Title
METABOLIC STUDIES WITH Fe59, Ca47, C11 IN VARIOUS DISEASES

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ERRATA

TO: All recipients of UCRL-9718

FROM: Technical Information Division

SUBJECT: Metabolic Studies with Fe^{59}, Ca^{47} and C^{11} in Various Diseases, by Thornton Sargent, dated May 1961.

Presented orally at the Symposium on Whole Body Counting sponsored by the IAEA Vienna, June 12-16, 1961.

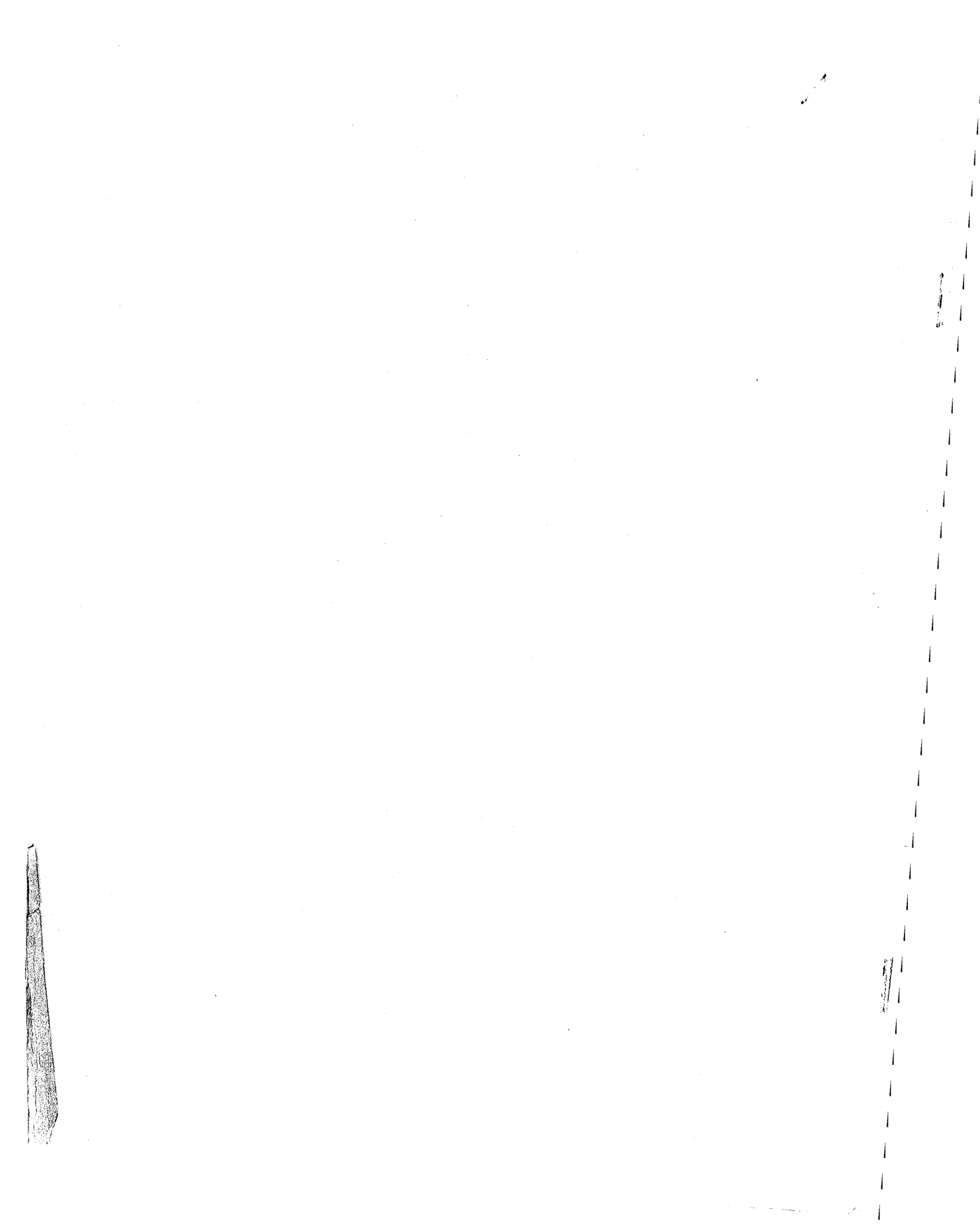
Please correct the following:

Page 8, line 18 should be corrected to read

The usual test for occult blood in stools is not a reliable measure of blood loss below 50 to 100 ml per day.

Page 14, line 16 up from bottom should be corrected to read

.....calcium activity, in a manner similar to that done by Pollycove (7) in iron kinetic studies.



For IAEA Vienna Symposium on Whole Body Counting - June 12-16, 1961

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METABOLIC STUDIES WITH Fe^{59}, Ca^{47} AND C^{11} IN VARIOUS DISEASES

Thornton Sargent

May 1961

Metabolic Studies with Fe^{59}, Ca^{47} and C^{11} in Various Diseases.

Thornton Sargent

Donner Laboratory, University of California,

Berkeley, California, USA

INTRODUCTION

The Whole Body Counter at Donner Laboratory of the University of California was constructed with a twofold purpose. The first is to provide Health and Safety monitoring service for the Lawrence Radiation Laboratory. The second is for medical research with isotopes in connection with the variety of diseases under investigation by the disciplines of Biophysics and Medical Physics at Donner Laboratory. The counter has been operating for about six months.

EQUIPMENT

The counter is of the Miller-Marinelli type. A shield of naval steel averaging about 16 cm thick encloses an area about 2.5 meters square and about 2 meters high. Magnetic fields in the steel have caused some difficulty by causing photomultiplier gain to vary with position in the room, necessitating recalibration for each change in location.

The NaI(TI) crystal is suspended from a commercial scissors-type x-ray tube crane, and can be positioned almost anywhere in the room. Subjects are placed on a one-meter arc couch which is convertible to a chair type geometry in a few seconds. This is being replaced by a stainless steel operating table which will permit other geometries as well. An extendable probe stand is also planned to hold other types of crystals for counting localized areas of the body.

To date the crystal used has been a 4 inch by 4 inch NaI(TI) with a single 5 inch Dumont photomultiplier. The background with this crystal is 380 cpm in the .08 to 2 mev range. A 9-3/8 by 4 inch crystal with five 3 inch tubes is presently being installed. A lining of 1/8 inch virgin lead is also in process of installation. The door is hinged and manually operated. Ventilation is forced air through an absolute filter; radon has not been a problem yet.

The pulse height analyzer is a 100-channel Penco with a ferrite core memory. Readout is by printer and point plotter.

SHIELDING STUDIES

Because of the enormous expense of handling and installing heavy plates of naval steel, and the high cost of new mill steel, we have been investigating less expensive materials for shielding, as have others. If building space is not a limitation, high density, high Z materials are not required. A lead lining would still be used to reduce low energy gammas and internal scattering.

Concrete does not seem very feasible, because although fairly clean silica deposits have been found, radioactively clean cement does not seem to exist. However, a local plant makes cement of fairly low activity from a bed of ancient sea shells; the activity found may be due to additives which could be replaced by purified chemicals.

In considering clean mineral deposits, the well-known cleanliness of dunnite was considered. It was found that asbestos is of the same ultramafic origin geologically as dunnite, and is readily available commercially. A block of asbestos bonded with 3% of polyvinyl acetate of the same diameter as our 4 inch crystal and 2 inches thick, was kindly prepared for us by the Asbestos Bonding Company of Napa, California. The density can be made almost as high as concrete. When counted in contact with the crystal it raised the background by only 2%, uniformly and not in the K^{40} or radium peaks. Any material placed in contact with a crystal within a large shield will raise the background by interacting with penetrating cosmic rays.

This material seems to be potentially good for shielding. Our next whole body counter shield will probably be built of it. The cost is only about $30 per ton, and a 50 ton shield would cost only $1,500 as opposed to $16,000 to $20,000 for steel. Labor costs have been estimated at an additional $2000. Bricks made of this material could be laid up in conventional fashion bonded with a plastic glue. A few steel beams would support the roof, and the usual lead lining would be used.

Such a shield would have the additional advantage of being a much better neutron shield than steel by virtue of its fairly large hydrogen content. At sea level with a total cosmic ray neutron flux of .04 n/cm^2 sec., the background produced in a 3 x 3 NaI(TI) crystal is probably of the order of 60 cpm, produced by the $I^{127}(n,\gamma)I^{128}$ reaction in the crystal (1). An asbestos shield would thermalize and capture most of these neutrons (a thin cadmium liner inside could be added for more complete capture), and in addition would not generate neutrons

from hard cosmic rays as high Z shields do. For whole body counters even at some distance from high energy accelerators, where fast neutron fluxes may be 4.8 n/cm^2 sec. and thermal fluxes 2.1 n/cm^2 sec. (1), such a shield would be even more important. Troublesome magnetic fields found in armor plate would be absent.

SAFETY MONITORING

The possible number of radionuclides to which personnel at the Lawrence Radiation Laboratory might be exposed includes literally the total number known, since any experimenter may make them at any time. So far 50 persons exposed to possible radionuclide poisoning involving 25 different isotopes have been counted. Only three had detectable burdens, involving Br^{82}, $Ru\text{-}Rh^{106}$, and Cs^{137}. Only the Cs^{137} was large enough to quantitate, and the excretion is being followed. A survey of accelerator crews, and chemists working with dangerous isotopes such as Am^{241} and Cf^{252}, is planned.

MEDICAL RESEARCH

A simple whole body counter can measure two things; oral absorption and the excretion rate of an isotope. If a shielded and collimated crystal is used, changes of activity in a specific area or organ can be followed. Such a probe will be installed in our steel room soon. For medical research, knowledge of uptake and elimination of an isotope in localized areas of the body, with simultaneous whole body counting, would be extremely valuable. Separate devices for localization studies have been in operation at this laboratory for some time; a 5-head scintillation counter used in ferrokinetic studies (2), a whole-body scanner (3), a pinhole scintillation camera and positron camera (4). Other devices for localization studies with greater sensitivity and geometrical resolution are planned. These will all be used in an integrated research program with the whole body counter.

The chief advantage of a whole body counter over present devices which measure localization is greater sensitivity. The latter devices require doses of 10 to 200μc of an isotope, while a whole body counter can accurately measure several orders of magnitude below this. To measure biological turnover times which are several times longer than the half life of the element used still requires a fairly large initial dose however. When a single known isotope is being used, and energy resolution is not as important as sensitivity, a liquid or plastic scintillator capable of giving high geometrical efficiency at reasonable cost may be the instrument of choice.

The studies with the whole body counter at Donner Labora-

tory reported here are the results of only the first several months of operation. Some interesting results have been obtained, nevertheless, and give some indication of future directions, potentialities, and limitations. Studies to date have been done with Fe^{59}, Ca^{47} and C^{11}; many others are planned.

Fe^{59}

This isotope has been widely used in studies of iron metabolism. A whole body counter can complement such studies by virtue of being able to measure the total radioiron in the body. This is important because normally one-third of the 4 grams of iron in the body is not in hemoglobin and cannot be measured by counting blood samples.

In all studies of Fe^{59} reported here counting was done with the subject on the one-meter arc couch. An element with complex and rapidly changing kinetics in the body such as iron is certain to produce counting rates very much dependent on localization and hence on time after injection, as will be seen later. The compartments occupied by iron at various times after injection cannot be equally distant from the crystal with the subject in the chair position, and hence that geometry could not give accurate results. All data are calculated on the basis of only the integral counts under the two Fe^{59} photopeaks.

1. Oral Absorption. This has been well studied previously (5), perhaps most elegantly by Hallberg (6) who measured radioiron found in blood after oral administration. The whole body counter offered a simpler method of measuring absorption.

A typical case of "iron deficiency anemia" has low hemoglobin concentration, a low concentration of plasma iron, and increased LIBC (Latent Iron Binding Capacity; the β_1-globulin, transferrin, which binds iron in the blood, having an increased number of unoccupied iron binding sites). Anemias of this type are almost always the result of blood loss, either acute or chronic. Complete inability to absorb iron is exceedingly rare. Various degrees of impairment of iron absorption, however, are frequently found and are of significance in regard to repairing the anemia.

Figure 1 shows absorption results in nine patients from the Donner Clinic with various forms of anemia. Between 5 and 10μc of Fe^{59} was given orally with a typical therapeutic dose of 30 mg of Fe^{++}. It was given in three forms: the customary $FeSO_4$ as a solution; a new preparation on the market containing ferrous gluconate and corn oil with a detergent which is claimed to increase iron absorption; and ferrous gluconate with corn oil as a control. (Fig. 1 refers to these latter two preparations as $FeSO_4$; they were actually gluconate.) A different form was

given on successive weeks, not less than 2½ hours after breakfast and 1½ hours before lunch. The subject was counted one week later, presuming all unabsorbed Fe^{59} had been eliminated. At the end of the series 10μc of Fe^{59} citrate calibrated against the oral doses was given intravenously, and the patient was counted one week later, to serve as the equivalent of 100% absorption.

The results are shown in Fig. 1. The symbols under each bar graph refer to the degree of the condition at the left; - means condition absent, i.e., normal; + means moderate, ++ severe. Anemia here refers to low hemoglobin concentration only; iron deficiency to low serum iron with high latent iron binding capacity. Blood loss was determined from further study, as described later.

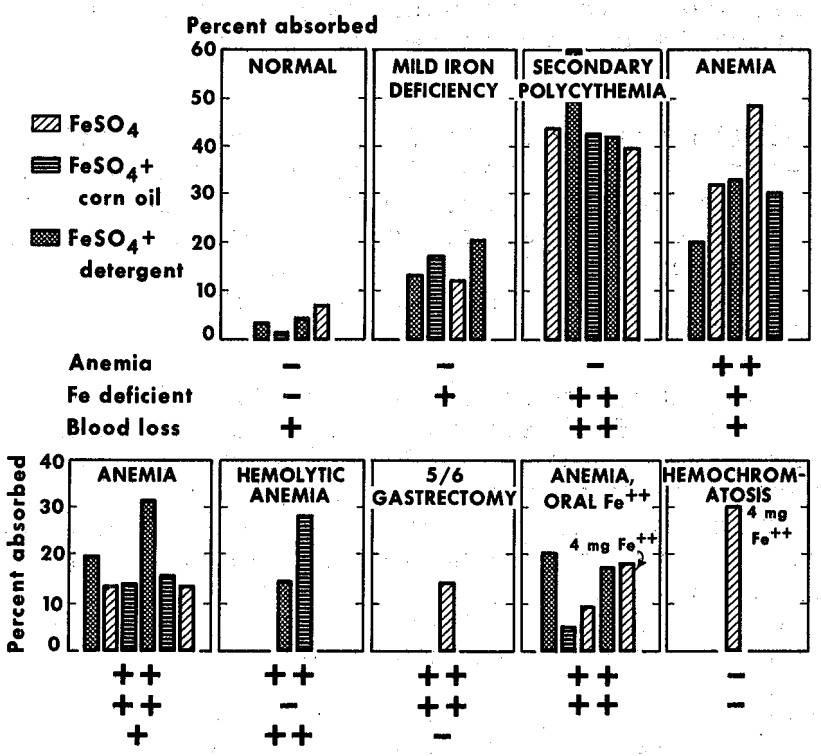
The first case, "normal" in Fig. 1, had been anemic but recovered with oral Fe therapy. A female approaching menopause, her iron loss as shown in Fig. 2 as "anemia-f" (although now actually recovered) was approximately 3% or 60 mg of iron per month, over twice the normal menstrual loss. This was probably the cause of her previous anemia, but whether this blood loss was entirely menstrual has yet to be determined. She was no longer anemic nor iron deficient, and her absorption was within the normal range found in other studies (5).

The case of "mild iron deficiency" was of undetermined etiology; blood loss has not been determined yet. With only moderately low serum iron and slightly elevated LIBC and normal hemoglobin, her absorption was increased, in normal physiologic response to iron depletion.

An elderly man with secondary polycythemia had just had a therapeutic venesection (++ blood loss), resulting in a very high LIBC, but with normal hemoglobin concentration. His iron absorption was the highest measured.

The next case shown, labelled "anemia", is a man with iron deficiency and low hemoglobin concentration, but only moderate increase of LIBC, yet absorption was quite high. He was found to have an iron loss (Fig. 2, "anemia-m") of about 6.2 mg per day, which is sufficient over an extended time to produce his anemia. He responded very well to intravenous dextran-bound iron.

The second case of "anemia" is a post-menopausal female with the same degree of anemia as the first. Although her LIBC was greater than the previous case, her iron absorption was less. This indicates a relative impairment of iron absorption even though it is distinctly greater than normal. Her blood loss (Fig. 2, "anemia-pmf") was about 60 mg per month.



Each oral dose = 30 mg Fe^{++}, except as indicated

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Fig. 1.

The case of hemolytic anemia is a pre-menopausal female with a low hemoglobin concentration. Although the serum iron was not low nor the LIBC increased (because of the hemolytic process), nevertheless her iron absorption was fairly high. She also showed considerable blood loss (Fig. 2 "hemolytic anemia + blood loss-f"), about 1200 mg of iron or 2400 ml of blood per month.

A man who had had a 5/6 gastrectomy with duodenal anastomosis 10 years previously was of interest; he had developed an anemia after repeated blood donations. Since iron is absorbed chiefly in the stomach and the duodenum, this man might have been unable to compensate for his voluntary iron loss because of his decreased ability to absorb iron in his remaining stomach area. In the one test, his absorption was found to be slightly above normal. This, however, is far less than the percentage absorption usually occurring with correspondingly high LIBC and low hemoglobin concentration. He also showed no blood loss, which in this case with a history of surgical removal of a peptic ulcer was a worthwhile finding. His anemia responded well to intravenous iron.

"Anemia, oral Fe^{++}" was a young woman with severe iron deficiency anemia who had been under oral iron therapy for several months without complete recovery at the time this study was begun. She was instructed to stop taking her iron two days before each test, with the expectation that this would be sufficient time to clear iron from the intestinal apoferritin. Her average absorption was below that expected for high LIBC and low hemoglobin concentration. She was then given a test with only 4 mg of Fe^{++}, equivalent to the iron in an ordinary meal. An anemic iron deficient person usually absorbs more than 60% of such a dose (5), yet she absorbed only 20%. (The percent absorption of a 4 mg dose is higher because the larger dose saturates the iron transfer mechanism in the intestine although it causes a greater total amount of iron to be absorbed.) She has now been removed from her oral iron for two weeks for a repeat absorption test. This is to determine whether the oral iron therapy actually blocked her intestinal absorption for more than two days, or whether she actually has impaired absorption. Further whole body counting will determine if blood loss is involved. She will be counted immediately before and after each menstrual period, to quantitate both menstrual loss and any other loss.

The case of latent endogenous hemochromatosis was a rare one in that it was detected in a young man before development of the usual signs and symptoms of the disease. Several of his relatives had died of the disease, which is considered to be hereditary. It is characterized by persistent uptake of iron from the diet far in excess of body requirements and a fixed rate of loss. This results in increased deposition of iron in

ferritin and hemosiderin until at middle age there is 30 to 100 times the normal amount. By the time the disease is usually detected, the iron burden is so large that iron absorption is at last suppressed and is frequently normal. In this early case we found that absorption of a 4 mg dose was 30%, well above the normal average of 10%, though his hemoglobin and serum iron were normal.

These iron absorption studies might best be considered as preliminary to more complete and exhaustive future studies. Some general results can be deduced, and some cautions and limitations outlined.

a) The amount of iron absorbed appears to vary over a rather wide range in an individual, sometimes by more than a factor of two. This variation might be due to contents of the stomach from the previous meal or the iron content of the previous meal. It is known that iron absorption can be almost doubled by the presence of ascorbic acid (6), and ordinary foods can bind iron and make it unavailable (2). Control of such factors might yield more reproducible results.

b) None of the three forms of iron tested produced higher absorption than any other, within the limits of variability observed; this result was also found by Hallberg (6).

c) Iron absorption increases with high LIBC and normal hemoglobin levels as in the secondary polycythemic, or with normal LIBC and low hemoglobin concentration as in the case of hemolytic anemia. Both factors are operative in the increased absorption of iron deficiency anemia. Some patients have impaired absorption in the presence of an iron deficiency which in other patients results in much greater absorption. In some the impairment is due to surgery which removed part of the iron absorbing areas of the intestinal tract and in others the cause is undetermined.

d) A whole body counter is a useful tool for study of cases of iron deficiency anemia refractory to oral iron therapy. A carefully designed series of tests can determine whether the patient is bleeding at a rate with which even a normally increased absorption could not keep pace, whether absorption is impaired and unable to replace a small regular blood loss, or a combination of both.

2. Iron Retention. It is generally accepted that in the absence of bleeding or pregnancy approximately 1.2 mg of iron is lost from the body per day. It should be much easier to measure this turnover with a whole body counter than by the previously used balance studies. This involves measuring a loss of 1% per month with an isotope with a 45 day half life, preferably for as long as a year. Such studies will be initiated at this

laboratory as soon as the large crystal is installed.

A simpler and clinically important measurement that we have done is determination of blood loss. Virtually every case of "iron deficiency anemia" seen so commonly by physicians must have been caused by blood loss. Diets will supply ten times the daily requirement of iron, except in pregnancy, lactation, or adolescence, where iron requirements are much higher. Blood loss in women may result from menstruation, which usually is compensated easily by increased absorption of iron from the diet. Menstrual blood loss, however, may be increased in many women without their being aware of it. If their diet is not rich in iron, a monthly net deficit may result in iron deficiency.

Blood may be lost in the gastrointestinal tract from a number of causes, such as oozing, surface abrasions or capillary dilatation, hemorrhoids, fissures, ulcerative conditions or malignant neoplasm. Iron deficiency anemia is an indication of previous blood loss, but determination of the rate of loss or even its presence is difficult by present methods. The usual test for occult blood in stools has a lower limit of sensitivity of 50 to 100 ml per day, and the test is not very reliable at that. Blood loss in any amount may be an indication of a condition relatively unimportant such as a mild mucosal friability, or as serious as a malignancy. Probably a relatively simple whole body counter could be developed as a clinical tool which would be valuable for this type of diagnostic study, using asbestos shielding described earlier, and one or two large plastic phosphors.

Of the nine cases studied for iron absorption, six have so far been followed to determine blood loss, and are shown in the right hand side of Fig. 2, with two additional cases. The patients with secondary polycythemia and hemolytic anemia (pmf) showed no loss, as expected. The patient with 5/6 gastrectomy also showed no loss, which was of value in his clinical management, and his anemia was probably the result of his extensive blood donation and moderately impaired absorption. The other four patients all showed varying degrees of blood loss, from rather moderate to considerable. The dashed line shows the expected normal loss for a male of 1.2 mg per day when total body iron is uniformly labelled. From 85% to 100% of radioiron is initially incorporated into circulating erythrocytes with a life span of 100 to 135 days; iron is not lost from this compartment unless blood loss occurs. When the iron is recycled after destruction of the first generation of labelled red cells, radioiron would begin to be lost by the known processes of desquamation, intestinal sloughing, a small loss in the breakdown of red cells and recovery of iron for new erythropoiesis. Some radioiron would be lost initially and again during recycling by the small loss of transferrin in the urine. A normally

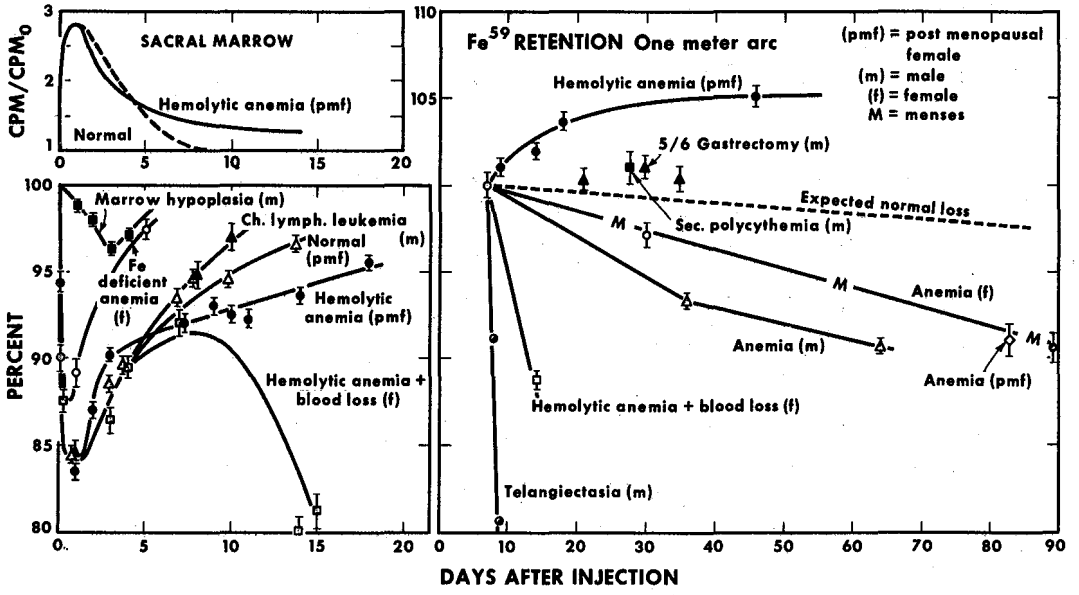

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Fig. 2.

menstruating female would lose not quite twice as much. The case of telangiectasia illustrates severe hemorrhage and will be discussed later.

The loss was calculated for each subject on the basis of his count seven days after the last oral or i.v. dose as 100%, on the assumption that incorporation of radioiron would then be virtually maximal. As will be seen below, this is not always a valid assumption.

The lower left side of Fig. 2 shows the whole body counts as a function of days after an intravenous injection of a tracer dose of Fe^{59}. The count at ten minutes after injection is taken as 100%. These patients were injected as part of iron kinetic studies (2), and only the two hemolytic anemias, f and pmf, also appear on the right side. Upper left is taken from the iron kinetic studies, and is the counts per minute taken over the sacral marrow divided by cpm. The counting rate cpm_0 is obtained by extrapolation of counts obtained after complete mixing taken as the time of injection, $t = o$. It is thus an indication of the increase of radioiron in the marrow relative to the maximum amount of marrow radioiron resulting from circulation of blood through the marrow, with the same time scale as lower left.

It can be seen that immediately after injection, within a few hours, the "whole body" count falls sharply, as virtually all the radioiron is taken up in the marrow. Then, after the iron becomes fixed for erythropoiesis, it is released over a period of several days back into the circulating blood. Although not followed far enough to be sure at this writing, it probably never returns to the 100% level, except possibly in iron deficiency because some of the iron will enter relatively fixed stores in bone marrow. Clearly, the gamma rays are absorbed more when the iron is in marrow inside bone than when it is circulating in peripheral blood with less tissue mass, on the average, intervening between the isotope and the crystal. The count in the sacral marrow is a mirror image of the lower curve, indicating that it is indeed the marrow sequestration which is responsible.

It can be seen that a simple assumption of equilibrium at seven days is not valid in all cases. In the case of iron deficient anemia, in which the marrow needs only more iron to make badly needed hemoglobin, the radioiron clears the marrow rapidly, reaching equilibrium in seven days. A normal patient had not reached equilibrium at 14 days. The patient with hemolytic anemia without blood loss had probably reached equilibrium by 30 or 40 days; the hemolytic process rapidly destroys red cells and hence the radioiron is then recycled through the marrow long before the end of the normal 115 day life span of these cells. The patient with hemolytic anemia and blood loss, on the other hand, lost iron so rapidly that the whole body count reached a

maximum of only 91% at one week and fell to 81% of her original count at two weeks. The apparently faster rate of marrow clearance of the case of chronic lymphatic leukemia is a consequence of the associated hemolysis and increased erythropoiesis present. The man with marrow hypoplasia fixed very little iron in the marrow and hence showed only a small dip; erythropoiesis occurred chiefly in splenic and hepatic soft tissues.

Thus for a simple iron deficient subject, who returns iron very quickly to the blood, a seven day interval may be a sufficient time to take as 100% for blood loss studies. Seven days is certainly not sufficient, without correction, for any subject with marrow abnormalities or blood loss, or even, apparently, for normals. In the iron absorption studies reported here, no such corrections were made. Clearly, the most accurate way to do an oral absorption study is to give the patient the intravenous "100% absorption" dose first, and count him every few hours the first day, then daily or every few days until equilibrium is reached. The patient should then be counted weekly until blood loss, if any, including menstrual, is established. However, for practical determination of iron absorption, this may be a small error. For instance, if iron absorption had been studied in the normal-pmf patient, whose whole body count increased another three percent between the 7th and 14th days, the value obtained for iron absorption would be in error by only 3% of the calculated fraction absorbed.

Accurate and well controlled absorption tests might be very useful, for instance, in screening members of a family with a history of endogenous hemachromatosis. In those who exhibited abnormally high absorption while still young, regular prophylactic venesections could be initiated early in life, thereby preventing the accumulation of abnormal amounts of iron and the consequent pathologic organ damage. Possibly this condition is due to a dominant gene of incomplete penetrance. A case of incomplete penetrance might express itself only as a mildly increased absorption, which would require very reliable absorption tests to detect. But, if such a condition could be detected, a better understanding of the genetic transmission of this disease could be obtained in such families. Prospective marriage partners of family members could even be checked to obviate the possibility of the mating of two persons affected subclinically by this condition.

3. Congenital Hemorrhagic Telangiectasia. This case was especially interesting because it presented an opportunity for some unusual studies. An elderly negro male had a long history of nose bleeding; many of his close relatives had died in early adulthood from massive hemorrhages of this sort. The condition is considered hereditary, and is caused by great distension and consequently fragility of mucosal capillaries, resulting in continual slow bleeding and frequent massive hemorrhages

throughout the gastrointestinal tract as well as in the nasal mucosa. In the past he had been frequently hospitalized in a state of shock and given as much as five units of whole blood on such occasions.

When referred to the Donner Clinic he was placed on a weekly regimen of 1500 mg of intravenous dextran-bound iron. With an average daily blood loss of 800 ml, or 200 mg of iron at a hemoglobin concentration of 7 gm per 100 ml, oral iron therapy was utterly incapable of replacing it. Although he was still weakened by depletion of blood volume in sudden hemorrhages, he was now able to maintain his hemoglobin and red cell volume by his own erythropoiesis, the enormous activity of which was indicated by reticulocyte counts as high as 50%.

After weekly doses of intravenous iron had brought his hemoglobin up to a reasonable value of 9 gm per 100 ml from its usual value around 4 gm per 100 ml, he was given an intravenous dose of 200μc of Fe^{59} for iron kinetics and whole body counting. At this time plasma iron concentration was high and considerable iron deposition in stores occurred as a result of the continued intravenous injections of iron. The results are shown in Fig. 3.

His retention curve, although not smooth because of intermittent gross hemorrhages, shows roughly three components. The first is the result of loss of radioiron in the circulating red cells. The second, less steep, undoubtedly represents labelled iron which originally went into storage as hemosiderin and ferfitin. Since the intravenous iron supplied more than enough iron for erythropoiesis, the excess, with which the radioiron mixed, went into stores. This second component then represents the turnover rate of his storage iron. After the 87th day the intravenous iron was discontinued. Subsequently his hemoglobin and plasma iron levels and erythrocyte indices demonstrated that his storage iron was rapidly exhausted, which was intended for the next part of the study. On the 87th day a blood volume determination and count of a blood sample showed only 0.088μc of Fe^{59} in circulating blood, but the whole body count gave a value of 1.9μc. A third component in the curve is now seen, which turns over with a very long half life in spite of the enormous demand for iron for erythropoiesis. This probably represents radioiron which has been incorporated into myoglobin and heme enzymes. By extrapolating this "fixed iron" component to time of injection, it is seen that about 2% of the injected Fe^{59} entered this compartment. When the fixed component is subtracted from the total curve, the stores component can be extrapolated and shows that 30% entered stores. When this is subtracted a curve for blood loss only is obtained, from which the value of 800 ml per day was obtained.

On the 111th day, with his hemoglobin down to 3.7 gm, the subject was relabelled with 250μc of Fe^{59}. With his stores

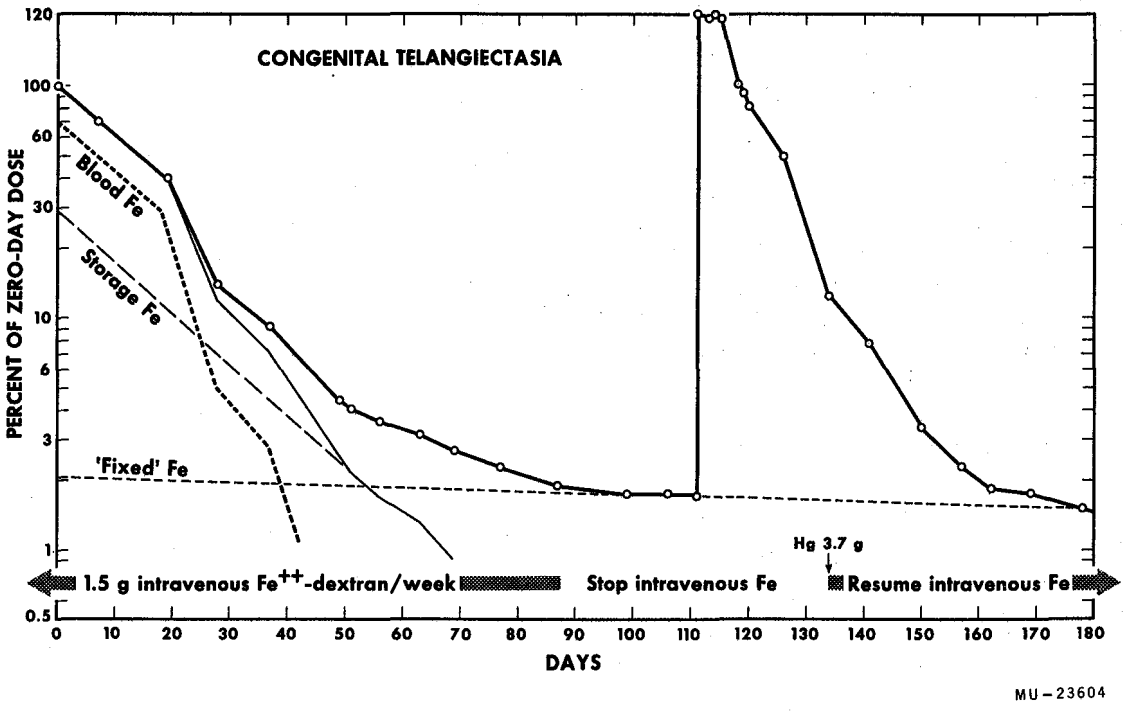
CONGENITAL TELANGIECTASIA
PERCENT OF ZERO-DAY DOSE
120
100
60
30
10
6
3
1
0.5
Blood Fe
Storage Fe
'Fixed' Fe
Hg 3.7 g
1.5 g intravenous Fe++-dextran/week
Stop intravenous Fe
Resume intravenous Fe
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180
DAYS
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Fig. 3.

exhausted, it was expected that virtually all of the isotope would be put into circulating blood. As the succeeding curve shows this was indeed the case. After a brief stay of only 4 days in the marrow, the radioiron entered the circulating blood and was rapidly lost in hemorrhages. Although there may be a small component due to stores between days 162 and 169, the level seems to have returned to the previous level of "fixed iron". When this level is subtracted, the loss curve closely approximates that obtained for blood loss only obtained in the first labelling.

This subject is now being followed without relabelling, his iron stores replenished by resumption of intravenous iron on day 134. We expect to be able to measure a turnover time for the iron in myoglobin and heme enzymes by continuing to count this patient. This will be very interesting since this is a measurement not yet made in man, made possible by the fortuitous combination of this unusual patient and a whole body counter.

Ca^{47}

This newly available isotope has already been used in studies of bone metabolism by several investigators (7,8) to determine accretion and exchange rates in various bone diseases. Whole body counting might be capable of providing as much information as can be obtained by serial serum sampling and urine and stool collections of a patient confined in a metabolic ward. To ascertain this it would be necessary to do both kinds of study simultaneously; this was not done in (7) since a whole body counter was not available, and was not done here because the subjects were out-patients and a metabolic ward was not available. Some studies of serum calcium and excreta were done here, but with limited accuracy, and at this writing the data has not been analyzed and correlated to the point where a comparison of the two methods can be made. Whole body counting has the advantage of simplicity of measurement and greater sensitivity, permitting the use of smaller doses.

Fig. 4 shows the results of whole body counting on a normal 32 year old male subject given 1.8μc of Ca^{47} intravenously. Percent retained is percentage of decay corrected counts with the count at 5 min. after injection equivalent to 100%. His diet was maintained at 900 mg ± 50 mg of Ca per day. It was not maintained at this level until the first day of the experiment, however, having been somewhat lower previously, so he may not have been in balance immediately. Daily urine and stools were collected, and counted with the 4 x 4 inch crystal in the steel room, using the total stool volume and a 500 ml sample of each 24 hour urine. These were compared to a standard equal to the injected dose and corrected for geometry and self absorption by dilution of the standard into varying volumes. Counts used in both excreta and whole body counting were those under the 1.31

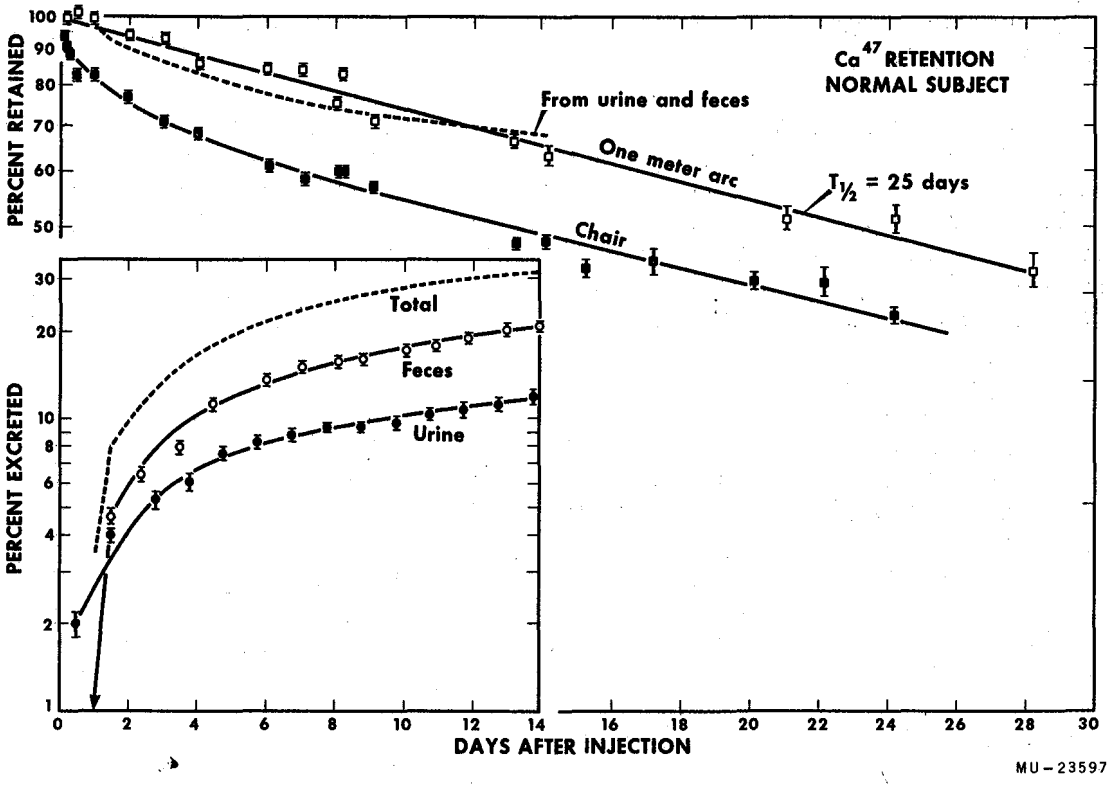
Ca47 RETENTION
NORMAL SUBJECT
From urine and feces
One meter arc
T½ = 25 days
Chair
Total
Feces
Urine
PERCENT RETAINED
PERCENT EXCRETED
DAYS AFTER INJECTION
MU-23597

Fig. 4.

mev photopeak of Ca^{47} from 1.2 to 1.8 mev.

A contaminant present in small percentage initially, but with a long half life and a gamma energy of 1.12 mev, was a problem at this low dose of Ca^{47}. The background initially measured in the subject was not adequate after most of the Ca^{47} had decayed because the upper part of the contaminant peak added counts to the Ca^{47} peak. This contaminant so far seems to have a half life of about 110 to 120 days, which does not fit either 84 day Sc^{46} or 245 day Zn^{65}, the most likely candidates. The .885 mev gamma ray of Sc^{46} was not observed, but should be equally abundant. There are also unidentified contaminant peaks at 0.32 mev, 0.50 mev, and a smaller one at 0.61 mev. In the cancer patient in Fig. 5, the 1.12 mev peak had a total half life of 30 days, while in the normal subject it appears to be much longer. The turnover will also be followed in the two cases of acromegaly. Chemical identification procedures will be attempted on the standard to aid in identifying the contaminants.

The normal subject was counted in both one-meter arc and chair geometries for comparison. The two are clearly not equivalent in all respects, yet the final slope attained in each is essentially the same. The initial drop in the curve for the chair position is most probably due to mixing of the Ca^{47} in the exchangeable pool as found in (7). By subtracting the extrapolated 25 day half time curve of the whole body count, one can obtain a component with a 2 day half time, which is approximately the same as found in (7). Certainly in the chair geometry the crystal is much closer to the general mass of circulating blood than it is to the average bone mass, which would account for the difference between the parallel sections of the chair and arc retention curves.

The inset of Fig. 4 shows the excreta measurements as cumulative percentage of injected dose excreted on the same time scale. When the total excreta curve is subtracted from 100%, the dashed line in the upper graph is obtained for total retention. The retention by excreta measurement is reasonably close to the one meter arc whole body count retention, probably within the error of the method.

Fig. 5 shows the results of Ca^{47} studies of four patients using chair geometry. The normal is the same as in Fig. 4. The case of acromegaly in a 35 year old man had been treated by irradiation of his pituitary. The other acromegalic was a 17 year old boy with a pituitary tumor, prior to pituitary irradiation. Both of these cases were treated under a program at Donner Laboratory in which patients are either partially or totally hypophysectomized with a cyclotron beam. The patient's head is rotated about the pituitary gland as a center through which the 910 mev alpha particle beam passes, producing maximum dose to the pituitary while giving a much smaller dose to surrounding tissue.

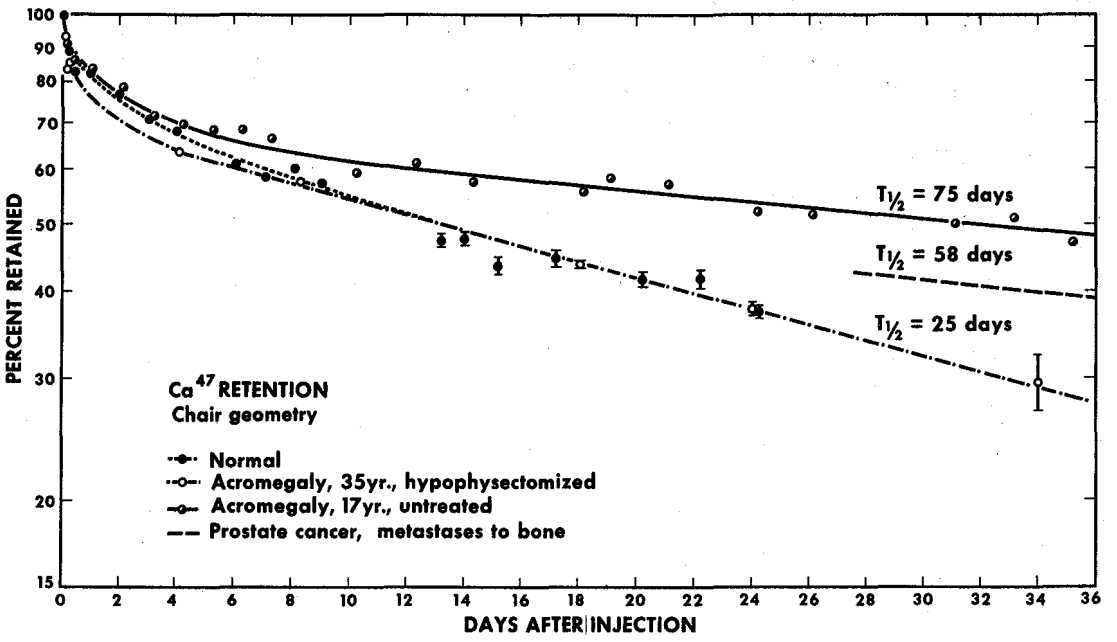
PERCENT RETAINED
100
90
80
70
60
50
40
30
20
15
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
DAYS AFTER INJECTION
T½ = 75 days
T½ = 58 days
T½ = 25 days
Ca47 RETENTION
Chair geometry
Normal
Acromegaly, 35yr., hypophysectomized
Acromegaly, 17yr., untreated
Prostate cancer, metastases to bone
MU-23600

Fig. 5.

Both of these cases received a dose calculated to suppress but not destroy pituitary activity.

Unfortunately, the whole body counter was not yet operating before the first case was treated, and the second case has not yet returned for study after his treatment. Thus comparison of turnover times is interesting but not conclusive. The calcium half time in the 17 year old boy is 75 days, three times that of the normal. Yet it is not possible to say whether this apparently greater rate of accretion of calcium is due to acromegaly or to the fact that the boy is still undergoing normal growth, i.e., whether a normal boy of his age would have the same or a faster turnover. The treated acromegalic shows a calcium half time in the body which is not distinguishable from the normal. It is hoped that this is an indication that his acromegaly has been successfully treated. The more rapid drop in the early part of the curve might however imply a faster removal of calcium from the exchangeable pool, or it may be related to the difference in geometry due to the subject's large size due to acromegalic giantism.

The fourth case, an elderly man with prostate cancer metastasized to bone, had been given Ca^{47} for another study 30 days prior to completion of the steel room. The Ca half time was calculated from the uncorrected decay curve and the known half time of Ca^{47}. This is not a very accurate measurement, but it does suggest greater retention of calcium in neoplasm involving bone as was found in (7).

By the use of shielded and collimated scintillator heads in addition to whole body counting, it should be possible to obtain additional valuable kinetic data on calcium levels in circulating blood and over bone, or over areas of bone suspected of unusual calcium activity, as done by Pollycove (7).

C^{11}

A high energy alpha particle beam from a cyclotron would be expected to produce a number of isotopes in organic material by nuclear reactions such as $C^{12}(\alpha,\alpha n)C^{11}$, $N^{14}(\alpha,\alpha n)N^{13}$, $O^{16}(\alpha,\alpha n)O^{15}$, $O^{16}(\alpha,pn)F^{18}$, and $Na^{23}(\alpha,\alpha n)Na^{22}$. The half lives of the product nuclei are 20.5 min., 10.1 min., 2.0 min., 112 min., and 2.6 years, respectively.

Since patients are treated regularly at this laboratory in the cyclotron hypophysectomy program mentioned earlier, they presented an opportunity to study such isotope production in humans by a 910 mev alpha particle beam. Eight ambulatory patients already chosen for hypophysectomy who were willing to cooperate in the study were chosen. Each received his cyclotron irradiation in a series of 6 equally divided doses, each delivered over a period of about 15 minutes.

On one of his treatment days, immediately after the cyclotron was turned off, the patient was brought to the whole body counter, and placed in the chair geometry. This usually required 15 to 20 minutes. He was then counted at intervals for about two hours. Counts in the 0.5 mev peak from positron annihilation gammas were observed. All of these patients had been given I^{131} uptake tests, and since .5 mev falls between the .364 and .637 mev peaks of I^{131} a background was obtained on each patient before irradiation.

Counts per minute in the .5 mev peak was plotted on a semilogarithmic scale as a function of time elapsed since the cyclotron was shut off (to the center of the counting interval), and results on five patients are shown in Fig. 6. The initial part of the curves decay with a half life averaging about 18 minutes. Then, one to one and one-half hours after the end of irradiation, the slope changes to a half life very close to the 20.5 minutes of C^{11}. If the 20.5 minute curve is extrapolated and subtracted from the 18 min. curve in the conventional manner, a half life of about 13 minutes is obtained. This could be 10.1 minute N^{13}, but it could also be the result of biological elimination of C^{11}. The biological half life alone would then be about 200 min. This is in reasonable agreement with the known 250 minute half time for the rate of disappearance in man of carboxyhemoglobin, the form in which CO is carried in the blood (9).

The beam dose for each patient was measured with an ionization chamber in the beam. Before entering the patient's head the beam was passed through an aperture chosen for each patient on the basis of x-ray pictures of the location and size of the pituitary. The actual energy delivered to each patient was then calculated in terms of gram-rads, that is, the area of the aperture times the average diameter of the patient's head times the beam dose in rads. We can then extrapolate the early portion of the curve to the cyclotron off time and get a value of cpm at the intercept. The intercept for each patient was then plotted against his dose in gram-rads and is shown in the inset of Fig. 6. By making the reasonable assumption that the curve passes through the origin, a fairly good correlation is seen. On the average about one count per minute from C^{11} has been produced by each gram-rad at the shut off time. Based on the positron emission from Co^{58} in a patient, an isotope decaying 100% by positron emission would produce a whole body count of 2.5×10^{-3} cpm per dpm in the chair geometry. Thus a yield of one cpm per gram-rad at shut off time is equivalent to 1.8×10^{-4} μc per gram-rad. A cross section of 750 mb calculated from this yield, when compared to a published value of 57.0 mb (11), suggests that some of the many assumptions required for the calculation are in error or that spallation reactions with nitrogen and oxygen also occur.

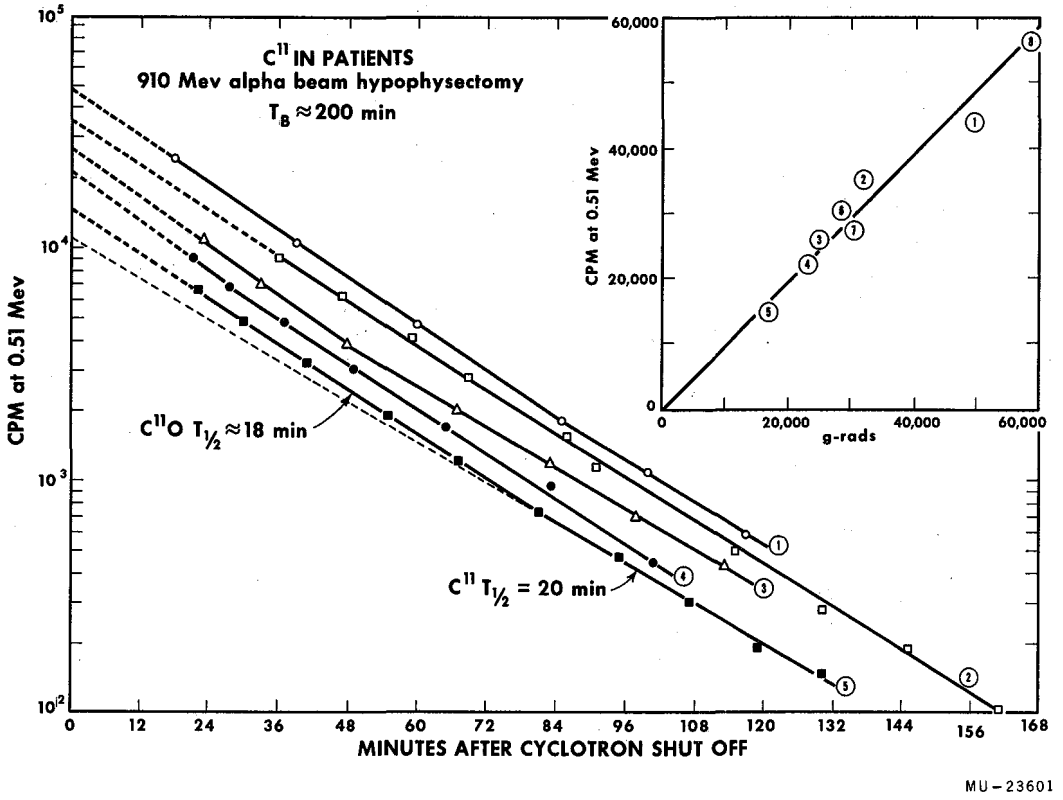
C11 IN PATIENTS
910 Mev alpha beam hypophysectomy
TB ≈ 200 min
C11O T½ ≈ 18 min
C11 T½ = 20 min
CPM at 0.51 Mev
MINUTES AFTER CYCLOTRON SHUT OFF
g-rads
MU-23601

Fig. 6.

This information might be useful in the event of a person accidentally exposed to the beam of a high energy particle accelerator. Considerable precautions are taken by operators of such machines to obviate the chance of such accidents, but they are always possible. A person so exposed might be struck by the beam in an area of his body at some distance from his film badge and thus the film would indicate only the dose from scattered radiation. If this person could be counted in a whole body counter within an hour or two of the accident, his dose in gram-rads could be obtained from the relationship found in Fig. 6. Reconstruction of the events of the accident could provide information as to area and volume of the body exposed, and the dose to various areas could be estimated. Duration of exposure and dose rate would have to be considered also, since they would determine what percentage of equilibrium had been reached when exposure ended. If the cross section for the particle and energy involved were much different from $\alpha,\alpha n$ reactions, another correction would have to be made. In the physics cave at the 184 inch cyclotron at Berkeley, at a beam current of one microampere, a person could receive a dose similar to that of the cyclotron patients in a few seconds. Although the mean lethal dose for total body radiation in man (neglecting RBE) would be about 3×10^7 gram-rad compared to about 2 to 6×10^4 gram-rad in the patients, a dose of the latter size delivered to a critical area such as the spinal cord, heart or brain could be serious.

To determine whether the change in slope of the C^{11} decay curve in patients was due to N^{13} or due to excretion of C^{11}, several of the patients were asked to come in for radioactive breath analysis on another of their treatment days. The breath analyzer used was of the type commonly used for C^{14} breath analysis. Air is passed through a helmet on the patient's head, through a $CaCl_2$ drier, an ion trap, an ionization chamber and an infrared CO_2 detector. A continuous record of radioactivity, CO_2, and specific activity of C^{14} in CO_2 is made. The machine is calibrated for C^{14}, but should also readily detect the 0.97 mev positron of C^{11}, although the ionization chamber would be quite insensitive to the anihilation gammas. The absolute amount of C^{11} would be difficult to determine, since a positron emitting gas of known activity would have to be used. On the basis of the ratio of the maximum β energies of the C^{14} and C^{11}, the machine should be 6.2 times as sensitive for C^{11} as for C^{14}.

Radioactivity was indeed found in the breath of these patients, although the amount was very small. It disappeared with a half life of about 20 minutes, presumptive evidence that it was C^{11}. Although this indicated a biological half life at least several times longer than 20 minutes, suggestive of CO, a further test was applied.

The analyzer was slightly modified, a trap containing "Ascarite" CO_2 absorber (active ingredient NaOH) being placed

after the $CaCl_2$ drier so that the air from the helmet could be passed either through the Ascarite trap or bypass it by turning a stopcock. Two patients were examined with this arrangement within 15 to 20 minutes after the end of their cyclotron treatment, with results shown in Fig. 7, a tracing of the record of the machine.

The first patient was started with the Ascarite bypassed, i.e. radioactivity in CO_2 being measured. The infrared spectrometer record of CO_2 shows the appearance of CO_2 when the patient was put in the helmet at 22 minutes. The variations on CO_2 reflect variations in the patient's respiratory minute volume which also appear in the record of radioactivity. At 42 minutes the stopcock was turned to pass the gas through the Ascarite, the successful removal of CO_2 being demonstrated by the fall of the CO_2 level to the background level or less than 0.7% of that in breath. The radioactivity record fell by a little less than half. The carbon-containing gas most likely to be present that would pass through the Ascarite trap is carbon monoxide. As will be described below, there is also a chemical basis for ascribing the activity to CO. The activity rose again when CO_2 was admitted again at 61 minutes.

The second patient was placed in the helmet with the CO_2 absorber in the circuit. The absorber was bypassed from 31 minutes to 50 minutes, and the admission of CO_2 increased the radioactivity to the same extent as it did in the first case. Removal of CO_2 again returned the activity to a level expected by extension of first part of the record.

Studies of the chemical reactions of recoiling C^{11} nuclei from the reaction $C^{12}(n,2n)C^{11}$ using benzene as a target (10) indicate that in tissue one might expect the ionized C^{11} nucleus to recombine to form CO and CO_2 in about equal amounts and about 5% to enter various complex molecules by recoil replacement reactions. The half time of CO_2 in the body is of the order of a few minutes, so any CO_2 formed directly during irradiation would have been cleared from the body before the patient was placed in the breath analyzer. Thus the $C^{11}O_2$ observed in Fig. 7 could be due to metabolism by the body of molecules into which C^{11} had entered by recoil. $C^{11}O$, however, by virtue of its long biological half life would survive long enough to be detected.

The presence of N^{13} still cannot be ruled out even if some of the C^{11} is exhaled as CO and CO_2. In a further experiment, a live rat was placed in the alpha beam and given a full body dose of 200 rad in 1 minute. He was placed in a holder which restrained him within 1/2 inch, in contact with the crystal in the whole body counter. The decay of the .51 peak was followed for eight hours, with results shown in Fig. 8. Since the animal readily survived this dose, the change in slope of the curve

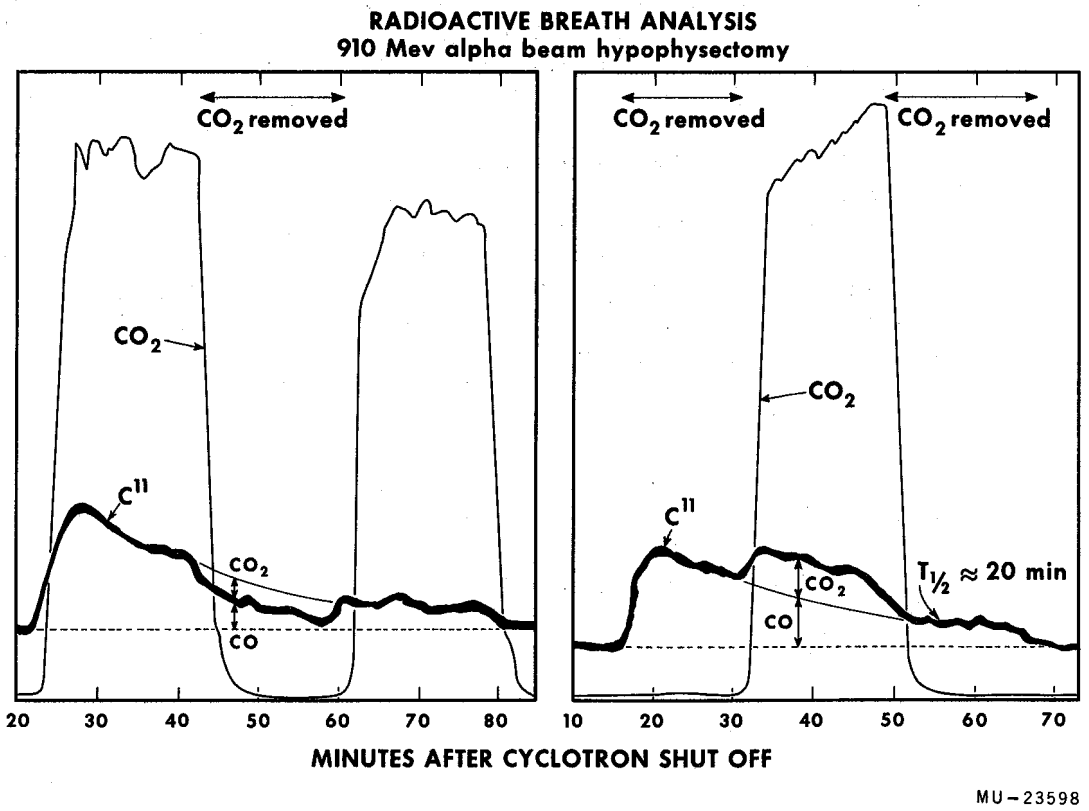
RADIOACTIVE BREATH ANALYSIS
910 Mev alpha beam hypophysectomy
CO2 removed
CO2 removed
CO2 removed
CO2
CO2
C11
C11
CO2
CO
CO2
CO
T½ ≈ 20 min
20 30 40 50 60 70 80
10 20 30 40 50 60 70
MINUTES AFTER CYCLOTRON SHUT OFF
MU-23598

Fig. 7.

from 17.0 min. to 20.5 min. could be considered the result of physiologic removal with a 100 minute half life.

To confirm this, the same animal was sacrificed 2 weeks later (having gained an additional 30% in weight), given the same dose and again counted. The results, with counts per minute corrected for the animal's additional weight, are also shown in Fig. 8.

Although the activities of the decay curves for the live and dead rat start at the same point, that of the rat when alive falls by an amount which could only be explained as that equivalent to the amount removed by exhalation. The difference between the two curves shows that approximately one-third of the C^{11} is removed by this process during the first 100 minutes, the remaining two thirds remaining relatively fixed in the body.

As further confirmation of the similarity of the activity decay curves of the alive and dead rat, except as differentiated by removal of C^{11} in breath, the two curves again coincide when the C^{11} has decayed leaving activity with a half life of 130 minutes. The extrapolated curve with this half life was subtracted from the total to yeild the 20.5 min. curve shown. This is undoubtedly 112 minute F^{18}, the half life of the curve perhaps lengthened by a trace of an isotope such as 3.9 hour Sc^{43} from $Ca^{40}(\alpha,p)Sc^{43}$. From the zero time intercept, the amount of F^{18} formed was about 0.1% of the C^{11}.

The steep portion of the early part of the decay curve indicates the presence of 2.0 min. O^{15}, or 10.1 min. N^{13}, or both. Evidence of Na^{22} was not seen.

As nearly as could be determined without collimation, by counting the patient with the crystal over various parts of the body the C^{11} was not concentrated at the site of production, i.e. the head. If 33% is produced as CO and at least part of the remainder as soluble molecules circulating in body fluids, this is to be expected. A pair of uncollimated crystals counting only coincident .5 mev gammas would be a good way to determine any localization with virtually zero background.

The excretion of C^{11} could be studied in much greater detail, even while the patient is being irradiated, by counting the exhaled air with a gas capillary plastic scintillator, which would give much higher counting efficiency. Two NaI crystals could be used on each side of a spiral coiled tube through which the gas passed, connected in coincidence as above. The extremely low background which would be produced by positrons from cosmic rays would probably give even greater sensitivity than the plastic phosphor. Such a study is not planned at present, however.

FUTURE STUDIES

The isotope studies reported here were done in the first six months of operation of the whole body counter; the isotopes and patients were readily available from other research already in progress. They are an indication of the kind of information which can be obtained readily with a whole body counter, but which is obtained only with great difficulty or not all by previous methods.

A new technique now being perfected in another research program under Dr. John Gofman at this laboratory is trace analysis by x-ray spectroscopy. Sensitivities of the order of a part per million can be obtained for almost all elements of the periodic table from aluminum to uranium. It is planned to study a great variety of disease conditions for abnormal levels of all of these elements. Isotopes which exist for many of these elements are well suited for use as tracers in humans, using the sensitivity available with a whole body counter. Several cyclotrons, a reactor and an excellent chemistry group are available to us to make them.

The whole body counter will thus be able to study the turnover of an element which is found or suspected to be involved in a disease process or in normal metabolism on the basis of the x-ray spectroscopy studies. Many such relationships are already well established, such as cobalt in pernicious anemia, iron in hemochromatosis, and copper in Wilson's disease. Undoubtedly there are many more, and the whole body counter is an ideal instrument for studying them.

Undoubtedly whole body counting will rapidly come into much wider use for fundamental research on human metabolism in both normal and disease states.

ACKNOWLEDGMENT

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