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MEDICAL AND HEALTH PHYSICS

QUARTERLY REPORT

January, February and March, 1951

May 8, 1951

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* Previous Quarterly Report: UCRL-1143, October, November and December, 1950.

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I THE METABOLIC PROPERTIES OF VARIOUS MATERIALS

J. G. Hamilton

Project 48A

Astatine Studies

K. G. Scott, C. W. Asling, Gretchen Thilo, Patricia Wallace, and D. C. Morrison

The astatine work has been continued at a vigorous level and now, with most of the data in hand, a manuscript is being prepared for publication.

In the last Quarterly Report, a summary was given of the work done in relation to radiation damage of astatine in the anterior chamber of the eye. Through an omission, the exact purpose of these experiments was not stated. Two factors were present which influenced the initiation of these experiments in collaboration with the Division of Ophthalmology. One was the possible therapeutic application to the treatment of retention cysts following removal of the lens in the treatment of cataracts. These cysts arise occasionally due to the fact that epithelial cells of the bulbar conjunctiva are carried down into the anterior chamber of the eye by the surgeon's scalpel. They then may proliferate and eventually form a thin-walled sac filled with clear fluid of a composition not too much unlike that of the aqueous humor, itself. As time progresses, these sacs increase in size so that they require aspiration every few weeks. This is a palliative measure, and as a rule after a few years the eye is destroyed due to the fact that this structure causes an occlusion of the Schlemm's canal which in turn produces a glaucoma with resultant destruction of vision. Surgical and roentgen therapy have been in the main ineffectual. When this laboratory was approached by a member of the Division of Ophthalmology for the purpose of exploring the possibility of treating these cysts by internal radioisotopes, a short-lived alpha active radioelement appeared to be by far the most desirable, as the walls of the cyst are of the order of 20 to 50 microns in thickness. With all of the data from the monkeys in hand and evidence that at a level below 50 microcuries there was no discernible radiation injury, it was felt that it would be safe going ahead with a clinical trial using 10 microcuries, a safety factor of 5 being added, another factor of from 5 to 10 being the difference in size between the monkey eye and the human eye, and finally, as was anticipated and demonstrated, the astatine would not diffuse from the sac as rapidly as it would from the anterior chamber itself, thus there was a hold-up factor which would permit a considerable fraction of the astatine to disappear due to radioactive decay. The details of the clinical trial and its present status will be discussed later.

The histopatholical changes seen in the eye of the monkey at the 75 to 220 microcurie dose level indicated a severe iritis, with the iris itself appearing shrunken and adherent to the cornea. The cornea showed evidence of atrophy and disorganization of its normal structure, the presence of large vacuoles, and a fragmented appearance, as well. The lens was of considerable

interest, as apparently the changes produced are comparable to those seen in severe radiation cataracts which include the presence of large numbers of water clefts and general disorganization of the lens structure. It is believed that this occurred as a result of the radiation of the equatorial cells which were within range of the astatine alpha particles and hence were severely damaged, this damage being presumably responsible for the changes in the lens itself which extended throughout this structure.

Technically, the production of astatine in such a manner that it was in a solution of isotonic saline with no other impurities, either chemical or radioactive, was troublesome, to say the least. The concentration had to be high inasmuch as the volume injected into the cyst was only 0.05 cc. Accordingly, the patient received 10 microcuries of astatine in sterile saline at pH 7. The retention cyst, whose volume was estimated to be of the order of 0.2 cc, was partially aspirated prior to the administration of the astatine. The procedure went very smoothly and, by use of a portable Geiger counter with a thin end window and a small collimating hole, it was possible to accomplish two things; first, to follow the apparent disappearance time from the cyst, and second, the rate of disappearance from the anterior chamber, itself. Also, a rough check could be made of the accuracy of the volume of fluid delivered to the cyst by using the end window counter and counting the x-rays from the astatine before and after injection at almost the same desired and comparable geometry. It was found that within an error of 15 to 20 percent all of the material administered entered the sac. Then by means of the collimating shield, it was apparent that the astatine was retained in the sac with a half-time disappearance rate of the order of 2 to 3 hours. These measurements were followed for approximately 10 hours. However, a precise evaluation of disappearance time from the eye as a whole was rendered difficult by the radiations from the material in the cyst, but it did appear to be somewhat more rapid than from the cyst itself. As a precautionary measure, prior to the admistration of the astatine, the patient received 500 milligrams of potassium iodide a day for one week, as it had been previously demonstrated that when rats are given comparable amounts of radio-iodine prior to the administration of astatine, the uptake of the latter is markedly reduced, usually by a factor of 5 or more. During the experiment, no astatine was detected in the thyroid. Two weeks have elaspsed since this was done, and the only finding to date is a minimal amount of corneal edema about the site of the injection, which is not surprising in view of the traumatic effect of this procedure. The mass of cells at the base of the cyst show possibly some inflamatory-like changes which are not marked. The cyst itself has shown no change in appearance, and secretion of fluid is apparently still continuing. To calculate the dosage received by the cells of the cyst wall and its base would be completely meaningless, as very possibly a considerable fraction of astatine may have become adsorbed on the inner wall of the cyst and its base. At any event, the patient will be followed for a considerable period of time to watch for possible atrophic changes in the cyst, which of course is the sole purpose of this procedure. Should no beneficial effects accrue, it is presently deemed undesirable to repeat this procedure.

The tracer aspect of our work with astatine has included thyroid uptake studies at varying intervals extending to 72 hours in rats in which a total of over 100 animals have been employed, as we observed that frequently there are variations in uptake. The average results are comparable to those

presented in the detailed overall tracer studies presented in the last Quarterly Report. Fig. 1 indicates average values, as well as the tendency for astatine to be released by the gland at the end of 24 hours. The dosages employed were below those which produced a significant diminution of radio-iodine, or showed apparent histopathological change at the 41-day time period. In other words, the possibility of radiation injury to the thyroid being responsible for this release does not seem great. The deduction from this is that probably half of the astatine is either not organically bound at all, or very loosely so. The observation that in an astatine uptake experiment on a rhesus monkey, 50 percent of the astatine was leached from the gland by the 80 percent alcohol used to fix this material.

One presumably normal rhesus monkey weighing approximately 4 kilograms was given 50 microcuries of astatine^{211} by intraperitoneal injection while under nembutal anesthesia in an attempt to follow the thyroid uptake by the in vivo technique of counting externally the emitted K x-rays. Unfortunately, difficulties were encountered with the scintillation counter and circuit employed for this purpose and what data were obtained were inconclusive. The animal was sacrificed 19.5 hours later and a number of tissues removed, which included blood, thyroid, liver, lung, small intestine and trachea. The latter was taken primarily to avoid the possibility of losing thyroid tissue which might not have been completely dissected from the trachea. From the data shown in Table I, it is obvious that approximately one-tenth of the thyroid was not dissected and weighed. At the time of sacrifice, the thyroid was not immediately removed from the trachea, but instead placed in 80 percent alcohol 30 minutes before the dissection. The values given here represent the total uptake before one lobe was removed for radioautographs. It can be seen that the astatine activity in the dissected thyroid tissue, trachea, and the 80 percent alcohol, totaled 22.6 percent of the administered dose. This represents a factor of 10 over the average uptake by the thyroid in the rat. This in part explains the radiosensitivity previously noted in the other monkeys, as manifested by the absence of thyroid tissue at the time they were sacrificed for the removal of the eyes. It might be noted in passing that the thyroid weighed approximately 2 grams. The astatine radioautographs are shown in Fig. 2. It will be noted that there is a non-uniformity in astatine collection in the different follicles which likewise is noted in the rat.

This surprisingly high uptake, as compared to the rat data, made us check very carefully our measurement techniques, which included calibration of the scintillation counter with the 60 kv x-rays emitted from an americium standard. So far as could be determined, there was no reasonable evidence to question this particular single experiment. However, this experiment is to be repeated as no tracer studies can be done in man until it is established beyond reasonable doubt that there would be no injury to the human thyroid.

One of the monkeys, which has not yet been sacrified, began to develop evidence of what we interpret to be a drastic impairment of thyroid function. Approximately 4.5 months after he had received 275 microcuries of astatine, it was noticed that the animal became lethargic, appeared cold to such a degree that it was necessary to install a small heater with a blower near his cage. When this was done, he spent practically all of his time squatting

TABLE I

THE ACCUMULATION OF At^{211} IN THE TISSUES NOTED BELOW IN A RHESUS MONKEY WHICH HAD RECEIVED 50 MICROCURIES OF At^{211} BY INTRAPERITONEAL INJECTION IN ISOTONIC SALINE. THE ANIMAL WAS SACRIFIED 19 HOURS LATER. VALUES ARE EXPRESSED IN PERCENT OF ADMINISTERED DOSE.

Thyroid	10.5 %
Trachea	1.83 %
Fixative	10.3 %
Blood	0.02 %/ml
Liver	0.04 %/gm
Lung	0.15 %/gm
Intestine	0.05 %/gm

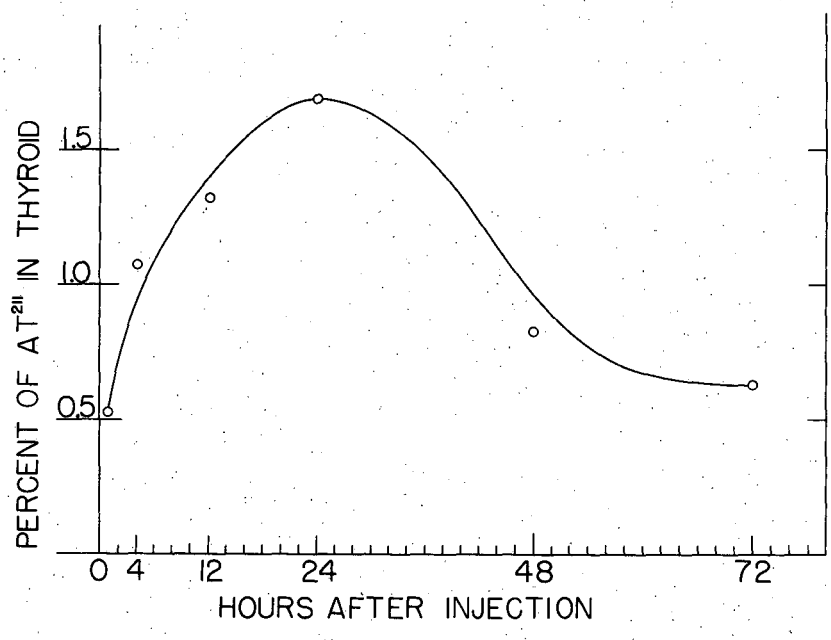


Fig. 1

The accumulation of At^{211} in the thyroid of normal rats weighing from 160 to 180 grams. The points shown here represent the average of approx. 10 rats per point with the exception of the 24 hour interval for which nearly 50 animals were employed. Extension beyond 72 hours was not practical in view of the fact that the dosage level necessary would have been well into the range of that which might have provoked radiation injury to the thyroid gland.

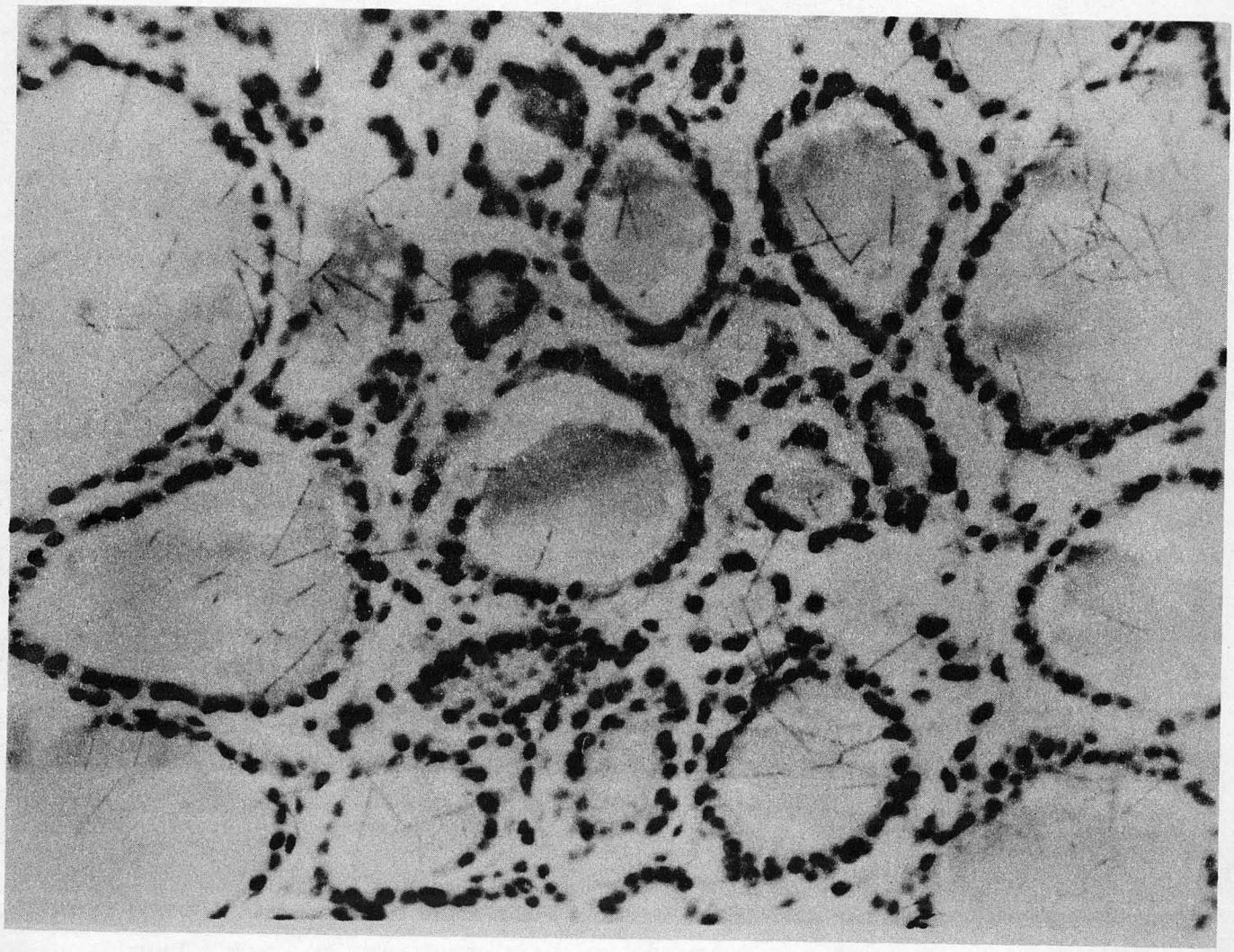


Fig. 2

Radioautograph on NTA stripping film of normal thyroid tissue from a young Rhesus monkey which had received 50 μc of At^{211} by intra-peritoneal injection 19 hours prior to sacrifice. The inhomogeneity of deposition from one follicle to another is indicated the the different numbers of alpha particle tracks which may be seen. (X 300)

or lying as close as possible to this heater. Before doing this, he literally sat for hours in a hunched-up and immobile position. Two normal animals have never shown any evidence of being cold, and the room in which they are maintained is kept at a degree ranging from 72-75°F. Other interesting manifestations included the appearance of heavy pouches beneath his eyes which were reminiscent of those seen in classical myxedema. Moreover, it was noted that bowel movements were less frequent and the fecal material more hardened than in the other 2 monkeys. That this apparent syndrome of simian hypothyroidism was not due to some unrelated effect could be readily ascertained if an attempt were made to take him from his cage, he was as difficult to manage as the other two. As time progressed, the degree of his apparent myxedema increased. Accordingly, we thought it of interest to secure close-up still photographs of both his face and that of his presumably normal companion in an adjoining cage. We are now in the progress of editing the film, as the two monkeys were not exactly cooperative for this procedure.

About four months after the onset of these apparent symptoms of myxedema, the animal began to show a gradual, but perceptible, remission of the various signs and symptoms described above, which would suggest that some of the few remaining epithelial cells seen in the thyroid tissue of the previous monkeys which had received comparable amounts of astatine, had regenerated to a sufficient degree to produce an appreciable amount of the thyroid hormone. For this reason, we are not planning to sacrifice the animal for some time yet.

In conclusion, the evidence seems very strong that two factors exist. First, the uptake of astatine is relatively very much greater in the monkey than in the rat. This is based on the findings in the first two monkeys at the time they were sacrificed and there was a virtual absence of thyroid present. In one instance, this was established further by means of determining the uptake of radio-iodine by the thyroid and a survey of the tissue by the radioautographic technique. The second point is the appearance of what is interpreted to be a myxedematous state in the third animal. A further factor is that an extreme degree of thyroid injury occurred when the dose level was of the order of 60 microcuries per kilo where comparable changes in the rat did not occur until the dose level was extended to about one millicurie per kilo.

A preliminary survey experiment was made with guinea pigs in which three animals weighing approximately 600 grams each were employed and at the end of 19.5 hours, the average accumulation by the thyroid was 1.34 percent, which is in the range observed with rats.

The possibility of iodine deficiency in the monkeys, as contrasted to the guinea pig and rats, is not too attractive as the monkeys are maintained on a variety of foods including liberal amounts of fresh vegetables grown in the Bay Area, which presumably are relatively much richer in iodine than comparable foods would be in more inland areas.

Several months ago, a large series of rats received At^{211} in varying doses by intravenous injection. The animals were divided into groups of five and the doses given to each group were divided into groups of five and the doses given to each group were of the following amounts: 0.5 μc, 1.0 μc, 5 μc, 10 μc, 50 μc, 70 μc, 100 μc and 150 μc. The animals used were plateaued females with a weight range of 160 to 180 grams, previously maintained on our

stock diet for several months so that relatively standard conditions would exist both as to the iodine intake and the diet as a whole. Forty-one days later, these animals were sacrificed. In the entire series, the thyroid and parathyroid were removed and serial sections prepared. The animals receiving 50 μc, 100 μc, and 150 μc had not only the thyroid removed, but a number of organs and tissues we deemed essential to observe the possibility of any histopathological change. These included kidney, stomach, small intestine, adrenal, ovary, salivary glands, spleen, pituitary, lymph nodes, heart, liver, bone marrow and lacrimal gland. The selection of this last tissue was made due to the fact that at the 150 μc dose level, one animal was observed to have profuse bleeding from the eyes which did not occur in the others, and there was no evidence of any mechanical injury or presence of hemorrhagic state. The selection at these three dose levels of tissues and organs other than thyroid and parathyroid arose from the following factors. First, a preliminary test had shown that with the order of one microcurie per gram of At^{211} in the rat, there was a marked depletion of both leukocytes and erythrocytes, and the animals would appear in a moribund state. As it turned out, in the 150 μc group two animals died, while the remaining three, though appearing very ill at about the 10th day, subsequently gained weight and returned to an apparently normal blood level. These trends were almost duplicated by weight curves taken during this interval. Thus, these were the factors which lead us to select the tissues enumerated above.

The histopathological material was first examined by Dr. K.G.Scott; then by Dr. John Z. Bowers, formerly Assistant Director of the Division of Biology and Medicine of the Atomic Energy Commission; and next by Dr. C. W. Asling, Assistant Professor of Anatomy who is primarily in the field of histology and morphology; and lastly, by Dr. Stuart Lindsay, Associate Professor of Pathology at the University of California School of Medicine. The following account is the consensus of opinion of all those concerned.

Small Bowel. Appeared to present no significant alteration of either the 100 or 150 μc level.

Lymph Nodes. At the 150 μc dose level, the general pattern of the lymph node was preserved, but there was noted widening of the sinusoids which contained pigment, neutrophilic leukocytes, and macrophages. The peripheral zone showed likewise marked leukocytic infiltration. The capsule appeared to be somewhat edematous. In general, it is felt that these deviations from normal were a secondary, rather than a primary, effect of the radiation possibly due to infection and breakdown of other tissues in the body not caused directly by radiation injury. In all three animals, there was no great variation in the degree of change noted at the 150 μc dose level. At the 100 μc value, similar effects were seen but were minimal.

Bone Marrow. Unfortunately, only one specimen was available from the three animals and this was the bone marrow taken from the vertebra. There was no apparent abnormality in that there was no discernible evidence of alteration of cellular activity or change in the differentiation of the various cell types present. The megakaryocytes appeared to be in normal number.

Stomach. There was no evidence of inflammatory reaction and the various cell types of the gastric mucosa appeared unchanged.

Lacrimal Gland. Here, considerable variation was noted in the effects at the 50, 100 and 150 μc dose levels, possibly due to the fact that there may have been varying amounts of astatine accumulated by this gland in the different animals. The general picture at the 150 μc dose level was that the acini were considerably smaller, somewhat more numerous, and the cells appeared to be smaller with less cytoplasm and possibly a greater variation in the size of the nuclei as compared to the normal. Areas of edema with infiltration by lymphocytes and polymorphonuclears were noted. A few mitotic figures were observed, where there did not appear to be any extensive regeneration. It seems quite likely that damage did occur to this tissue and that regeneration had been completed at the time the animals were sacrificed, though this point of necessity must remain somewhat speculative. There appears to be a significant increase in a dark brown staining pigment in the astatine-treated animals as compared to the control. This effect showed a significant treatment with increasing dosage although again there are variations of considerable magnitude in this tissue secured from the different rats. The evidence at hand does suggest the possibility of some changes which may have been produced directly by the action of the alpha particles from the astatine. It might be noted that nominally this gland would have been selected at the time the animals were sacrificed and the material collected for histopathological study. However, at about the 20th day following administration of the astatine, one of the animals in the 150 μc group began to show quite pronounced bleeding from both eyes and no evidence could be found that this was produced by any mechanical injury.

Salivary Gland. This organ showed no alteration that could be construed to be of significant deviation from the normal.

Spleen. The 150 μc group showed evidence of significant changes in the spleen. The lymphoid follicles were somewhat smaller, germinal centers were absent, large clumps of leukocytes were noted about the central arteries but without germinal centers. The total amount of lymphoid tissue present per unit area would appear to be the same, but its arrangement was somewhat different from the normal as indicated above. The other components of splenic tissue appeared to be normal. The 100 μc splenic tissue did not show any marked deviation from normal other than the presence of more pigment than is found with the control animals.

Kidney. In the 150 μc group there appeared to be a slight thickening of the basement membrane of the glomeruli and an increase of hyalin tissue within it. The proximal convoluted tubules evidenced less closely defined cell borders than appear in the controls. Here again, the increased pigment was noted as in so many other tissues. In general, it might be summarized that the changes found at this dose level were at a minimal degree and at the lower dose levels nothing was seen to suggest evidence of change from the normal histology of this organ.

Ovary. At the 50, 100, 150 μc dose levels, no definitive alteration could be observed.

In conclusion, aside from the lacrimal gland and the spleen at the

highest dose level, no changes of importance were found in the specimens examined except for the thyroid gland.

Thyroid. A large share of time was devoted to going over the thyroid sections and, as has been indicated before, dose levels in the rats whose weight ranged from 160 to 180 grams extended from 0.5 to 150 μc of At^{211} to each animal by intravenous injection. The first indication of any possible changes was noted in one of the 10 μc group, the epithelium appearing slightly larger with some desquamation; but in general, it was our feeling that there was nothing that could be definitely assessed as changes that might have resulted from radiation effects. At the 50 μc level there was great variation between different animals; in some, there was apparently very little functional capacity to take up iodine as indicated by the radioautographs prepared, since it will be noted that these animals received 10 μc of radio-iodine one day before sacrifice. Attention should be drawn to the fact that the uptake of radio-iodine in this group averaged about one-fifth that of the normal controls. However, the thyroids from some animals in the 50 μc group showed less marked destruction with a correspondingly greater uptake. This apparently is a function of the variation in astatine accumulation by the thyroid in the rat which has been described earlier. In general, the pattern may be described as follows. There appears to be partial destruction of groups of cells without acinar formation and with some pleomorphism. In general, it may be said that the thyroid tissue appears to be attempting to form new follicles and bears resemblance to the histopathological observations noted in thyroiditis. There was no evidence of any change in the structure of the parathyroid. The thyroids from those animals receiving 100 μc showed a more extensive degree of injury than noted in those that were given half the amount. There were to be noted occasional follicles, usually large, which would appear to be not too severely damaged, capable of accumulating radio-iodine, which apparently had been present prior to the administration of the astatine. These usually lie about the periphery of the gland and from the radioautographs observed with astatine, this is not surprising as it has been noted that follicles of this character and position frequently accumulate practically no astatine and the irradiation from the surrounding ones apparently was not sufficient to cause marked injury of the epithelial cells of those follicles which were preserved. The histopathological changes produced by the administration of 150 μc of astatine were, as was to be expected, somewhat more marked. Inasmuch as serial sections were taken, it is possible to gain a good estimate of the actual number of surviving follicles, and in the case of the thyroids of three surviving animals, there were only seven follicles which apparently survived complete destruction. The gland itself was shrunken, there were present many cells with ovoid nuclei which were possibly fibroblasts. The few remaining epithelial cells tended to be arranged in cords and small groups within which occasionally could be seen a very small amount of colloid-like material. Occasional mitotic figures were noted and a few "Hürthle"-like cells were observed. This might arise conceivably from excessive stimulation of the remaining few epithelial cells by the thyrotropic hormone. There was evidence of very little residual inflammatory reaction although some pigment was present which was not surprising in light of the marked degree of damage to the thyroid. The parathyroid appeared completely normal.

The thyroid tissue from a monkey which received astatine by intra-ocular injection in the anterior chamber of the eye had grossly no thyroid tissue visible through the parathyroid could be discerned at the time of sacrifice four months later. This animal received 200 μc of radio-iodine, and in view of the rapid disappearance within the anterior chamber of the eye, the effective dose was probably not significantly less than had it been given by intravenous injection. The histological material and radioautographs revealed a few cell clumps which had accumulated radio-iodine which had been given 24 hours before sacrifice. The tissue as a whole presented loosely arranged fibrous tissue with chronic inflammatory cells, macrophages and some pigment. There were again present considerable number of fibroblasts. Occasionally, cells which resembled thyroid epithelial cells had arranged themselves in small acinar structures which contained colloid-like material and accumulated radio-iodine.

It should be pointed out that there are several factors in this particular experiment that differ from the more extensive and detailed studies with rats. In the first place, the dose of astatine^{211} given was approximately 40 μc per kilo as contrasted to a value of roughly one millicurie per kilo in the case of the rats. As will be noted from the figures, the uptake level at the 24 hour interval in the rat averaged about 1.5 percent in the large series of studies that were made, while in the case of another monkey, the uptake of astatine^{211} in the corresponding time interval was 10.5 percent. This, of course, does not give information as to the level of uptake between 1 and 18 hours which is the period that the thyroid would receive most of its irradiation, as by 24 hours there had been a diminution of astatine^{211} in any tissue and organ by a factor of 8 due to radioactive decay. Another consideration is that the monkey was sacrificed approximately 4 months following the administration of the astatine as contrasted to 41 days in the case of the rats. The apparent greater sensitivity of astatine to the thyroid of the monkey cannot be readily explained, and more work of this character in such animals must be done to establish more firmly the reliability of this observation.

Attention should be drawn to the fact that Doctor Lindsay was a co-author in a experiment somewhat comparable to that from this laboratory in which I^{131} was used in the rat. This work, which appeared in the January issue of ENDOCRINOLOGY, was done with Doctor I. L. Chaikoff and his colleagues. Much of the histopathology, particularly the interpretation of thyroid changes was Doctor Lindsay's responsibility to this problem. We were able to secure a few of the sections of Doctor Chaikoff's radio-iodine treated rats at comparable time intervals. At a very loosely approximated dose level, as an estimation, dosimetry of radio-iodine even in a large animal such as man is at best a situation in which numbers have only a relative meaning. In a small animal such as the rat, where radiation leakage is added to the irregular deposition of radio-iodine, matters are still worse. With these limitations in mind, however, the ultimate changes effected to the thyroid tissue appear not to be too different, although on first hand examination of the radio-iodine material, there appeared to be great dissimilarities which arose from difference in histological techniques, staining, etc. In the thyroid tissue, the only really significant variance between the two is that the follicles at the periphery of the gland in the case of astatine would appear to be more prone to escape destruction since we have observed by

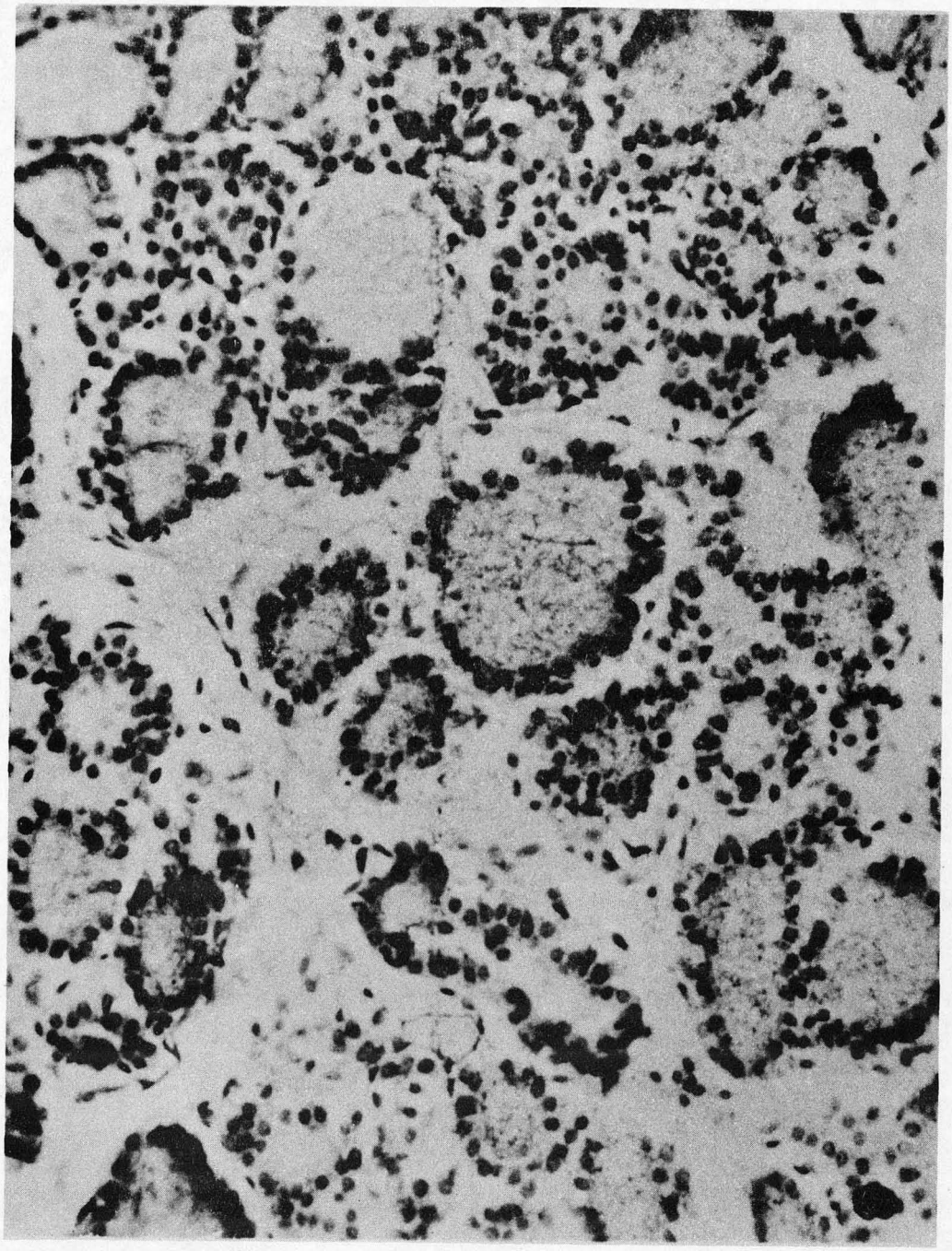
radioautographic techniques that astatine has a tendency to accumulate to a smaller degree in the large follicles which characteristically are seen at the periphery and poles in the thyroid of the rat.

It may be pertinent at this point to present some additional radioautographs which, at least in part, indicate a correlation between the character of deposition of both radio-iodine and astatine in the thyroid and observations of radiation injury produced by the latter, and in particular, the variability of accumulation of these two radioelements from one follicle to another. In addition, there are presented two photomicrographs of severely damaged thyroid tissue from one of the monkeys, which includes the radioautograph. It may be noted in passing that in all instances the radioautographs presented from Figs. 2 to 7, the technique was to employ stripping film, NTB being employed for all of the radio-iodine radioautographs with the exception of Figs. 2, 4 and 5 in which NTA stripping emulsion was used.

Figs. 3, 4, and 5 are presented to serve as an orientation of the variability of the deposition of both astatine and radio-iodine in the thyroid of the rat. A similar irregularity has been shown previously in Fig. 2 which presents the deposition of astatine in the thyroid of the normal young rhesus monkey. Figs. 6 and 7 are photomicrographs of thyroid tissue of a monkey whose gland was severly injured by the administration of 197 μc of astatine 4.5 months before the animal was sacrificed.

One of the outstanding observations is the irregularity of accumulation of both radio-halogens by the different acini. In both the monkey and the rat, particularly in the latter, there is a tendency for the larger follicles not to accumulate as much of either of these radioelements as in the case of smaller and presumably metabolically more active follicles. This correlation is relative, not absolute, as not infrequently the larger acini show a predilection for either one or both of these two radioelements. This would explain the persistence of a few large follicles in the rat thyroid after almost complete destruction of the gland has taken place following the administration of the higher dose levels of astatine.

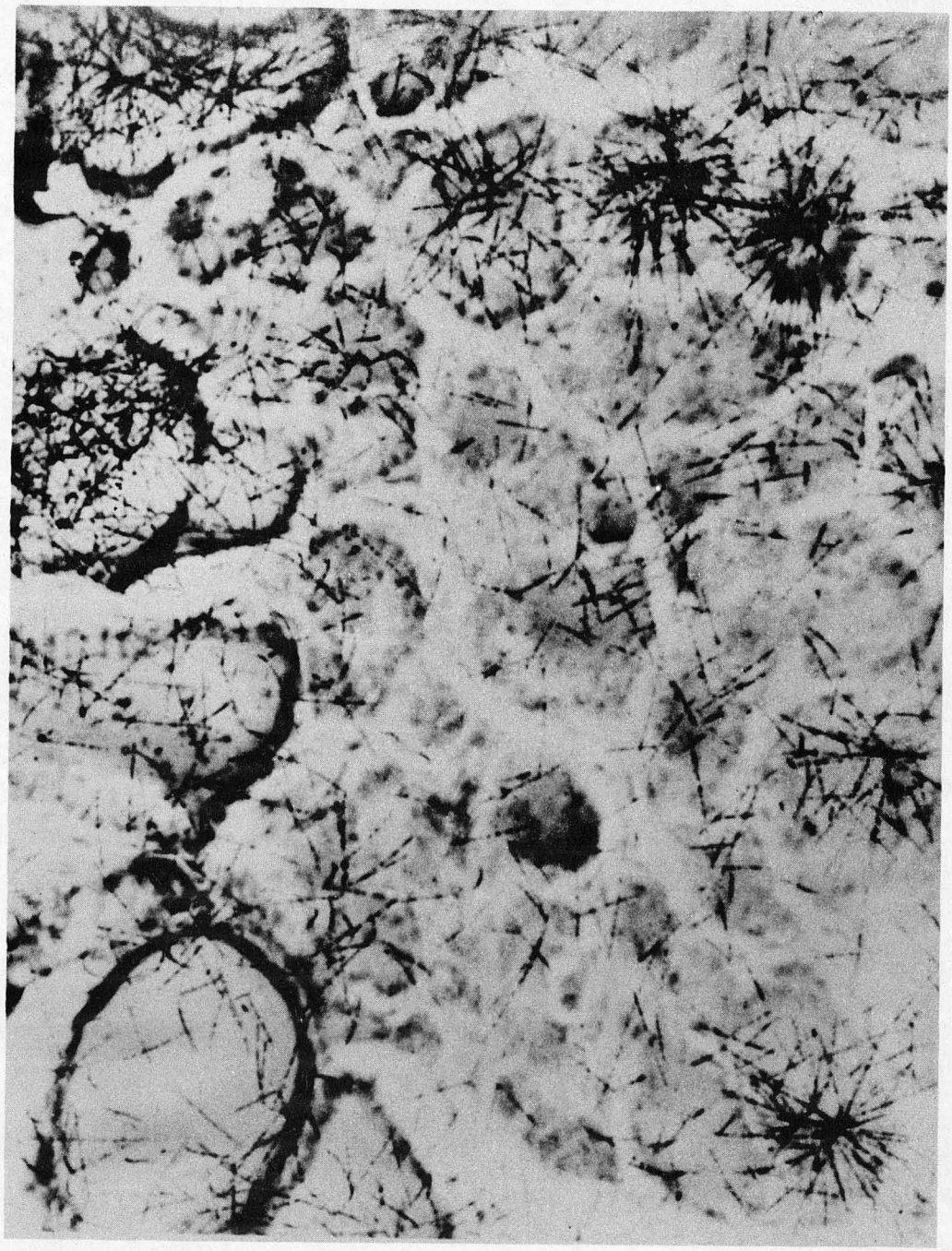
Figs. 6 and 7 are of particular interest, for this monkey had received 197 μc of astatine and then 4.5 months later 100 μc of radio-iodine was given and the animal sacrificed 24 hours later. As has been indicated earlier, no thyroid tissue discernible as such, either directly to the eye or under the dissection microscope, was to be found. The parathyroids, however, were visible; and as carefully as possible this region was dissected out, fixed in 80 percent alcohol, and paraffin sections prepared in the routine manner. NTB stripping film was employed to secure the radioautographs, and it will be noted that there are four areas in Fig. 6 which show evidence of radio-iodine accumulation. In both Figs 6 and 7, it will be noted that there was no normal follicular structure, this point being much clearer to direct visual observation than with the photomicrograph, due to the obscuration of the underlying cells by the silver granules of the irradiated portion of the emulsion. Only a few regions of this character were seen in the entire specimen, and the total uptake of radio-iodine by the thyroid was of the order of a few tenths of a percent of the administered dose, as contrasted to the order of 20 percent in normal monkeys. As has been indicated in the previous discussion, occasional rosettes of what appeared to be follicular cells were



ZN 51

Fig. 3

Radioautograph of radio-iodine deposition in normal thyroid tissue of a rat which had received 10 μc of radio-iodine by intravenous injection 24 hours prior to sacrifice. The variability of deposition in the follicles is quite evident. (X 300)



ZN 52

Fig. 4

Radioautograph obtained using NTA stripping film of normal thyroid section from a rat which received 50 μc of At^{211} by intravenous injection and was sacrificed 18 hours later. The marked variability in deposition is evident and there would appear to be a tendency for greater accumulation in the colloid as compared to the follicular cells. Caution must be exercized in these interpretations for all of the astatine radioautographs on the basis of possible translocation due to leaching.

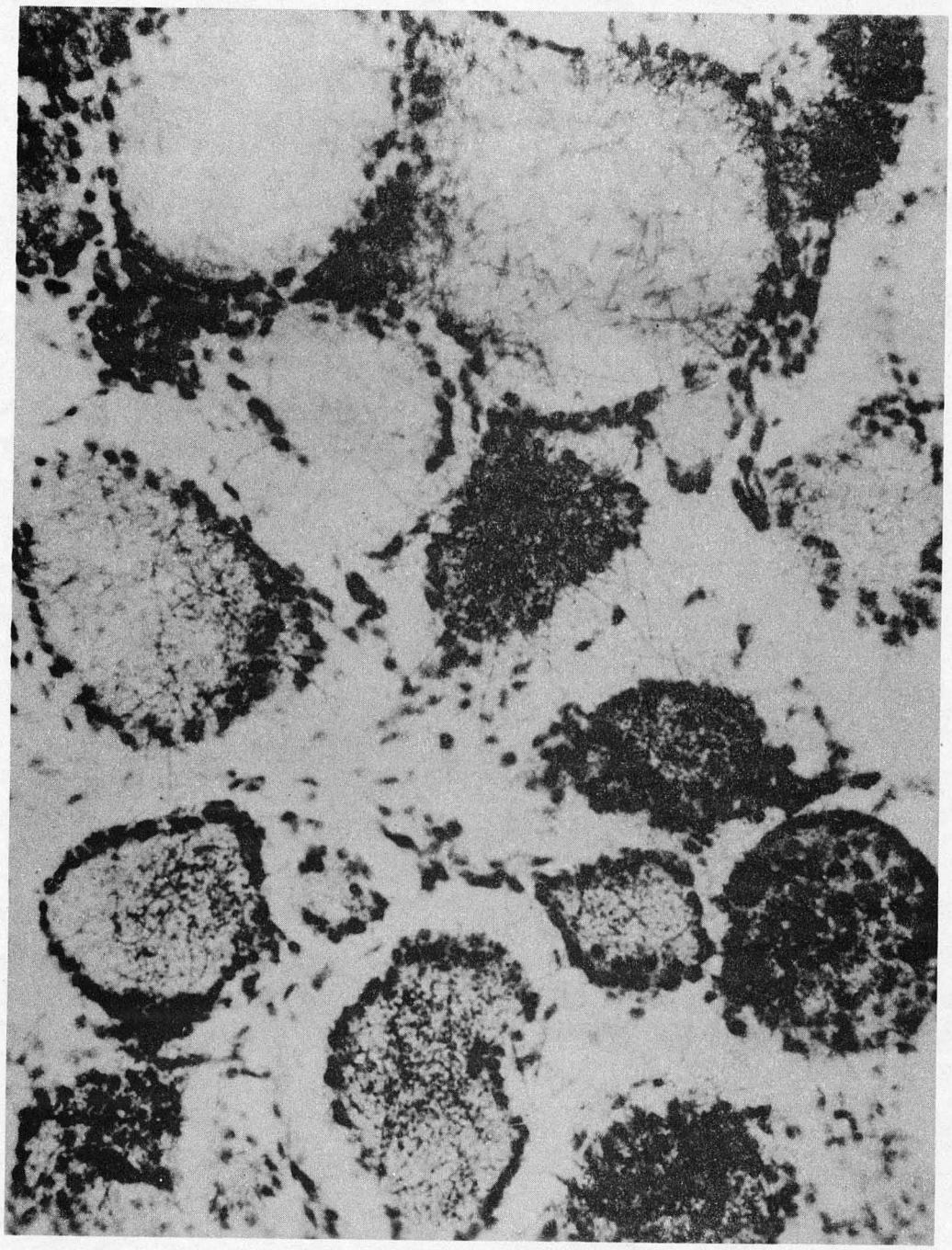


Fig. 5

ZN 53

Radioautograph obtained on NTA stripping film of normal thyroid tissue from a rat which was sacrificed 18 hours after intravenous administration of 50 μc of At^{211} and 4 μc of I^{131}. The accumulation of radio-iodine as indicated from the fine granular darkening, and the presence of At^{211} can be seen from the alpha particle tracks. There appears, not too clearly in this particular photomicrograph, a non-uniformity of both iodine and astatine collection which seem to be uncorrelated with one another. (X 300)

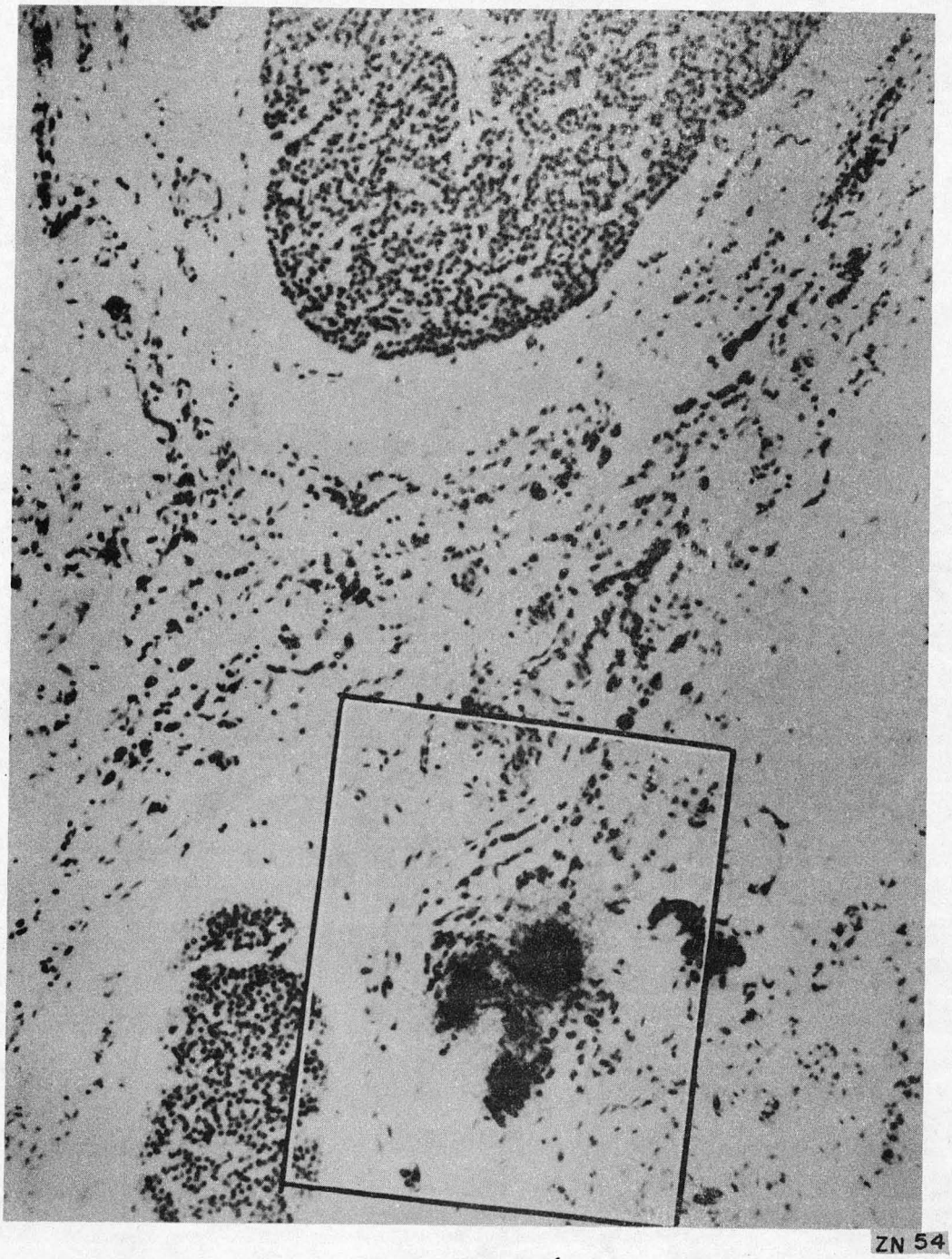
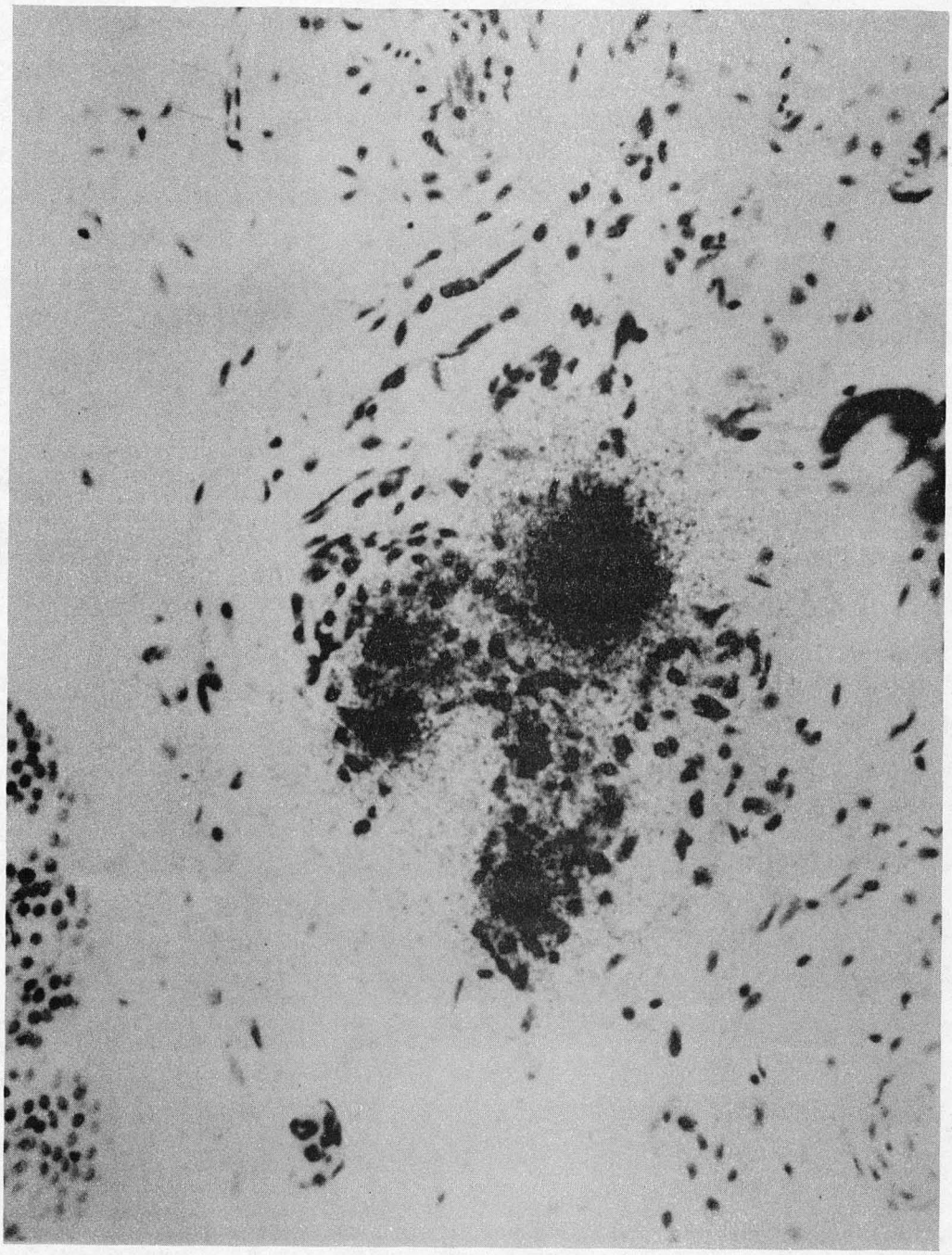


Fig. 6

Radioautograph of paratracheal tissue and parathyroid gland of a yound Rhesus monkey 4.5 months subsequent to the intraocular injection of 197 μc of At^{211}. Twenty-four hours prior to sacrifice the animal received 100 μc of radio-iodine by intraperitoneal injection. The radioautograph was prepared using NTB stripping film and it will be noted that the normal thyroid tissue has been replaced by loose fibrous structure of a small region delineated in the box as shown in the capacity to accumulate radio-iodine. (X 120)



ZN 55

Fig. 7

High power magnification (x 300) of the region outlined in Fig. 6. It is apparent that the tissue which accumulated radio-iodine was not organized into normal follicles. The adjacent tissue consisted chiefly of fibroblasts, occasionally pigmented macrophages and a few cells, not clearly shown here, that are presumably severely damaged follicular cells. In both Figs. 6 and 7 the parathyroid tissue showed not visible evidence of injury.(X 300)

observed. Some evidenced capacity to accumulate radio-iodine, others did not. Rarely, it was possible to see aggregates of 2 to 5 cells enclosing a lightly staining homogeneous and eosinophilic material which may represent thyroid colloid. This picture suggests a beginning regeneration of follicular cells. The apparent slight remission of the myxedematous-like signs in the monkey described earlier may well be a result of further proliferation of follicular tissue and concurrent synthesis of thyroid hormone.

The major difference between the astatine and radio-iodine treated rats was the marked destruction of the parathyroid and adjacent structures in Dr. Chaikoff's experiment. In our studies, the effect was limited only to thyroid tissue. Basically both in the rat and the two monkeys, the appearance is that of a thyroiditis. There was nothing that very specifically looked like radiation injury per se. At later time intervals the picture may be quite different.

Tracer Studies

K. G. Scott, H. Foreman, and Josephine Crowley

Ruthenium^{97}. The carrier-free Ru^{97} was in isotonic saline at a pH of approximately 5 and presumably a mixture of the +3 and +4 valence states was given to rats in groups of three. The dosages given and tissues removed are indicated in Table II. It will be noted that the primary organ of concentration for this carrier-free radioelement is the kidney which correlates with work done earlier. This may be in part related to the fact that the kidney is the chief channel of elimination.

Molybdenum^{99}. Carrier-free Mo^{99} in the hexavalent state in isotonic saline was administered to 3 rats by the intravenous route. They were sacrificed 2 hours after the injection. A previous 4-hour experiment presented in the preceding report indicated that over 30 percent had been eliminated by the kidneys. At the 2-hour interval, there was very little excretion as will be noted in Table III. The main difference between the 2 and 4-hour intervals is that the soft tissue content in the main is somewhat higher as might be expected.

Palladium^{103}. Preparation of carrier-free Pd as $PdCl_2$ in isotonic saline at pH 4 was carried out by procedures reported earlier. The rats were divided into groups of 3 and the time intervals between administration by vein and sacrifice were 4 hours, 1 and 7 days. The dosage and list of tissues obtained appear in Table IV. It is of interest to note that at 4 hours nearly 60 percent had been excreted in the urine and that thereafter excretion proceeded at a relatively slower rate. This trend was noted with the carrier-free Ru. Likewise, the other observation is the relatively high concentration in the kidney at the 7-day period, whereas practically all of the other tissues and organs subjected to assay, with the exception of the liver and spleen, had been traces of the administered radioelement.

Rhodium^{105}. Similar studies have been done with carrier-free radio-rhodium, this radioelement having been prepared and isolated by procedures described elsewhere, and it was given in isotonic saline at a pH of about 4 in the +3 valence state, presumably as $RhCl_3$. The data are presented in Table V. Here again, there are trends which are reminiscent of Ru and Pd, namely prompt appearance of a considerable amount in the urine with a diminishing rate of excretion from the kidney thereafter. However, fecal excretion plays a greater role in the elimination of this radioelement. Again, the organ of highest concentration is the kidney, but there tends to be at the 6 day interval a considerable fraction of the administered amount present in most of the soft tissues subject to assay.

Terbium^{160}. We were fortunate in securing Tb^{160} of an extremely high degree of specific activity which was of the order of 10 $\mu c/\mu g$. In this particular experiment, the Tb^{160} was administered as $TbCl_3$ in isotonic saline at about pH 4 by both the intramuscular and intravenous routes. Twelve rats were employed for this particular experiment and were divided into four groups. Two groups received the Tb^{160} by intramuscular injection. In one of the groups, 6 milligrams of sodium citrate was added to the solution injected

TABLE II

THE DEPOSITION OF CARRIER-FREE RU^{97} IN THE RAT 1, 4, AND 7 DAYS AFTER INTRAVENOUS INJECTION. VALUES ARE CORRECTED FOR RECOVERY AND ARE EXPRESSED IN PERCENT OF ADMINISTERED DOSE. EACH RAT IN THE 1, 4, and 7 DAY GROUPS RECEIVED 10, 30, AND 50 MICROCURIES OF RU^{97}, RESPECTIVELY.

Organ	1 day		4 days		7 days	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Heart	.49	.52	.34	.40	.31	.39
Lung	2.16	.73	1.25	.67	1.12	.65
Spleen	.48	.56	.82	.73	.72	.70
Blood	9.14	.55	1.62	.11	.88	.06
Liver	7.12	.85	6.02	.52	5.35	.55
Kidney	3.29	1.67	3.97	1.76	3.27	2.04
Stomach	.60	.43	.41	.35	.37	.37
Stom. Cont.	.13	-	<.01	-	.01	-
Sm. Int.	2.11	.48	1.95	.37	1.47	.31
Sm. Int. Cont.	.51	-	.14	-	.14	-
Lg. Int.	1.45	.56	1.24	.42	1.14	.39
Lg. Int. Cont.	1.89	-	.15	-	.17	-
Pancreas	.70	.35	.26	.36	.31	.30
Skeleton	8.00	.45	6.09	.39	5.12	.34
Muscle	26.3	.22	19.7	.20	18.5	.19
Skin	17.7	.46	18.3	.46	13.9	.40
Fat	-	.31	-	.10	-	.17
Brain	.03	.02	<.01	<.01	<.01	<.01
Eyes	.03	.18	.03	.12	.04	.12
Pituitary	<.01	-	<.01	-	<.01	-
Gonads	.68	.21	.64	.22	.69	.26
Thyroid	.02	-	.02	-	.02	-
Adrenal	.03	-	.04	-	.02	-
Lymph Gl.	-	.62	-	.41	-	.54
Urine	16.0	-	29.1	-	38.8	-
Feces	1.16	-	8.01	-	7.64	-

TABLE III

THE DEPOSITION OF CARRIER-FREE Mo^{99} IN THE RAT 2 HOURS AFTER INTRAVENOUS INJECTION. VALUES ARE CORRECTED FOR RECOVERY AND EXPRESSED IN PERCENT OF THE ADMINISTERED DOSE. EACH RAT RECEIVED 2.6 MICROCURIES OF Mo^{99}.

Organ	% per organ	% per gram
Heart	.27	.26
Lung	1.02	.44
Spleen	.97	.64
Blood	5.89	.32
Liver	29.4	2.45
Kidney	5.84	1.67
Stomach	.82	.52
Stom. Cont.	.84	-
Sm. Int.	5.89	1.02
Sm. Int. Cont.	2.10	-
Lg. Int.	1.65	.52
Lg. Int. Cont.	.29	-
Pancreas	1.21	.62
Skeleton	12.27	.58
Muscle	16.08	.13
Skin	14.2	.29
Fat	-	.38
Brain	.10	.06
Eyes	.04	.19
Pituitary	<.03	-
Gonads	.71	.23
Thyroid	.01	.35
Adrenal	.06	-
Lymph. Gl.	-	.54
Urine	.30	-
Feces	.03	-

TABLE IV

THE DEPOSITION OF CARRIER-FREE Pd^{103} IN THE RAT 4 HOURS, 1 DAY AND 7 DAYS AFTER INTRAVENOUS INJECTION. VALUES ARE CORRECTED FOR RECOVERY AND ARE EXPRESSED IN PERCENT OF THE ADMINISTERED DOSE. EACH RAT IN THE 4 HOUR, 1 AND 7 DAY GROUPS RECEIVED 0.3, 0.5, and 2.0 MICROCURIES OF Pd^{103}, RESPECTIVELY.

Organ	4 hour - 0.3 μc		1 day - 0.5 μc		7 day - 2.0 μc	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Heart	.17	.18	.06	.07	<.01	-
Lung	4.88	1.68	1.43	.94	.05	.03
Spleen	.42	.49	.66	.69	.21	.19
Blood	7.63	.45	.80	.06	<.05	<.01
Liver	8.23	.79	8.76	.93	3.72	.34
Kidney	6.76	3.02	8.47	4.49	5.15	2.32
Stomach	.09	.07	.08	.06	.01	<.01
Stom. Cont.	.07	-	.02	-	<.01	-
Sm. Cont.	.67	.15	.34	.07	.08	.01
Sm. Int. Cont.	1.78	-	.23	-	.05	-
Lg. Int.	.48	.15	.22	.07	.12	.02
Lg. Int. Cont.	1.89	-	.45	-	.01	-
Pancreas	.22	.07	.09	.06	.05	.02
Skeleton	1.92	.10	1.04	.06	.27	.01
Muscle	2.10	.01	1.35	.01	.50	.01
Skin	1.64	.03	1.28	.03	.41	.01
Fat	-	.02	-	.02	-	.01
Brain	.04	.02	.02	.01	<.01	<.01
Eyes	.01	.03	.08	.24	<.01	<.01
Pituitary	<.01	-	.01	-	<.01	-
Gonads	.19	.07	.14	.06	.06	.02
Thyroid	<.01	-	.03	-	<.01	-
Adrenal	.01	-	.01	-	<.01	-
Lymph Gland	-	.12	-	.08	-	.03
Urine	60.6	-	70.6	-	75.9	-
Feces	.15	-	3.81	-	13.4	-

TABLE V

THE DEPOSITION OF CARRIER-FREE Rh^{105} IN THE RAT 2 HOURS, 1, 2, and 6 DAYS AFTER INTRAVENOUS INJECTION. VALUES ARE CORRECTED FOR RECOVERY AND ARE EXPRESSED IN PERCENT OF THE ADMINISTERED DOSE. EACH RAT IN THE 2 HOUR, 1, 2, and 6 DAY GROUPS RECEIVED 2, 2, 4, and 6.5 MICROCURIES OF Rh^{105} RESPECTIVELY.

Organ	2 hours - 2 μc		1 day - 2 μc		2 days - 4 μc		6 days - 6.5 μc	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Heart	.30	.30	.24	.27	.22	.22	.15	.15
Lung	1.51	.56	1.16	.52	.74	.41	.66	.32
Spleen	.39	.18	.34	.32	.51	.40	.64	.55
Blood	11.4	.61	6.47	.45	5.01	.31	1.25	.08
Liver	5.41	.34	3.92	.40	4.34	.35	2.65	.27
Kidney	3.03	.87	2.89	1.61	3.41	1.37	3.36	1.42
Stomach	.48	.30	.44	.25	.31	.24	.22	.20
Stom. Cont.	.03	-	.02	-	.12	-	<.01	-
Sm. Int.	1.33	.25	1.13	.25	1.10	.15	.82	.16
Sm.Int.Cont.	1.23	-	.62	-	.41	-	.10	-
Lg. Int.	1.24	.32	2.57	.39	.90	.27	.89	.27
Lg.Int.Cont.	.25	-	.66	-	.69	-	.28	-
Pancreas	.67	.28	.35	.30	.23	.22	.14	.22
Skeleton	8.76	.39	4.07	.24	5.29	.28	3.03	.15
Muscle	18.6	.16	13.4	.14	15.1	.13	11.7	.11
Skin	21.4	.43	14.4	.52	21.6	.47	17.5	.40
Fat	-	.23	-	.15	-	.08	-	.14
Brain	.05	.03	.03	.02	.03	.01	<.01	<.01
Eyes	.04	.12	.05	.14	.04	.15	.02	.08
Pituitary	<.01	-	<.01	-	<.01	-	<.01	-
Gonads	.49	.14	.55	.23	.46	.17	.47	.19
Thyroid	<.01	-	.01	-	.01	-	<.01	-
Adrenal	.04	-	.02	-	<.01	-	.02	-
Lymph Gland	-	.50	-	.61	-	.37	-	.37
Urine	23.3	-	35.2	-	29.1	-	35.5	-
Feces	.03	-	11.4	-	10.4	-	20.6	-

also in UCRL-1437

into each of two rats. The other two groups received the Tb without the addition of the citrate. The same procedure was performed for the two intravenous groups and all twelve animals were sacrificed at the end of 256 days following the administration of this radioelement. It will be noted in Tables VI and VII that the skeleton is the principal organ of retention, and that excretion is about equally divided between the urine and feces. Two points of interest are apparent here. First, under the conditions of these experiments, the route of administration and the presence or absence of citrate as a complexing agent made no significant variation in the retention and excretion of this radioelement. Second, it will be noted in comparing this data with that secured at shorter time intervals, the skeleton is the only structure in which there is a high and prolonged degree of accumulation. This, of course is a characteristic that has been noted in other members of the lanthanide rare earths, yttrium, actinium, thorium, protactinium, plutonium, americium and curium.

Rhenium^{183,184}. Some time ago, some tracer studies were done with carrier-free radio-rhenium which had been prepared and isolated by methods already described. We felt it desirable to repeat these studies and with particular reference to the thyroid uptake. The time interval and dosages appear in Table VIII as well as the tissues selected for assay. It will be noted that the thyroid accumulation at four hours is 3.5 percent per gram. On studying the data carefully, we feel that this is significant and a little surprising in view of the fact that rhenium is chemically very different from the halogen series, though it is a member of the seventh group of the periodic table. To the best of our knowledge, it was in the +4 valence state when given as carrier-free $ReCl_4$. Interestingly, there was at the four hour time interval a rather large amount in the stomach and stomach contents which is reminiscent of the behavior of carrier-free radioiodine and astatine. Another noteworthy point is the initial high accumulation in skin both as to the total amount and on a percent per gram basis. The excretion is by way of the kidney, but unlike the Pd and Pt group of metals, very little was retained by the kidney, the value falling to less than 1/10,000th of the administered dose at the end of 4 days.

Iridium^{190,192}. Tracer studies in the rat were set up with carrier-free $Ir^{190,192}$. Table IX summarizes the dose levels, mode of injection, time intervals, and tissues assayed for rats in groups of three. The behavior of the carrier-free Ir, which was in the form of $IrCl_3$, follows much the pattern exhibited by Ru, Rh, and Pd with the exception that carrier-free Ru is not eliminated in the feces to a comparable degree observed with Rh and Pd. Here again, spleen and liver at comparable time intervals show a fairly high accumulation, but the highest, again is the kidney.

Lead^{203}. An attempt was made to determine the effect of calcium ethylenediaminetetraacetic acid (CaEDTA) on the deposition of carrier-free Pb^{203} in the divalent state. Three groups of rats, three rats to a group were employed. The carrier-free Pb^{203} was administered by the intravenous route in isotonic saline at pH 4. As has been indicated from earlier work, the skeleton is the chief organ of accumulation, though the kidney approaches comparable values on a per gram basis. As will be seen from Table X, one group of 3 rats was sacrificed at 24 hours, a second group at 6 days, and a third group which had received 3 percent CaEDTA by oral ingestion on the 4th, 5th and 6th days was sacrificed at 6 days. No impressive effect upon either the distribution in the various tissues, including skeleton, or the excretion was noted. It would appear to be desirable to pursue this a little further when time, facilities and man power would permit.

TABLE VI

THE EFFECT OF A COMPLEXING AGENT ON THE DEPOSITION OF TERBIUM IN THE RAT USING Tb^{160} AS A TRACER 256 DAYS AFTER INTRAMUSCULAR INJECTION. VALUES ARE CORRECTED FOR RECOVERY AND ARE EXPRESSED IN PERCENT OF ABSORBED DOSE. EACH RAT RECEIVED 0.6 MICROGRAMS OF TERBIUM, 5 MICROCURIES OF Tb^{160}, AND 3 RATS EACH RECEIVED 12 MICROGRAMS OF SODIUM CITRATE.

Organ	Intramuscular		Intramuscular + Citrate	
	% per organ	% per gram	% per organ	% per gram
Heart	.02	.02	.03	.02
Lung	.12	.05	.14	.04
Spleen	.06	.06	.05	.04
Blood	.11	<.01	<.01	<.01
Liver	.26	.02	.20	.06
Kidney	.20	.07	.79	.29
Stomach	.02	.01	.04	<.01
Sm. Int.	.07	<.01	.06	<.01
Lg. Int.	.05	<.01	.03	<.01
Pancreas	<.01	<.01	<.01	<.01
Skeleton	35.6	1.63	29.4	1.30
Muscle	1.30	.01	1.48	.01
Skin	1.45	.02	1.66	.03
Fat	-	<.01	-	<.01
Brain	.01	<.01	.01	<.01
Eyes	.01	.02	.01	.02
Pituitary	<.01	-	<.01	-
Gonads	.17	.06	.08	.03
Thyroid	<.01	-	<.01	-
Adrenal	.01	-	<.01	-
Lymph Gland	-	.02	-	.06
Urine	25.0	-	35.8	-
Feces	35.5	-	30.2	-

TABLE VII

THE EFFECT OF A COMPLEXING AGENT ON THE DEPOSITION OF TERBIUM IN THE RAT USING Tb^{160} AS A TRACER 256 DAYS AFTER INTRAVENOUS INJECTION. VALUES ARE CORRECTED FOR RECOVERY AND ARE EXPRESSED IN PERCENT OF THE ADMINISTERED DOSE. EACH RAT RECEIVED 0.6 MICROGRAMS OF TERBIUM, 5 MICROCURIES OF Tb^{160} AND 3 RATS EACH RECEIVED 6 MILLIGRAMS OF SODIUM CITRATE.

Organ	% per organ	% per gram	complexed with citrate % per organ	complexed with citrate % per gram
Heart	.04	.03	.02	.02
Lung	.10	.04	.09	.03
Spleen	.04	.04	.04	.04
Blood	<.01	<.01	<.01	<.01
Liver	.24	.02	.19	.02
Kidney	.39	.17	.58	.18
Stomach	.04	<.01	.02	<.01
Sm. Int.	.08	<.01	.06	<.01
Lg. Int.	.02	<.01	.02	<.01
Pancreas	<.01	<.01	<.01	.01
Skeleton	39.7	1.90	39.0	1.76
Muscle	1.28	.01	.71	.01
Skin	1.04	.02	.78	.02
Fat	-	-	-	<.01
Brain	.01	<.01	.02	.01
Eyes	<.01	.02	<.01	.01
Pituitary	<.01	-	<.01	-
Gonads	.05	.02	.09	.02
Thyroid	<.01	-	.01	-
Adrenal	<.01	-	<.01	-
Lymph Gland	-	.02	-	<.01
Urine	30.9	-	29.7	-
Feces	26.0	-	28.7	-

TABLE VIII

THE DEPOSITION OF CARRIER-FREE $Re^{183,184}$ IN THE RAT 4 HOURS, 1, 2, 4, AND 16 DAYS AFTER INTRAVENOUS INJECTION. VALUES ARE CORRECTED FOR RECOVERY AND ARE EXPRESSED IN PERCENT OF THE ADMINISTERED DOSE. EACH RAT IN THE 4 HOUR, 1 AND 2 DAY GROUPS RECEIVED 2 MICROCURIES OF $Re^{183,184}$. THE RATS IN THE 4 AND 16 DAY GROUPS EACH RECEIVED 4 MICROCURIES OF $Re^{183,184}$.

Organ	4 hour-2 μc		1 day - 2 μc		2 day - 2 μc		4 day - 4 μc		16 day-4 μc	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Heart	.06	.10	<.01	-	<.01	-	<.01	-	<.01	-
Lung	.31	.15	.01	<.01	<.01	-	<.01	-	<.01	-
Spleen	.06	.06	<.01	-	<.01	-	<.01	-	<.01	-
Blood	3.14	.23	.13	<.01	.02	<.01	<.01	-	<.01	-
Liver	1.14	.12	.10	<.01	.05	<.01	.03	<.01	.02	<.01
Kidney	.24	.12	.01	<.01	<.01	-	<.01	-	<.01	-
Stomach	1.66	1.26	.06	.06	.01	<.01				
St. Cont.	10.53	-	.28	-	.01	-				
Sm. Int.	.57	.12	.04	<.01	<.01	-				
Sm. Int. Cont.	.90	-	.05	-	<.01	-	.02	<.01	<.01	-
Lg. Int.	.37	.11	<.01	-	<.01					
Lg. Int. Cont.	.34	-	.01	-	<.01					
Pancreas	.10	.09	<.01	-	<.01					
Skeleton	.99	.08	<.01	-	<.01	-	<.01	-	<.01	-
Muscle	3.70	.04	.20	<.01	.17	<.01	.03	<.01	.08	<.01
Skin	26.06	.80	4.30	.12	7.10	.17	3.28	.08	.90	.03
Trachea	.06	.10	<.01	-	<.01	-	<.01	-	-	-
Fat	-	.06	<.01	-	<.01	-	<.01	-	<.01	-
Brain	.01	.01	<.01	-	<.01	-	<.01	-	<.01	-
Eyes	<.01	.03	<.01	-	<.01	-	<.01	-	-	-
Pituitary	<.01	-	<.01	-	<.01	-	<.01	-	-	-
Gonads	.14	.05	<.01	-	<.01	-	<.01	-	<.01	-
Thyroid	.07	3.5	<.01	-	<.01	-	<.01	-	-	-
Adrenal	<.01	-	<.01	-	<.01	-	<.01	-	-	-
Lym. Gl.	-	.14	<.01	-	<.01	-	<.01	-	-	-
Urine	49.5	-	92.0	-	90.2	-	94.4	-	93.8	-
Feces	.06	-	2.76	-	2.45	-	2.25	-	5.18	-

TABLE IX

THE DEPOSITION OF CARRIER FREE $Ir^{190,192}$ IN THE RAT 4 HOURS, 1, 4, AND 17 DAYS AFTER INTRAVENOUS INJECTION. VALUES ARE CORRECTED FOR RECOVERY AND ARE EXPRESSED IN PERCENT OF THE ADMINISTERED DOSE. EACH RAT IN THE 4 HOUR, 1, 4, AND 17 DAY GROUPS RECEIVED 7, 14, 14, AND 21 MICROCURIES OF $Ir^{190,192}$ RESPECTIVELY.

Organ	4 hour - 7 μc		1 day - 14 μc		4 day - 14 μc		17 day -21 μc	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Heart	.46	.40	.23	.24	.21	.21	.12	.12
Lung	1.59	.74	.87	.39	.65	.29	.26	.12
Spleen	.51	.49	.69	.69	.82	.77	.73	.72
Blood	11.6	.64	6.83	.40	2.80	.17	.41	.02
Liver	19.0	1.79	20.5	2.41	17.7	1.98	6.71	.61
Kidney	5.78	2.55	4.25	2.13	4.46	2.17	3.00	1.46
Stomach	.36	.23	.29	.20	.21	.16	} .13	.06
Stomach Cont.	.21		.02		.07			
Sm. Int.	1.15	.25	1.36	.20	1.03	.15	} .33	.03
Sm. Int. Cont.	.99		.64		.11			
Lg. Int.	1.12	.25	1.06	.34	.36	.12	} .31	.04
Lg. Int. Cont.	1.45		2.07		.65			
Pancreas	.46	.16	.23	.24	.20	.17	.12	.11
Skeleton	2.29	.10	3.28	.17	2.78	.15	1.31	.07
Muscle	6.52	.06	5.90	.05	6.12	.05	3.84	.03
Skin	6.87	.13	10.7	.23	8.96	.19	6.17	.13
Fat	-	.05	-	.07	-	.04	-	.02
Brain	.05	.03	.02	.01	.02	.01	<.01	<.01
Eyes	.02	.06	.02	.07	.02	.06	.01	.03
Pituitary	<.01	-	<.01	-	<.01	-	<.01	-
Gonads	.33	.12	.39	.13	.29	.15	.32	.13
Thyroid	.02	-	<.01	-	<.01	-	<.01	-
Adrenal	.02	-	.02	-	.02	-	.02	-
Lym. Gland	-	.18	-	.20	-	.26	-	.16
Urine	38.9	-	38.8	-	33.9	-	56.5	-
Feces	.36	-	1.92	-	18.6	-	19.7	-

TABLE X

THE EFFECT OF FEEDING VERSENE (CALCIUM SALT OF ETHYLENEDIAMINETETRAACETIC ACID) OF THE DEPOSITION OF CARRIER-FREE Pb^{203} IN THE RAT 6 DAYS AFTER INTRAVENOUS INJECTION. VALUES ARE CORRECTED FOR RECOVERY AND ARE EXPRESSED IN PERCENT OF THE ADMINISTERED DOSE. EACH RAT IN THE 1 DAY GROUP RECEIVED 3.3 MICROCURIES OF Pb^{203}. EACH RAT IN THE TWO 6 DAY GROUPS RECEIVED 6.7 MICROCURIES OF Pb^{203}. VERSENE WAS ADMINISTERED TO ONE SIX-DAY GROUP IN FOOD 4 DAYS AFTER INJECTION OF Pb^{203}.

Organ	1 day		6 days		6 day (versene on day 4-6)	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Heart	.13	.18	.04	.05	.01	.01
Lung	.45	.31	.18	.07	.10	.05
Spleen	.33	.65	.51	.65	.21	.33
Blood	6.66	.52	.81	.06	.84	.06
Liver	8.32	1.15	1.96	.24	2.08	.29
Kidney	14.2	8.55	3.17	1.83	2.03	1.26
Stomach	.15	.16	.02	.02	.03	.03
Stom. Cont.	.07	-	.02	-	<.01	-
Sm. Int.	1.27	.29	.08	.02	.07	.04
Sm. Int. Cont.	1.29	-	.14	-	.08	-
Lg. Int.	.96	.43	.10	.04	.14	.05
Lg. Int. Cont.	4.13	-	.34	-	.49	-
Pancreas	.41	.42	.04	.05	.03	.06
Skeleton	28.0	1.73	28.2	1.91	32.9	2.14
Muscle	2.20	.03	.46	<.01	.82	.01
Skin	2.08	.08	.81	.03	.93	.03
Fat	-	.05	-	-	-	-
Brain	.07	.04	.02	.01	.03	.05
Eyes	.02	.06	<.01	<.01	<.01	<.01
Pituitary	<.01	-	<.01	-	<.01	-
Gonads	.03	.29	<.01	<.01	<.01	<.01
Thyroid	.02	-	<.01	-	<.01	-
Adrenal	.03	-	<.01	-	<.01	-
Lym. Gl.	-	.13	-	<.01	-	.02
Urine	16.5	-	25.5	-	26.9	-
Feces	12.7	-	37.5	-	32.1	-
Uterus	-	-	-	-	.23	.21

Chelating Experiments

Harry Foreman

Experiments to determine the effect of prolonged administration of CaEDTA and Fe-3 are continuing. As was indicated in the last progress report, animals fed on a stock diet containing 10 percent CaEDTA died at the end of 10 days. The findings in these animals were described previously. Animals fed on a stock diet containing 5 percent CaEDTA are still alive at the end of 16 weeks of the experiment. During this time, they had diarrhea for the first month and from then on the stools became formed but very soft. They showed no gross long-term ill effects. After three weeks the animals apparently became adjusted to the diet and from then on they had a steady intake of 12-13 grams of food per animal per day. This would represent an intake of 600 milligrams of CaEDTA daily. However, the actual amount absorbed is not known. In this time period the animals gained on the average of 15 grams each, the same as the control animals which received no chelating agent in their food.

Animals fed on a diet containing 10 percent Fe-3 are still alive after 16 weeks. Shortly after (1 day) the start of the experiment, they developed diarrhea which has continued to the present time. Except for this diarrhea, the animals showed no apparent ill effects until 7 weeks after the start of the experiment when the condition of the fur of the animals became affected. The fur became progressively more ruffled, matted and soiled with excreta. At this time, the animals were placed on the same diet with the Fe-3 omitted. In one day the diarrhea stopped, and by 3 days the fur returned to normal appearance. After 2 weeks, the animals were again started on a 10 percent Fe-3 diet. The diarrhea returned the following day. Five weeks later, one of the animals developed a spasmotic generalized tremor which has persisted to the present. The daily food intake was an average of 12 grams per animal per day, equivalent to approximately one gram of Fe-3 intake per day. The animals have lost an average of 10 grams weight in this time period.

Animals fed on a diet containing 5 percent Fe-3 showed no ill effects except for the development of the diarrhea. The average food intake was 12-13 grams per day. The change in weight varied greatly among the different animals ranging from a loss of 11 grams in the case of one animal to a gain of 8 grams in the case of another.

Animals fed on a diet containing 3 percent CaEDTA showed normal food intake, had normal but slightly soft stools and had the same weight gain as control animals. There are no grossly apparent ill effects from this type diet. Animals fed 3 percent Fe-3 likewise show no ill effects.

An experiment has been set up to determine the effect of CaEDTA and Fe-3 on the excretion of plutonium after it has been fixed in the skeleton for a long period of time, i.e., the simulation of a situation of chronic plutonium poisoning in humans. Six groups of 3 rats each were injected intravenously with approximately 1.2×10^4 C/S of Pu^{238} (Pu^{238} was used because of high specific activity in order that only a small quantity of plutonium need be used). The animals were placed on a stock diet for one month. At the end of that time, two of the groups were placed on a diet containing 3.5 percent CaEDTA, two groups on a diet containing 3.5 percent Fe-3, and the other two groups were kept as controls and remained on the stock diet. The excreta are being collected and assayed for the plutonium content. The experiment is still in progress and results will be reported later.

Radiochemistry

W. M. Garrison, H. R. Haymond, J. D. Gile and D. C. Morrison

Carrier-Free $Ru^{97,103}$ from Molybdenum. The Ru^{97} was prepared by the reaction, Mo(α,xn) and the Ru^{103} by Mo^{100}(α,n). After bombardment, the Mo target (ca 2 gm) was fused with 25 gm KOH-KNO_3 and the resultant melt was extracted with 100 ml water. After centrifugation, the basic solution was transferred to an all-glass distilling flask and the carrier-free $Ru^{97,103}$ was volatilized by passing Cl_2 through the solution at the boiling point. The Ru activity was collected in a trap filled with 12 N HCl. The radio-technetium isotope produced concurrently by (α,pxn) reaction was retained in the residue. The distillate was evaporated to a small volume, treated with excess KOH and the $Ru^{97,103}$ was redistilled as above. The final HCl solution of carrier-free $Ru^{97,103}$ was evaporated to dryness on added NaCl. In a chemical separation using milligram quantities of Mo, Ru and Rh, the activity followed the Ru quantitatively. Decay and radiation characteristics agreed with published values.

Carrier-Free $Rh^{101,102}$ from Ruthenium. The $Rh^{101,102}$ was prepared by the reaction Ru(d,xn). The Ru target (ca 0.5 gm) was fused with 15 gm Na_2O_2 and the melt was digested with 10 ml concentrated HCl. After centrifugation, the acid solution was made basic (ca 6 N in KOH) and the target Ru was volatilized by passing Cl_2 through the heated solution. The residue, containing the carrier-free $Rh^{101,102}$ was acidified, 5 mg of Fe^{+3} was added and the $Rh^{101,102}$ was co-precipitated on $Fe(OH)_3$ with the addition of KOH. The $Fe(OH)_3$ containing the Rh activity was dissolved in 6 N HCl and Fe was separated by ether extraction. The HCl solution of $Rh^{101,102}$ was evaporated to dryness on NaCl. The activity followed Rh quantitatively in a separation of milligram quantities of Ru, Rh and Pd. The decay and radiation characteristics agreed with published values.

Carrier-Free $Ir^{190,192}$ from Osmium. The $Ir^{190,192}$ was prepared by the reaction Os(d,xn). The Os powder (ca 0.5 gm) was dissolved in 35 ml aqua regia and volatilized with the addition of excess HNO_3. No residue was visible, but most of the activity remained in the dish. From this was fumed 2 ml H_2SO_4 to remove any Re activity produced by (d,α) reaction. The H_2SO_4 was volatilized completely and the carrier-free $Ir^{190,192}$ was redissolved in 2 ml, 12 N HCl. The resultant solution was evaporated to dryness on NaCl. The decay and radiation characteristics of the radioactive Ir agreed with published values.

Carrier-Free F^{18} from Oxygen. A procedure has been developed for the production of F^{18} by the reaction O^{16} (α,pn) using liquid water as the target substance. The target chamber consisted of a 50 ml round bottom flask one side of which was drawn in to give a "window" having an average thickness of approximately 5 mil over the bombarded area. A condenser was attached to the top of the cell through a tapered joint. With this arrangement, a 40 Mev helium ion beam at a current as high as 10 μa could be used. The yield of F^{18} was found to be approximately 1 mc/ μa-hr.

Carrier-Free Pb^{203} from Thallium. The Pb^{203} was prepared by the (d,2n) reaction on thallium. The Tl_2O_3 (ca 0.5 gm) target material was dissolved in 20 ml dilute HNO_3 and Tl^{+3} was reduced with SO_2 to Tl^{+}. Five milligrams of Fe^{+3} was added and the solution was made basic with NH_4OH. The Pb^{203} co-precipitated quantitatively on the $Fe(OH)_3$. Fe was separated by ether extraction from 6 N HCl.

The activity followed Pb quantitatively in a separation of milligram quantities of Pb, Hg and Tl. The half-life and radiation characteristics agreed with published values.

Other Radiochemistry. The following carrier-free radioactivities were separated from cyclotron targets using previously reported procedures: Cr^{51}, $K^{42,43}$, Pd^{103} A^{37}, At^{211}, W^{181}, $Mo^{93,99}$ and $Re^{182,184}$.

Radiation Chemistry. Work has started on the radiation chemistry of dilute aqueous solutions. A preliminary study has been made of the radiation induced reduction of CO_2 in 1 M $FeSO_4$. This work, done in cooperation with Professor Calvin and Dr. Benson, is reported below.

While it has long been known that high energy radiations can cause organic decomposition and oxidation, it seemed useful to us to demonstrate that conditions could be found in which high energy radiations could induce the reduction with water of carbon dioxide and the ultimate creation of polyatomic molecules (other than simple polymerization of monomers) of carbon, oxygen, sulfur, hydrogen and nitrogen.

EXPERIMENTAL

The general technique employed in this investigation was to bombard air-free aqueous solutions of C^{14} labelled CO_2 in a closed system with and without the addition of ferrous sulfate. The bombardments were made using the 40 Mev helium ion beam of the 60-inch cyclotron at Crocker Laboratory. To detect the amount and nature of the reduction products, chemical separations were made on the bombarded solution after the addition of carrier amounts of formic acid, formaldehyde, and methyl alcohol. These were separated as solid derivatives and assayed for C^{14} activity. In most of the bombardments one millicurie of from 5 to 9 percent C^{14} labelled CO_2 was used. This made it possible to detect the reduction of approximately one part in 10^6.

Target Assembly. A diagram of the target assembly is shown in Fig. 8. The aqueous solutions were bombarded in an all-glass target cell (A) which consisted essentially of a 50 ml pyrex flask one side of which was drawn in to give a window (B) having an average thickness of approximately 5 mil over the bombarded area. The cell had a volume of 12 ml. It was connected to a glass manifold (C) which in turn was connected through stopcocks to a 100 ml product gas storage bulb (D) to a 25 ml CO_2 reservoir (E) to a mercury manometer (F) and to an outlet (G) through which the entire system could be evacuated. The assembly was supported on a bracket (H) which was fastened to the bell-jar type target (I). The helium ion beam was brought out of the cyclotron vacuum through a 1.5 mil aluminum foil (J) and was delimited in cross section by the aperture (K) in plate (L). The target window was cooled by means of an air stream which entered at (M) and emerged through the aperture (K). The beam current was monitored through the electrode (N). With the all-glass target cell, it was necessary, because of the non-uniform thickness of the window, to calculate the number of ion-pairs produced from the amount of Fe^{+2} oxidation, assuming the same ion-pair yield for this reaction in the glass cell as was obtained in the cell having the platinum window. With the latter target cell, it was possible to estimate within a few percent the energy loss of the helium ions in penetrating the 1.5 mm aluminum foil, 10 cm of air

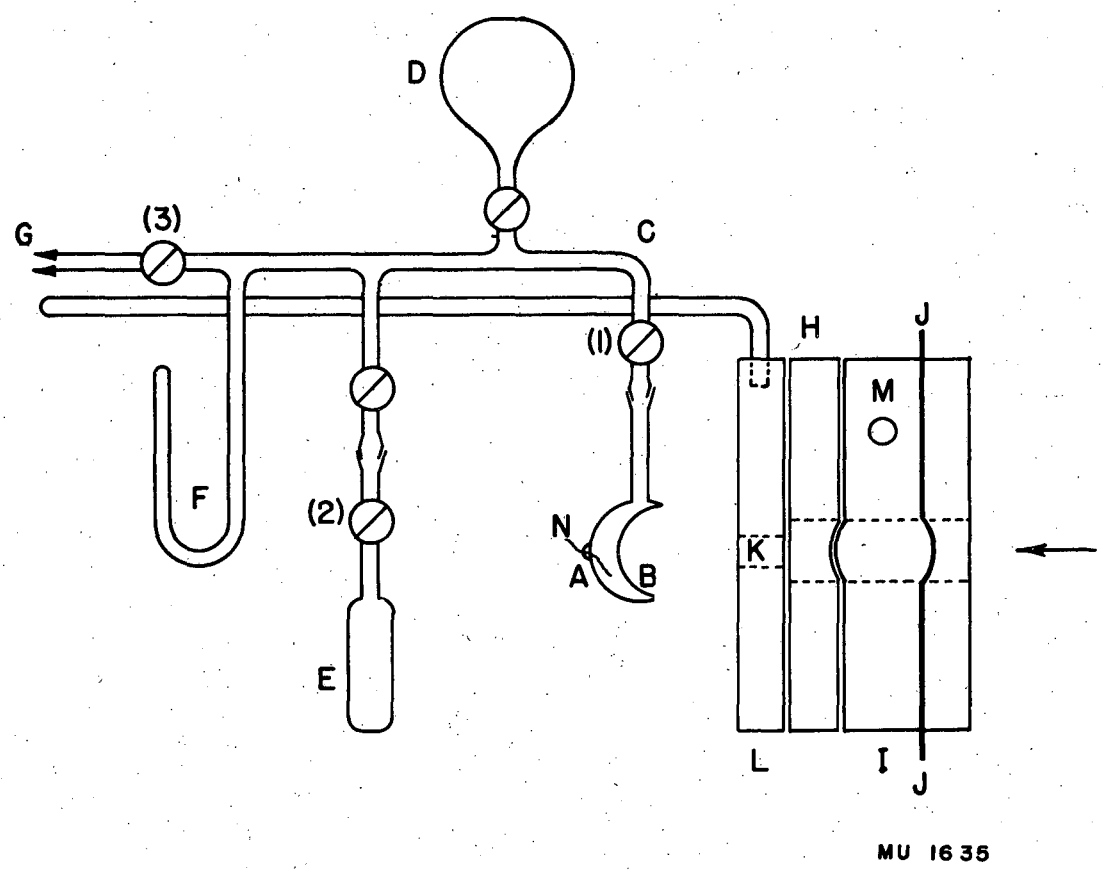


Fig. 8

path, and the one mil platinum window.

Bombardment Procedure. The target cell was first flushed with nitrogen, then filled with triple-distilled deaerated water, or deaerated one molar ferrous sulfate solution at a pH of approx. 3.5. The water was deaerated by boiling and then allowed to cool in a glass-stoppered vessel which had been filled with nitrogen gas. The one molar ferrous sulfate solutions were prepared by adding a known weight of ferrous sulfate. After the target cell was filled, it was immediately connected to the manifold which was then evacuated until roughly 5 percent of the target solution had been evaporated. Stopcock (1) was then closed and the manifold was evacuated including the product gas storage bulb and that portion of the manifold to stopcock (2) which was connected to the CO_2 reservoir containing approximately one millicurie of from 5 to 9 percent C^{14} labelled CO_2. After the evacuation was complete, the manifold was isolated by closing stopcock (3). Stopcocks (1) (2) were then opened and the CO_2 was allowed to equilibrate with the target solution. The target cell was then bombarded with a 0.5 μa beam of 40 Mev helium ions. Bombardment data for each of the experiments are summarized in Table XI.

Chemical Procedures. After bombardment, the target cell was allowed to stand for from 1 to 2 hours to permit the induced radioactivity to decay out. Stopcocks (1) (2) were closed and the target cell was removed from the manifold. The solution was then treated with sulfuric acid to dissolve the ferric hydroxide and adjusted to pH 1. The unreacted $C^{14}O_2$ was stripped with nitrogen and recovered in NaOH solution. After most of the high specific activity $C^{14}O_2$ had been removed, the solution was flushed with tank CO_2 which was discarded. A sample of the solution was withdrawn at this point for ferric ion analysis.

To the remainder of the solution was added formic acid, formaldehyde and methyl alcohol carriers in amounts to give 100 milligrams of the isolated product, i.e., barium formate, methone derivative of formaldehyde, and barium carbonate prepared from the CO_2 formed on oxidation of the methyl alcohol fraction.

The pH of the solution was then adjusted to 7 and the formaldehyde and methyl alcohol were distilled in vacuo. The distillate was treated with methone solution in 50 percent excess and acidified. This precipitated the methone-formaldehyde derivative and the methyl alcohol was separated from this mixture by a second vacuum distillation. The methyl alcohol distillate was wet oxidized with a chromium trioxide-sulfuric acid mixture containing potassium iodate and the evolved CO_2 was recovered as barium carbonate. In none of the bombardments was this barium carbonate fraction active.

The methone-formaldehyde precipitate was filtered off, washed and dissolved in sodium hydroxide. The solution was acidified and the precipitate centrifuged, washed, redissolved in NaOH, and reprecipitated. This procedure was repeated and then the methone-formaldehyde reaction product was recrystallized twice from acetone-water. A sample of the purified methone-formaldehyde product was counted.

The residue from the first distillation containing the formic acid was acidified to pH 1 and distilled in vacuo. The distillate was titrated to phenolphthalein endpoint with a saturated barium hydroxide solution after flushing with CO_2 followed by nitrogen. The precipitate of barium carbonate which formed was

TABLE XI

Bomardment	(1)	(2)	(3)	(4)‡	(5)‡
Cell Window	glass	glass	glass	platinum	glass
Solute	$C^{14}O_2+FeSO_4$	$C^{14}O_2+FeSO_4$	$C^{14}O_2$	CO_2+FeSO_4	CO_2+FeSO_4
Volume of solution (cc)	12.0	12.0	12.0	13.0	12.0
Gas volume, manifold product gas bulb (cc)	145.	145.	145.	145.	145.
Initial concentration of $C^{14}O_2$ in solution (M)	6.8×10^{-5}	8.2×10^{-5}	1.0×10^{-5}	8.2×10^{-5}	$\sim8\times10^{-5}$
Number of CO_2 Molecules dissolved in H_2O phase	5.5×10^{17}	6.7×10^{17}	8.3×10^{17}	6.2×10^{17}	$\sim7\times10^{17}$
Partial pressure of $C^{14}O_2$ in gas space (mm Hg)	2.4	2.9	3.6	2.7	~3
C^{14} activity (mc)	1	1	1	none	none
C^{14} in CO_2(%)	9.0	6.0	4.8	none	none
Energy of helium ions exterior cell (Mev)	36.	36.	36.	36.	36.
Bombardment current (μa)	0.5	0.5	0.5	0.5	0.5
Total bombardment (μa-hr)	0.75	0.042	0.042	0.13	0.13
Number of ion-pairs produced in solution (assuming 32.5 e.u/i.p.)	7.8×10^{21}	4.4×10^{20}	4.4×10^{20}	3.1×10^{21}	1.3×10^{21}
Hydrogen pressure after bombardment (mm Hg)	208.	8.	(*)	(*)	(*)
Number of hydrogen molecules produced	1.1×10^{21}	$.43\times10^{20}$	(*)	(*)	(*)
Initial concentration of Fe^{+2} (M)	0.80	0.80	none	0.77	0.80
Concentration of Fe^{+3} after bombardment	0.23	(*)	none	0.10	(*)
Number of Fe^{+3} atoms formed	1.5×10^{21}	(*)	none	6.2×10^{20}	(*)
Ion-pair yield for Fe^{+3} formation	0.20	(*)	none	0.20	(*)
Total C^{14} activity in the HCOOH fraction (μc)	0.21	1.32	8.7×10^{-3}	inactive	inactive
Number of CO_2 molecules reduced to HCOOH	2.2×10^{16}	1.5×10^{17}	1.2×10^{15}	(*)	-
Fraction of dissolved CO_2 reduced to HCOOH	0.04	0.22	1.4×10^{-3}	(*)	-
Ion-pair yield for HCOOH formation	2.9×10^{-6}	3.4×10^{-4}	2.8×10^{-6}	(*)	-
Total C^{14} activity in the HCHO fraction (μc)	5.7×10^{-3}	8×10^{-3}	inactive	inactive	inactive
Number of CO_2 molecules reduced to HCHO	6.4×10^{14}	9×10^{14}	(*)	(*)	(*)
Ion-pair yield for HCHO formation	0.82×10^{-7}	2.1×10^{-6}	(*)	(*)	(*)

(*) not determined
‡ control bombardments

centrifuged off. The supernatent containing the barium formate was evaporated to approximately 0.5 ml and while warm, was treated with absolute ethyl alcohol which precipitated crystalline barium formate. This was redissolved in water and recrystallized in this manner four times.

A fraction of original solution which had been removed for iron determination was acidified with 6 N H_2SO_4 and titrated with standard solution of potassium permangante. A second fraction of this solution was reduced with SO_2 and titrated with $KMnO_4$ after the excess SO_2 was removed by boiling. The Fe^{+3} concentration in the target solution was calculated from the difference in titre.

In Table XI, bombardments 1, 2, and 3 were made using the all-glass cell. Bombardment 4 was made using the cell having a one mil platinum window. With this cell, the helium ion beam incident on the solution had an energy of 36 Mev. The number of ion-pairs produced in bombardments 1, 2, and 3 were calculated assuming that the ion-pair yield for ferric ion oxidation obtained in bombardment 4 was also obtained using the all-glass target cells. This assumption is considered reasonable since the energy loss in the glass and platinum windows were of the same order of magnitude.

To insure that $HC^{14}OOH$ and $HC^{14}HO$ were actually produced by helium ion bombardment, the following additional control experiments were made: (1) A sample of the original unbombarded target solution containing $C^{14}O_2$ and $FeSO_4$ was retained at approximately 30°C for one week and then processed in a manner identical to that used in separating the HCOOH, HCHO and CH_3OH fractions in the bombarded samples. No C^{14} activity could be detected in these fractions from the unbombarded solution indicating that reduced C^{14} compounds were not present in the original solution or formed by a metabolic process involving mold or other organisms. (2) A blank bombardment (No.5) was made without added $C^{14}O_2$ and the isolated HCOOH and HCHO carriers were inactive. (3) Mass absorption curves run on active barium formate produced in the radiation reduction of $C^{14}O_2$ were identical with those obtained using known samples of active barium formate prepared chemically and having the same specific activity and counting geometry. (4) No decay could be detected in the activity of the radiation producted $HC^{14}OOH$ and $HC^{14}HO$.

DISCUSSION

An examination of Table XI demonstrates unequivocally that it is quite possible to reduce appreciable quantities of carbon dioxide to formic acid by means of water through the agency of radiation. In fact, it appears that approximately one-fourth of the dissolved carbon dioxide was reduced in experiment No.2. Whether or not the formic acid is further reduced to formaldehyde or whether the formaldehyde has its origin in a direct reduction of carbon dioxide still remains to be demonstrated.

The actual ion-pair yield is certainly no optimal even in the experiment No. 2 in view of the large excess of the number of ion-pairs produced over the number of molecules of carbon dioxide in the solution. Presumably, this reduction is achieved by means of the secondary hydrogen atoms resulting from the radiolysis of the solvent. The actual amount of reduction observed is clearly still only the resultant of the reduction and oxidation reactions. The back reactions, i.e., the oxidation reactions, are presumably minimized by the destruction

of the hydroxyl radicals by their reaction with ferrous ion.[1,2,3]

Whether or not carbon-carbon bonds and carbon-nitrogen bonds can be formed and more highly organized structures created under the influence of high energy radiations is at present under investigation.

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II BIOLOGICAL STUDIES OF RADIATION EFFECTS

J. H. Lawrence - in charge

Project 48A-1

Biological Effects of Radiations on Animals (184-inch Cyclotron)

C. A. Tobias

Irradiation of the pituitary gland of young male rats was continued. The results indicate irradiation has more effect on growth hormone and thyrotropic hormone production than on the production of ACTH and sex hormone. With 30,000 rep to the pituitary, the production of growth hormone as judged by tibial cartilage width and tail length measurements completely ceases for at least two months, and the size of the pituitary gland decreases by a factor of 20 compared to normal. At the same time, however, the size of the adrenal gland remains almost normal and the size of the testes is slightly below normal, indicating continued activity of the ACTH and sex hormones. The work is being continued by extending the observations for a longer time range.

Activation Analysis.

J. C. Weaver and R. L. Dobson

It appears that cobalt is chiefly distributed in the nuclear fraction of cells. Human red blood cells show almost ten times lower cobalt concentration (about 10^{-9} g/g cells) than other tissues. There is a possibility that the cobalt disappears when the red cells become enucleated in the bone marrow. To test this hypothesis, activation analysis of bone marrow and of the blood of avian species is studied, where it is known that the nucleus is retained in the red blood cell.

Acceleration of Multiply Charged Ions

C. A. Tobias

In view of the current and future interest in the biological effect of nuclei with high specific ionization, considerable time was devoted to the study of possible methods of acceleration of multiply charged nuclei that would reproduce at ground level the possible biological effects from cosmic rays at high altitude and provide many important developments in nuclear physics and chemistry. A new method for injection of the ions in the cyclotron has been proposed which should enable the acceleration of nuclei even with atomic number $Z = 25$. The study is being continued and will be reported later in more detail.

Double Nucleated Lymphocyte Problem

J. Gofman and H. Jones

Additional determinations have been made of the normal incidence of double-nucleated lymphocytes in the peripheral venous blood of two dogs. It is now planned to carry out the low-dose fast neutron irradiation of the animals in the "cave" using neutrons generated by the external beam of the 184-inch

cyclotron. In this way a rather homogeneous wide-angle beam will be available to insure uniform dosage.

The Rate of Transfer of Fe^{59} Tagged Red Cells from the Peritoneal Space to the Lymph and Thence to the Blood

Rex Huff

Previous investigations have demonstrated the fact that blood in the peritoneal cavity is absorbed in some manner and eventually varying fractions of the total initially present in the peritoneal cavity is found in the circulating blood. The mechanism has been shown to involve transfer to the lymphatic system and thence to the blood. This paper concerns the quantitative aspects of this transfer as evidenced in highly inbred Curtis Dunning male rats when they were given 1.0 ml of homologous blood in a single "instantaneous" intraperitoneal injection.

Methods. Forty rats were injected intraperitoneally with 1.0 ml of blood of which the cells had been previously tagged with Fe^{59} by injecting donors with Fe^{59}. Four rats were sacrificed at the following time intervals after injection: 0.25 hr., 0.50 hr., 1.0 hr., 2.5 hrs., 6 hrs., 9.5 hrs., 12.5 hrs., 17 hrs., and 24 hrs., and two at 48 hours and 72 hours.

Sacrifice was by decapitation. Blood was collected in a beaker in which a small amount of heparin had been dried. A 1 or 2 ml sample was pipetted to a 4 ml vial for counting. The abdomen was then opened and all areas were washed carefully with 500 ml of saline. The washings were collected in a porcelain pan, transferred to a 1000 ml beaker and counted, with a crystal counting system used for analyzing large samples. Recovery from the peritoneal cavity was found to be complete when the procedure was carried out 1-2 minutes after injection. Contamination of the peritoneal cavity from Fe^{59} tagged blood within the animal was checked in animals not having intraperitoneal injection but having highly tagged blood and was found to be insignificant.

The quantity of tagged cells in the circulating blood of each animal was calculated on the basis of the analyzed concentrations and a blood volume assumed/unit weight on the basis of previous determinations in this laboratory. The calculation was as follows:

$$X = \frac{1}{\frac{1}{Ay} - \frac{1}{A}}$$

where X = fraction of injected tagged cells in the circulating blood.
y = counting rate/ml of circulating blood.
A = assumed blood volume.

This method assumes that the plasma of the injected blood is transferred to the animal's blood and remains there for the duration of the study.

Results. The loss of the tagged cells from the peritoneal cavity approximated an exponential function with time such that 0.0815 of the cells

present disappeared each hour as shown in Table I. The appearance of the tagged cells in the blood could be analyzed as a function of time. The function was a sum of two exponentials of which one exponential had a time constant identical to that of the peritoneal function.

The data may be given as follows:

$$X_1 = e^{-.082\ t}$$
$$X_3 = 1 - 1.26\ e^{-.082} + .26\ e^{-.410\ t}$$

where the amount injected is considered unity, X_1 the fraction of the material in the peritoneal cavity at any time, X_3 the fraction of the injected material in the blood at any time and t in hours.

An interposed compartment is indicated by the second term in the expression for blood. The following model was hypothesized:

$$X_1 \qquad\qquad X_2 \qquad\qquad X_3$$

If there were more than one interposed compartment the blood data would be a polynomial of exponentials providing the transfer from each was first order.

If the model assumption is granted, an expression may be obtained for X_2 since all material not accounted for by X_1 and X_3 is in X_2. Thus, $1 - (X_1 + X_3) = X_2 = 0.26\ e^{-.080\ t} - 0.26\ e^{-.41\ t}$. Since there is no return flow of any of the compartments the rate constants are the time constants. There is no difficulty in identifying λ_2 since λ_1 appeared separately in X_1. This indicates that 0.41 of the lymph containing the absorbed red cells enters the blood stream each hour. Tobias has given a general solution to this type of three compartment systems in which the coefficients of the exponentials appear in terms of the time constants. The data from this study show that the coefficients fit the solution given by Tobias for such a model.

Summary. When 1 ml of homologous tagged blood is injected instantaneously intraperitoneally into Curtis Dunning male rats it is transferred from the peritoneum to the lymph at a rate such that 0.082 of what is present is moved/hour The circulating blood receives each hour 0.41 of the tagged cells present in the lymph. The data do not indicate the presence of any other mechanism for the transfer than has been previously postulated. The transfer is complete without apparent cell damage.

Studies with C^{14} Labeled Stilbamidine

N. I. Berlin

Further efforts to saturate the liver mechanism for concentrating stilbamidine are in progress. In a preliminary study several A Strain mice with sarcomas were given intraperitoneal injections of 0.4 mg non-radioactive stilbamidine daily for six days. On the 7th day the usual dose of 0.4 gm C^{14} labeled stilbamidine was given intraperitoneally. Four days later the mice were autopsied and tissues analyzed. High concentrations of activity were found in the livers. These concentrations were in the same order of

TABLE I

THE TRANSFER OF TAGGED HOMOLOGOUS ERYTHROCYTES FROM THE PERITONEAL SPACE TO THE BLOOD OF CURTIS DUNNING MALE RATS

Group No.	Rat No.	Time after Injection	Corrected Blood Volume cc	Fraction of Cells in Peritoneal Cavity (P)	Fraction of Cells in Blood (B)	Sum of Blood and Peritoneal Fractions (P B)	1 - (P B)
1	1,2,4	15 minutes	10.11	0.999	0.006	1.005	-0.005
2	5-8	30 minutes	9.58	0.992	0.007	0.999	0.001
3	9-12	1 hour	10.69	0.926	0.028	0.954	0.046
4	13,14 16	2-1/2 hrs	10.13	0.895	0.061	0.956	0.044
5	17-20	6 hours	10.99	0.703	0.283	0.986	0.014
6	21-24	9-1/2 hrs	10.63	0.418	0.483	0.901	0.099
7	25-28	12-1/2 hrs	11.31	0.360	0.648	1.008	-0.008
8	29-32	17 hours	10.96	0.238	0.791	1.029	-0.029
9	33-36	24-1/4 hrs	10.74	0.072	0.794	0.866	0.134
10	37,38	48 hours	12.09	0 030	0.976	1.006	-0.006

magnitude as are found following a single injection of stilbamidine in tumor bearing mice. The indication from this preliminary run is that the multiple injections of normal stilbamidine did not diminish the capacity of the liver for concentrating the subsequent dose of labeled stilbamidine.

Metabolism of C^{14} Labeled Glycine

N. I. Berlin

Study of the C^{14} content of the urine of four patients followed for three or more months shows that there is a long term component of urinary excretion with a half-time of approximately 40 days. This may be the fourth component of excretion that was postulated, but instead of being pulmonary it is renal. Since approximately 90 percent of the carbon in the urine is in the form of urea, it is highly probable that represents the excretion of the C^{14} as urea. Separation of the urea by means of differential enzymatic action to determine this point is being undertaken. At the same time an apparatus is being built for the direct filling of a 500 cc ionization chamber with exhaled carbon dioxide. The greater sensitivity of the 500 cc ionization chamber should permit extension of the study of the breath excretion into the lower range of specific activity; i.e., less than 0.04 d/min/mgm $BaCo_3$. This should permit resolution of the fourth component of pulmonary C^{14} excretion if it exists.

Iron-Turnover in Normal Persons and in Patients Having Various Blood Disorders

Rex Huff

It is possible, by external means to detect the movement of Fe^{59} within the human body. This has important significance in showing that individuals with certain hematopoietic disorders have abnormalities in the turnover mechanism of Fe^{59}. These aberrations are particularly evident in the spleen, liver and bone marrow.

Plasma and red cell turnover of radio iron has been the topic of a previous report[1] and many groups have studied the red cell uptake. The additional information gained by the *in vivo* serial measurements of radioactivity in tissue other than in plasma and red cells is a useful adjunct to the understanding of hematologic disorders.

A small number of cases have been studied by the *in vivo* technique and each disease is represented by too few cases to make generalizations; however, the deviations from normal are so remarkable that a report of the methods and findings was thought desirable.

These studies have been made possible by the recent construction of reliable high efficiency scintillation counters. Detailed descriptions of the construction and performance of these counting devices have been published by Anger[2,3].

Methods and Materials.

Counting Equipment. In Vivo Counter. Fig. 1 illustrates the counting head used for *invivo* counting. It employs a thallium activated sodium iodide crystal, a 2.5 cm

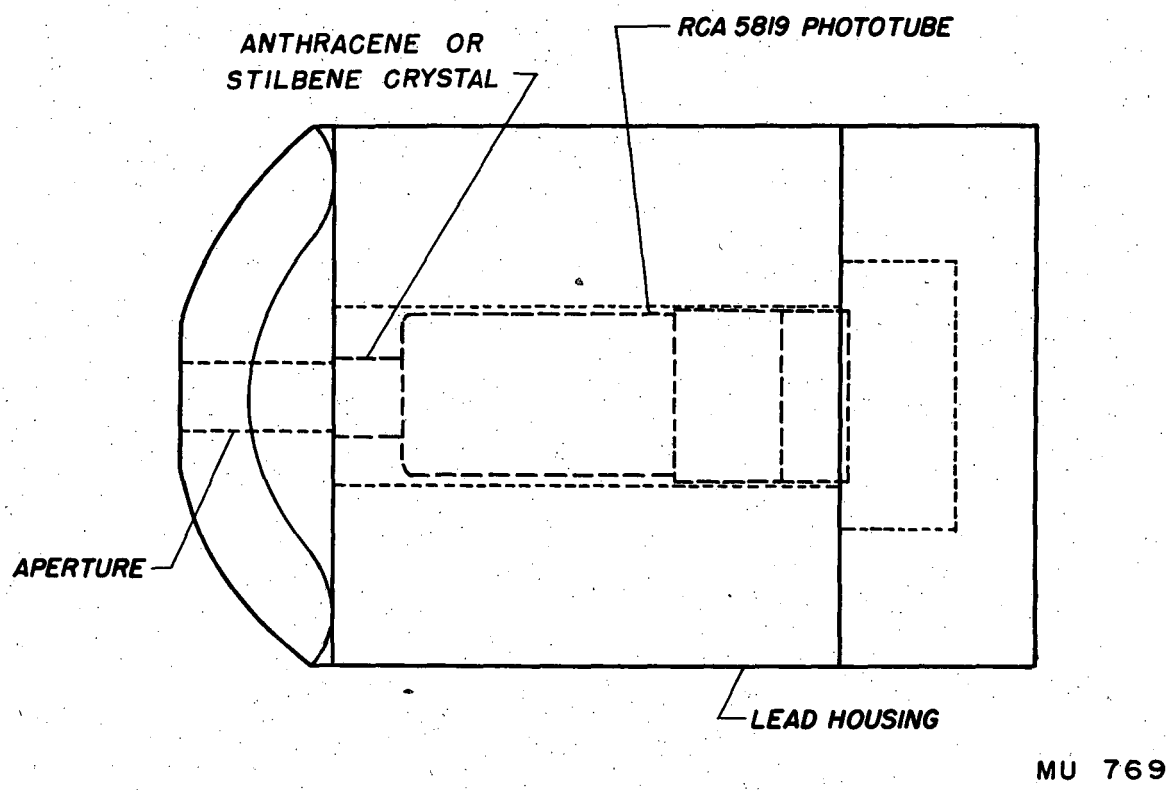
ANTHRACENE OR
STILBENE CRYSTAL
RCA 5819 PHOTOTUBE
APERTURE
LEAD HOUSING
MU 769

Fig. 1

cube which lies in contact with the end window of a photomultiplier tube (RCA 5819). The side of the crystal toward the patient is shielded against beta radiation.

A hydraulic lift is used to provide vertical mobility in a horizontal plane within a limited range. An adjustable height rotatable stool for the patient adds to the range of positioning. The counter output is coupled to the scaler unit which is of the usual design. The unit also contains the regulated high voltage supply, intended for a G-M tube, which is arranged to supply the photomultiplier.

The integrating circuit in connection with a Leads and Northrup Speedomax recording millivoltmeter comprises a point-plotting linear counting rate recorder with several ranges. The numerous body positions counted, and the length of time of the studies, required considerable cooperation by the patient and close surveillance by the operator. In order that bed patients can be studied, and so that ambulatory patient investigations are facilitated, a mechanized multiple port in vivo counter with simultaneous multi-channel recording is being built.

The Vial Counter. Fig. 2 shows the crystal counter arrangement for receiving the 4 ml screw cap vials (Kimble glass 60910) containing cell or plasma samples. The shape and the reflective aluminum container of the crystal surrounding the vial provide a highly efficient geometry. The operation of the "RCA 5819" with less than 1 count per minute of the background attributable to phototube noise is satisfactory for our work. The tube is operated at less than maximum voltage and cooling or special circuits are not necessary. The shielding consists of 6 mm aluminum box housing 5 cm of iron, and 10 cm of lead.

The overall efficiency of the vial counter is 17.5 percent for Fe^{59} gammas. This is approximately the efficiency attained with the usual end window Geiger tubes for betas of Fe^{59} when electrodeposited samples are analyzed. The saving in time and effort of this method over the old method of analyzing Fe^{59} is inestimable.

Radio-iron. The radio-iron was prepared for injection by combining it with the iron binding beta-globulin fraction IV-7. (COHN)* The iron binding capacity of this protein was measured by a method similar to that used by Surgenor et al.[4] Usually only about one-half of the available unsaturation was utilized. The iron isotope was obtained by deuteron bombardment of cobalt in the 60-inch cyclotron in Berkeley and by a neutron bombardment of enriched Fe^{58} from Oak Ridge.[5] The cyclotron produced iron is free of Fe^{55} and only this was used in normal subjects. The most recent cyclotron bombarded samples have shown high specific activity, 10 microcuries/microgram Fe, because of the careful chemistry in preparation of the target and the separation chemistry following the exposure**. The samples from the enriched Fe^{58} have a slightly lower specific activity and contain small amounts of Fe^{55}.

The amount of Fe^{59} injected varied from 7 to 45 microcuries. Because of radio decay the total amount of iron and protein given each subject varied considerably; however, in no instance was an amount given which would detectably

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* The Globulin IV-7 was provided through the courtesy of Cutter Laboratories, Berkeley.

**We wish to thank Mr. Rayburn Dunn of the Radiation Laboratory, University of California, for the radiochemistry.

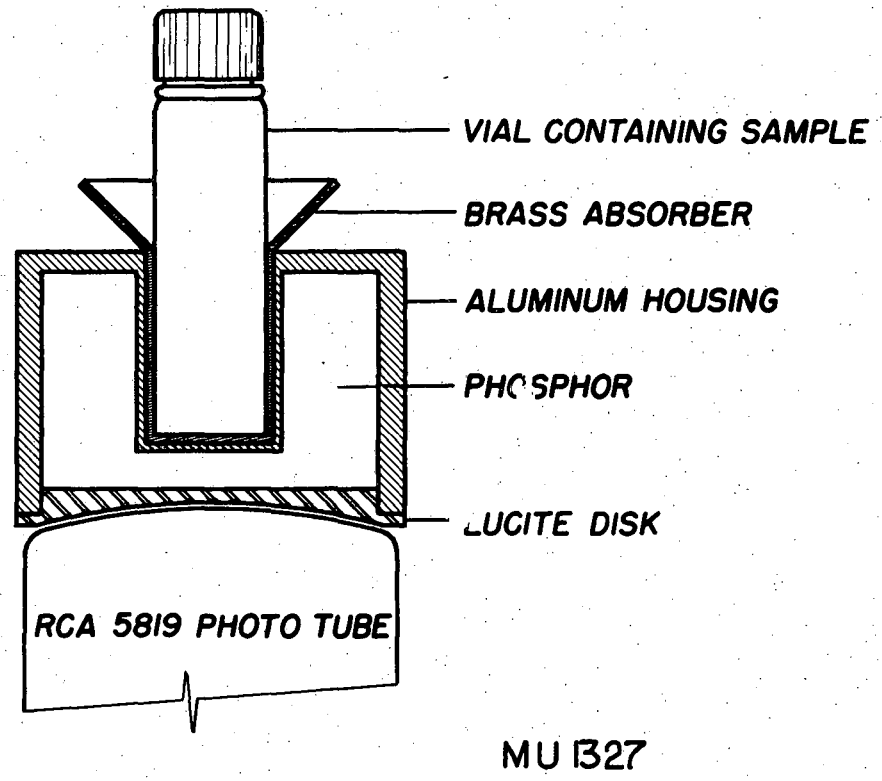

VIAL CONTAINING SAMPLE
BRASS ABSORBER
ALUMINUM HOUSING
PHOSPHOR
LUCITE DISK
RCA 5819 PHOTO TUBE
MU 1327

Fig. 2

change the total plasma iron concentration. The tracer was kept as a ferric chloride solution. An aliquot of this solution was prepared by reduction with a small amount of thioglycolic acid and neutralization with dilute sodium hydroxide to a pH of approximately 7. This mixture was added to an aqueous solution of the Globulin IV-7 and was allowed to stand over night. The mixture was then filtered through a Seitz filter and transferred to sterile serum vials.

Injection and Blood Sampling. The tracer-globulin solution was injected into the antecubital vein as rapidly as possible. Blood samples were taken at approximately 10 and 45 minutes, and 1.5, 3, 5 and 12 hours during the first day. During the ensuing 2 weeks from 7 to 10 samples were taken. Thereafter blood was drawn at 7 to 14 day intervals. The blood was centrifuged at 2500 rpm for 20 minutes and the packed cell volume recorded. Plasma was taken from one or more samples for the determination of total plasma iron by the method of Kitzes et al.[6] From some normal subjects all specimens from the first 4 hours were analyzed. No significant changes in the total iron were noted in this period. From all blood samples 2 ml of plasma were pipetted to the 4 ml medicine vials for radio analysis in the apparatus shown in Fig. 2. The cells from early samples were washed in saline two or three times. After the last washing an amount of saline equal to the packed cell volume was added to the centrifuge cone. Four ml of the cell suspension was pipetted to the medicine vials which were then centrifuged and analyzed in the same manner as the plasma samples.

Plasma radioactivity data were plotted on semilogarithmic paper and the extrapolated concentration at zero time was used to determine the plasma volume. Total blood volume and red cell volume were computed using the average hematocrit from the first day's samples. Volume data obtained by the P^{32} tagged cell method, when available, were used in calculating the fraction of injected Fe^{59} in cells, since we have observed that the red cell volumes obtained by the iron method sometimes give an apparent red cell uptake greater than 100 percent. The data so obtained, the fraction of Fe^{59} in red cells, plasma, and blood were plotted as a function of time as illustrated in Fig. 3

Body Surface Counting Rate (BSCR). In general, four principal positions were used in counting tissue volumes thought to be representative of blood, spleen, marrow and liver. With the subject on a stool, the counting aperture was placed against the skin overlying the site. The precordium was used as the site most likely to reflect changes in a representative volume of blood. A left oblique placement over the costal cartilages with the counter pointed in the direction of the mid-mediastinum was chosen in order to minimize the contribution of radiation from marrow either in ribs, vertebra, or sternum.

The right posterior axiallary line between the 9th and 10th ribs, with minor variations, if percussion indicated, was the position used as representative of liver. In this instance the counter was pointed anteriorly and medially so that the counting axis passed through the anterior midline at the skin surface. An analogous position for a spleen tissue volume was chosen on the left side except when the spleen was palpable. Palpable spleens were counted through the anterior or lateral abdominal wall.

For a site overlying a representative volume of marrow, the counter was placed over the sacrum and pointed directly anterior, with the patient standing.

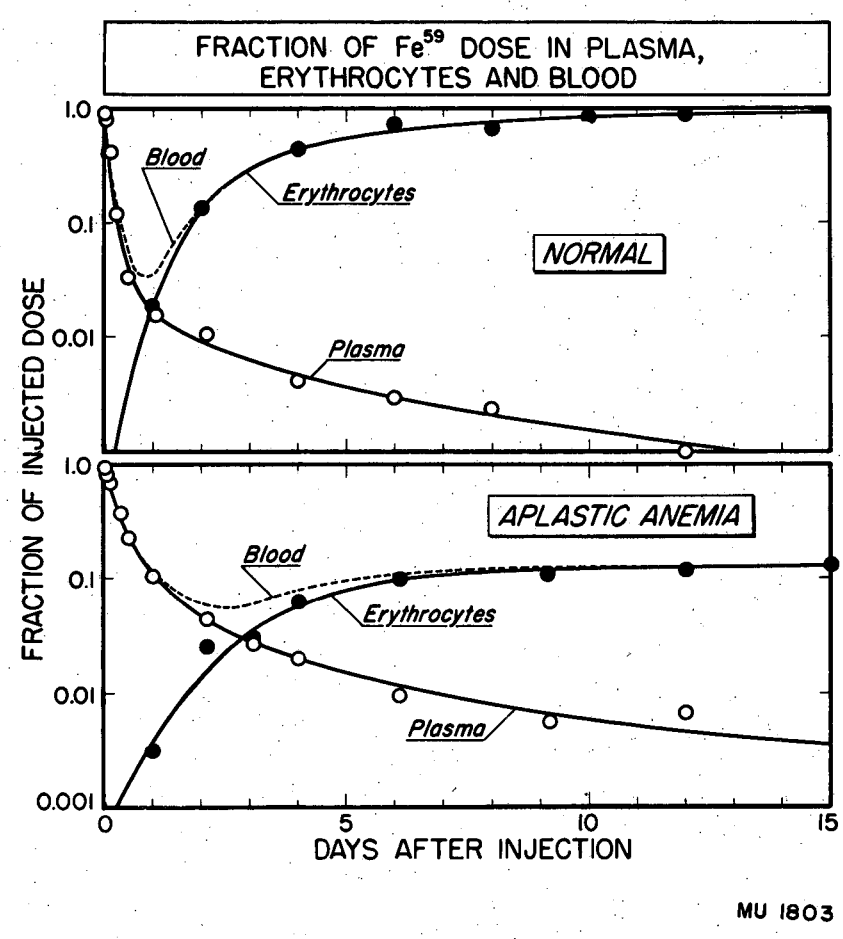

FRACTION OF Fe59 DOSE IN PLASMA, ERYTHROCYTES AND BLOOD
1.0
0.1
0.01
Blood
Erythrocytes
NORMAL
Plasma
1.0
0.1
0.01
0.001
APLASTIC ANEMIA
Blood
Erythrocytes
Plasma
FRACTION OF INJECTED DOSE
0
5
10
15
DAYS AFTER INJECTION
MU 1803

Fig. 3

The level used for the sacrum was located by palpation of the posterior spines of the ilium. Other positions were used at irregular intervals in an exporatory manner. Counting rates varied considerably with the areas and with time. Usually the range was from 500 to 5000 c/m. Each position was counted long enough so that at least 1500 counts were recorded -- usually more counts than this were recorded.

A typical study was as follows. First, the various positions to be counted were marked out on the patient's body. Body background counting rates were determined in almost all patients and in all who had had other iron turnover studies recently. The injection was made with the patient sitting in the precordial position. An initial count was made over the precordium; then the other positions were counted sequentially as rapidly as possible. If the changes were very slow, for example a plasma clearance of Fe with a half-time of 5 hours, readings were made at approximately hourly intervals; however, if the major changes were occurring in the first hour, a plasma half-time of 10 minutes, positions would be counted as rapidly as was possible. As indicated above, these changes with time of the body surface counting rates were recorded on a Leeds and Northrup Speedomax recorder. In most instances these records were plotted again on semilogarithmic paper as a rate per microcurie of injection mixture for more careful analysis. The curves of the marrow, liver and spleen site counting rates were extrapolated to zero time and this extrapolated point was taken as the "blood background body surface counting rate" and was used in correcting the "body surface counting rate" to a count which represented the true tissue counting rate. It was assumed the entire counting rate at extrapolated zero time represented radioactivity in the blood of the underlying tissue and that the contribution of blood in the tissue to the counting rate was proportional to the amount of the radio iron in the blood at the time (Fig. 3).

Thus:

$$\left[BSCR_t = t\right] - \left[(BSCR_t = 0) \times (\text{Fraction } Fe^{59} \text{ dose in blood } t = t)\right] = BSCR_{tissue}$$

Localization survey of torso at the time of minimal blood tracer concentration. When the changes in the body surface counting rates over the three tissue sites were minimal and the blood tracer concentration had declined to a minimum (Fig. 3), skin surface counting rates were obtained from many points on the torso and in some instances from the limbs. In order that some uniformity in the selection of points for counting might obtain, the following procedure was used. With a wax pencil, 6 body levels were marked on the subject:

1. The supraclavicular notch
2. 1 cm above the manubriosternal juncture
3. 10 cm below the manubriosternal juncture
4. 2 cm below the xiphisternum
5. 2 cm above the umbilicus (unless the abdomen was pendulous; then midway between levels 4 and 6)
6. 2.5 cm below the anterior superior spine of the ilium.

Eight counting points were used at each of these levels. In each instance the counter receiver was pointed at the vertical curved axis just anterior to the vertebral column. The anterior position was in each instance considered 0 degrees and the posterior 180 degrees with rotation to the subject's left, as

illustrated in Fig. 5. Thus, 90 degrees and 270 degrees were at the left and right midaxillary lines repectively. All in vivo counting rates were plotted as counts per minute per microcurie injected. The data from the multiple torse examination were plotted on polar coordinates as illustrated in Fig. 5. From 2 to 3 hours were required for taking the counting rates at these 48 points. During this time there was usually very little change in the combined cell and plasma levels which often represented less than 0.05 of the administered dose (Fig. 3).

Problems Associated with In Vivo Counting. At first thought, the detection of an isotope by external means would appear to be a very useful method in studies of metabolism. However, more careful consideration of the method brings out numerous problems which may lead at first to a very pessimistic outlook regarding the value of the method.

Among the major problems which have existed in the past is the necessity for large doses of tracer because of the very low efficiency of G-M tubes for gamma-rays. These doses have exceeded the maximal allowable tissue dose of radiation, at least instantaneously. Now, however, with the use of equipment as described here with an efficiency of 55 times greater than similar G-M tube equipment, it is possible to restrict the tissue radiation to less than the maximum allowable dose rate. Moreover, the rise in background counting rate is not nearly proportional to the rise in efficiency; in fact, there is an increase in the ratio efficiency/background counting rate of 11 compared to similar G-M equipment*.

Ideally the data accrued from an in vivo study should be applicable to the determination of rates of movement of the homologue of the tracer isotope from one body compartment or tissue to another. Tobias[7] has pointed out a method whereby rate constants can be calculated from a given set of data; however, it requires that all of the tracer be accounted for at many times. Any other method depends on estimation and approximation. The difficulty of accounting for all of the tracer all of the time by in vivo methods, and describing it a as a function of time, is apparent when some of the variables are considered. In a particular subject, with the counter at a carefully selected site, so as to discriminate against compartments having a different metabolism, many of the variables become constants. For example, with good reproduction of positioning in the same patient, radiation absorption of the intervening tissue becomes constant. Another major variable is the fraction of the tissue or compartment being studied which the counted volume represents. This also can be controlled in a single subject study so that it is a constant. Even though the turnover of tracer iron results in rather discrete separation into compartments, the problem of distinguishing these individual compartments may be great. However, it has been possible in this study to describe the various compartments as a function of time such that they represent a constant fraction of the tracer in the particular tissue. This has been facilitated by knowing and using the fraction of material

* The efficiency mentioned above for the in vivo counter is 2.9 times that of the crystal used at the beginning of the present studies. For convenience in comparisons between patients, all graphs included in this paper are plotted in counts/minute/microcurie at the old efficiency.

present in the blood as a function of time to further enhance the delineation of compartments such as marrow, liver, and spleen. It is probable that other methods can be used to estimate the fraction of the tracer present in an organ or tissue at one time so that a multiplicative may be applied to the data which is now a function of time, on a relative basis, so that it will then describe the exact fraction of the tracer in the compartment as a function of time.

Despite these hindrances to approaching the ideal situation in the analysis of iron in vivo tracer data, the uniformity of the data from normal subjects and their comparison to the abnormal affords a description of diagnostic patterns which an be useful in the classification and understanding of erythropoietic disease.

Cases Studies.

Normal Subjects. 5 normal young male adults, ages 26 to 32, 1 normal female, age 31, and 1 normal male age 72 were studied.

Abnormal Cases. These were of varying age and sex, and had polycythemia vera (7), secondary polycythemia (1), erythroleukemia (1), myelofibrotic myeloid metaplasia (1), Myelogenous leukemia (4), lymphatic leukemia (2), refractory anemia (5) and multiple myeloma (2).

Synopses of case reports of patients whose Fe^{59} data are described in most detail are presented.

Case 1. J. M. Aplastic anemia.

This 57-year-old married white man was referred with diagnosis of aplastic anemia.

A history was obtained of an anemia, refractory to all the usual methods of therapy and requiring 36 blood transfusions since its initial detection in April, 1947.

Physical Examination: Physical examinations failed to disclose any notable findings except moderate bronzing of the skin.

Laboratory Examinations:

RBC	2.96 - 4.02 million per cm mm
Hematocrit	27 -35
Hemoglobin	8.6 - 10.9 grms per 100 ml.
Platelets	140,000 - 218,000 per cm. mm.
Reticulocytes	0.1 - 0.9 percent
WBC	8000 - 13,600 per cm. mm.
Neutrophilic Granulocytes	51 - 81 percent
Lymphocytes	20 - 49 percent
Moncytes	2 - 13 percent
Eosinophilic Granulocytes	1 - 3 percent
Fecal urobilinogen excretion	75, 76 mg per day

Sternal Marrow Aspiration on 2/20/50 showed only 5.8 percent of the nucleated cells to be of the erythroid series.

Fe^{59} studies were started June 15, 1950.

Course: The course of the disease has been constant. Repeated transfusions have been necessary. Recovery following appendectomy was prolonged and febrile.

Case 2. E. P. Polycythemia vera:

This 41-year-old married woman was referred with the diagnosis of polycythemia vera. She had had no radiation therapy.

Physical Examination: Physical examination disclosed facial plethora, conjunctival injection, and a spleen so large it almost entered the pelvis.

Laboratory Examinations:

RBC	6.72 - 866 million per cu. mm.
Hematocrit	61 - 68
Hemoglobin	14.2 - 17.7 grams per 100 ml blood
Platelets	110,000 - 300,000 per cu. mm.
Reticulocytes	1 - 4 percent
WBC	20,000 - 38,000 per cu. mm.

The differential white cell counts showed the following ranges:

Segmented neutrophilic granulocytes	48 - 63 percent
Band neutrophilic granulocytes	8 - 19 percent
Metamyelocytes	0 - 6 percent
Myelocytes	4 - 15 percent
Progranulocytes	0 - 2 percent
Myeloblasts	0 - 2 percent
Eosinophilic Granulocytes	3 - 8 percent
Basophilic Granulocytes	1 - 13 percent
Lymphocytes	4 - 15 percent
Monocytes	0 - 2 percent
Nucleated Red Cells	0 - 5 per 100 WBC
Uric acid	5.0 mg percent
Total protein	8.0 gram per 100 ml.
Albumin	4.4 gram per 100 ml.
Globulin	3.6 gram per 100 ml.
Fecal urobilinogen excretion	158 mg per 24 hours

Blood Volume, with P32 labeled cells, was 5851 (97.5 ml/kg)

Total Red Cell Volume	3276 (54.6 ml/kg)
Plasma Volume	2457 (40.9 ml/kg)

Aspiration specimens of the bone marrow were unsatisfactory.

A report on the sternal marrow biopsy was as follows: "The marrow shows a marked diffuse fibrosis. Numerous megakaryocytes are seen. There are a fair number of lymphocytes, eosinophils and fairly well-matured cells of the myeloid series. Diagnosis: Myelofibrosis." No treatment was given and the course is not changed. Fe^{59} studies were started 6/28/50.

Case 3. H. E. Myelofibrosis with myeloid metaplasia.

This 47-year-old woman was referred with the diagnosis of myelocytic leukemia. She was given deep roentgen therapy to an enlarged spleen (87 r) on July 28, 1950. On August 4 this dose was repeated and on August 11, 1950 44.4 r was given. The spleen size was reported to be smaller than on August 14. At no time was any radiation directed to marrow. Fe^{59} studies were started on 8/31/50.

Physical Examination: The spleen was enlarged and the lower border was at the umbilicus.

Laboratory Findings:

On 8/23 the blood count was:

RBC	4.68 million per cu. mm.
Hemoglobin	10 grams per 100 ml.
Platelets	130,000 per cu. mm.
WBC	13,800 per cu. mm.
Reticulocytes	3.5 percent

The differential count was:

Segmented Neutrophilic Granulocytes	47 percent
Band Neutrophilic Granulocytes	16 percent
Myelocytes	8 percent
Progranulocytes	3 percent
Myeloblasts	2 percent
Lymphocytes	23 percent

Sternal marrow aspirations on two occasions contained no marrow only peripheral blood. On 9/2/50 a biopsy of sternal marrow was taken, and the report on the microscopic examination of the sections (decalcified) was:

"The sections of the bone show almost complete replacement of the medullary tissue by fibrous tissue, which is rather vascular. A few isolated cells, which appear to have come from the myeloid series, are seen. There is no question about the diagnosis of this marrow. Diagnosis: Myelofibrosis."

Course: From August, 1950, to October, 1950 the blood counts showed first a decline to normal values of total white cells. The hemoglobin remained at 10 grams. In September the hemoglobin on several determinations was 8 grams, but the total WBC declined from 5900 - 6400, to 3900 - 4200. Iron studies were started August 31, 1950.

Case 4. P.N. Erythroleukemia

This 60-year-old, male was referred in September, 1946. About two weeks before his first visit here, he developed a severe ecchymosis and swelling at the left elbow, subsequent to a very minor blow to the arm. He had had no

radiation therapy.

Physical Examination: The left elbow and forearm were swollen, slightly edematous and ecchymotic. The spleen was firm, non-tender and it extended 8 - 10 cm. below the left costal margin on inspiration. It was estimated to be several times the normal size. The liver was not enlarged.

Laboratory Examinations:

The initial red blood counts were 7.74 to 8.42 million per cu.mm. The range in values in blood examinations were as follows:

Hemoglobin	8.3 to 17.7 grams per 100 ml.
Reticulocytes	0.20 - 10.2 percent
Platelets	290,000 to 340,000 per cu. mm.
Hematocrit	28 - 63
WBC	9,200 - 42,000 per cu. mm
Segmented	58 - 88
Bands	3 - 23
Eosinophils	1 - 10
Metamyelocytes	0 - 7
Lymphocytes	1 - 30
Monocytes	0 - 6
Nucleated red cells	0 - 5 per 100 WBC

On 9/24/46 the marrow differential showed:

Segmented Neutrophilic Granulocytes	25
Band Neutrophilic Granulocytes	16.6
Eosinophils	0.6
Basophils	0.4
Neutrophilic myelocytes	21.6
Eosinophilic myelocytes	1.0
Myeloblasts	0.6
Lymphocytes	0.6
Monocytes	0.4
Nucleated red cells	32 percent

Clinical Course: With some venesections, P^{32} and Y^{90}, he was maintained in health with a less leukemoid hemogram throughout 1947 and until about mid-1948, when more myelocytes appeared. Later, promyelocytes and myeloblasts appeared and the RBC had declined gradually so that in late 1948 and early 1949 , it varied between 3 and 4 million per cu. mm., with 8 grams of hemoglobin per 100 ml. The white counts were not lower and varied from 20,000 to 7,000 per cu. mm. The marrow exhibited a shift to more immature myeloid forms.

During 1949, the liver enlarged and the spleen increased in size, and he was given a transfusion. His blood picture now suggested the late anemic phase sometimes seen in polycythemia vera. The marrow aspiration was unsatisfactory because of dilution with peripheral blood.

By May of 1050 he had become gradually worse. His strength and endurance diminshed. He noted some spontaneous ecchymoses similar to those at the onset of his illness two years previous. The spleen and liver increased in size and he had intermittent enorexia. He worked less steadily at his job. After each blood transfusion he felt temporarily improved.

Unfortunately the blood volume was not determined in the polycythemic phase. When it was done the initial picture had changed to that of an anemia. On 9/30/49 the blood volume using P^{32} labeled cells was 4453, or 64.1 ml/kg body weight. The total red cell volume was 1425 or 20.5 ml/kg body weight. On 2/10/50 the blood volume was 5914 or 83.9 ml/kg but the total red cell mass was 1656 ml. or 23.5 ml/kg. Fe^{59} studies were started 5/31/50.

Case 5. E.S Chronic myelogenous leukemia

This 44-year-old married woman was referred with the diagnosis of chronic myelogenous leukemia in September, 1950. An enlarged spleen, an elevated white cell count and a sternal marrow examination led to the diagnosis of chronic myelogenous leukemia.

Physical Examination:

The arms showed some residual discolorations from old ecchymoses. The spleen was firm, non-tender and palpable about 2 - 3 cm below the costal margin in the left anterior axillary line. No lymph node enlargement was found.

Laboratory Examination:

The range in values in blood examinations were as follows:

RBC	3.3 - 3.6 million per cu. mm.
WBC	106,000 to 141,000 per cu. mm.
Hemoglobin	7.4 to 8.5 grams per 100 ml.
Platelets	215,000 to 420,000 per cu. mm.

The blood volume studies by the P^{32} tagged cell method were recorded as follows:

Blood Volume	4387 cc. or 72.5 cc/kg
Total Red Cell Volume	1163 or 19.2 cc/kg
Plasma Volume	2895 or 47.9 cc/kg

Course: She returned to her physician with arrangements for small doses of radioactive phosphorus. Fe^{59} studies were started 9/12/50.

Case 6. C.H. Refractory anemia

This 72-year-old male was first discovered to have an anemia in October, 1949.

Physical Examination: Liver edge 4 - 6 cm. inferior to costal margin at end of deep inspiration. Spleen enlarged on deep inspiration extended 4 cm. inferior to costal margin in the mid-axillary line.

Laboratory Examinations:

The blood data showed the following ranges.

RBC	1.80 - 3.94 million per cu. mm.
Hematocrit	19 - 36
Hemoglobin	7.5 - 12.1 gms. per 100 ml.
Platelets	260,000 - 844,000 per cu. mm.
Reticulocytes	0.1 - 15.7 percent
Nucleated Red Cells	0 - 16 per 100 WBC
WBC	6,600 - 45,000 per cu. mm.
Segmented Neutrophilic Granulocytes	18 - 66 percent
Band Neutrophilic Granulocytes	1 - 35 percent
Metamyelocytes	0 - 1 percent
Progranulocytes	0 - 3 percent
Myeloblasts	0 - 0 percent
Eosinophilic Granulocytes	0 - 22 percent
Basophilic Granulocytes	0 - 6 percent
Fecal Urobilinogen Excretion	154 - 299 mg. per day
Coombs Test Negative	

On February 9, 1950, the pathologist's report of a marrow biopsy was as follows:

"The bone marrow shows a rather moderate increase in cells. Large numbers of megakaryocytes are seen. There are also a large number of myelocytes and nucleated red cells. This slide shows essentially the same pattern that we saw in the previous bone marrow biopsy. Diagnosis: Hyperplasia of bone marrow."

Course: The course has not been altered except for a lower requirement of blood by transfusion following splenectomy. The numerous young red and white cells appeared in the blood during treatment with Cortisone. He has received all of the various vitamins and liver concentrates as well as crude liver parenteral injections and raw liver by mouth. Fe^{59} studies started 6/21/50.

Case 7. C. H. Multiple myeloma

This 65-year-old male was referred 1/9/50. About 5 months before a hard swelling arose just above the left ankle. A biopsy was done and the microscopic examination (reported in the laboratory data below), as well as roentgenograms of bones disclosed multiple myeloma. He had lost 15 to 20 pounds in weight during the past year. At the time he was first examined here he complained of an unremitting pain in the right elbow.

Physical Examination: The skin had a slight icteric tint. He was thin and the muscles were flabby. The spleen was palpated 1 - 2 cm. beneath the costal margin and a larger than usual area of splenic dullness found. Bone tenderness was found over the distal end of the right humerus and the left ulna.

Laboratory Examinations. Prior to any therapy, the range in RBC per cu. mm. was 2.97 to 4.00 million.

Hemoglobin	8.4 to 10.9 gm. per 100 ml.
WBC	5,400 to 10,400
Reticulocytes	0.6 to 9.7 (average of 10 determinations was 3.5)
Hematocrit	19 - 28

The differential white count was within the normal range. On 1/9/50 the aspirated sternal marrow was found to have 43 percent nucleated red cells and 5.2 plasma cells. On 9/6/50 the sternal marrow contained 61 percent nucleated red cells and 0.8 percent plasma cells. Fe^{59} studies were started 11/16/50.

Only once was the test for Bence-Jones protein in the urine positive. A month later and on many subsequent tests none has been detected.

Total protein 4.92, albumin 2.92, globulin 2.00 gm. per 100 ml. plasma on 1/11/50.

Icteric index	25 units on 7/24/50 and 8/8/50
Serum bilirubin	0.4 mg. per 100 ml. blood
Fecal urobilinogen excretion	860 mg. per 24 hours.

Course: Treatment with urethane and P^{32} apparently did not alter the course. He has not required transfusions.

Results and Discussion.

Certain deviations from the normal may be evident during the first 15 minutes after the injection of the tracer iron and can be seen on the paper emerging from the counting rate recorder. As the 4 positions are "counted" repeatedly, excessively rapid disappearance of the tracer from the blood, or an unusually rapid rise in counting rate over marrow, spleen or liver may be seen. A rapid and excessive rise in counting rate over liver or spleen is

accompanied by a partial or complete failure of the marrow counting rate to rise. This is illustrated in Fig. 4a, 4b, 4c, 4d, and 4e. These figures are drawings of the counting rate recorder data of the first few hours from a normal subject and 5 patients. An estimate of the ability of the bone marrow and other tissues to accumulate radio iron is possible in the first few hours. Fig. 4a shows the counting rates in counts per minute per microcurie over sacrum, spleen, liver and precordium in a normal male, 32 years old. The data from the remaining normal subjects deviate very slightly from those shown in Fig. 4. The sacral position was the only position showing a rising counting rate with time. The counting rate over liver was practically constant with time while that over the precordium and spleen fell to a near plateau level relatively soon. Since mixing times are short compared to the removal rates, it is reasonable to assume that body surface counting rates over a tissue during the first few minutes represent the radioactivity which is contained in the perfusing plasma. Similarly in 10 to 14 days 80 to 100 percent of the radioactivity is again contained in blood, but at this time in the red cells. Thus in these very early periods and the late periods, nearly all of the injected dose is in the blood first in the plasma and later in the red cells. The contribution of blood radioactivity to the gross body surface-counting rates is nearly the same at these two times except possibly in such tissues as may have different hematocrits. Thus a constant body surface counting rate with time during a period when the radioactivity in blood is known to be decreasing, indicates that the tissue is accumulating radioactivity. This is the situation which is usually found with the liver, in contrast to the spleen where the counting rate as a function of time is almost identical to that over the precordium.

In contrast to the rising sacral counting rates seen in normal individuals, nearly constant sacral counting rates are seen in the cases represented in Figs. 4b, 4c, 4d, and 4e. Case 3, Fig. 4d, shows a decrease in counting rate with time. This is the type of curve which might be expected in the absence of marrow erythropoiesis. Reference to the case descriptions shows the coincidence of a low counting rate over sacrum with the marrow biopsy findings of hypoplasia of aplasia of the erythroid series, and at times of fibrosis.

The extraordinarily high serial rise of counting rates occurring in the splenic areas as demonstrated in Fig. 4c and 4d, Cases 2 and 3 were not necessarily expected although there was splenomegaly in both cases. This similarity in the two cases is in contrast to the finding of polycythemia in the one and anemia in the other. The ability of the spleen to undergo a functional metaplasia so that a significant accumulation of tracer iron occurs in the organ with a decline reciprocal to the subsequent appearance of the iron in the red cells has been demonstrated in rats given total body x-radiation with spleen protected where the marrow was obliterated.[8] That this possible cause of a change in splenic iron metabolism is not concerned in these patients is indicated by the absence of a history of exposure of their marrow to ionizing radiation. The spleen counting rate of the case represented in Fig. 4b is intermediate and represents some ability of the spleen to accumulate iron. The splenomegaly is not always associated with splenic removal of radio iron from plasma was demonstrated in a case having chronic lymphatic leukemia whose spleen filled half the abdominal cavity.

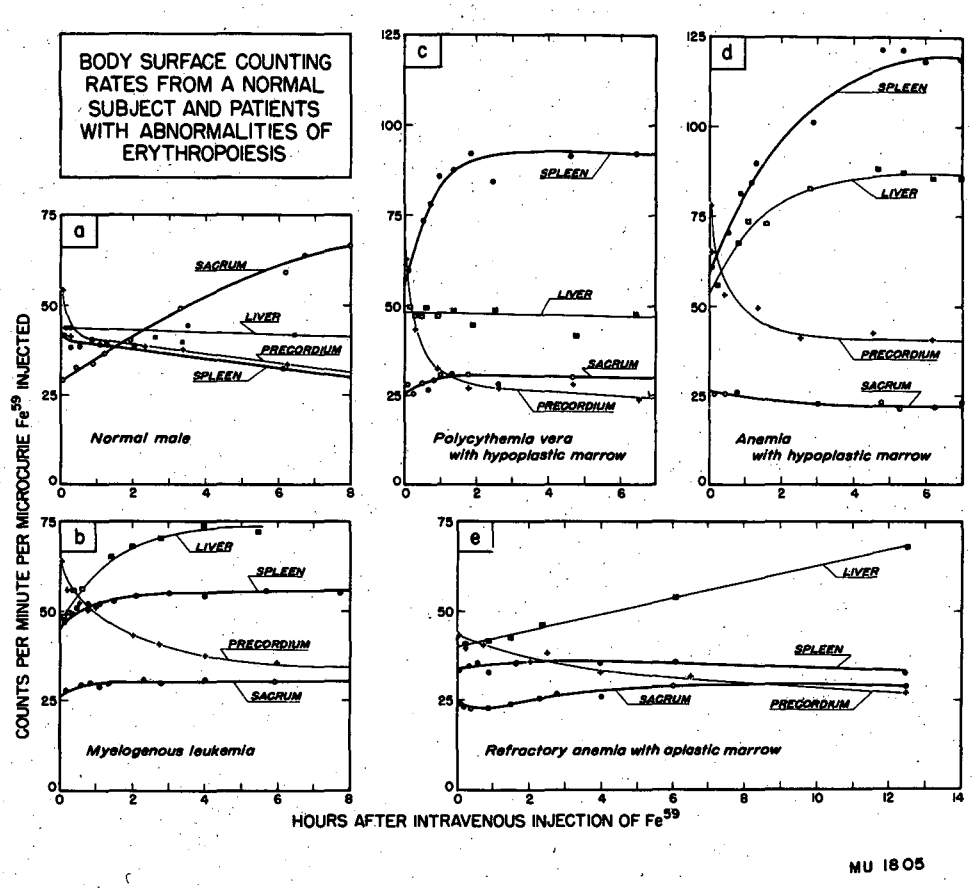

BODY SURFACE COUNTING RATES FROM A NORMAL SUBJECT AND PATIENTS WITH ABNORMALITIES OF ERYTHROPOIESIS
a
SACRUM
LIVER
PRECORDIUM
SPLEEN
Normal male
b
LIVER
SPLEEN
PRECORDIUM
SACRUM
Myelogenous leukemia
c
SPLEEN
LIVER
SACRUM
PRECORDIUM
Polycythemia vera with hypoplastic marrow
d
SPLEEN
LIVER
PRECORDIUM
SACRUM
Anemia with hypoplastic marrow
e
LIVER
SPLEEN
SACRUM
PRECORDIUM
Refractory anemia with aplastic marrow
COUNTS PER MINUTE PER MICROCURIE Fe59 INJECTED
HOURS AFTER INTRAVENOUS INJECTION OF Fe59
MU 1805

Fig. 4

Fig. 4e, Case 1, is illustrative of the great changes in the rates of turnover of iron which are associated with some cases of refractory anemia. Casual comparison of the several figures indicates the unusual difference in the rates at which the various iron avid tissues accumulate and discharge the tracer. Fig. 4e Case 1 demonstrates the situation wherein usual marrow ability to deplete the plasma of tracer is replaced by a very slow removal by liver.

The desirability of knowing more accurately the tissue distribution of tracer when it was nearly absent from blood led to the system described in methods "e" of body profile counting rate and its associated polar plot.

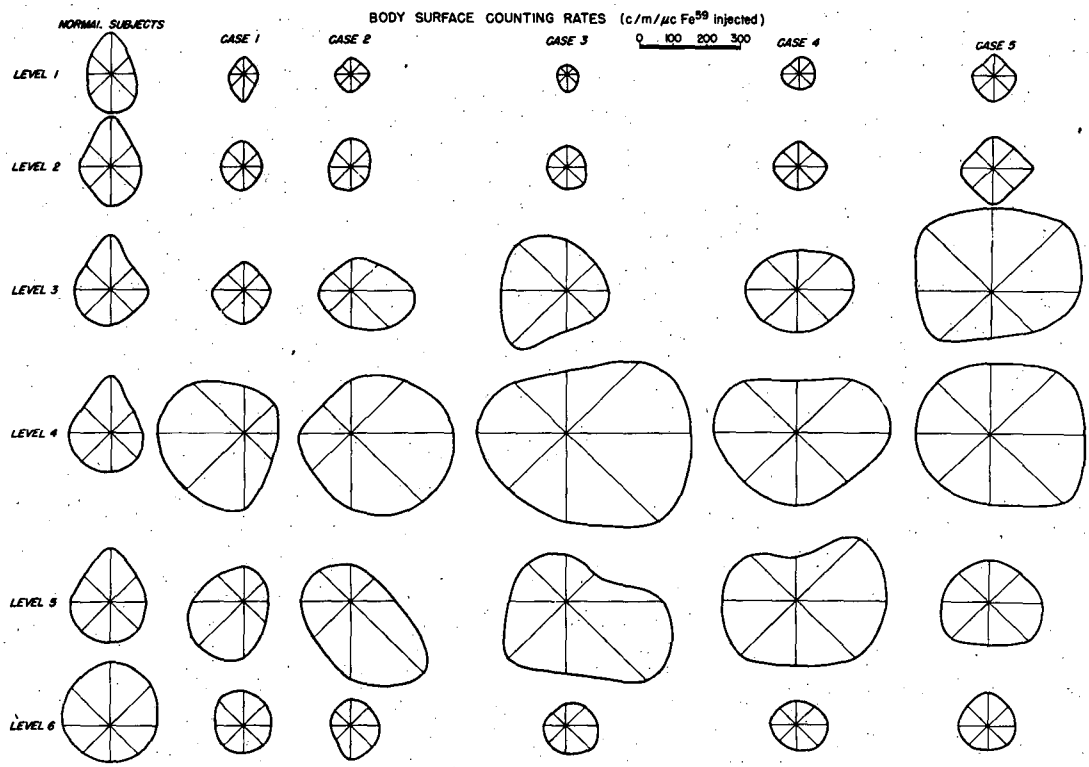
Fig. 5a through 5g are reproductions of the polar plots from the various cases.[1,2,3,4,5,7] The average values of 5 normal male adults are represented in Fig. 5a. The extensive and careful investigations of Mechanik[9] as well as the contributions of others concerning the distribution of red bone marrow in the body indicate that the vertebra and the pelvis contain the major portion in the adult. The sacrum was estimated by Mechanik to contain approximately 100 gms. of hematopoietic marrow and this of course is found in a small volume relatively near the body surface. It was for this reason, as well as the fact that the marrow volume within the cone of acceptance of the counter crystal would be relatively free of spleen and liver influence, that the sacral position was chosen to determine marrow counting rates as a function of time. This site is below the aortic bifurcation and the vena cava; thus some blood is avoided.

The shape of the uppermost levels with the posterior projection indicates the association of vertebrae with the iron isotope. The subsequent finding of the tracer incorporated in the red cells is an indication of a normally functioning marrow. The normal distribution of tracer as evidenced by the polar plot is no assurance that the patient has a normal iron turnover; for in the subsequent periods some individuals show a failure of the counting rates over marrow to fall to normal and a failure of the erythrocyte tracer level to rise (Case 6).

In the third and fourth levels of the plots of the normal cases the shape is similar. There is a slight asymmetry with a right preponderance which probably indicates the normal liver iron turnover. That the third and fourth levels are not more asymmetrical shows that the major stream of iron from plasma is normally to bone marrow. Finally, the larger area of the lowermost level shows that this region in the 23-33 age group probably contains a considerable portion of the red marrow of the body. That this distribution may not hold for all age groups is indicated by the finding of a contracted sixth level in a 72-year-old normal male.

In the early studies it was thought worthwhile to explore the head and the extremities as well as the torso; however, the counting rates of the skull and the extremities in the adult subjects were so low that the project was abandoned in all but special cases. It was then noted that a marrow hyperplasia with more diffuse skeletal distribution is manifested by contracted polar plots of the torso (Fig. 5, Case 7).

The grotesque figures from Cases 2, 3, 4, and 5 exemplify the aberrant iron kinetics which are associated with certain abnormalities in hematopoiesis.


BODY SURFACE COUNTING RATES (c/m/μc Fe59 injected)
NORMAL SUBJECTS
CASE 1
CASE 2
CASE 3
CASE 4
CASE 5
0 100 200 300
LEVEL 1
LEVEL 2
LEVEL 3
LEVEL 4
LEVEL 5
LEVEL 6

MU 1799

Fig. 5

Fig. 5, Case 1, the same patient as shown in Fig. 4e (aplastic anemia), demonstrates the relative failure of tracer to accumulate in marrow areas and negligibly in spleen. The fraction of tracer appearing in red cells in this case never exceeded 0.12 of the injected dose and the liver counting rate increased slowly with time and never decreased. Fig. 5, Case 2, the same as Fig. 4c, shows the major rapid transient accumulation of tracer in the splenic region, with very little in the normal marrow areas and no rise in counting rate. That this abnormal location of iron was associated with red cell production in the spleen is indicated by the high fraction of the tracer dose which appeared in red cells and is confirmed by the polycythemia and the morphologic finding of myelofibrosis.

Fig. 5, Case 3, shows the presence of the majority of the tracer in spleen but a significant amount in liver. Fig. 4d, Case 3 illustrates the failure of marrow to concentrate the isotope. Indeed the counting rate over marrow declines. The pattern differs from myelocytic leukemia in which evidence of hepatic and splenic metaplasia as well as some marrow function exists. A bone marrow biopsy was reported as showing almost complete replacement of the normal medullary tissue by fibrous tissue. The maximum fraction of the tracer which was present in the erythrocytes at any one time during the first two weeks of study was 0.6. The concomitant drop in counting rates over spleen and liver with a rise in erythrocyte concentration of tracer indicated that both the liver and spleen were involved in erythropoiesis. The asymmetry of the polar plot (Fig. 5, Case 3) with a left preponderance indicates that the spleen was the more important for this purpose.

Fig. 5, Case 4 (Erythroleukemia), again shows a failure of the expected temporary rise in the normal marrow locations. Instead the tracer had rapidly accumulated almost equally between the liver and the spleen. The subsequent appearance of approximately 0.4 of the tracer dose in red cells, with a concomitant fall in counting rate over the spleen and a constant counting rate over liver shows erythroid metaplasia of the spleen and storage function in the liver. Repeated marrow aspirations during the course of the illness indicated a diminishing marrow cellularity.

The previous discussion of results is illustrative of the usefulness of the application of in vivo Fe^{59} methods in following the immediate movement of the tracer from plasma to the various tissue sites.

A more comprehensive insight is gained of the functional significance of the particular tissue distribution if plasma and red cell concentrations as well as BSCR's are continued for a longer period of time. Fig. 3 illustrates the plasma and red cell radio iron as a function of time after administration (15 days). In addition a third curve is shown which represents the sum of the fractions of radio iron in plasma and red cells, i.e., the fraction of the injected dose in blood. It was possible to determine the time in each case for doing the localization survey of the torso (the time when the sum of the fractions of radio iron in red cells and plasma was at a minimum) by counting the plasma and red cell samples as soon as they were drawn.

The third curve was used for another purpose--the correction of body surface counting rates for the contribution of blood (see methods). Examples of the counting rate as a function of time over the several body positions are illustrated in Figs. 6,7, and 8. The data presented in these figures is the

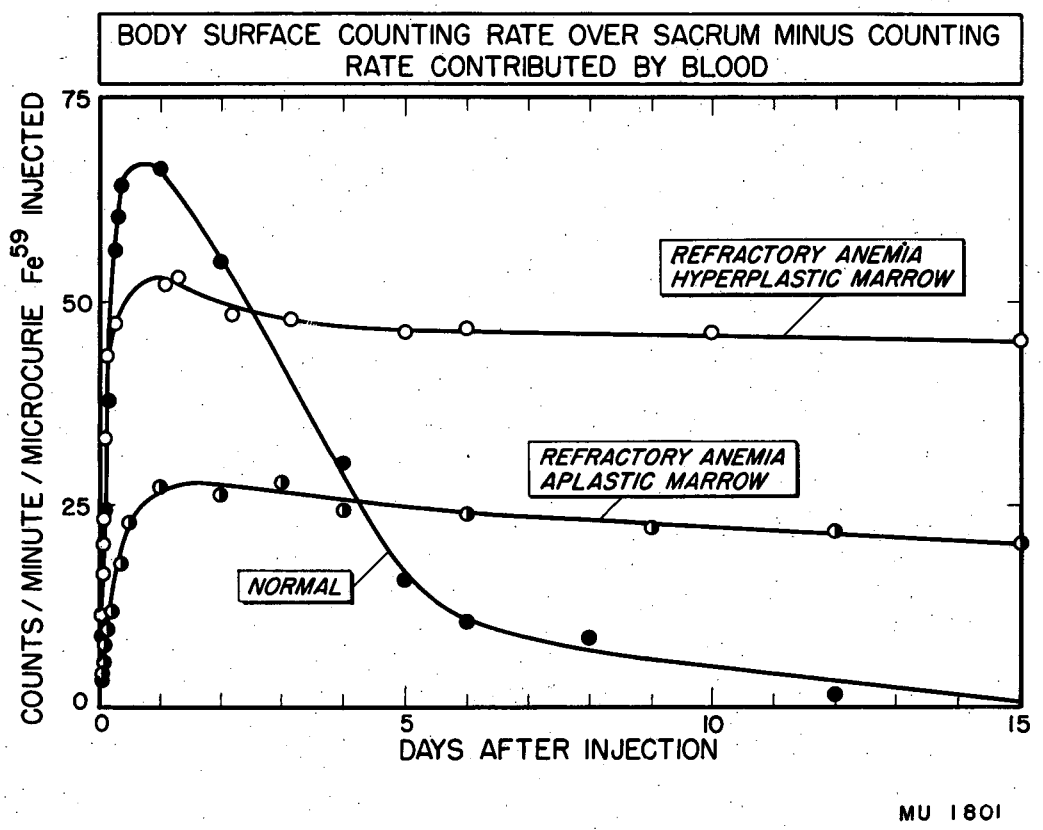

BODY SURFACE COUNTING RATE OVER SACRUM MINUS COUNTING RATE CONTRIBUTED BY BLOOD
COUNTS / MINUTE / MICROCURIE Fe59 INJECTED
75
50
25
0
0
5
10
15
DAYS AFTER INJECTION
REFRACTORY ANEMIA HYPERPLASTIC MARROW
REFRACTORY ANEMIA APLASTIC MARROW
NORMAL
MU 1801

Fig. 6

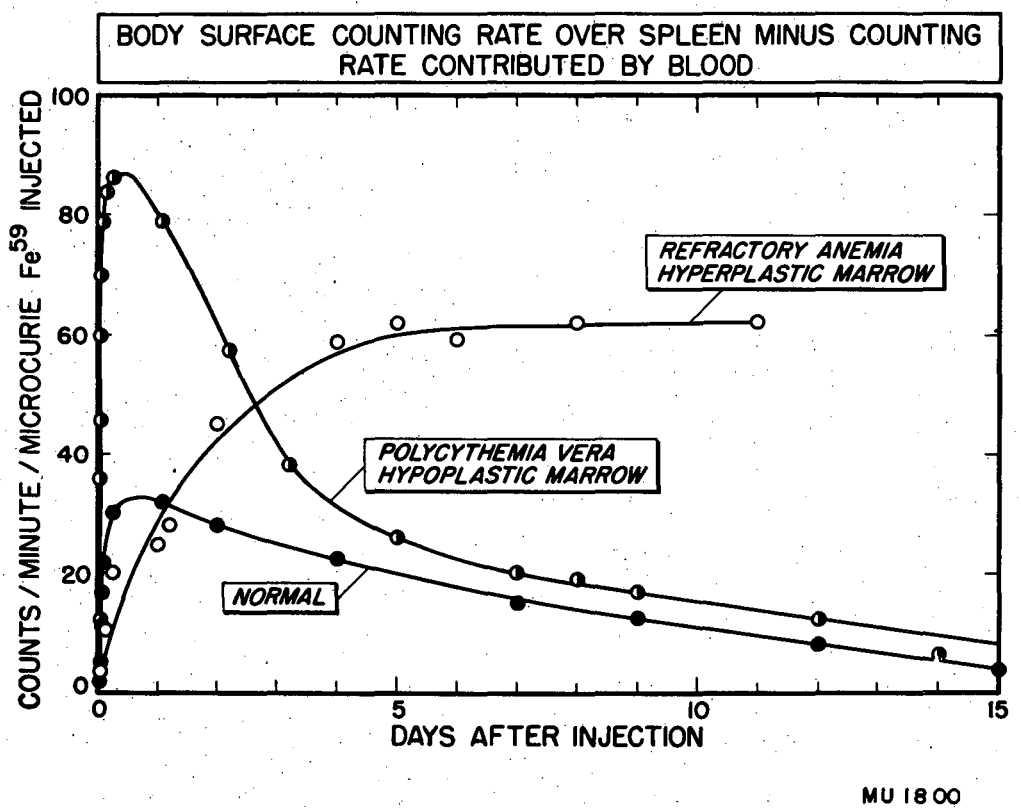

BODY SURFACE COUNTING RATE OVER SPLEEN MINUS COUNTING RATE CONTRIBUTED BY BLOOD
COUNTS / MINUTE / MICROCURIE Fe^{59} INJECTED
100
80
60
40
20
0
0
5
10
15
DAYS AFTER INJECTION
REFRACTORY ANEMIA HYPERPLASTIC MARROW
POLYCYTHEMIA VERA HYPOPLASTIC MARROW
NORMAL
MU 1800

Fig. 7

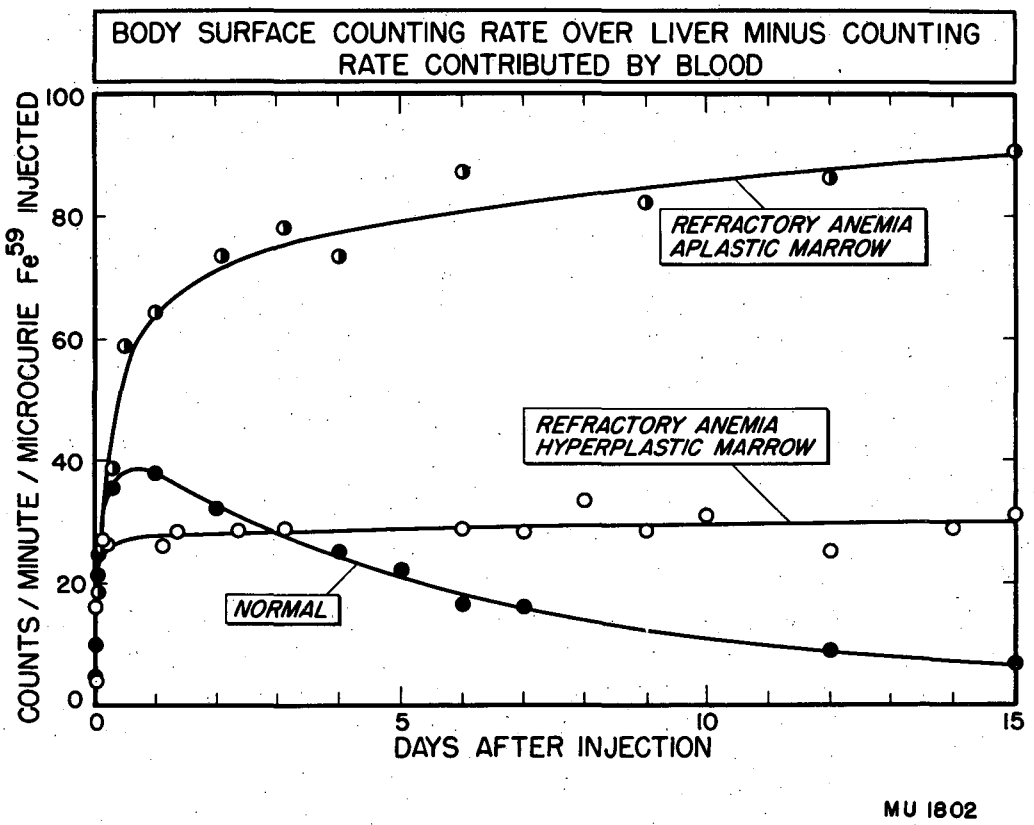

BODY SURFACE COUNTING RATE OVER LIVER MINUS COUNTING RATE CONTRIBUTED BY BLOOD
COUNTS / MINUTE / MICROCURIE Fe^{59} INJECTED
100
80
60
40
20
0
0
5
10
15
DAYS AFTER INJECTION
REFRACTORY ANEMIA APLASTIC MARROW
REFRACTORY ANEMIA HYPERPLASTIC MARROW
NORMAL
MU 1802

Fig. 8

counting rate (per microcurie injected) minus the counting rate contributed by blood. Fig. 6 shows such data from the representative marrow site. Included are three cases--a normal male adult, a patient with refractory anemia and a hyperplastic marrow (Case 6) and a patient with refractory anemia and an aplastic marrow (Case 1). The curve from the normal subject, in its time concentration relationship, resembles very closely similar data from animals which have been obtained by serial tissue sampling[8,10]. Comparison with Fig.3 shows the concomitant rise in red cell concentration of tracer and the fall in marrow counting rate in the normal case. It is not surprising that the counting rate over the marrow site of Case 6 (refractory anemia, hyperplastic marrow) rose to a fairly high level, almost normal. That it did not rise as high as the normal is possibly associated with the involvement of the long bones in a significant accumulation of radio iron. That this is probably true is borne out by the finding of slightly contracted polar plots. Also, it was expected that the marrow of the aplastic anemia patient (Case 1) could accumulate very little iron. The surprising point was the failure of these counting rates to fall subsequently. This phenomenon need not be considered a static one, i.e., it is not necessarily indicative of iron accumulation in the marrow without discharge. Instead it may indeed indicate a very rapid turnover through marrow with a high equilibrium level. Indirect evidence that the latter is probably true is gained from other considerations of this and other cases. For example, such findings are usually associated with a "secondary" rise in spleen counting rates. Such a case is illustrated in Fig. 7. This rise is secondary in that it does not occur as the iron is initially cleared from the plasma but only subsequent to its appearance in marrow. Case 7 having multiple myeloma and a severe anemia had data of this nature and had additional evidence of extraordinary erythropoietic hyperfunction in that the marrow showed 65 percent of the cells to be of the rubricytic series and that the stool urobilinogen excretion was greater than 860 mg/day. Definite marrow aplasia without erythroid metaplasia is distinguished by a slow plasma iron turnover in contrast to the large quantities of iron turned over by cases with erythroid metaplasia or cases with rapid cell destruction without erythroid metaplasia. Marrow aplasia in the case studied here was associated with transfusion hemosiderosis. There have been observations showing that abnormal marrow may contain hemosiderin granules in such patients. A possible reason for the marrow counting rate not being near zero 2 weeks after injection in Case 1 may be related to non-erythrogenic medullary iron function.[11] The high marrow equilibrium level in Case 6, however, need not be related to iron storage since it was associated with high splenic secondary counting rates and hyperplastic marrow that resembled that of a case previously described in which there was evidence of faulty hemoglobin production and numerous siderocytes.

From Fig. 8 a comparison may be made of net counting rates over spleen in a normal subject and in 2 patients. The abnormal cases are 2 and 6. The curve of Case 2 is remarkably similar in time concentration relationship to the normal marrow curve. The drop in counting rate over the spleen of this case was associated with a rise in the erythrocyte tracer concentration to near normal levels. This type of spleen curve has been called primary in that it rises as the concentration of the injected radio iron in plasma falls, and falls as the erythrocyte concentration of tracer rises. Another type of curve associated with spleen distinctly different from the primary type is illustrated by the data of Case 6 (Fig. 7). In this instance a great rise in counting rate over spleen occurs following or simultaneously with a fall in counting rate over sacrum and concomitantly with a subnormal rise in erythrocyte concentration of radio iron. This type of splenic radio iron curve has been called secondary. Despite the

fact that these body surface counting rates over spleen are corrected for the contribution of counting rate by isotope in the circulating blood (see methods) they are higher at 3-10 days than they are when all of the isotope is in the plasma, i.e., just after injection.

Convincing evidence that the primary type of spleen curve as seen in Case 2 is the result of splenic erythroid metaplasia are the facts that this patient (see Case 2 report) had myelofibrosis and polycythemia (red cell mass, 54.6 ml/kg), and that nearly all of the tracer was in red cells within 10 days after the dose was given. That areas other than biopsied sternum and the serially followed representative marrow site may be contributing significantly to red cell production is ruled out by the evidence shown on the polar plots (Diagram 2) which indicate the presence of the major portion of the dose of radio-iron in spleen.

Corroborative data showing that the secondary type of spleen curve is associated with excessive cell destruction is given in the case reports 6 and 7 which had this type of spleen curve. The pertinent data are anemia, marrow hyperplasia, reticulocytosis, high iron turnover rates, near normal uptakes of radio-iron in marrow, high stool urobilinogen excretion and a high icteric index. The extirpated spleen of Case 6 showed not evidence of erythroid metaplasia but did show hyperplasia of the reticulum and large blood depots. There was no evidence of excessive deposits of iron pigments in the spleen. It weighed 585 grams. The routine findings in these two individuals which are ordinarily associated with the diagnosis of hemolytic anemia are different in that they are clear-cut and definite in Case 7, but questionable to absent in Case 6. In contrast plasma iron turnover rates are increased in both cases and both cases show the secondary spleen curve. We believe this affords a method for distinguishing anemias or abnormal formation from those of abnormal destruction. The findings of a more rapid than normal iron turnover, a secondary spleen curve, the absence of excessive urobilinogen excretion, and the absence of high serum bilirubin and reticulocytes go together to indicate an abnormality or failure in the formation of hemoglobin and the rapid destruction of abnormal cells. This type of case could well be called an anemia of abnormal red cell formation even though apparent cell life is short. A well known example of this type is pernicious anemia.

The second type of case in which the usual findings indicating abnormally rapid destruction and formation of erythrocytes are present with excessive iron turnover and a secondary spleen curve could be considered as having an abnormally rapid destruction of red cells, not the result of an inherent abnormality in formation but existing independently of erythrogenesis except for the stimulation it affords.

In Fig. 8 is presented the body surface counting rate over liver minus the contribution to the counting rate due to blood perfusing the counted volume from a normal subject and from 2 patients with refractory anemia. The curve from the patient with an aplastic marrow differs from the normal in that it rises beyond the expected value and after 12 - 14 hours continues to rise, albeit at a slower rate, while that of the normal tends to decrease slowly. In contrast, the patient having the hyperplastic marrow shows a failure of the counting rate over liver to reach the normal value and then tends to increase slowly.

The case with an aplastic marrow illustrates the failure of the major exit of plasma iron to marrow to function and the substitute of the liver as the major pathway. The gradual slow gain in counting rate over liver is in accordance with the well known function of liver to effect net gains in iron when there is a failure in red cell formation or there is introduced into the body extra iron. The patient having the hyperplastic marrow demonstrates that the major exit of the iron from the plasma may continue to be marrow in the presence of severe anemia and abnormalities in medullary morphology. In fact, the diversion of iron to this type of marrow is possibly greater than normal and accounts for the failure of counting rate over the liver to rise initially to the normal value. The rising counting rates over liver in the later days indicates net gains of iron which are occurring. Measurements over this patient's liver were continued for 70 days at which time the counting rate over liver was higher than over any other area.

Summary.

The in vivo kinetics of intravenously injected Fe^{59} tracer doses were observed by external scintillation counting over marrow, liver and spleen as well as by analysis of plasma and erythrocytes. Thirty patients and seven normal subjects were studied.

The quantity of tracer in the plasma and cells was plotted as a function of time during a period of two weeks. The relative counting rates representing the other three compartments, marrow, liver and spleen, free from the counting rate contributed by blood, were plotted in a similar manner. In order to assess the significance of a tissue counting rate a survey of the torso was made when the combined plasma and erythrocyte level was minimal. Polar coordinate plots of the relative counting rates of 6 cross-sectional torso levels indicated the relation of the spleen, liver or marrow counting rates to those over the remainder of the body.

The kinetics were remarkably uniform in young male adults. The tissue of major importance in depleting the plasma of the tracer was the bone marrow. The bones of the pelvis seemed to be particularly capable of removing the tracer. The time required for the plasma depletion was quite uniform. This major initial transient accumulator, the bone marrow was observed to simultaneously discharge the tracer as it appeared in the circulating erythrocytes. A minor initial transient accumulation occurred in the liver. However, this was seen to feed back into the major pathway--plasma to marrow to red cells.

In the patients abnormalities in the initial direction or destination as well as in velocity were observed.

In subjects having known marrow hyperplasia without splenic or hepatic abnormality, for example, secondary polycythemia, the velocity from plasma to marrow was increased and the marrow volume included the extremities as was evidenced by the lesser area of the normally shaped torso polar plots.

When the major initial transient accumulator was spleen, there was uniformly markedly increased velocity. Such patients showed microscopic evidence of lack of erythrogenic tissue in the bone marrow. If the initial transient major accumulation was divided among liver, marrow and spleen or liver and spleen alone, the velocity was also great.

These abnormal initial depletions of Fe^{59} from the plasma might be followed by a variety of kinetics. The spleen might turn over the tracer with the same time constant as marrow normally does and into the identical second major transient accumulator--the circulating red cells. Such abnormal kinetics were observed in a patient with polycythemia vera and one with anemia. The spleen was seen to act as the major initial remover of iron from the plasma and to also retain the tracer at high concentrations in one case.

Also the liver was never observed to be the major remover of iron from the plasma and to discharge it into cells; however, it with the spleen might share in various portions the delivering of tracer to the second major compartment, the red cells.

A sub-normal velocity was associated with the exclusive hepatic removal of tracer from plasma. For example, 48 hours might be required to deplete the plasma of tracer to a level that would be achieved in 1 - 2 hours in a case where the spleen or bone marrow was the receptacle. However, exlusive hepatic removal was not associated with plasma iron turnover rates which were markedly below normal. When the liver was the major primary accumulator, it continued to maintain high levels for many days and no secondary wave appeared in any of the other four compartments studied.

Failure of the normal second major compartment, the circulating erythrocytes, to exhibit the wave of Fe^{59}, was associated with all variations in the preceding kinetics, that is, plasma to spleen to liver or to bone marrow. An interesting partial failure of the erythrocytes to accumulate tracer even though the marrow depleted the plasma or Fe^{59} occurred (in a patient with refractory anemia and hyperplastic marrow) with the appearance of a third receptacle, the spleen. In one of these patients who presented the usual findings of excessive hemoglobin production and destruction, the velocity of Fe^{59} from plasma to marrow was great and there was no other tissue collecting the tracer. The marrow appeared to only partially empty itself of tracer, moreover the erythrocytes did not accumulate their normal portion and simultaneously splenic counting rates rose over a two to four day period. Finally an abnormal equilibrium was established in which the levels in spleen and marrow were very high while that of the erythrocytes was unusally low.

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III HEALTH CHEMISTRY AND PHYSICS

Health Chemistry

N. Garden

Monitoring. Monitoring duties are increasing with expanding work with radioactive materials. It has been deemed advisable to take additional precautions by routine monitoring over greater areas--salvage, shops and guard shacks. The enlarged requirements for air monitoring has necessitated a revised procedure for greater efficiency. Adjacent soils downwind from the area of work with large amounts of long-lived alpha emitters are being examined. This group is also assisting in surveying some hazardous though non-radioactive conditions such as mercury vapor. The standby portable air exhaust assembly with 700 CFM capacity has been completed. It is equipped with CWS No.6 filter paper to remove airborne radioactive particles with greater efficiency from the air.

Activity Handling Group. The disadvantages of the first model of the decontamination chamber have now been well determined after a year's operation and a modified design is developing through the engineering stages. In spite of the difficulties, decontamination has proceeded and the backlog of contaminated equipment, which had accumulated over a two year period prior to the construction of the chamber, has now been cared for.

A Navy barge towed by a tug boat is being used for radioactive waste disposal, since the mine cable layer is no longer available. The barge, a former mud or sand dumper, has trap doors which open in the middle of the barge. The drums and concrete blocks of waste are loaded on the trap doors. Problems of the present system include lack of capacity for drums, due to the sloping sides of the trap doors. This condition is apt to jam the drums on dumping. Transferring crew members from the tug to the barge at the dumping grounds for the dumping operation during rough weather is difficult. Both conditions are being corrected.

Equipment Development Group. Equipment for processing pile-irradiated animal tissue was completed and is awaiting the delivery from the bombardment. Other equipment completed includes a hydrogenation unit for use in magnetic susceptibility work, seven new lead glass windows for use with gloved and lead-shielded boxes, a calorimeter system to be used on experiments on properties of metals in the transuranic elements, and miscellaneous new and modified small parts. The new features for the Berkeley box, described in the last Quarterly Report, which included lining the inside surface with polyethylene film, the installation of a single panel which contains all inlet-outlet fixtures, the collapsible features or section-like compartments introduced into the box design, and the new sliding door feature, have all proved satisfactory.

Health Physics

B. J. Moyer

Linear Accelerator. Since the recent overhaul of the linear accelerator, a survey of the fast neutron and gamma fields has been made. At present both the fast neutron and gamma fields exceed the tolerance level in many working areas, with the exception of the control room, by as much as five times. The proton beam current at the time this survey was made was about 6×10^{-8} amps. The results of this survey can best be seen by referring to the accompanying diagrams. The working areas in this building extend along the north side of the building running east and west. This machine has been running quite steadily at this level so it can be seen that additional shielding is needed. A concrete wall 50 ft. x 7 ft. x 1 ft. or its equivalent is to be erected along the north side of the linear accelerator running east and west to provide the necessary shielding in this area. (See Fig. 1 and 2) In other areas where personnel just pass through, interlocking barriers will be used.

Statistical Summary of Monitoring Program. Survey instruments maintained.

1. B-γ ionization chamber	33
2. Victoreen 263 meters	19
3. I.D.L. portable survey meters	20
4. Cutie pies	3
5. Recording γ-intensity meters	16
6. Victoreen proteximeters	3
7. Fast neutron proportional counters	5
8. Slow neutron proportional counters	10
9. Balanced chamber (slow neutron survey instrument)	2
10. Balanced chamber (fast neutron survey instrument)	1
11. Special tissue wall survey instrument	1
12. Fast neutron proportional (portable)	1

Personnel meters in use:

1. Total people covered with film badges	1850
2. Total man days coverage with pocket chambers	2075
3. Total man days coverage with pocket dosimeters	3150
4. Total man days coverage with pocket chambers (SN)	2557

Cases of weekly exposures above 0.3r:

Weekly film exposure above	184" Area	60" Area	Linear Accelerator	Building 52 Area	Chemistry	Total
0.3r	9	6	11	72	8	106
0.5r	0	2	2	38	2	44
1.0r	0	1	0	11	2	14
1.5r	0	1	0	0	2	3
2.0r	0	0	0	0	0	0

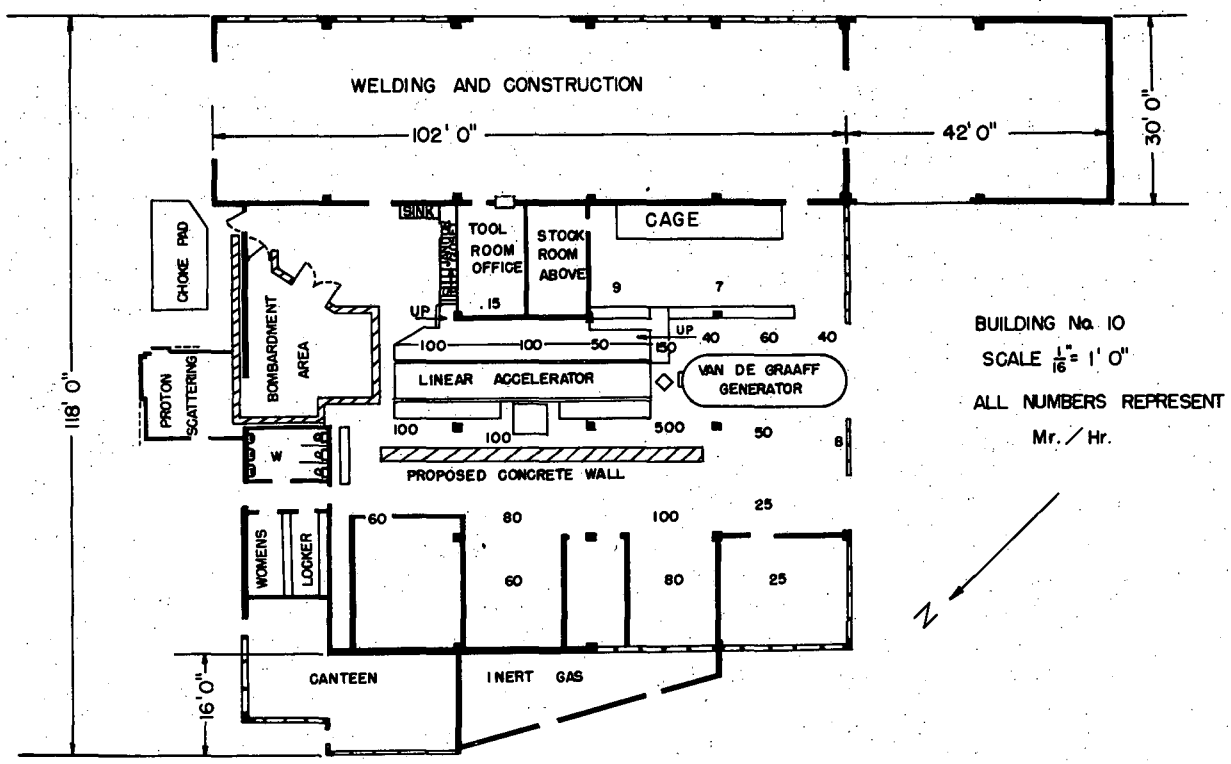
WELDING AND CONSTRUCTION
102' 0"
42' 0"
30' 0"
118' 0"
16' 0"
CHOKE PAD
PROTON SCATTERING
BOMBARDMENT AREA
SINK
TOOL ROOM OFFICE
STOCK ROOM ABOVE
CAGE
UP
15
9
7
100
100
50
50
40
60
40
LINEAR ACCELERATOR
VAN DE GRAAFF GENERATOR
100
100
500
50
8
W
PROPOSED CONCRETE WALL
WOMENS
LOCKER
60
80
100
25
60
80
25
CANTEEN
INERT GAS
BUILDING No. 10
SCALE 1/16" = 1' 0"
ALL NUMBERS REPRESENT
Mr. / Hr.
N

FIG. 1

MU 1804

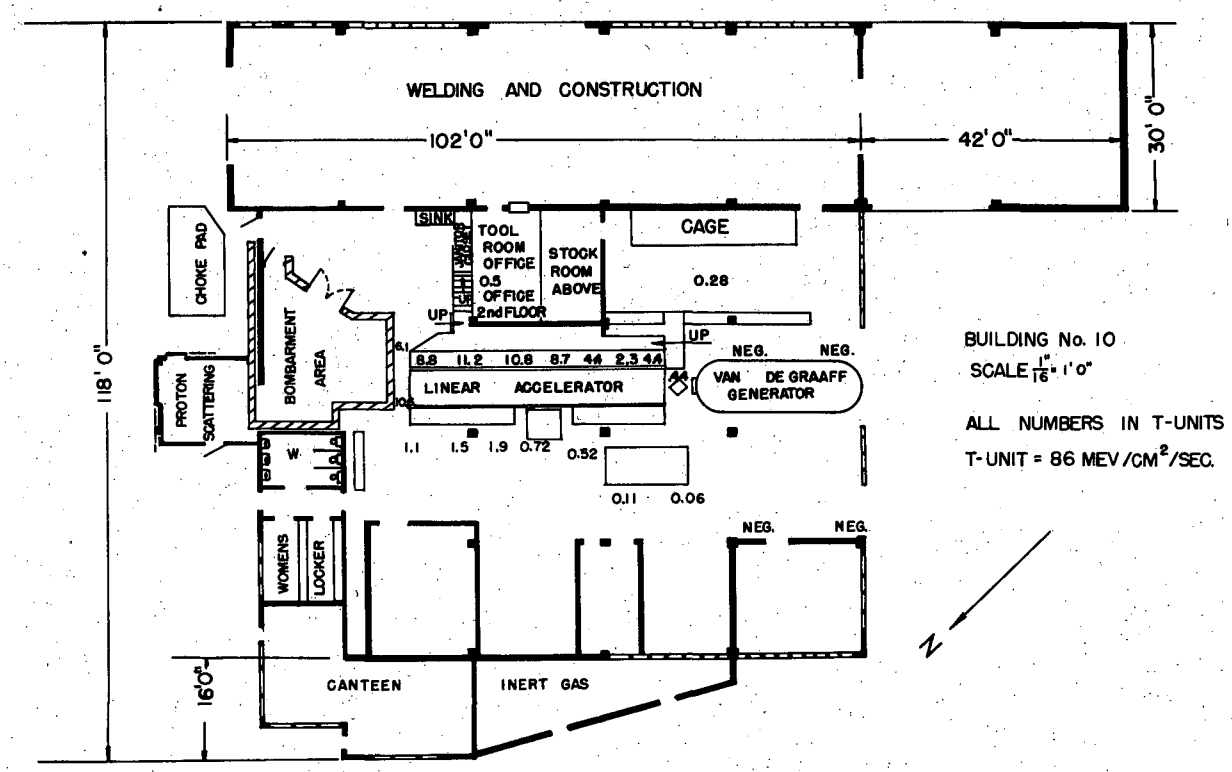
WELDING AND CONSTRUCTION
102'0"
42'0"
30'0"
118'0"
16'0"
CHOKE PAD
PROTON SCATTERING
BOMBARMENT AREA
SINK
TOOL ROOM OFFICE
0.5 OFFICE 2nd FLOOR
STOCK ROOM ABOVE
CAGE
0.28
UP
UP
6.1
6.8 11.2 10.8 8.7 4.4 2.3 4.4
LINEAR ACCELERATOR
4.4
NEG.
NEG.
VAN DE GRAAFF GENERATOR
10.6
W
1.1 1.5 1.9 0.72
0.52
0.11 0.06
NEG.
NEG.
WOMENS
LOCKER
CANTEEN
INERT GAS
N
BUILDING No. 10
SCALE 1/16" = 1'0"
ALL NUMBERS IN T-UNITS
T-UNIT = 86 MEV/CM²/SEC.

FIG. 2

MU 1806

CONFIDENTIAL