Patients

Our standard procedure is to separately measure the left foot and both hands of each patient. For activation of the foot the patient is positioned on a specially constructed stretcher. The foot is strapped on the acrylic holder attached to the stretcher and placed over the collimator opening. Because of the small thickness of the foot one can get adequate penetration with neutrons of even lower

energy than those from ^{252}Cf. To lower the radiation dose by degrading the effective energy of the neutrons, a 10cm thick plastic premoderator is placed in the direct path from the source to the foot. Neutrons scattering from the walls of the collimator are relatively unobstructed. A backscatterer of 5cm acrylic is placed over the foot. The thermal neutron flux uniformity as measured in a phantom of a foot is given in Table 2. The highest value is about 70% above the lowest. This would make absolute measurements difficult but should not affect the ability to follow serial changes in an individual. After irradiation the patient is rolled to the next room for counting.

For the hand measurements, the subject's hand is placed in an acrylic sheet over the collimator opening. Reproducible positioning is obtained by having the patient slide his hand against 2 plastic pins positioned between his fingers. The irradiation conditions for the hand are similar to those for the foot but with more premoderator and a narrower collimator opening. The irradiation time for the foot is 3 minutes and for the hand 6 minutes. In each case the region is counted for 15 minutes. The neutron exposure is approximately 70 mrad to the foot and 60 mrad to the hand.

III. Results

A. Cebus Monkeys

During the control period each region of each monkey was measured 3 times. As an indication of the precision of the technique the per cent standard deviation was calculated for each group of three measurements. The range and mean of these values are given in Table 3. The

standard deviations expected from counting statistics alone are typically 1.5 per cent for the lower body and 2.5 per cent for the spine.

To determine the calcium change during the experiment a line was fitted to a plot of the measured values versus time. From the slope of this line the per cent change during the experimental period was calculated. These changes for the lower body are given in Table 4. The experimental period was 16 weeks except for 2 monkeys (E4 and C3) where it was 12 weeks. Mondey C3 died from heat stroke following air conditioning failure during transport. Monkey E4 died from unknown causes. Upon necropsy the adrenals were atrophied, presumably due to the corticosteroid administration. The only indications of change in the lower body region are the 11% and 8% losses in experimental monkeys E1 and E3. There is no indication of loss in the spinal region, Table 5.

B. Renal Dialysis Patients

To test the repeatability of the technique and to obtain baseline values for the patients, five patients were measured twice at a one week interval. The observed differences are shown in Table 6. Each measurement has a one standard deviation uncertainty due to counting statistics of approximately 2%. The only case where the two measurements differed by more than 5% was the 10% for the right hand of patient BJW. Table 7 shows the change observed in 4 patients over a 6 month period. In patient WKJ there is a slight indication of calcium loss and in patient MN a strong indication of a gain in calcium.

IV. Discussion

The clear and substantial calcium loss we had anticipated in the corticosteroid treated monkeys did not occur. Our expectations of large calcium losses were based partially on the work of Jaffe et al.(1972) with rabbits. Over a 9 week period they administered about 4mg/kg body weight/day of costisone. They noted evidence of generalized osteoporosis both histologically and roentrographically by the 5th week. In our experiment no substantial calcium loss was seen in spite of dose rates starting at 2½ times and going to almost 10 times that of Jaffe et al. The explanation for this probably lies in the adreno-cortico function of cebus monkeys and possibly new world monkeys in general.

Yamamoto et al. (1977) have compared the plasma cortisoteroids of new world marmosets to macaque monkeys and found the levels over 5 times as great in the marmosets. Another small new world monkey (the squirrel monkey) has been studied by Brown et al. (1970) and been found to have very high plasma cortisol levels and a turnover rate of 30mg per day. These studies in addition to our own work have led us to conclude that the cebus monkey is not a good model for studying the effects of corticosteroids on calcium balance in man.

At relatively low radiation risk regional activation of the extremities provides a method of monitoring calcium changes in renal dialysis patients. Calcium changes greater than about 5% should be detectable with the current procedures. We plan to continue this study with a substantially expanded patient population and hopefully will be able to report further in the future.

Weeks	mg/kg Body Weight/day	Corticosteroid
1 through 5	0	
6 through 11	10	Cortisone
12 through 15	15	Cortisone
16 through 21	35	Cortisol

Table 1. Corticosteroid dosage to experimental monkeys

Region	Range of % S.D.	Mean % S.D.	Expected % S.D.
Lower Body	.8 to 5.0	2.8	1.5
Spine	1.2 to 4.5	2.5	2.5

Table 3. Control period calcium measurement precision in 8 cebus monkeys. Each region was activated 3 times.

Experimentals		Controls	
Monkey	% Change	Monkey	% Change
E1	-11	C1	-4
E2	2	C2	-2
E3	-8	C3	-3
E4	-4	C4	4

Table 4. Lower body calcium change in cebus monkeys. The experimental monkeys received daily corticosteroid injections. The experimental period was 4 months except for monkeys E4 and C3 where it was 3 months.

Experimentals		Controls	
Monkey	% Change	Monkey	% Change
E1	1	C1	3
E2	4	C2	-1
E3	-1	C3	4
E4	0	C4	5

Table 5. Spinal calcium change in cebus monkeys. The experimental monkeys received daily corticosteroid injections. The experimental period was 4 months except for monkeys E4 and C3 where it was 3 months.

APPENDIX F

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REVISED MANUSCRIPT

TOTAL BODY SODIUM, CALCIUM, AND CHLORIDE MEASURED CHEMICALLY AND BY NEUTRON ACTIVATION IN GUINEA PIGS

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Running head: Total Ca, Cl, Na by neutron activation

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The material in this manuscript has not been submitted for publication elsewhere.

ABSTRACT

Total body sodium, calcium, and chloride measured chemically and by neutron activation in guinea pigs. H.P. Sheng, R.A. Huggins, C. Garza, H.J. Evans, A.D. LeBlanc, B.L. Nichols, and P.C. Johnson. Department of Physiology, USDA/SEA Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Texas Children's Hospital, Technology Inc., Houston, TX 77030, and Johnson Space Center, Clear Lake, Texas. Body sodium (Na), calcium (Ca), and chloride (Cl) of guinea pigs weighing between 227-600 g were measured by total body neutron activation analysis (TBNAA) followed by chemical analysis (CA) on 12 to 17 animals. Paired t test was used to compare any differences in the results obtained by the two methods. There was no significant difference in the results for the three elements. The means and standard deviation for Ca (g/100 g body weight) 1.070 $\pm$ 0.132 (TBNAA) and 1.107 $\pm$ 0.125 (CA); for Na 0.149 $\pm$ 0.019 (TBNAA) and 0.143 $\pm$ 0.021 (CA); and for Cl 0.126 $\pm$ 0.009 (TBNAA) and 0.132 $\pm$ 0.024 (CA). Neutron activation analysis alone, in a series of 27 animals, gave means (g/100 g body weight) and standard deviation of 1.110 $\pm$ 0.084 for Ca, 0.120 $\pm$ 0.009 for Cl and 0.153 $\pm$ 0.011 for Na. Total body neutron activation analysis has potential usefulness, particularly in longitudinal studies in the same animal, because of its accuracy and the rapidity and ease with which the measurements can be made.

Index terms: Total body, sodium, calcium, chloride, neutron activation, guinea pigs.

The first experimental use of neutrons to produce radioactivity in living animals, according to Nagai et al. (17), probably was by Kellerholm and his associates. They irradiated lambs with thermal neutrons and measured the induced ^{128}I activity of the thyroid. Anderson et al. (2) in 1964, however, reported the first use of neutron activation analysis in the human for the measurement of total body Na, Ca, and Cl. This report soon was followed by studies of other investigators who explored the accuracy and reproducibility of neutron activation in measuring total body Na, Cl, P, Mg, Ca, N, and O (2,3,8,10,11,13,15,16).

The reproducibility and accuracy of total body neutron activation (TBNAA) have been measured repeatedly with phantoms. The accuracy of TBNAA varies depending on the element measured; the error is within 1-4%. For example, the mean differences between results obtained by neutron activation analysis and known concentrations in a phantom are reported to be within 1.1% for Ca, 1.8% for Na, 2.1% for Cl, and 3.9% for P (1,4,8,9). The reproducibility of TBNAA in measurements on humans essentially is the same as that with phantoms. Nelp et al. (18) measured Ca in three cadavers and obtained a coefficient of variation for repeated measurements that ranged between 0.6 and 1.6%, whereas Cohn and colleagues (8,9) estimated a reproducibility for Na, Cl, and Ca in the living human of less than $\pm$ 4% in the same patient.

There are few published comparisons of TBNAA and chemical analysis (CA) on the same animal. Two experiments have been reported on rats in which total body Ca was measured by TBNAA and by CA. Shukla and Cohn (21) used three rats in their comparison of the two methods and the mean value for Ca was 6.3% higher for the chemical method than for TBNAA. Evans et al. (12) used four rats and reported a mean precision of $\pm$ 1.6%. Other elements have been

studied: Nagai and colleagues (17), using six mice, measured nitrogen by TBNAA and CA and reported excellent agreement between the two methods; Biggen and Morgan (3) reported comparable results for nitrogen with two mice; and magnesium was measured in three rats by TBNAA and CA by Dombrowski et al. (10) but the reproducibility was poor, ± 16%, with an average difference of approximately 13% between the means of the two methods.

The present experiment was designed to assess the potential usefulness of TBNAA in repetitive experiments in the same animal, particularly for balance studies in growing animals. The accuracy of TBNAA in measuring total body Na, Ca, and Cl was tested by comparing the values obtained with those determined by chemical analysis.

MATERIALS AND METHODS

Guinea pigs whose body weights were between 170 and 600 g were used for the comparison of the two methods. The neutron source was 1 mg Californium 252. A plastic phantom approximately the size of the guinea pigs used was filled with a water solution of Na, Ca, and Cl at concentrations established for the "reference body" (5). Repetitive irradiation of the phantom was done and the standard deviations of the means were calculated. To achieve uniform activation, the phantom and a plastic cylinder containing the live guinea pig were rotated at 6 rev/min with an irradiation time of 15 min. The gamma rays emitted from the radioactive isotopes ^{49}Ca, ^{38}Cl, and ^{24}Na were detected by two large sodium iodide crystal detectors (11 1/2" x 4" in dimension). The detector and animal were surrounded with a heavy shield of lead bricks. Three to five counts of 1000 sec each were made on the guinea pigs. For analysis of the data, seven peaks were used in each detector: Ca: 3084 keV; Cl: 1643,

2167, and 1643 + 2167 keV; Na: 1369, 2754, and 1369 + 2754 keV. In a few early experiments the dose of radiation received by the guinea pigs at a single exposure was 11 rem, but for the later and majority of experiments the dose was 7 rem. The dosages used on the guinea pigs, however, cannot be extrapolated to human subjects because of differences in body size and geometry of the two species. A complete description of the apparatus used was published by Evans, LeBlanc, and Johnson (12).

The following experiments were done: 1) The phantom was irradiated seven times; the means of the results for each element were calculated and the appropriate mean was used as the "normalization constant". These calibration factors derived from phantom irradiation were applied to the measurement of total body Ca, Cl, and Na. 2) The reproducibility of the measurement of TBNAA was tested by calculating the standard deviation of Ca, Cl, and Na from repetitive irradiation of the phantom. Also, three guinea pigs were killed with ether and frozen, and the reproducibility of TBNAA was tested by irradiating each guinea pig daily for four days and calculating the standard deviation for each of the three elements. 3) Total body Ca and Na were measured by TBNAA in 17 guinea pigs and Cl in 12. They then were killed with ether, and the three elements were measured chemically. Their body weights were between 227 - 600 g. 4) Finally, total body Ca, Cl, and Na were measured by neutron activation in 27 guinea pigs weighing between 170-375 g.

After the guinea pigs were killed, a midline incision was made from the manubrium to the pubis, to expose the viscera, and the cranium was cut open. The animals were dried at 105°C in an oven to a constant weight. After drying, the guinea pigs were pulverized to a fine powder. Fat was extracted from two aliquots of the dried powder with diethyl ether in a Soxhlet

apparatus, and the fat-free samples were dried to a constant weight. The fat-free dried samples were analyzed for Cl using a Buchler-Cotlove chloridometer. Another two aliquots of dried tissue were ashed in a muffle furnace at 500-550°C until a constant weight was reached. The ash was analyzed for Na and Ca with an atomic absorption spectrophotometer. The reproducibility of the chemical methods for measuring Na and Ca was tested by dividing four ashed samples into three fractions, analyzing for the two elements, and calculating the mean, the standard deviation, and the coefficient of variation (CV) of the results. The source of the statistical methods used to analyze the data was Snedecor and Cochran (22).

RESULTS

The standard deviations for total body Na and Ca obtained by CA was 0.06 g/100 g of ash (CV = 2%) and 0.43 g/100 g of ash (CV = 1.5%), respectively. In a similar experiment, Cl was measured on several series of fat-free dried tissue with a standard deviation of 0.004 g/100 g and a CV of 3.1%.

Repetitive irradiations of the phantom yielded coefficients of variation of 1.2% for Ca and Na and 0.7% for Cl. These coefficients of variation were in good agreement with those calculated from the counting statistics: 1.3% for Ca, 0.6% for Na, and 0.5% for Cl.

Table 1 shows data on repetitive total body neutron activation analysis on frozen guinea pigs and the excellent reproducibility of the method. Further, the original data from this table were used to make a best estimate of the error variation in the measurements (22). By this estimate the error variation in repetitive measurements with TBNAA was 3.0% for Ca, 1.0% for Cl, and 2.2% for Na.

The comparison of the results between the two methods on 17 guinea pigs for Ca and Na, and 12 guinea pigs for Cl showed that there were no statistically significant differences between them (Table 2).

Measurement of total body Ca, Cl, and Na by neutron activation in a larger series of guinea pigs that weighed between 170 and 375 g gave mean values (g/100 g body weight) of 1.110 for Ca, 0.120 for Cl, and 0.153 for Na (Table 3).

DISCUSSION

In the present experiment no statistical differences were found between the results obtained by TBNAA and CA. The small error variation achieved by TBNAA, the rapidity of analysis, and the ease with which the measurements can be repeated make it an excellent method for the study of growth and nutrition in small laboratory animals. Also results obtained in these studies confirm the potential applicability of TBNAA in experiments that previously required cross-sectional designs. Figure 1 illustrates the number of paired observations required to detect differences of 5 and 20% at two points in time (T_1 and T_2) assuming that the measurements at T_1 and T_2 have correlation coefficients of 0.75 or 0.90. Estimates of the number of paired observations needed to achieve a power of 0.70 and 0.95 are included. In cross-sectional experiments where the coefficient of correlation is assumed to be 0, at any power function, the number of animals needed to measure a 5% or 20% difference is significantly greater (Figure 1). Even if one assumes a correlation between paired animals used in cross-sectional design comparable to those assumed here, the number of animals required still would be twice those needed for a longitudinal experiment.

Recently Ziegler et al. (23), using data in the literature in which only 22 fetuses met their criteria, calculated the body composition of a "Reference Fetus" at gestational ages from week 24 through 40. The authors commented on the need for more body composition data on fetuses of accurately dated gestational age for the development of a more satisfactory "Reference Fetus". Although TBNAA at its present stage of development will not provide data for all the electrolytes needed, it should be able to determine total body Na, Cl, and Ca in a fetus accurately and rapidly and without its destruction.

Of the three elements measured in this experiment, only Ca has been measured in small animals in a similar experiment. The result in Shukla and Cohn's study on three rats (21) was 6.3% lower for TBNAA than for chemical analysis. Also, when the data for their rats are expressed as g/100 g body weight, it appears that total body Ca is appreciably less than for the guinea pig; the mean value for the rat was 0.785 g/100 g body weight, while for the guinea pig it was 1.110. Evans et al. (12) also compared neutron activation and chemical analysis in rats. They reported that activation analysis underestimated the Ca content of the whole body from 0.5% to 4.8% compared to chemical analysis. When they used a correction factor of +2.1%, the variability from the mean ranged from -1.5 to +2.7%. Their ratio for the 4 rats was 1.336 g/100 g body weight (unpublished data), a figure higher than that of Shukla and Cohn for the rat and higher than the ratio obtained in this experiment for the guinea pig (1.110 g/100 g body weight). Published values on mature guinea pigs are not available for total body Na and Cl measured by chemical analysis for comparison with the present data from TBNAA and CA.

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TABLE 1. *Mean total body calcium, chloride, and sodium of frozen guinea pigs measured by neutron activation on four successive days*

GP#	B. Wt. g	Ca g/100 g B. Wt.	Cl g/100 g B. Wt.	Na g/100 g B. Wt.
1	271	1.219 ± 0.021*	0.160 ± 0.002	0.183 ± 0.003
		1.7%†	1.3%	1.6%
2	281	1.178 ± 0.022	0.127 ± 0.001	0.143 ± 0.002
		1.9%	0.8%	1.4%
3	203	1.224 ± 0.054	0.144 ± 0.001	0.171 ± 0.005
		4.4%	0.7%	2.9%

*Standard deviation.

†Coefficient of variation.

TABLE 2. *Total body calcium, sodium, and chloride of the guinea pig measured by neutron activation and chemical analysis*

	Ca (g/100 g B. Wt.)*		Na (g/100 g B. Wt.)*		Cl (g/100 g B. Wt.)†	
	TBNAA‡	CA§	TBNAA	CA	TBNAA	CA
Mean	1.070	1.107	0.149	0.143	0.126	0.132
Standard deviation	0.132	0.125	0.019	0.021	0.009	0.024
Paired t test		- 1.18		0.979		- 0.947
P value		0.225		0.342		0.364

*17 guinea pigs

†12 guinea pigs

‡Neutron activation

§Chemical analysis

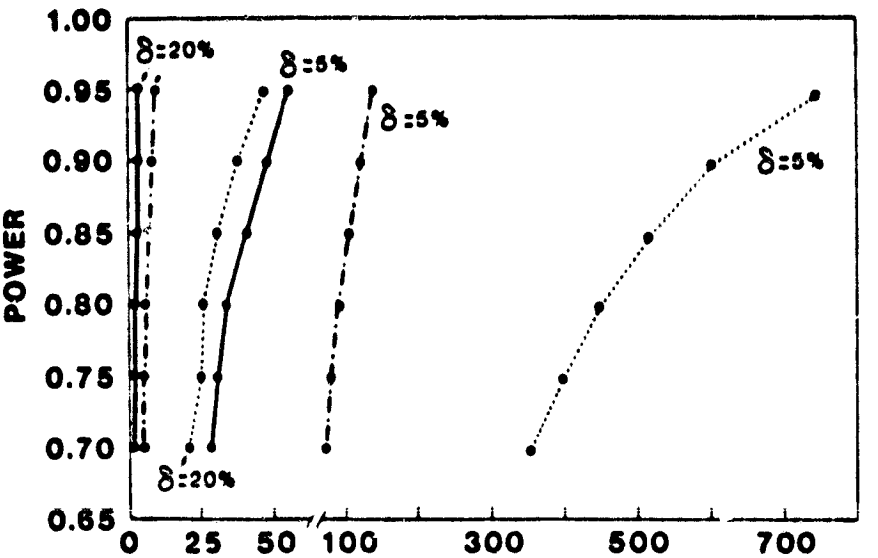
TABLE 3. *Total body calcium, chloride, and sodium of 27 live guinea pigs measured by total body neutron activation analysis**

	Ca	Cl	Na
Mean (g/100 g b. wt.)	1.110	0.120	0.153
Standard deviation	0.084	0.009	0.011

*Body weights: 170 - 375 g

FIGURE LEGEND

Figure 1. Estimates of the number of paired observations obtained at T_1 and T_2 required to achieve specific levels of power. Power curves have been calculated for the detection of differences of 5 and 20% (δ). A coefficient of correlation of 0.75 was assumed for the two broken lines, 0.90 for the two solid lines, and a 0 correlation for the dotted lines. δ is the magnitude of the % change to be detected.


1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
POWER
δ=20%
δ=5%
δ=5%
δ=5%
δ=20%
0
25
50
100
300
500
700