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SELECTIVE BETA IRRADIATION OF THE LYMPHATIC SYSTEM USING INTERNALLY ADMINISTERED Y^{90} DTPA: KINETICS, DOSIMETRY AND BIOLOGICAL EVALUATION

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Contents

ABSTRACT

That homotransplantation of tissues following total-body irradiation, though successful in mice and rats has been extremely difficult to achieve in large mammals such as dogs and man may reflect the inadequacy of radiating modalities currently used in preparing a large mammal for a homograft. In order to provide for the relatively selective irradiation of the tissues responsible for the homograft rejection response while minimizing the exposure of other tissues, we have developed a technique using internally administered Yttrium-90 chelated with DTPA (diethylene-triamine-pentaacetic acid). By use of continuous intravenous recycling of the urine containing the excreted Y^{90}-DTPA a method has been developed in which dosimetry is controllable and the excretion of the radioactivity from the body following the cessation of urine recycling is rapid, thereby permitting bone marrow transplantation within 24 hours following the procedure. The clinical, hematologic and pathologic postirradiation responses of the animals receiving Y^{90}-DTPA by this method were observed for each group of animals studied. Dogs given sublethal doses of radiation generally had a benign clinical course, the only remarkable finding being a selective lymphopenia without depression of granulocytes, platelets, or reticulocytes. Lethally irradiated dogs showed depression of all formed blood elements but severe

depression of lymphocytes was most prominent. Autologous bone marrow is capable of repopulating hematopoietic and lymphopoietic tissues with associated survival of lethally irradiated dogs. Successful homologous bone-marrow transplantation apparently has been achieved between unrelated beagle dogs. Thus, a new radiation procedure has been developed which offers greater selectivity in suppressing the activity of lymphatic structures, and presumably the homograft rejection response, than do methods presently in use. The results suggest the usefulness of this procedure in the treatment of malignancies of lymphatic structures and in the preparation of large mammals for transplantation of homologous tissues.

I. INTRODUCTION

For more than a decade many investigators have successfully performed tissue homotransplantations in mice and rats whose immune responses had been obtunded by supralethal total-body irradiation (1, 2, 3). However, in large mammals such as dogs, monkeys, and man successful homotransplants following total-body irradiation have been difficult to achieve (4, 5, 6, 7). Only a small percentage of attempts at this procedure in the large mammals have met with success (8, 9).

Undoubtedly many factors are operative in producing poor homotransplantation results in large mammals while allowing for favorable results in small mammals. However, we have focused our attention on the particular problem of delivering large radiation doses to the tissues responsible for the immune response. It was felt that a major difficulty encountered with many tissue transplantation studies in man and other large mammals was the inadequacy of presently available techniques to deliver significant radiation dosage to cells of their immune systems (10).

We have therefore attempted to develop a technique, using an internally administered isotope, for delivering high doses of radiation to the tissues responsible for the immune response without resulting in the inevitable death of the animal from other forms of radiation damage. This paper deals with the details of this technique and its applicability to the problems of bone-marrow transplantation.

II. THE EXPERIMENTAL PROBLEM

A. A Brief Discussion of the Results Obtained in Bone-Marrow Transplantation Following Total-Body Irradiation in Man, Monkeys, and Dogs

A report of cures in murine leukemia (11) using total-body irradiation and bone-marrow homografts suggested the possible use of this procedure in treating human leukemias. This possibility has stimulated the performance of virtually all the studies using bone-marrow transplants in man. Although it seemed improbable that sufficient radiation could be delivered to eradicate all the leukemic cells (12), it was hoped that the graft of bone marrow cells would be sufficiently competent immunologically to produce specific antibodies against the leukemic cells (11). Hypothetically, leukemic cells surviving the radiation exposure would be attacked by the immunologically active cells of the homograft.

Kurnick et al. (13), McGovern et al. (14), and Newton et al. (15) obtained and stored bone marrow from leukemic patients in remission. Subsequently they reinfused the stored autologous bone marrow into patients after the patient had been given a course of supralethal total-body irradiation. In this case it was hoped that the leukemic cells might indeed be destroyed by the radiation and that the patient would then be saved from death by radiation-induced hematopoietic failure by the infusion of presumably

"leukemia-free" autologous bone marrow collected during remission. As could be expected from considerations of the known exponential killing function of mammalian cells following radiation exposure (16), the doses of radiation given these patients was inadequate to destroy the leukemic cells and the patients eventually had recurrences of their disease. However, these studies did help to establish the ability of autologous bone marrow to re-establish bone marrow function after radiation injury in man.

Similar studies were carried out using isologous bone marrow. Cases of leukemic patients having identical twins were studied by Thomas et al. (17) and others (18, 19). The leukemic patients were given supralethal total-body irradiation and then were given an infusion of normal isologous bone marrow from their twins. Some temporary remissions occurred, but no cures were obtained. These studies demonstrated the ability of isologous bone-marrow transplants to repopulate the bone marrow in lethally irradiated humans.

Hope for the usefulness of this approach in the treatment of leukemias was then focused on the use of homologous bone-marrow transplants. It appeared that isologous bone marrow cells did not discriminate between leukemic and normal cells and did not produce antibodies against the leukemic cells. Possibly, immunologically active cells in a homograft might better be able to make antibodies against the host leukemic cells. Thomas et al. (20), Mathé et al. (21),

and others (5, 22) have attempted homologous bone-marrow infusions following supralethal total-body X- and gamma-radiation in leukemic patients. The results, thus far, have been singularly discouraging. Even following massive doses of conditioning radiation, the homografted cells did not grow well in the human hosts. The majority of such transplantation attempts have yielded no indication of any growth of the bone marrow homograft in the host (5). A few cases have been reported where some evidence of partial growth of the homograft cells was obtained. Mathé published a report of four cases of leukemia treated by this method in which evidence of growth of the transplanted cells was seen (21). However, from a therapeutic point of view even these cases were failures. The patients appeared to succumb to a combination of damage done by the leukemic process, the high levels of conditioning irradiation, and the immunologic attack of the donor cells against the host.

The complexity of the leukemic process in the patients studied by using radiation and bone marrow prevent a clear-cut evaluation of the problem of successful homologous bone marrow "takes" in humans. Although the largest bulk of human data on bone-marrow transplants involves patients with leukemia, a few cases are recorded of bone-marrow transplantation in relatively normal patients accidentally exposed to total-body irradiation. Mathé et al. (23) reported on six Yugoslavian workers exposed to what was

originally estimated to be 400 to 1000 rads of total-body neutron and gamma radiation. These figures have subsequently been revised to much lower levels. Homologous bone-marrow infusions were performed on five of these patients at 27 to 36 days following the radiation. There was some evidence of a take, but in less than a month following the bone-marrow infusion all evidence of bone-marrow homograft growth disappeared.

Even though the problem of achieving bone-marrow homografts in humans with presently available techniques is still poorly defined, nevertheless it would appear that the results generally have been quite disappointing.

Studies performed in primates and dogs further support the expectation that long-term bone-marrow homograft "takes" will be difficult to achieve in the large mammals by use of presently available methods for delivering the radiation (24, 25, 26, 27, 28). Rothberg et al. (24) failed to achieve evidence of any homologous bone marrow takes in chimpanzees after total-body irradiation. In his report, Rothberg emphasizes the difficulties in getting homograft takes in primates. Investigators in Holland (25) evidently have been able to achieve a reasonable number of bone-marrow homograft successes in monkeys. However, the majority of their animals died in 1 to 3 weeks after the irradiation, presumably as a result of direct irradiation damage and the effects of the donor-immune cells attacking

the host. Working with dogs, Thomas et al. (26) have
been able to achieve a fair amount of success in trans-
planting homologous bone marrow between closely related
animals. In purebred beagles he has managed to get
homograft takes between mother-and-son and sister-brother
combinations. His results in bone-marrow homografting
between unrelated beagles have been much more disappointing.
Only one of a total of five dogs attempted in this series
showed evidence of growth of the graft (26). This
animal had previously been splenectomized, and had received
ACTH, a large dose of irradiation (1200 r), and a very
large dose of bone marrow (12.5 billion cells). The
animal survived 31 days and then succumbed to an interstitial
nephritis. Cole (27) has been unable to get definite takes of
bone-marrow homografts in dogs by radiation conditioning
alone, and has now resorted to radiation plus the use of
various drugs. Porter and Couch (6) found some evidence
of homologous bone-marrow takes in mongrels following
supralethal X-irradiation, but the mean survival time
of the dogs treated with homologous bone marrow was
actually lower than the untreated control group.

In general, it may be said that the ease in bone-
marrow homografting seen in rodents following total-body
irradiation has not been experienced in performing this
procedure in the larger mammals, such as dogs, monkeys, and
man. Not only has it been very difficult to get bone-marrow

homografts to grow between unrelated members of the same species in the larger mammals, but the few animals in which successful homotransplants of this type have occurred have survived for only short periods of time after irradiation. These deaths in the "successful bone-marrow homotransplants" may have been due in part, to radiation damage suffered by nonimmunologically active systems, such as the gastrointestinal tract, since in general, the conditioning radiation dose was quite high in these experiments.

B. Discussion of the Use of Internally Administered Isotopes for Selective Irradiation of the Cells of the Immune System

Some insight into the problem of successful bone-marrow transplantation in mice but concomitant failure of this procedure in dogs can be gathered from certain dosimetry considerations.

The LD_{50} dose for hard X-rays given in a single exposure in mice is between 400 and 600 r, and that in dogs around 325 r (29). Many of the successful homotransplantation experiments in mice have been done by using single-exposure doses in excess of 600 r, usually 900 to 1000 r (30). Dogs given this high dosage of hard X-rays as a single dose frequently succumb to a radiation-induced gastrointestinal syndrome and, in general, show a morbidity which complicates the interpretation of the transplantation experiment (31, 32).

Thus, at least with the use of presently available high-voltage X-ray machines, we are faced with the inability to achieve rapidly delivered high radiation doses to the critical immune tissues of the dog because of an apparent adverse species difference in radiosensitivity of critical nontarget organs, such as the gastrointestinal tract.

Examination of the dosage delivered to the bone marrow of mice has furnished us with yet another clue to the problem of radiation "adequacy". It was pointed out by Spiers as early as 1949 (33) that small cavities surrounded by bone absorb more radiation energy than similar small cavities situated at equivalent depths in the body but not surrounded by bone. Considerations of this effect, plus attenuation of the X-ray beam passing through the thick bony cortex of large mammals, have led us to the conclusion that the bone marrow of mice receives over 16% more radiation than the bone marrow of dogs given identical exposures to a 250-kv X-ray beam (see Appendix A). It would therefore appear that even if modalities were available for administering a perfectly homogeneous total-body radiation dosage to large mammals we still might be unable to deliver such high doses as given to the bone marrow and other tissues of the mouse.

Such considerations have led us to the development of a procedure for delivering high doses of radiation with a degree of selectivity for the target organs while sparing the nontarget organs.

The "target" organ, in this case, is the cells responsible
for the foreign tissue rejection response. It is not known
with certainty exactly which cell type is responsible for
this function. Present evidence strongly suggests an
association between the lymphocyte and this homograft
rejection response (34, 35, 36, 37, 38). However,
regardless of their cell type it is probable that these
cells are widely dispersed throughout the body. Although
lymph nodes and spleen probably contain the large bulk of
the antibody-producing cells, there are probably many of
these entities outside these organs which carry on this
function (39, 40).

Investigators have used colloids of radiogold, administered
parenterally, to selectively irradiate phagocytic cells and
presumably the immune system (41). The mortality of these
animals given colloidal radiogold is uninfluenced by the
administration of isologous or homologous bone marrow, in-
dicating the inadequacy of this material for use in
marrow transplantation studies. The use of colloids for
destruction or suppression of the immune response faces two
main difficulties. First, phagocytosis and the production of
antibodies appear to be two distinct functions, and the
cells responsible for the latter may not be in close
proximity to cells responsible for the former (41, 42). Thus,
destruction of relatively radioresistant phagocytic cells may
not markedly influence the antibody response (42, 43). Second,

colloids of different sizes are removed from the blood by
different components of the RE (reticulo-endothelial) system:
very large particles are removed in the capillary bed in the
lungs, next largest by the liver and spleen, and much
smaller particles are removed by the bone-marrow component
of the RE system (44). Indeed, many tissue phagocytes may
not even be exposed to intravascularly injected colloids.
It is obvious that only a carefully balanced mixture of
colloids of different sizes could approach a uniform
distribution and irradiation of most of the phagocytic
cells, and even under the best of circumstances, a large
number of cells of this type would not be exposed to irradi-
ation.

Radioisotopes of elements having a wide distribution
in the body have also been considered. Radiopotassium (45)
has been used in mice for delivering total-body irradiation,
but since potassium has a rather homogeneous intracellular
distribution, little selectively of irradiation in terms of
damaging specific cell types can be achieved. Radiosodium,
on the other hand, in general has a distribution in
extracellular fluid spaces, and since structures such as
lymph nodes, which are known antibody-producing centers,
are rich in extracellular fluid, we could hope to achieve
some selectivity in irradiating the cells of the immune
system by use of such an isotope. However, since sodium

is cleared slowly from the body, we would have to wait until
most of the radiosodium had disappeared via its physical
decay before homografting of cells could be attempted.
Should homograft cells be introduced prematurely, they
could possibly receive a lethal or inhibitory dose of
radiation from residual radioactive sodium which would
prevent growth of the graft. These difficulties could be
avoided by using a very short-lived isotope, but of course
this introduces formidable technical difficulties.

The characteristics that we should require in an
isotope to be administered internally for the purpose of
preparing the organism for a tissue homograft should be
as follows: First, there should be some selectivity in
irradiating the cells responsible for the homograft re-
jection response. Since there was no known unique metabolism
carried out by these cells which could have been utilized
in irradiation of these cell types with absolute specificity,
we attempted to reach the target cells through some non-
specific means which would give us relative specificity in
irradiating the cells in question. Since antigens probably
contact the antibody-producing cells in some component of the
extracellular fluid space, we made the assumption that these
cells must be bathed to a large extent by the constituents
of the extracellular fluid. If, in general, there is more
extracellular fluid in the tissues containing the immune

cells than in the tissues not responsible for the immune response, then we could achieve some selectivity in irradiation of these cells by using a modality which distributes itself in the extracellular fluid compartment. Secondly, it is important that the material selected release its radiant energy locally, so as to maximize the selective advantage of its distribution pattern. Thus, the isotope must be either a pure beta or a pure alpha emitter. A gamma emitter would be undesirable not only because of its long range in tissue but also because of the radiation hazard to attendant personnel tending the animal or patient during the irradiation procedure. Thirdly, the radioactivity must be rapidly removed from the body after the irradiating procedure so that homografting may be attempted in reasonable proximity to the radiation episode. Fourthly, the isotope chosen must be readily available in large quantities in a pure form and must have a convenient half life -- one long enough that it can be readily handled without excessive physical decay and one short enough to avoid an excessive hazard in the event of an accident.

No isotope came to mind that possessed all these characteristics. Y^{90} chelated with DTPA (diethyline triamine pentaacetic acid), however, fulfilled most of the above mentioned criteria quite adequately (46, 47). DTPA is a chelating agent that in general distributes itself

in the extracellular space (46) except for increased renal concentration. It is excreted rapidly from the body in the urine as an exponential function of time, with a half life in rats of approximately 35 minutes after a single intravenous injection (46). Yttrium is a lanthanide which is very strongly bound by the DTPA chelate (47). One of its isotopes, Y^{90}, is a pure beta emitter with a half life of 64.4 hours (48). The beta particles of Y^{90} have an E_{max} of 2.26 Mev. and E_{mean} of 0.9 Mev. (49). The Y^{90} can be obtained in fairly pure form by separating it from Sr^{90}, its parent compound (50). Sr^{90} is a readily available, inexpensive isotope found abundantly in the waste of U^{235} reactor piles. The reactions involved are as follows:

$$Sr^{90} \dashrightarrow Y^{90} + \beta + (neutrino),$$
$$Y^{90} \dashrightarrow Zr^{90} + \beta + (neutrino).$$
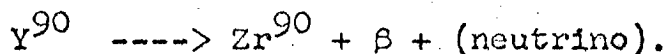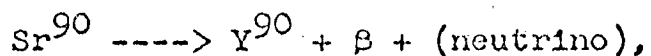
The zirconium-90 is stable (49).

The Y^{90} chelated with DTPA thus represents a material which closely fulfills the criteria enumerated previously, and it was chosen for the purpose of administering total-body irradiation for suppression of the homograft rejection response.

III. THE EXPERIMENTAL PROCESS

A. Preparation of the Y^{90}-DTPA for Use in the Dog

1. Introduction

Several methods are available for the separation of Y^{90} from it's parent material, Sr^{90} (50). We adopted a modification of a technique used by the Oak Ridge Laboratories which they had found satisfactory for the separation of curie quantities of this material (51). The procedure involves the use of an organic phosphate (di-(2 ethyl-hexyl)-phosphoric acid) to remove the Y^{90} from the Y^{90}-Sr^{90} equilibrium mixture. Steps are then taken to minimize the amount of Sr^{90} contamination and to bring the Y^{90} into a convenient soluble form.

2. Materials and Methods

The diagram in Fig. 1 summarizes the essential experimental steps in the extraction procedure. The di-(2 ethyl-hexyl)-phosphoric acid in toluene is washed three times with 0.1 normal HCl to remove any free phosphate or other materials readily soluble in aqueous solutions; 40 ml of this solution is added to a clean 100-ml cone containing 40 ml of the Sr^{90}-Y^{90} mixture. The mixture is stirred for 20 minutes at a speed which insures dispersion of the two phases. The phases are allowed to separate for 10 minutes. The aqueous phase (0.1 $\underline{N}$ HCl) contains the
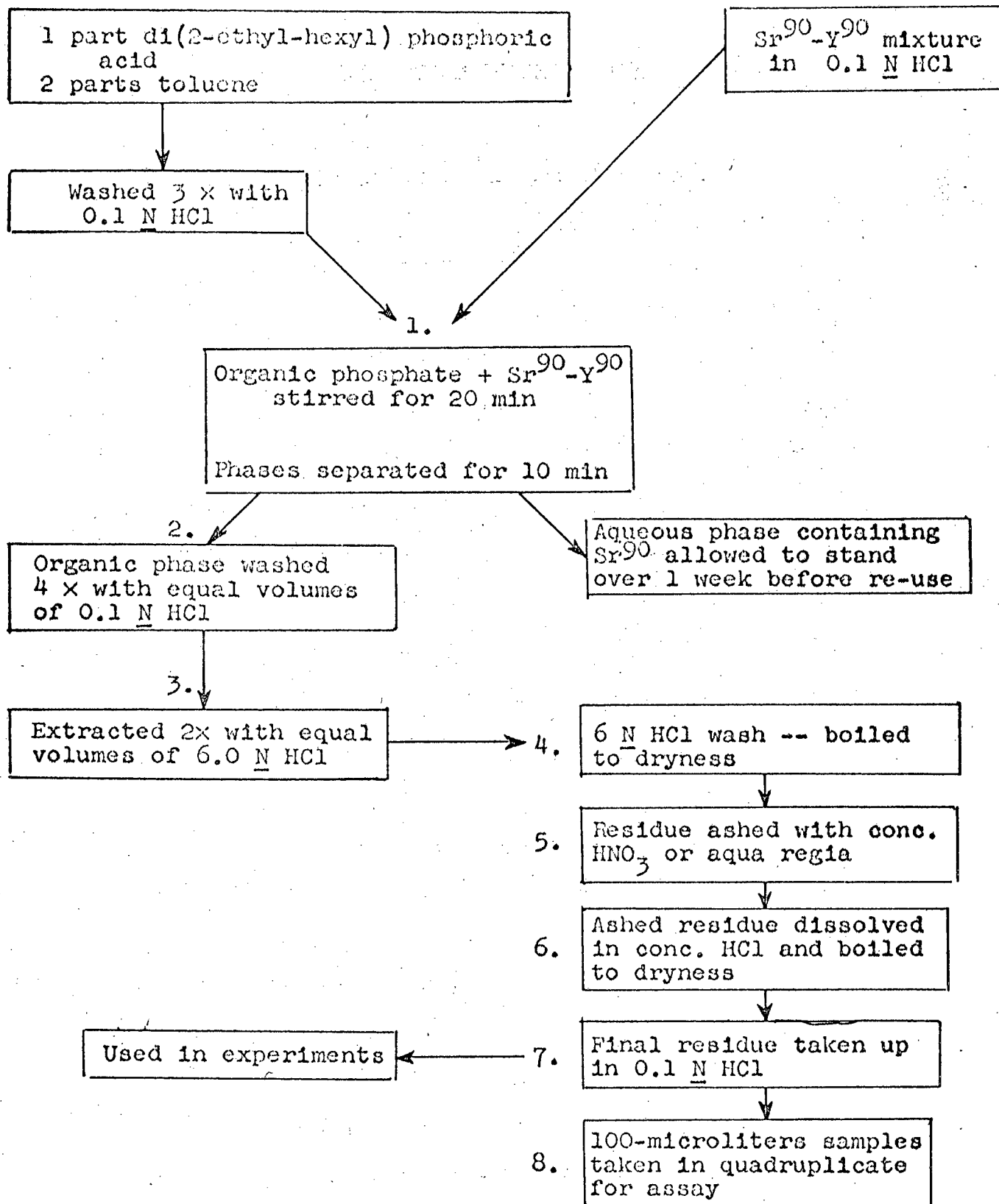
Fig. 1. Procedure for separation of Y^{90} from Sr^{90}-Y^{90} mixture

Sr^{90} and is removed. The organic phase containing the Y^{90} is washed four times with equal volumes of 0.1 $\underline{N}$ HCl to remove any Sr^{90} which might be found contaminating the organic phase. In each wash the phases are stirred for 10 minutes and allowed to separate for 5 minutes before the phases are separated for the next wash. The washed organic phase is then extracted twice with equal volumes of 6.0 $\underline{N}$ HCl, stirring for 15 minutes each time. The phases are allowed to separate for 10 minutes. The aqueous phases of the two extractions are pooled in a 400-ml round-bottom flask and boiled to dryness. The residue is then ashed with either concentrated HNO_3 or aqua regia until a white or tan residue remains. This residue is redissolved in concentrated HCl and boiled to dryness to convert the inorganic salts to the soluble chloride form. The final residue is then taken up in 15 to 25 ml of 0.1 $\underline{N}$ HCl and this solution is filtered through four thicknesses of No. 40 Whatman filter paper.

Four 100-microliter aliquots are removed for assay. Each 100-microliter aliquot is placed in a 5-ml volumetric flask containing 0.1 $\underline{N}$ HCl. Serial dilutions are performed independently for each sample until an appropriate concentration for assay is achieved. All dilutions are carried out by using 0.1 $\underline{N}$ HCl as the diluting fluid. Samples of 500 microliters of the final dilution are plated out in

steel planchettes and dried. The sample counts are compared to a Nuclear Chicago Sr^{90}-Y^{90} equilibrium mixture standard fitted with an aluminum absorber of greater than 200 mg/cm^2 thickness to absorb all the Sr^{90} beta particles. This procedure, recommended by S. B. Garfinkel, of the National Bureau of Standards (52, 53), provides for a long-lived standard (half life of Sr^{90} = 27.7 years) for a short-lived isotope (half life of Y^{90} = 64.6 hours) (49). The assay planchettes are fitted with aluminum absorbers exactly similar to those used on the standard so as to afford comparable attenuation of the Y^{90} beta particles in the standard and the assay samples. All counting is done on a Nuclear Chicago gas-flow counter fitted with a micromil window having a surface density less than 150 μgm/cm^2. A minimum of 5000 counts is performed on each assay sample with a standard deviation of 1.4% for each count and a standard deviation for the mean of the counts of 0.7%. A total variation in counts between the four samples of less than 10% was considered acceptable.

The physical decay of the Y^{90} was calculated from the time of assay to the time of the irradiation procedure by the decay equation $N_t = N_0 e^{-\lambda t}$, using λ = 0.01076, and t in hours.

In preparation of the Y^{90} to be given to the animal during the irradiating procedure, the calculated volume of

Y^{90} solution is pipetted into a 40-ml cone containing
0.5 ml of a 25% solution of DTPA plus enough distilled
water to make a total final volume of 22 ml. The final
pH of this solution varies from 2 to 4. This solution is
then drawn into a special container, described below,
where it awaits use in the irradiation procedure.

Several technical problems arose in the use of the
procedure outlined above, two of which warrant discussion.
We discovered that there was a significant solubility of
the organic phosphate-toluene organic phase in the 6.0 $\underline{N}$
HCl acid phase occurring during the extraction step No. 3.
This resulted in an organic contamination of the residue
when the 6.0 $\underline{N}$ HCl extract was boiled to dryness (step
No. 4). Frequently, repeated time-consuming wet ashings
of this organic material were required, using concentrated
HNO_3. In the latter extractions, aqua regia replaced the
HNO_3, and more efficient removal of the organic contaminants
was obtained. In all the early extractions, no addition
of carrier yttrium was attempted and the final product
was carrier-free. However, handling and assaying of the
carrier-free material was associated with many technical
problems and in later experiments, carrier in concentration
of 10 mg% yttrium nitrate was added to the 6 $\underline{N}$ HCl used in
extracting the organic phase (step No. 3). Prior to the
addition of the carrier, the molar ratio of DTPA to yttrium

was on the order of 10^5. The addition of 10 mg% carrier to
the 6.0 $\underline{N}$ HCl extracting solution dropped the molar ratio
to 10^1. Since it is probable that increases in the molar
ratio of DTPA with yttrium above 1.2:1 probably does not
significantly effect the pharmacology of the chelate
complex (47), we did not expect any changes in the action
of the material in reducing the molar ratio to 10^1. That
this was indeed the case was borne out by comparable
urine and blood clearances of the material at the different
molar ratios when it was administered to dogs.

3.   Physical Facilities and Radiation-Protection
Devices Used in Preparing the Y^{90}-DTPA

All procedures outlined in the flow sheet (see Fig. 1)
were carried out in a specially designed enclosure. This
enclosure consisted of a main chamber which is essentially a
modified Berkeley glove box surrounded by 1.5 in. of
lead shielding, with an adjoining side chamber made out
of plastic-covered plywood. This side chamber acted as an
intermediate antechamber for passage of materials into and
out of the main chamber. The two chambers are connected by
a sliding plywood door and a hinged lead door. The inner
chamber is fitted with three motors provided with disposable
glass stirring rods.

The inner chamber also contains 90-ml pipettes mounted on a circular bracket which can be rotated by a control located at the top of the box. Each pipette is connected to a control switch which allows the individual pipettes to be filled by manipulations outside the box. All heating procedures are performed in a round-bottom flask placed in an electric heating sock inside the box. Gases from both inner and outer chambers are exhausted and filtered through a standard radioactive exhaust system. Liquid wastes inside the box are poured into an open funnel which connects with a gallon jug placed in a separate compartment beneath the main chamber. The jug containing the liquid waste can be processed periodically without entering the main chamber. Dry radioactive waste from the chamber is placed into a cylindrical cardboard container and then the container is passed into an adjoining compartment located behind the main chamber. Here the cardboard container is allowed to fall into a shielded plastic bag and the plastic bag is then sealed. The sealed, airtight lower portion of the bag containing the dry radioactive waste can then be processed in a routine manner.

4. Discussion and Conclusion

By use of the facilities described, 20 to 40 curies of Sr^{90}-Y^{90} mixture can be kept in the box and extracted with minimal radiation hazard to personnel. The radiation seen

from the outside of the lead-enclosed box is mainly brehmsstrahlung, and amounts to approximately 4 to 5 mr/hr. During the routine extraction procedure, it is never necessary for personnel to be exposed to the full radiation field emanating from the doors leading to the inner chamber. The field at these doors has not been accurately measured, but is in excess of 50 r/hr.

More than 35 extractions of Y^{90} from Sr^{90} have been performed by using the protocol described above. The efficiency of extraction of Y^{90} from its parent Sr^{90} has been approximately 40%. Sr^{90} contamination in the final Y^{90} solution was on the order of 10^{-4} to 10^{-5} Sr^{90}:Y^{90} in six of the extracts analyzed for Sr^{90}. The radiation-protection protocol devised for the extraction procedure has been successful to the extent that personnel involved in the procedure have never recorded exposures greater than 200 mr/wk. The procedure described is a workable method for producing large quantities of Y^{90} with minimal contamination by Sr^{90}. Y^{90} can be made carrier-free or with carrier in any desired volume of solution. A procedure is described for accurate assaying of the material. The health hazards involved in performing the extraction procedure have been adequately handled by the methods described.

B. Pattern of Clearance of Y^{90}-DTPA from the Body

1. Introduction

The work of Foreman (46) offers some evidence suggesting that the DTPA chelate generally has a distribution among the extracellular fluids of the body excluding the cerebrospinal fluid. Foreman (46) and Kroll et al. (47) report a simple exponential rate of clearance of the material in the rat via the urine following a single intravenous injection of C^{14}-labeled DTPA. The half life of this excretion is short, amounting to approximately 35 minutes in the rat.. The DTPA appeared to be metabolically inert, being excreted exclusively as the unchanged DTPA with no evidence of breakdown or modification by the body. Toxicologic studies in rats (46) indicate that when DTPA is administered in doses greater than 62.5 mg/kg body weight/day hydropic degeneration is noted in the renal tubules. These changes appeared to be reversible upon cessation of administration of the drug.

The following studies were performed to see whether the carrier-free Y^{90}-DTPA prepared by us (Sec. III A) of this report) was cleared from the blood by the kidneys in a fashion similar to that described for C^{14}-labeled Ca-DTPA, and to further extend the observations on the excretion pattern of this material from the body. A study was also performed to determine the nature of the Y^{90}-DTPA appearance and clearance characteristics from the lymphatic fluid.

2. Materials and Methods

Carrier-free Y^{90} extracted in the manner outlined in Sec. III A was chelated with an excess of DTPA (molar ratio greater than 10:1) and given to purebred beagle dogs between 6 months and 2 years of age by a single intravenous injection. Multiple blood and urine samples were taken at different times after the injection.

The thoracic ducts of adult Long-Evans strain rats were cannulated by Dr. John Schooley of the Donner Laboratory (54). Both jugular veins of these rats were exposed and the Y^{90}-DTPA solution was injected into the right jugular vein. Multiple samples of blood and lymph were taken from the left jugular vein and thoracic duct at intervals following the injection. All samples were plated on stainless steel planchettes, dried, and counted in a Nuclear Chicago gas-flow counter using a micromil window.

3. Results

Figure 2 shows a typical blood-clearance curve and cumulative urine-appearance curve obtained from a beagle dog following a single intravenous injection of Y^{90}- DTPA. The concentration of Y^{90} is plotted as the ordinate on a semilog scale, and time after the injection of the material is plotted on the abscissa. The blood clearance curve for the first 7 hours after the injection of the

Y^{90}-DTPA can be broken down into two simple exponential components, given by the equation

$$\text{Concentration} = Ae^{-\lambda_1 t} + Be^{-\lambda_2 t}.$$

The values for λ_1, λ_2, A, and B can be obtained by extrapolating to time zero the linear portion of the curve determined between the 3rd and 7th hours postirradiation. The intercept of this line with the ordinate gives a value for B, and from the slope of the line the value of λ_2 can be determined. By subtracting the values of this line from the observed values on the curve we determine a second line whose intercept with the ordinate is A, and from its slope the value of λ_1 can be determined (see Fig. 2).

The exponential function of $Ae^{-\lambda_1 t}$ is interpreted to represent the phase of mixing of the Y^{90}-DTPA in the fluid volume it will come to occupy. The $t_{1/2}$ of this first exponential function varied between 7 and 15 minutes in the various experiments performed. This indicated that this mixing phase is quite rapid and is essentially complete 1 hour after intravenous injection of the activity. The function B $\exp(-\lambda_2 t)$ is interpreted to represent the actual removal of the Y^{90}-DTPA from the body.

The curve on the right of this figure depicts the cumulative clearance of the Y^{90} activity from the body via the urine. It is readily seen that this takes the general form of the blood clearance curve. Both the absolute amount and the rate of urinary clearance of activity correspond
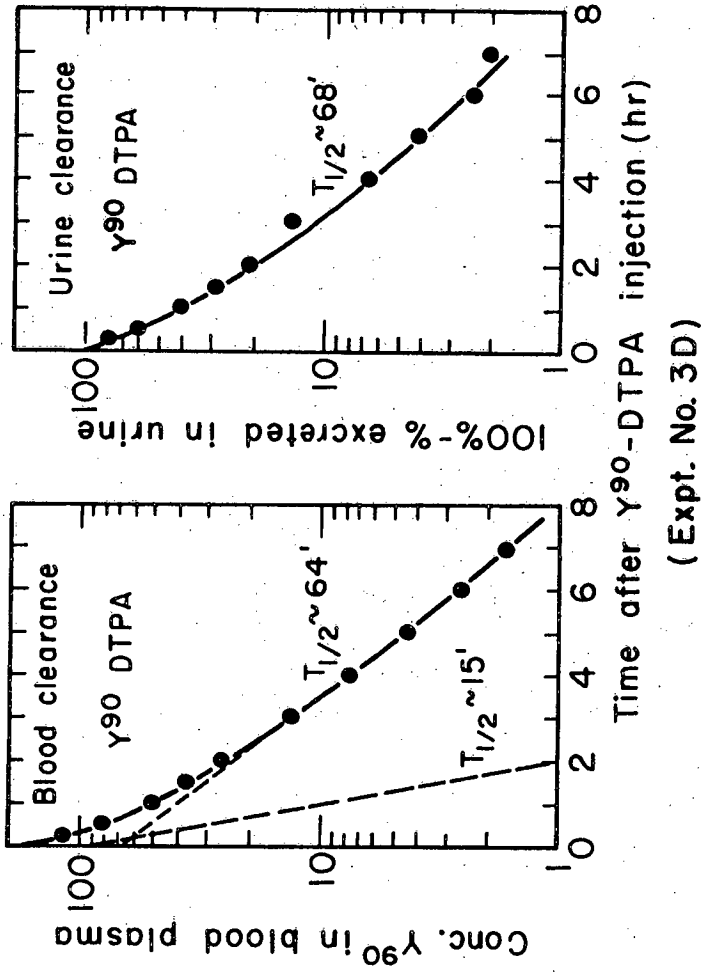
MU-24044

Fig. 2. Blood clearance and cumulative appearance in the urine of Y^{90}-DTPA in the beagle dog.

closely to the amount and rate of clearance of activity from the blood. This parallelism, plus the fact that more than 95% of the total amount of injected material has been excreted in the urine within 7 hr after injection, clearly identifies urinary excretion as the major route of loss of Y^{90}-DTPA from the body.

Analysis of data similar to that presented in Fig. 2 indicated that the Y^{90}-DTPA distributed itself in a calculated body fluid space larger than that occupied by the extracellular fluid space alone. On the assumption that specific concentration of the Y^{90}-DTPA did not occur in any tissue, the fluid volume in which the Y^{90}-DTPA equilibrates amounts to 30 - 40% of the body weight. Foreman (46) found that the equilibrium volume of carbon-14-labeled calcium DTPA, assuming no specific tissue concentration, amounted to approximately 30% of the body weight in the rat. That the assumption of uniform distribution of Y^{90}-DTPA without specific tissue concentration is only approximate is suggested by the tissue distribution studies presented in Sec. III D of this report.

Figure 3 presents the results of a typical experiment in rats, showing the appearance of activity in thoracic duct lymph and blood plasma following an intravenous injection of DTPA. It can be seen that the activity in the lymph rises sharply after the injection of the activity, reaching and surpassing the level of Y^{90}-DTPA activity in the plasma
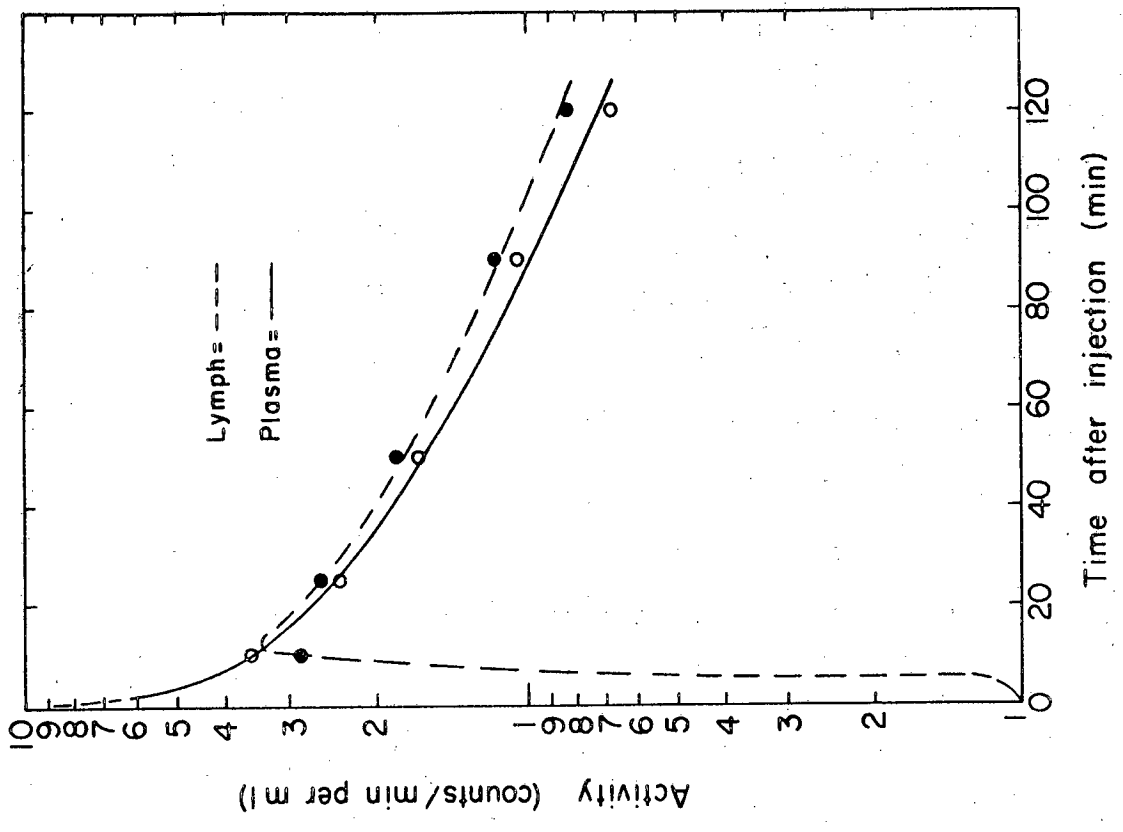
Fig. 3. Y^{90} -DTPA clearance from the lymph and plasma of a rat.

MU-23999

After about 10 min. The rate of clearance of activity
from the lymph after this time closely parallels the clearance
from the plasma. That the activity in the lymph is somewhat
higher than the activity in the plasma is to be expected
from the mode of clearance of the activity from the plasma.
Some of these differences may also be a result of simplifi-
cations in data plotting.

4. Discussion

Plasma clearance after a single intravenous injection of
Y^{90}-DTPA prepared according to the method given in Sec. I
can be described as the sum of two exponential processes.
These processes are rapid equilibration of the material in
the body space it will eventually come to occupy and the
rapid excretion of the material from the body via the
urine. Soon after intravenous injection of the Y^{90}-DTPA
activity appears in the lymph and rapidly reaches levels
of activity comparable to those in plasma. This rapid and
nearly complete excretion of the material from the body is
advantageous in that it permits rapid removal of the
isotope from the body after the irradiating procedure is
terminated. However, this also makes it difficult to
achieve the constant levels of activity for a prescribed
time period that are necessary for accurate dosimetry in an
irradiating technique.

C. <u>The Irradiation Procedure: Intravenous Recyling of</u>

<u>Urine for the Maintenance of Constant Levels of Y^{90}-</u>

<u>DTPA in the Body for Prescribed Time Periods</u>

1. <u>Introduction</u>

It is apparent from considerations of the rapid

removal of Y^{90}-DTPA from the body via the urine (as

described in Sec. III B of this report) that, in order

to achieve a constant level of radioactivity in the tissues

during an irradiating procedure, one must have a constant

infusion of the Y^{90}-DTPA into the body at a rate equal to

its rate of excretion. Calculations indicated that this

would require extraordinarily large quantities of Y^{90}-DTPA.

in order for effective quantities of radiation to be

delivered. It would also require continual assaying of the

urine and blood during the procedure to determine the amount

of activity in the body at a given time. Such a procedure

should be applicable in man, but did not appear to lend

itself to ready performance in the large number of animal

experiments necessary to establish the values of the

technique.

The problem of infusing the material at a rate exactly

equal to its rate of excretion appeared formidable, but

an audacious yet simple solution made itself obvious. If

we could continuously reinfuse intravenously the Y^{90}-DTPA

excreted in the urine as it was excreted, the rate of infusion

would by necessity equal the rate of excretion, and a constant

level of radioactivity in the tissues of the body could
be obtained. This seemingly unprecedented concept, upon
further evaluation, indeed had some precedent. Zondek
and Black gave large amounts of urine parenterally to mice,
rats, and rabbits and noted no toxic effects (55). In
their article reference is also made to benign effects of
urine administered intramuscularly in humans. Many reports
attest to the innocuity of urea; indeed, at present
intravenous urea infusions are used as a diuretic in con-
trolling ascites in humans (56). Although the kidney is
the critical organ in controlling fluid and electrolyte and
acid-base balance, it is probable that the body could
compensate for a brief disruption of renal function. This
gains support from the clinical observations in anuric
patients, who show few clinical signs of illness during
the first few days of anuria (57).

It thus seemed plausible that disruption of normal
urinary excretion by intravenous urine recycling would
probably be well tolerated for short periods of time.
It was then hoped that continuous intravenous infusion
of urine would indeed produce constant levels of radioactivity
in the body during the recycling procedure.

2. Materials and Methods

Figure 4 is a schematic representation of the urine-
intravenous recycling system. The dog's urethra is catheter-
ized with a No. 8 or a No. 10 dog urethral catheter. This
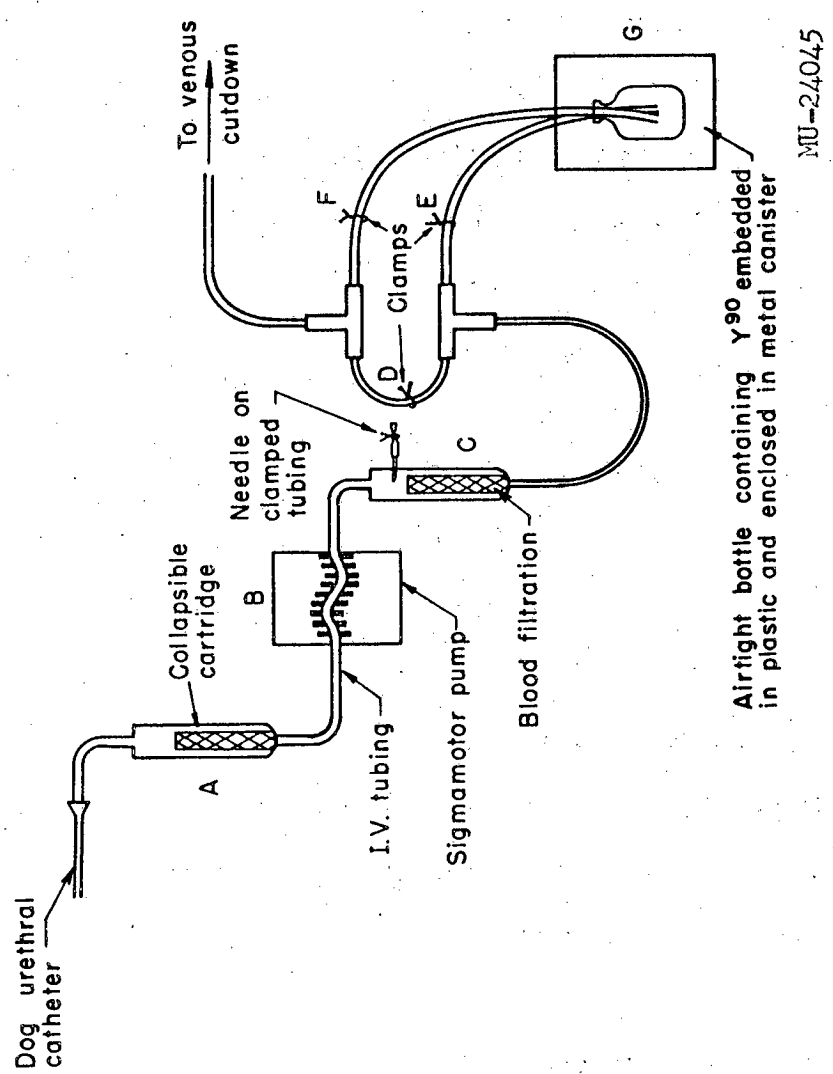
Fig. 4. Schematic representation of urine intravenous recycling system.

catheter has numerous openings along the distal 5 inches of
its length to allow for drainage of the bladder from
alternative openings in the event that the main apertures
become occluded during the procedure. The urine then
flows through a blood filtration cartridge, A, by virtue
of decreased intraluminal pressures created by the milking
action of the fingers of a sigmamotor pump, B. The urine
is then pumped through a second blood filtration cartridge,
C, and from there passes into an intravenous indwelling
polyethelene tube in the animal's right forepaw. Irradiation
is begun by placing Clamp D over the tubing and opening
clamps E and F leading to the specially designed Y^{90}-DTPA
container, thereby forcing the urine to be pumped through
container G and thus washing the radioactivity into the vein.

The construction of container G, along with the details
of its use, are depicted in Fig. 5. The container is made
from a 2-oz jar with four pieces of polyethylene tubing
extending into the jar through holes drilled in the lid.
Three of these pieces of polyethylene tubing extend to the
bottom of the jar, the fourth just protrudes beneath the
surface of the lid. An airtight seal around the pieces of
polyethylene tubing and lid of the jar is effected by a
polyepoxy resin which is poured into the upper portion of
the jar during its preparation. The jar is placed in a
close-fitting plastic container, which is then placed in a
brass container to assure adequate shielding.

End in extraction box

Clamp

Stainless steel tube

To evacuating syringe

Thick polyethylene tubing

Lid with holes in top

Epoxy resin seal of tubes and lid of jar

3-oz jar

40-ml cone in box containing measured amount Y^{90}-DTPA

To afferent limb of recycling system

To efferent limb of recycling system

Heat sealed

Heat sealed
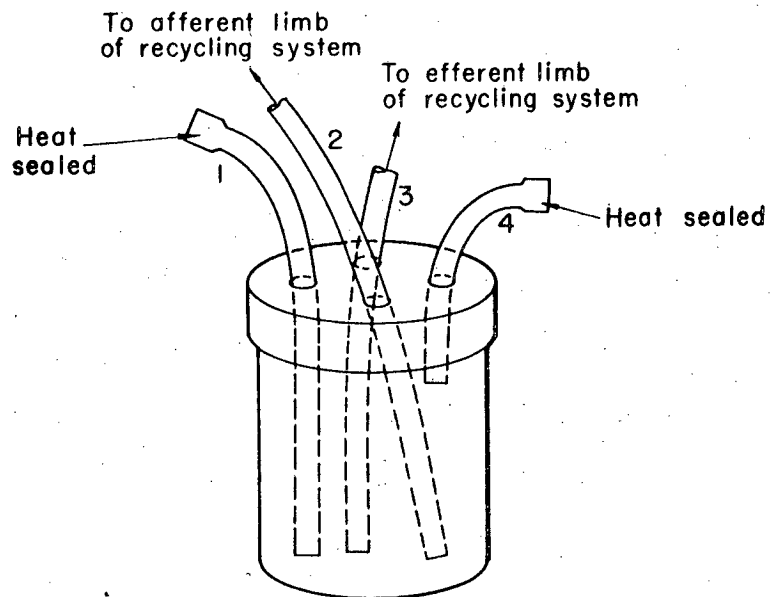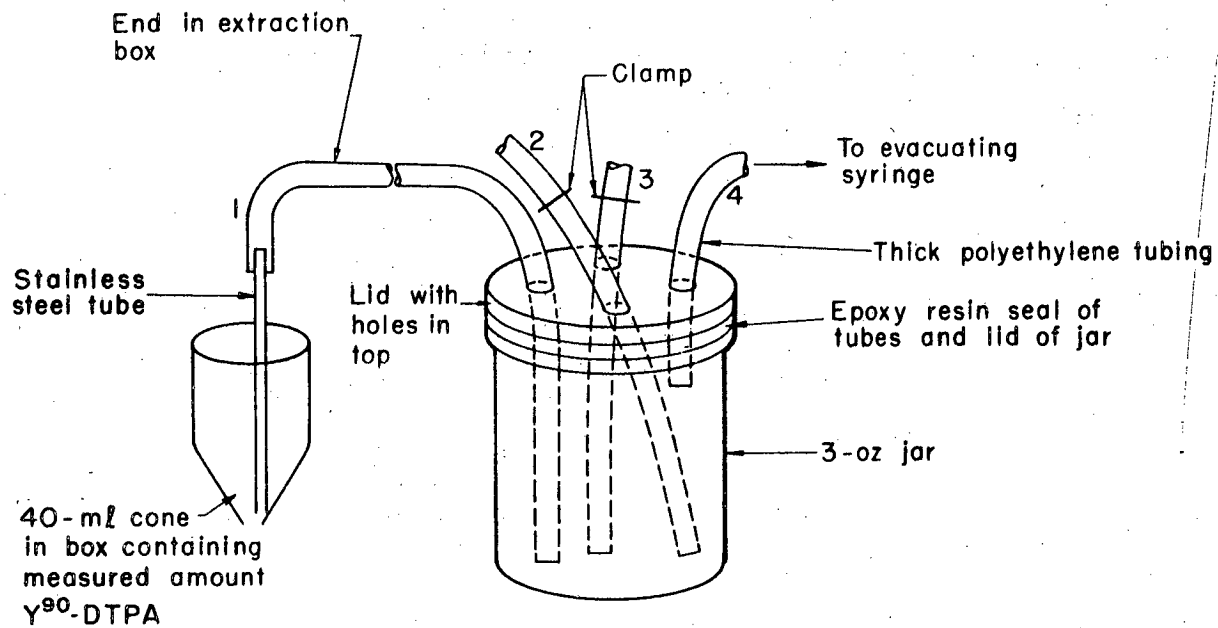
MUB-737

Fig. 5. Y^{90} -DTPA container.

The Y^{90}-DTPA is prepared as described in Sec. III A
and is placed in a 40-ml cone inside the inner chamber of
the extraction box. The jar with its shielding is placed in
the antechamber of the extracting box described in Sec. III A. 3,
and the tubing (1) in Fig. 5, fitted with a piece of stainless
steel tubing, is passed through the door connecting the two
chambers into the inner chamber where it is placed into the
bottom of the cone containing the Y^{90}-DTPA. Line 4 (Fig. 5)
is fitted to a syringe, and upon withdrawal of the plunger of
the syringe one jar is evacuated and the DTPA is drawn into
the jar. Once the isotope is within the inner jar lines
No. 1 and No. 4 are sealed. The container is sealed and
transported to the area where the animal is to be irradiated,
where lines 2 and 3 are attached to the recycling tubing
unit just distal to clamps E and F (Fig. 4).

Consideration of the rapid clearance of Y^{90}-DTPA, its
excretion in the urine, and subsequent high concentrations
of radioactivity found in the urine led us to induce a
diuresis during the irradiating procedure. Since the
rate of excretion of the DTPA is independent of the volume
of urine produced, we felt that an increase in the volume of
urine production would effectively decrease the concentration
of Y^{90}-DTPA in this fluid and consequently decrease the dose
of radiation to those tissues intimately associated with
urine production and excretion. To achieve the diuresis,

agents such as intravenous mercuhydrin, urea, and dextrose
infusions were used.   It was soon discovered that
intravenous mercuhydrin could be deleted from the regimen
without seriously effecting the diuresis as long as the
water and osmotic diuresis provided by the urea and dextrose
solutions were optimal. All experiments described in this
section were performed using a 500-ml intravenous infusion
of 30 g urea in 5% dextrose and 0.2% NaCl solution to
initiate a diuresis before the administration of the Y^{90}-
DTPA. Urine outputs of 7 to 15 ml/min were obtained by
using this procedure throughout the time of the recycling
phase.

Purebred male beagle dogs 6 months to 2 years old
were used in all experiments. Experimental animals were
kept under light nembutal anesthesia during the entire
process.

During the recycling procedure the animal is placed in
a specially modified Berkeley box, Fig. 6. He is suspended on
a plastic hammock with his legs protruding through holes in the
plastic.

The recycling of the urine containing the Y^{90}-DTPA is
continued for 6-hr, after which the recycling is stopped
and the excreted urine is collected in a large jug for 2hr.
Following this the animal is placed in a special holding
cage, Fig. 7, where he is allowed to freely excrete the
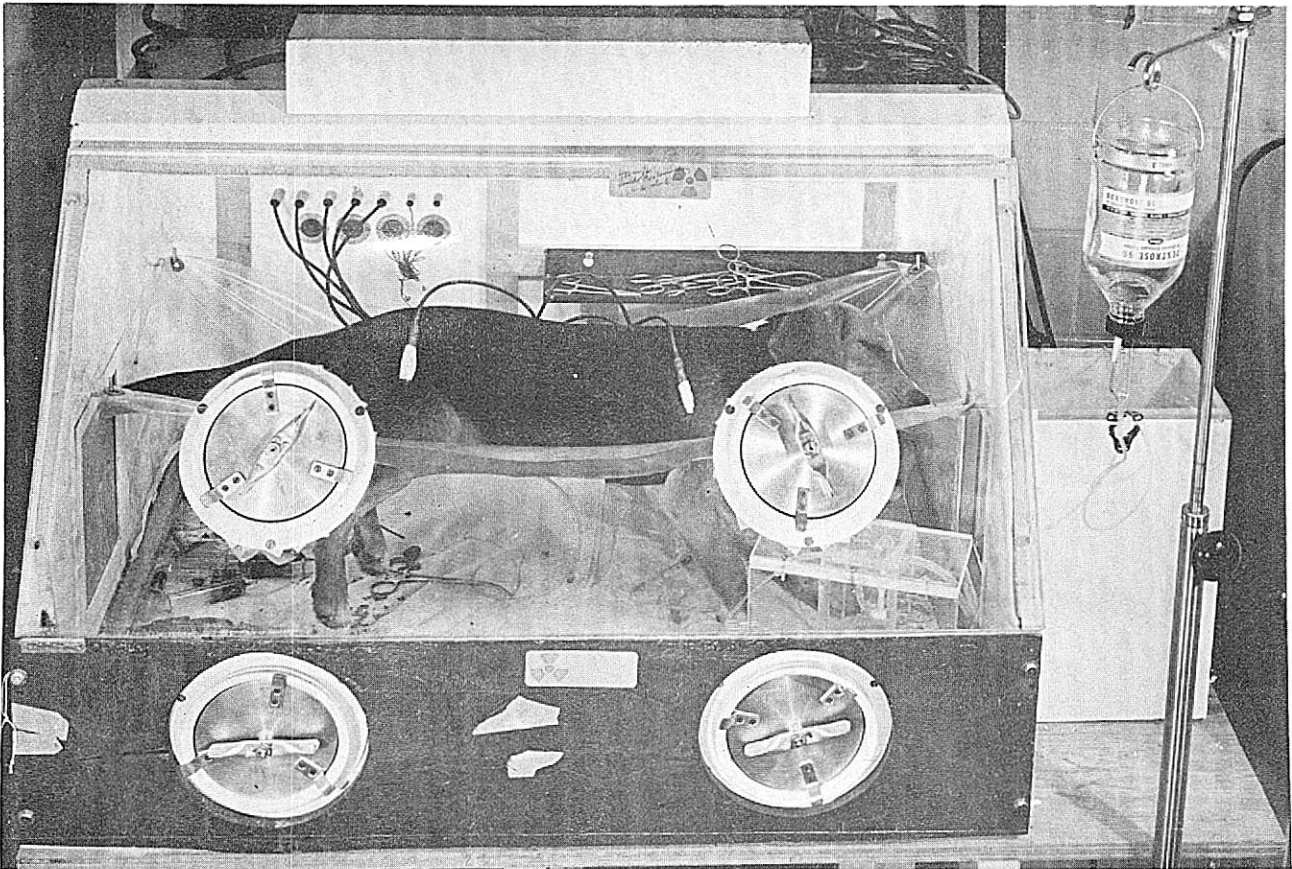
ZN-2990

Fig. 6. Dog-holding cave for irradiation procedure.
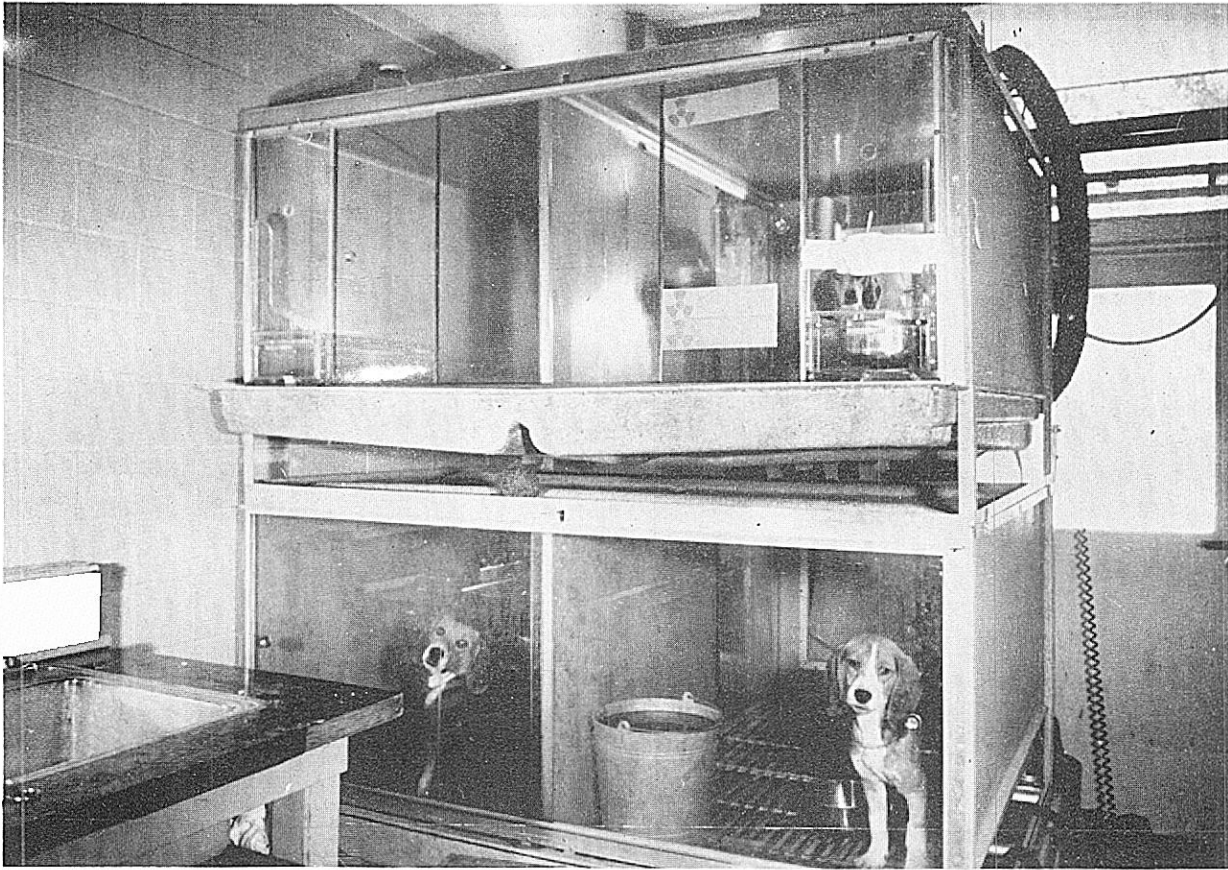
ZN-2989

Fig. 7. Cages modified for Y^{90} DTPA dogs.

radioactivity remaining in his body after the end of the procedure. (These fiberglass cages were designed by Dr. A. C. Andersen of the University of California, Davis, and manufactured by the Kirschner Manufacturing Company, of Vashon, Washington. We have modified them to suit our purposes.) The front of each cage unit has been fitted with a feeding tray. The tray containing the food and water pans can be pulled forward and the food and water can be added without entering the contaminated inner cage. The cages are periodically washed with a high-pressure hose and detergent, and the liquid waste from the individual cages is drained into a 100-gallon metal container beneath the cage. (The accumulated radioactive waste in this large container is weekly pumped into large double-walled 40-gallon drums and the liquid waste is made into concrete and disposed of in a routine manner.)

3. Discussion of Results

Figure 8 shows a result obtained from experiments performed in the manner outlined above. The figure represents the relationship of plasma activity with time, during and after the recycling procedure. The plasma activity level varied somewhat around a constant level during the recycling period, and in this experiment the recycling was stopped at 6-1/2 hours. It is obvious that the urine recycling
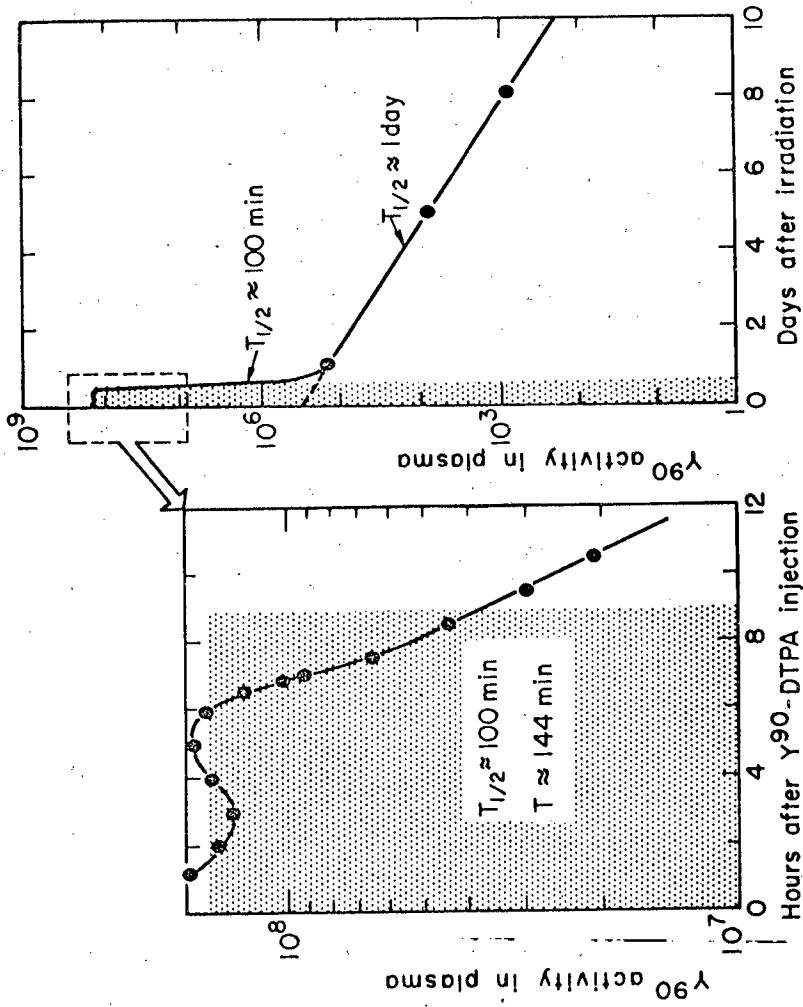
MU-24043

Fig. 8.   Plasma activity as a function of time during and following recycling.

does provide for the maintenance of high levels of activity
in the body for a specified time. After recycling is
stopped, the Y^{90}-DTPA is cleared from the body during the
first 24-hr postirradiation period as an exponential
function with a half time in this experiment of approximately
100 min. After the first day there is a break in the curve
and the small amount of remaining activity is cleared from
the plasma at a much slower rate. The urinary clearance
of the Y^{90}-DTPA after the termination of urine recycling
is reasonably constant in the different beagle dogs used in
this procedure. In 12 dogs, the mean clearance constant
was $\lambda = 0.00808$ min^{-1} with a standard deviation of 0.00233 min^{-1}.
We can thus say that the equation $N_t = N_0 e^{-\lambda t}$ accurately
describes the clearance of the Y^{90} clearance from the body
of the dog during the first 24 hours (N_t = total amount of
radioactivity remaining in the body of the dog at t hours
after cessation of the urine recycling, N_0 = total amount
of radioactivity in the body at the instant the urine
recycling is discontinued, $\lambda = 0.00808$ min^{-1}, t = time in
min). Thus, the radioactivity remaining after 24 hr in the
body of the animals if this rate of clearance remains steady
during the first 24 hr is <0.005%. This result is encouraging
in view of the desireability of minimizing the level of
residual radioactivity in the body present 24 hr after the
radiation procedure.

Table I. Urinary excretion constants for Y^{90}-DTPA following intravenous urine recycling

Dog number	Time (t) of urine collection (min)	Portion of given dose in urine at t (%)	Urine excretion constant, λ (min^{-1})
X-43	120	55.9	.00683
X-50	120	61.8	.00800
X-48	120	60.2	.00767
2 A	110	52.3	.00673
X-49	135	67.4	.00830
W-8	135	76.2	.01067
W-4	120	80.2	.01350
W-9	135	58.2	.00644
R-10	135	51.5	.00533
R-9	135	51.5	.00533
W-7	135	70.3	.00896
R-12	120	67.0	.00925

X_{λ} = .00808 min^{-1}

$S_{x\lambda}$ = .00233 min^{-1}

To date 48 dogs have been irradiated by this procedure. All dogs receiving sublethal levels survived the procedure itself. The first 24 hr after the recycling procedure, the dogs are somewhat hypoactive, owing to the prolonged nembutal anesthesia. However, even during this early period they usually eat and drink well. In subsequent days, the animals appear and act perfectly normal. The oldest dog in this series has been exposed to two urine-recycling procedures with low levels of Y^{90}-DTPA, 15 months and 6 months prior to this writing. He appears normal in all respects at this time.

The use of the equipment and procedures described under Materials and Methods has kept personnel radiation exposure well below the maximum permissible occupational dose allowed by the Atomic Energy Commission. When 200 to 500 mC of Y^{90} is being given to the animal, only 5 to 6 mr/hr is noted at the surface of the lucite front of the irradiating cave. When this amount of activity is being given to the animal, 1 to 2 r/hr is noted at the portholes of the cave when the porthole covers are removed. At the end of the urinary recycling period and after the dog's urine has been collected inside the irradiating box for 2 hr, the dog has 3 to 6 r/hr coming from his body surface. By 24 hr after the irradiating procedure the animal has so thoroughly excreted the activity that approximately

50 to 200 mr/hr is noted at his body surface (much of this represents contamination of the animal's fur). On the second and third days postirradiation, the radiation at the body surface of the dog is usually 10 to 25 mr/hr. Usually by 2 weeks postirradiation, the dog shows no evidence of contamination with radioactive material, and can be treated as a nonradioactive animal if desired.

4. Conclusions

A procedure has been described which achieves relatively constant high levels of activity in the body during a specified period of time with subsequent single exponential clearance of more than 99% of the material from the plasma during the first 24 hr. These features provide a relatively simple basis for dosimetry estimation. The low levels of radioactivity remaining in the body one day after irradiation should not be sufficient to damage tissues transplanted after this time. The urinary-intravenous recycling procedure is benign and appears to produce no clinically evident acute or chronic malfunction.

D. Tissue Distribution Patterns of Y^{90}-DTPA During Intravenous Urine Recycling: Dosimetry Considerations

1. Introduction

In the preceding section a characteristic curve of activity in plasma during and after the urine recycling

procedure was presented (Fig. 8). Since the ordinate of this curve represents counts per unit time per ml of plasma, and the abscissa represents time, it is obvious that the area under the curve obtained is a measure of the total number of "counts" or beta particles released in a ml of plasma. Since the average energy of the Y^{90} beta particles is known (0.90 Mev) (49), we can determine the energy of all the beta particles released in a ml of plasma. If we assume that equilibrium conditions prevail, then the number of beta particles losing their energy in the ml of plasma is equivalent to the number of beta particles originating in the same ml of plasma. The amount of energy released in the unit volume of plasma in question equals the product of the total number of beta particles released and the average _____ of each beta particle:

$$E_{rel} = \left[\int_{t=0}^{t=\infty} a(Y^{90}) dt \right] \left[\bar{E}_{\beta Y90} \right],$$

where

$\underline{E_{rel}}$ = energy released in a unit volume of plasma,

$\underline{a(Y^{90})}$ = disintegrations per unit time due to Y^{90} in unit volume of plasma

$\underline{\bar{E}_{\beta Y90}}$ = average energy of $Y^{90}\beta$.

The value of $\int_{t=0}^{t=\infty} a(Y90) dt$ can be estimated from Fig. 6 as follows. During the time of recycling (0 time to the end

of recycling) the area under the curve equals the average activity (in disintegrations per unit time) in the blood during this time, times the time of recycling. After recycling has been discontinued the curve is essentially a simple exponential for the next 24 hr, after which only negligible levels of Y^{90} are left in the body. [Blood activity levels at the end of 24 hr are less than 1/1000 of their values during their recycling phase. The maximum number of beta particles discharged in the plasma by the residual Y^{90} after the first 24 hr represents less than 0.08% of the total number of beta particles discharged during the first 24 hr, assuming, at worst, no further excretion of Y^{90} (i.e., the remaining Y^{90} decaying by its physical half life of 64.4 hr). Thus, more than 99% of the dose is administered during the first 24 hr after recycling.] Since the clearance of Y^{90} from the body during the first 24 hr is essentially a simple exponential function, we can estimate the number of beta particles released in the body after the recycling procedure is complete by multiplying the mean excretory time (T) by the blood activity levels at the end of the recycling phase (corresponding to the beginning of the excretory phase):

$$\int_{t=end\ of\ re-cycling}^{t\sim\infty} a(Y^{90})dt \quad \left[T\right] \left[a(Y^{90})o\right],$$

where:

T = mean excretory time (in min),

$a(Y^{90})o$ = plasma activity at end of recycling (in dpm).

Since the disintegrations/min per ml of plasma at the beginning of the excretory phase is approximately equal to the average disintegrations/min per ml of plasma during the recycling phase, the equation for the total number of particles emitted per ml of plasma becomes

$$\int_{t=0}^{t} a(Y^{90})dt = \int_{t=0}^{t \,=\, \text{end of recycling}} a(Y^{90})dt + \int_{t=\text{end of recycling}}^{t} a(Y^{90}) \, dt$$

$$= \left[a(\overline{Y^{90}}) \right]\left[T_{rec} \right] + \left[a(\overline{Y^{90}}) \right]\left[T \right] ,$$

where

$a(\overline{Y^{90}})$ = average plasma activity during recycling (in dpm),

T_{rec} = time of urine recycling.

This is equal to the shaded retangular area shown in Fig. 8. The total amount of energy released in a ml of plasma, then, is

$$E_{rel} = \left[a(\overline{Y^{90}}) \right]\left[T_{rec} + T \right]\left[\overline{E_{\beta Y^{90}}} \right]$$

This expression may be used to calculate the total energy released per ml of plasma (and assuming equilibrium state, the amount of energy absorbed in a ml of plasma), when the recycling procedure described in the preceding section is used.

If there were a constant ratio of activity in a given tissue to the activity in plasma, we could then determine the amount of energy released per gram of tissue by formulating

a simple ratio

$$E_T = (R)_t (E_{rel}),$$

where:

E_t = energy released in a gram of a given tissue,

R_t = ratio of activity in a gram of tissue to activity in 1 ml of plasma,

E_{rel} = energy released in 1 ml of plasma.

Thus, if the ratio of activity in 1 gram of tissue to the activity in 1 ml of plasma, the average level of plasma activity, the time of recycling, and the mean clearance time were known in a given experiment, we could estimate dose to the individual tissues of the body. In addition, if there were a constant relationship between the average plasma levels of activity achieved and the total amount of injected material, we could further estimate dose on the basis of the amount of activity injected, without having determined specific plasma levels in a given experiment.

Close control of the dose administered can therefore be obtained by initially performing the irradiation procedure, using tracer levels of Y^{90}-DTPA on each animal (or man) and determining the level obtained in the blood when a given dose is administered and the mean excretory time (T) for the individual animal or man. The dose could then be controlled by modifying the recycling time or the total amount of activity injected.

In order to simplify dosimetry in dogs, we have developed a standardized radiation procedure. Urine recycling is maintained for 6 hr on all dogs. Since the rate of urinary excretion of the Y^{90}-DTPA after recycling was fairly constant in a series of dogs studied (see Table I), the value for the mean excretion time was taken as 124 min ($\tau = \frac{1}{\lambda} = \frac{1}{0.0080}$) in the dosimetry calculations for all dogs. The total number of beta particles released in a cubic centimeter of plasma then becomes equal to the product of the mean activity during recycling (in disintegrations per min) and the mean effective radiation time (i.e., $T_{rec} + \tau = 484$ min). Variations in dosage to tissues then can be effected in this system by modifying the dose of Y^{90}-DTPA administered.

The following work was performed to evaluate the cricital parameters listed above, in order to provide a basis for tissue dosimetry with the radiating procedure described in the preceding section.

2. Materials and Methods

Purebred male beagles were irradiated by the procedure outlined in the preceding section in a manner analogous to that to be used for the proposed supralethal irradiation experiments, with the exception that tracer levels of Y^{90}-DTPA were employed. The animals were killed, after specified

periods of urine recycling, with a large dose of nembutal given intravenously. Death was almost instantaneous. The animals were autopsied immediately. The animals' tissues were sampled in triplicate and placed in stainless steel radioassay planchettes. Weight of individual tissues were determined by subtracting the tare weights from weights of the planchettes with tissue. The tissues were then wet ashed by adding fuming nitric acid to the planchettes and slowly heating under an infrared lamp for approximately 12 hr. The planchettes containing the ashed tissues were then counted in a Nuclear-Chicago gas-flow counter with micromil window. Absolute assays of the Y^{90} were performed as described in the foregoing sections.

3. Results

The various tissues in 10 dogs were assayed for Y^{90} activity after 3 to 4 hr of urine recycling of Y^{90}-DTPA. Table II lists the average ratio of activity per gram of tissue to activity per ml of plasma in the number of experiments noted. Next to the average values for the tissues in question are listed standard deviations of the individual tissue values from the mean. Dogs done in a different series and sacrificed after 6 to 8 hr of urine recycling gave results substantially the same as those given above, indicating that the distribution pattern seen in these dogs is a stable one and remains essentially unchanged during the recycling phase.

Table II. Ratio of Y^{90}-DTPA activity in tissue to plasma (cpm/gm: cpm/ml) during urinary recycling and dosimetry estimates.

Tissue	Number of experiments performed	Mean ratio of activity in tissue to plasma (cpm/gm: cpm;ml)	Standard deviation of mean ratio	Estimated radiation dose in tissue per μC/ml of plasma (rads ± 1 S.D.)
Lymph node	10	0.489	0.068	7.56 ± 1.05
Thymus	2	0.323	--	4.99 ± --
Spleen[a]	7	0.260	0.097	>4.02 ± 1.50
Bone marrow	6	0.281	0.061	<4.34 ± 0.94
Surface of bone marrow cavity				~ 2.17+
Gastric mucosa	3	0.534	0.128	8.26 ± 1.98
Ileum	7	0.331	0.112	5.12 ± 1.73
Colon	3	0.463	0.130	7.16 ± 2.01
Salivary glands	4	0.299	0.114	4.62 ± 1.76
Pancreas	4	0.194	0.028	3.00 ± 0.43
Liver	7	0.295	0.084	4.56 ± 1.30
Bile	4	0.257	0.207	3.97 ± 3.20
Feces	4	1.013	0.926	15.66 ± 14.32

Table II -- continued

Renal cortex	7	1.888	0.692	29.19 ± 10.70
Renal medulla	7	1.161	0.250	17.95 ± 3.87
Bladder wall	4	0.957	0.384	14.80 ± 5.94
Urine	7	4.028	2.490	62.27 ± 38.49
Lung	7	0.641	0.117	9.91 ± 5.94
Heart	7	0.253	0.076	3.91 ± 1.17
Adrenal	4	0.334	0.051	5.16 ± 0.79
Thyroid	4	0.328	0.083	5.07 ± 1.28
Spinal cord	7	0.024	0.009	0.37 ± 0.14
Spinal fluid	6	0.034	0.008	0.53 ± 0.12
Bone	3	0.102	0.010	1.58 ± 0.15
Tracheal cartilage	4	0.495	0.085	7.65 ± 1.31
Skin	7	0.531	0.094	8.21 ± 1.45
Fat	7	0.240	0.176	3.71 ± 2.72
Skeletal muscle[a]	7	0.191	0.072	2.95 ± 1.11
Testis	4	0.156	0.077	2.41 ± 1.19
Aqueous humor	4	0.069	0.013	1.07 ± 0.20

[a] Assayed after blood drained from pulp; thus dosimetry represents a lower limit because of the loss of Y^{90}-DTPA-rich plasma prior to radioassay.

The last column in Table II represents an estimation
of the radiation dosage delivered to the various tissues
for each μC of Y^{90}-DTPA found in a ml of plasma during the
recycling procedure. The calculation assumes that an
equilibrium state exists in each tissue, an assumption
that is only approximate in tissues having small dimensions.
It is obtained as follows: For each μC/ml in plasma 15.5
rads is given up per ml of plasma during the entire
irradiation procedure $\left[2.22 \times 10^6 \text{dpm}/\mu\text{C} \times 484 \text{ min (effective} \right.$
radiation time$) \times 0.90$ Mev/disintegration $\times 1.6 \times$
$\left. 10^{-6} \text{ erg/Mev} \times 10^{-2} \text{ rad/erg} \right]$. The estimates of rads
given up per gram of tissue, then, is this number times the
experimentally determined ratio of activity per gram of
tissue to activity per ml of plasma.

The first group of tissues listed in Table II are
those associated with lymphopoiesis and hematopoiesis.
Because of the large body of evidence indicating that
lymphatic structures and primarily lymph nodes contain the
cells responsible for the tissue hemograft rejection response,
we were interested in delivering high doses of radiation to
these tissues (34, 35, 36, 37, 38). The lymph nodes showed
high levels of activity in all experiments, and the estimated
radiation dose delivered to these structures ranked high
among the various tissues of the body. The spleen probably
receives a dosage in excess of that shown in the table,
because of the variable amount of blood found in this organ

when the dog is alive. The dosage estimate given for the
spleen is a minimal one because the tissue was assayed
after the blood had been allowed to drain from the pulp.
The estimation of dosimetry to bone marrow by an internal
beta emitter posses many problems. In the other soft
tissues of the body, cells at the surface of the organ
are being bombarded by beta particles arising from the
body of the organ as well as beta particles arising from the
adjacent soft tissues and connective tissues. Thus, for
example, the cells at the surface of a lymph node adjacent
to skin would receive a dosage intermediate between that
of the equilibrium dose in skin and the equilibrium dose in
the center of the lymph node. In the bone marrow the cells
on the outer boundary of the marrow adjacent to the bone
receive a flux of beta particles from only one side, i.e.,
from the marrow cavity. The bone itself contains little
Y^{90}-DTPA, and it effectively shields out the beta particles
arising in the soft tissues on the outside of the bone. We
may roughly estimate the dosage to marrow cells lying on the
bony cylinder surrounding the marrow cavity as approximately
1/2 the equilibrium value in the center of the cavity, i.e.,
1/2 of 4.34 rads/μC per ml of plasma, or 2.17 rads/μC per
ml of plasma. The presence of bone trabeculae in the marrow
cavity further complicates estimates of radiation dose to the
marrow.

The next group of tissues is representative of the gastrointestinal tract. Initial experiments measuring the level of Y^{90}-DTPA in the ilium indicated that the gastrointestinal tract would receive approximately 1/3 less radiation than the lymph nodes. However, further experiments demonstrated that certain tissues of the gastrointestinal tract contained a selectively higher concentration of the Y^{90}-DTPA than did the ilium. Specifically, the gastric mucosa near the pylorus received somewhat higher and the colon slightly lower dosages than lymph nodes. In addition, certain stool samples were found to contain higher concentration of Y^{90}-DTPA than the plasma. These findings indicated that the GI mucosa may actively secrete a small amount of Y^{90}-DTPA above and beyond that appearing in the stools by passive diffusion during the DTPA chelates' equilibration in the extracellular body fluids. However, this result may simply reflect selective water resorbtion by the colon with subsequent concentration of the unresorbed Y^{90}-DTPA. The salivary glands, pancreas, and liver did not show any remarkable concentration of the radiating material.

As was expected, the tissues of the urinary system received high radiation exposures. The renal cortex contained higher concentrations of the Y^{90}-DTPA than did

the renal medulla, suggesting that the material may be actively secreted by the proximal or distal tubules. The concentration of isotope in the urine was quite variable because of the varying diuresis obtained in each dog and the subsequent variation in renal concentration.

It was interesting that the lung receives a significantly high radiation dosage. The desirability or undesirability of this effect is difficult to assess. The lungs are a reticuloendothelial bed of a sort and are known to phagocytize inhaled particulate matter and to trap infused leukocytes that had been manipulated in vitro. Their role in the complex process of antibody formation is difficult to evaluate in humans but they have been shown to be a significant source of antibody in the rabbit (59). Irradiation of lung tissue may be desirable in preparation for bone marrow homotransplantation.

The heart receives a relatively low radiation dose.

Both the adrenal and the thyroid glands receive a significant but not unusually high radiation dose.

It has already been reported (46) that DTPA is not found in the cerebrospinal fluid after a single injection of this chelate. Even with the sustained blood levels of the Y^{90}-DTPA complex obtained in these experiments, the chelated yttrium is not found in appreciable amounts in

the cerebrospinal fluid or the substance of the spinal cord.
We may conclude that the blood-brain barrier represents an
efficient though probably incomplete deterrent to the
passage of Y^{90}-DTPA.

Bone is found to contain small quantities of the Y^{90}-
DTPA after 3 to 4 hr of sustained levels in the blood.
Tracheal cartilage contains a significant level of
activity under these conditions. These findings probably
reflect the differences in the rate of equilibration of
extracellular materials in the extracellular water of
cartilage and bone (60).

Skin contains reasonably high levels of the chelate
complex, probably related to its extracellular fluid
content, although some specific mechanism for holding the
material may be operative. Fat and skeletal muscle are low
in activity. It is significant that the testes receive low
radiation exposures in this procedure. Aqueous humor also
has low concentrations, probably reflecting some inability
of this material to pass by the cells of the anterial uveal
tract.

4. Discussion

In general, we may conclude that the distribution of the
Y^{90}-DTPA is complex. Although it generally may be thought of
as having an extracellular fluid distribution, many tissues

contain concentrations of this material that cannot be explained simply on the basis of their extracellular fluid content. The lymph nodes, representing one of the primary targets for the radiation, do indeed have large concentrations of the chelate complex. The urinary system receives high doses of radiation but since it is relatively radio-resistant this is not prohibitive. The present evidence suggests that the human kidney can receive up to 2300 r before lasting damage becomes obvious (61, 62, 63, 64, 65, 66, 67, 68). The bone marrow, because of its encasement in bone, receives relatively low radiation exposures. However, the role of the erythroblasts, myeloblasts, and megakaryocytes in antibody production are questionable. Other elements such as lymphocytes, plasma cells, mononuclear cells and reticulum cells, found in the bone marrow to varying degrees may be significant in the homograft-rejection response. Central nervous system elements, muscle, and fat receive notably low levels of radiation. If the majority of the gut receives radiation exposures similar to that calculated for the ilium, then we may say that target organs such as the lymph nodes receive significantly higher dosage upon use of this procedure than does the gut. However, the higher concentrations seen in the gastric mucosa and colon cloud the issue. It thus becomes difficult to evaluate the effects of this procedure on the gut from these dosimetry calculations alone.

We can estimate the dosage to various tissues in a given experiment simply by multiplying the values obtained from the last column of Table II by the average blood levels obtained during the 6-hr recycling period. However, the performance of multiple blood sampling in the large number of experiments needed to analyze the parameters of this procedure represents a formidable task. It would be convenient to be able to estimate dosimetry to various tissues solely on the basis of dosage of Y^{90}-DTPA administered to the animal. Table III is an analysis of the average plasma concentration of isotope obtained during the 6-hr recycling period in eleven dogs receiving various dosages per unit of body weight. On the bottom of this chart we see that an average plasma level of 5.543 μC per ml of plasma is obtained for each mC administered per pound of body weight. The standard deviation of this mean is $S = \pm 1.807$ μC.

Thus, we may estimate radiation dosage to various tissues by accepting that 5.543 μC per ml of plasma will be the average plasma concentration achieved for each mC of Y^{90}-DTPA administered per pound of body weight, and then use this figure in the previously described equations to get the dosage to the tissue in question. Such calculations involve two main errors. The first is the variability of the plasma levels achieved when a given dose of activity is given to the animal. The second is the variability of the ratio of

Table III. Relationship of blood plasma levels of Y^{90}-DTPA to administered dose.

| Dog no. | Dose of Y^{90} administered | | Average blood plasma level per mC/lb during recycling (μC/ml plasma) |
	Total amount injected (mC)	mC/lb body wt injected	
X-49	254.0	14.11	5.763
W-8	303.4	12.91	1.448 *
W-4	339.0	12.33	8.301
W-5	440.0	14.92	5.295
W-9	305.5	13.58	6.646
R-10	246.9	14.52	4.384
R-9	230.8	12.12	7.686
R-6	226.4	14.15	4.657
W-7	578.0	24.08	5.345
R-5	328.5	13.14	5.483
R-12	342.4	14.27	5.961

Over-all average blood level of Y^{90} obtained by administration of 1 mC/lb body wt $= 5.543 \times 10^{-3}$ mC/ml plasma $\pm$ S $= 1.807$ mC/ml plasma

* This low value represents inadequate urine recycling in this particular experiment, however, this figure was included in the calculations to avoid introducing the bias of the investigator.

the total activity in a gram of tissue to the total
activity in a ml of plasma. Obviously, the first-mentioned
variable is eliminated when multiple blood sampling is
performed during the procedure.

5. Conclusions

Distribution patterns of the Y^{90}-DTPA, after maintenance
of constant levels of activity in the body by urine recycling,
have been described. The basic pattern of distribution of
the chelated Y^{90} in the body is somewhat variable.

Methods for estimating dosimetry to various organs using
the Y^{90}-DTPA distribution data have been presented. These
estimates of dosimetry are best when based on observed blood
activity levels during recycling. A more convenient but
less exact estimation can be made on the basis of dose
administered per unit body weight. The second method, is
advantageous when dosimetry in a large series of experiments
is being considered. At best, however, both methods are
approximations.

E. **High-Level Irradiation of Dogs with Yttrium-90-DTPA**

   Intravenous Urine Recycling procedure: Definition

   of the Sublethal and Lethal Dose Ranges and

   Analysis of Radiation Effects

   1. Introduction

The radiation procedure described in the foregoing

sections was unique, and directly comparable data were

not available for evaluation of the sublethal and lethal

dosage ranges and the radiation pathology encountered with

this technique. It was necessary to determine the dosage

levels in given tissues that would result in death of

the organism and the pattern of pathologic changes that

would be encountered at various dosage levels.

It was our hope that this technique would provide for

virtual destruction of the immune systems of the body with

sufficient selectivity that the animal would not die from

irreparable damage to other organ systems. Specifically,

we hoped to achieve high radiation dosage in the lymphatic

structures but to relatively spare such nontarget organs as

the mucosa of the gastrointestinal tract. Our attention

was focused on these lymphatic tissues because of the

large mass of evidence pointing to these structures -- in

particular the lymphocyte -- as responsible for the homograft

injection response (34, 35, 36, 37, 38).

In order to acquaint ourselves with the sublethal and

lethal levels of radiation as well as the pathologic changes

occurring at these dosage levels, we performed experiments using varying amounts of administered Y^{90}-DTPA. The animals' courses were followed clinically and hematologically. Gross pathologic and histologic changes were studied in the animals that succumbed during the experiment.

2. Materials and Methods

The radiation procedure employed was the same as described in foregoing parts, except that larger doses of Y^{90} DTPA were administered. All animals were purebred male beagles between 6 months and 2 years of age. All animals were given daily injections of penicillin and streptomycin (600,000 units penicillin, 3/4 g streptomycin) during the first 4 weeks following irradiation. For hisotlogic studies tissues were fixed in Bouin's solution and embedded in paraffin or celloidan. Hematoxylin and eosin, Pollack's trichrome, and methylene blue stains were used. Platelets were counted in duplicate with phase-contrast microscopy. Blood creatinines were determined by using the technique of Folin and Wu (69).

3. Experimental Results

Table IV summarizes the clinical and pathological results obtained in eight dogs given sublethal total-body radiation with Y^{90}-DTPA. Figures 9 and 10 summarize respectively the body-weight and hematologic changes seen in these animals.

Table IV. Summary for sublethally irradiated dogs surviving more than 6 weeks.

Dog number	Dose administered		Estimated dose to lymph nodes (rads)	Clinical history	Survival status	Gross pathology	Histological pathology
	Total mC	mC/lb body wt					
180603	90.7	3.63	152	Appeared normal throughout observation period.	>2 mo		
2B1a-II	95.0	4.24	178	Appeared normal throughout observation period.	>6 mo		
Snapper	108.0	4.24	178	Appeared normal throughout observation period.	>6 mo		
100	68.3	4.49	188	Hypoactive and moderately anorexic during first week after radiation.	>6 mo		
203	69.96	4.66	195	Moderately dehydrated and hypoactive on 5th day, bloody mucoid diarrhea and emesis on 6th day. Appeared normal from 11th day until preterminally when he became markedly dehydrated and hypoactive.	Died at 51 days	Markedly emaciated and dehydrated, melena in small bowel but not colon, bloody material in stomach. Some lymph nodes appeared fibrotic.	Lymph nodes from neck, mediastinum, mesentery all remarkably hypoplastic with cellularity <20%. Spleen shrunken ∼30% cellularity
3-D[c]	108.8	4.96	208	Appeared normal throughout observation period.	>6 mo		
2-B	139.9	6.50	<272[a]	Appeared normal throughout observation period.	>2 mo		
W-6	223.0	13.94	b	Appeared normal throughout observation period.	>2 mo		

a) Urine recycling inadequate, dosage to lymph nodes probably significantly less than 272 rads.
b) Accident during urine recycling precluded dosimetry estimations.
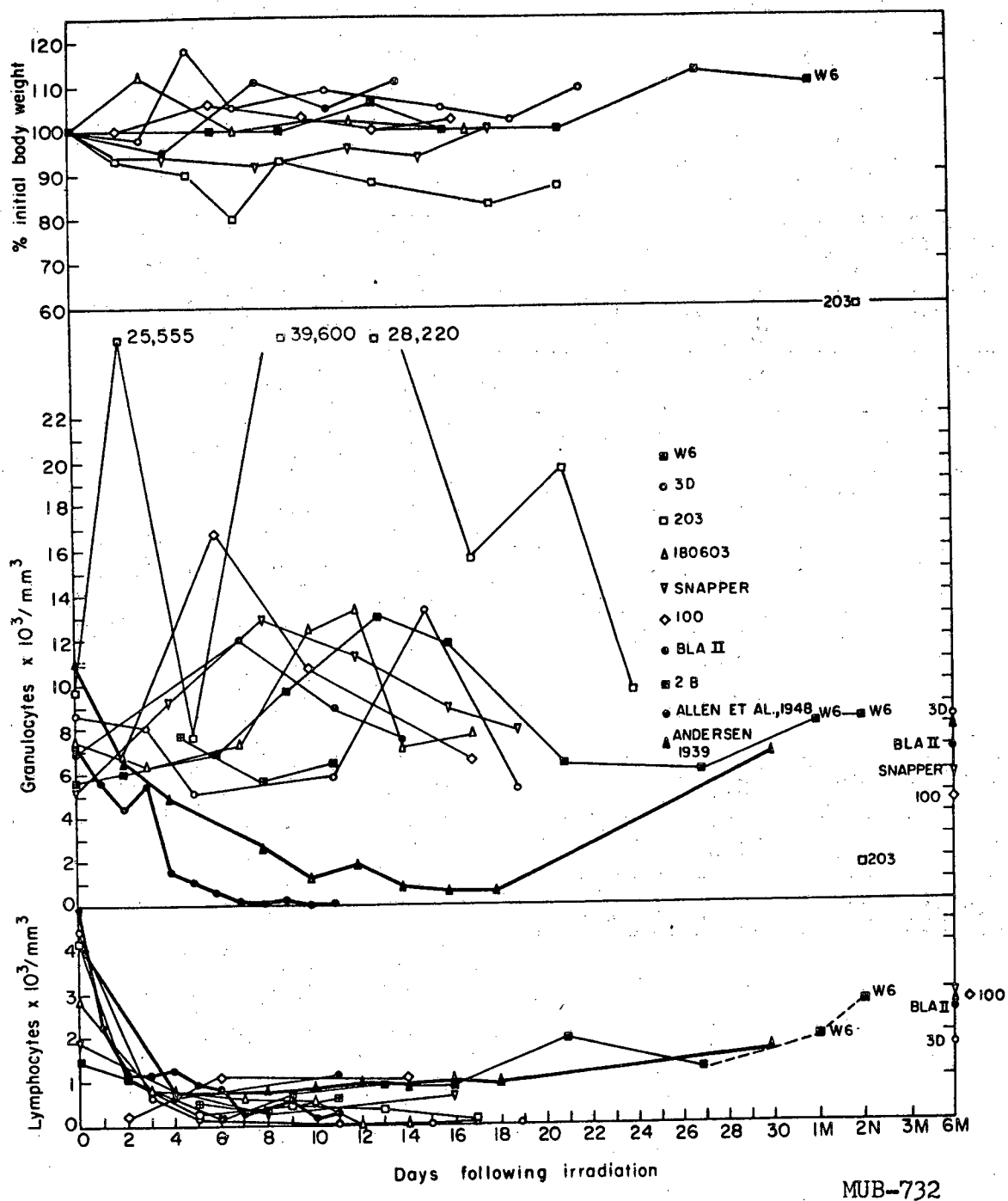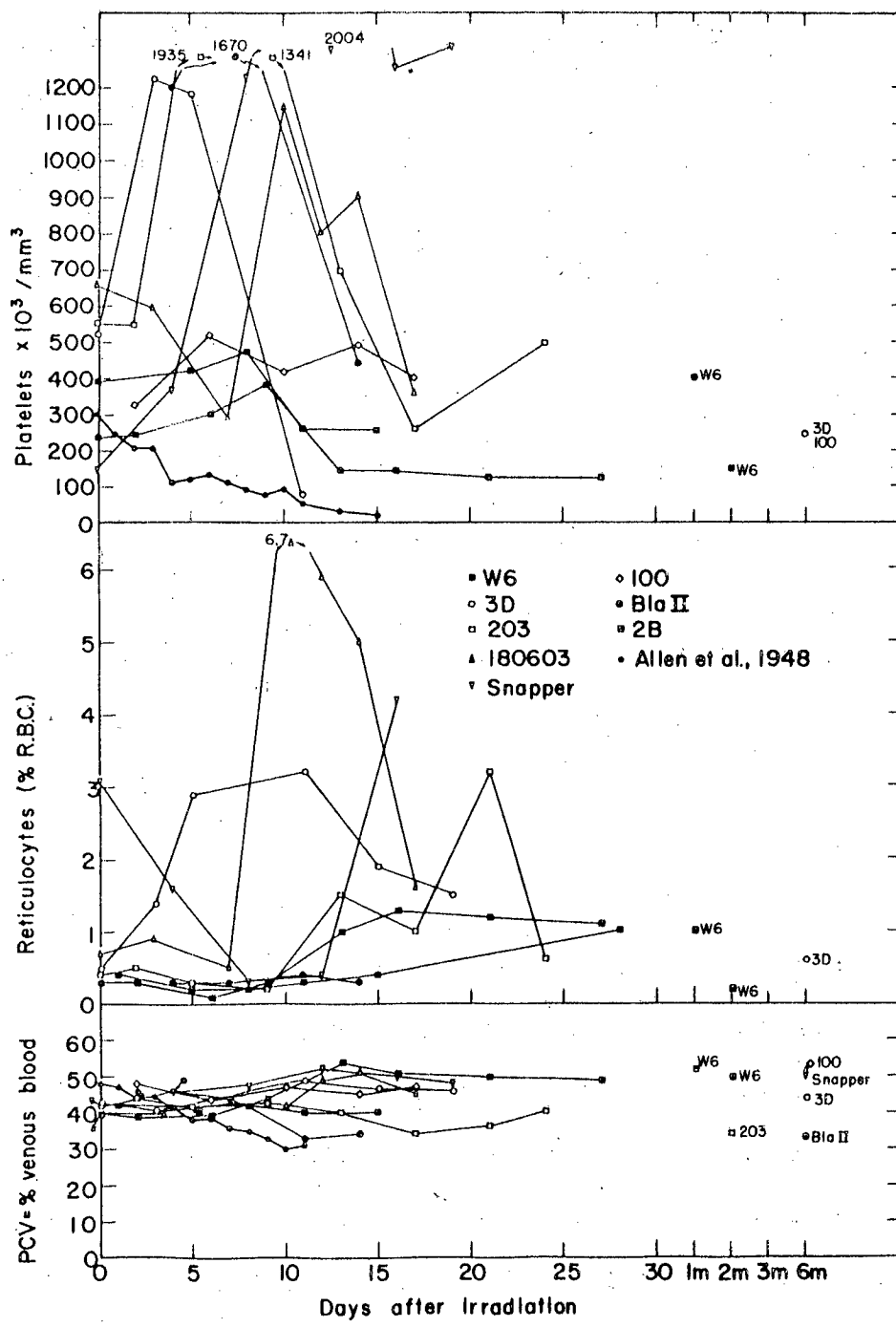c) Had been previously irradiated sublethally - 15 mo prior to this writing.

Fig. 9. Changes in body weight, lymphocytes, and granulocytes in sublethally irradiated dogs.

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Fig. 10. Changes in platelets, reticulocytes, and packed cell volume in sublethally irradiated dogs.

The first three dogs listed in Table IV received estimated
dosage to lymph nodes of 152 to 178 rads (see previous
section for method of dosimetry estimation). Clinically,
they appeared normal and showed no significant weight loss
during the observation period. Hematologically, the only
dramatic change seen was marked depression of the lymphocytes.
Dog No. 100 received an estimated 188 rads to the lymph
nodes and had an unremarkable course other than being
moderately anorexic and hypoactive during the first radiation
week. He showed no weight loss, and lymphopenia was the
only remarkable postirradiation finding. Dog No. 203
received an estimated 195 rads to the lymph nodes and was
the only animal in this series that showed any unequivocal
clinical effects resulting from the radiation. He became
hypoactive and anorexic and showed weight loss at the end
of the first week. Bloody mucoid diarrhea and emesis were
noted at this time. These signs subsided by the end of the
second week and the animal appeared relatively normal though
somewhat thin until a week before his demise, at which
time he became increasingly anorexic and hypoactive. During
the last three weeks of his life, the animal received no
special care and was allowed to run free in the dog colony,
where distemper was endemic. The animal died 51 days after
radiation and the post mortem examination revealed hypo-
plasia of both lymph nodes and spleen.

Dog No. 3-D received an estimated 208 rads to the lymph nodes and, similarly to the first three dogs in this table, appeared normal throughout the observation period. Hematologically, only a lymphopenia was remarkable. This dog had received a course of sublethal radiation by the same technique 6 months previously. At the time of this writing he has survived more than a year since the first radiation exposure, and more than 6 months since the second radiation exposure. Dogs 2-B and W-6 both received higher administered doses (in terms of millicuries per pound) than did the other dogs in this series. But both had difficulties during the urine recycling procedure that precluded tissue dosimetry estimations. Neither of these dogs showed clinical or hematological abnormalities other than lymphopenia. It is noteworthy that the clinical course of the dogs in this series was quite benign; only two of the eight animals studied showed any symptoms or signs of radiation damage. Only one dog (Dog No. 203, who subsequently died) showed any significant weight loss. No dramatic changes in the packed cell volume were noted. The granulocytes, platelets and reticulocytes showed no significant fall, and in several cases there seemed to be a postirradiation rise in their values. Thus the decrease in the number of circulating lymphocytes was the only significant change noted in these animals after radiation. This finding is in keeping with the

dosimetry estimations in the previous section and supports the notion that this procedure results in an effective degree of selective irradiation of lymphatic structures.

In Figs. 9 and 10 the magnitude of the hematologic changes seen by using this procedure are compared to those obtained in dogs after exposure to supervoltage X-rays by Allen (70) and Andersen (32). Allen gave 25 mongrel dogs 450 r from a 200-kv X-ray machine (a dosage which in his hands gave 100% mortality); Andersen gave a large series of purebred beagle dogs (comparable to those used in these experiments) a midline dose of 300 r from a 250-kv X-ray machine. The dose of 300 r represented an LD 60/30 (60% lethality in 30 days) in his experiments. He had 14 dogs which survived the acute radiation syndrome and made hematopoietic recovery. The averaged hematologic values for these 14 survivors given 300 r and for the 25 mongrels given 450 r are plotted on the graphs in Figs. 9 and 10.as heavy lines. It is apparent that the dogs in our series receiving internally administered Y^{90}-DTPA had lymphocyte depressions generally greater than that seen in the dogs given 300 r, and roughly paralleling the depressions seen in the dogs given 450 r. As has been noted, the animals receiving Y^{90}-DTPA had estimated dosages to lymph nodes generally between 150 and 210 rads. It would thus appear that biologically our estimated rad dosage to lymph nodes is

roughly equivalent to more than twice its value in exposure dose (expressed in roentgens) to 200 to 250-kv X-rays. It is possible that some of this discrepancy can be accounted for by noting that many lymph nodes in the body are shielded from external radiation by bony structures (e.g., submaxillary and submandibular lymph nodes). It is probable that these shielded lymph nodes receive significantly lower doses of radiation than that calculated for "midline structures." These protected nodes could probably repopulate the empty lymphatic spaces in the body following radiation; similar phenomena are known to occur with shielding of spleen, bone marrow, and Peyer's Patches.(71, 72). When Y^{90}-DTPA is internally administered all the lymph nodes of the body are rather uniformly radiated (see previous section of this work). It is interesting that the dogs of Allen and Andersen show profound decrease in granulocytes and platelets which after a few days parallels the decrease lymphocytes. Granulocytopenia and thrombocytopenia were not seen in the dogs given Y^{90}-DTPA in this series.

Table V summarizes the clinical and pathologic findings in the dogs receiving lethal radiation with Y^{90}-DTPA and dying more than 10 days after the radiation. Figures 11 and 12 summarize the changes in body weight and blood elements respectively of these dogs. In general the clinical course of most of these animals was characterized by weight loss

Table V. Summary of lethally irradiated dogs surviving more than 10 days

Dog number	Dose administered Total mC	Dose administered mC/lb body wt	Estimated dose to lymph nodes (rads)	Clinical history	Time of death after radiation (days)	Gross pathology	Histological pathology
Brutus	338.6	8.91	373	Normal first 8 days. Last 3 days anorexic, excessive drooling, hypoactive.	11	Hemorrhagic diathesis with purpuric lungs, bladder, G-I bleeding, hemorrhagic lymph nodes, spleen, bloody pleural effusion (about 400 ml).	Lymph nodes and spleen markedly hypoplastic, focal necrosis and loss of kupfer cells in liver, some disruption of edges of mucosa in small bowel, bone marrow aplastic, damaged testis.
490	265.1	10.00	419	Normal first 8 to 9 days, moderate weight loss last 4 days. Active until day of death.	13	Hemorrhage into basal portion of lung -- complete post mortem examination not performed.	
David	315.5	13.50	566	Fifth day anorexic and bloody, mucoid stools. Refused food for last 5 days of life.	12	Hemorrhage in lungs, and colon, hemorrhagic lymph nodes and spleen.	Spleen hypoplastic, bone marrow aplastic, liver areas of focal necrosis. Mucosa of G-I tract intact, some submucosal hemorrhage in colon.
Joe	306.0	14.20	595	Occult blood appeared in stool 5th day and continued for about 1 month; drooling after 12th day. Pen. and strep. discontinued on 46th day, following which animal's condition slowly deteriorated.	61	Lymph nodes small and scarce, roundworms in G-I lumen; scattered scars on kidney -- general loss of subcutaneous tissue.	Hemosiderin in bone marrow, liver. Lymph nodes fibrotic, polymorphonuclear leukocyte infiltration in lymph nodes seen. Lungs show hemorrhage, edema, bronchopneumonia and interstitial pneumonitis.
X-43	291	15.3	641	Hypoactive and anorexic from 2nd day, bloody diarrhea beginning 5th day. Progressive dehydration.	11	Death from dehydration and G-I bleeding -- complete post mortem examination not done.	
M I	-	-	786[a]	Hypoactive from 5th day. Melena from day 6, frank red blood in stools from day 8, anorexic from day 15.	24	Small pale lymph nodes and spleen, blood in distal small bowel and colon, petechiae in bladder.	Cellularity of spleen and lymph nodes near normal, germinal centers present. Liver shows focal areas of necrosis with inflammatory cell infiltrate.
2-A	365.5	19.24	806	Hypoactive and anorexic from 11th day. Melena preterminally.	14	Hemorrhagic lymph nodes and bone marrow and lungs, petechiae in spleen, heart, pericardium and bladder, dark blood in colon, hematuria.	Marked hypoplasia and fibrinoid degeneration of lymph nodes, spleen and bone marrow, hemorrhage in kidneys, bladder, lung.
R-14	517	19.51	1015[b]	Moderately hypoactive from 9th day. Anorexic preterminally.	12	Lymph nodes hemorrhagic, lungs and bladder purpuric, petechiae in skin, renal cortex, pleura, peritoneum in pelvis, stomach and colon. Mucosal ulcers in duodenum, hematuria.	Lymph nodes and spleen hemorrhagic and markedly hypocellular. Lungs hyperemic and edematous, bone marrow acellular. Marked damage to glomerulae and tubules of kidneys. Partial slough of G-I mucosa.

a) Dosimetry on the basis of blood levels of Y^{90} activity during recycling.
b) Recycling inadequate, dosage to lymph nodes an undetermined amount less than 1015 rads.
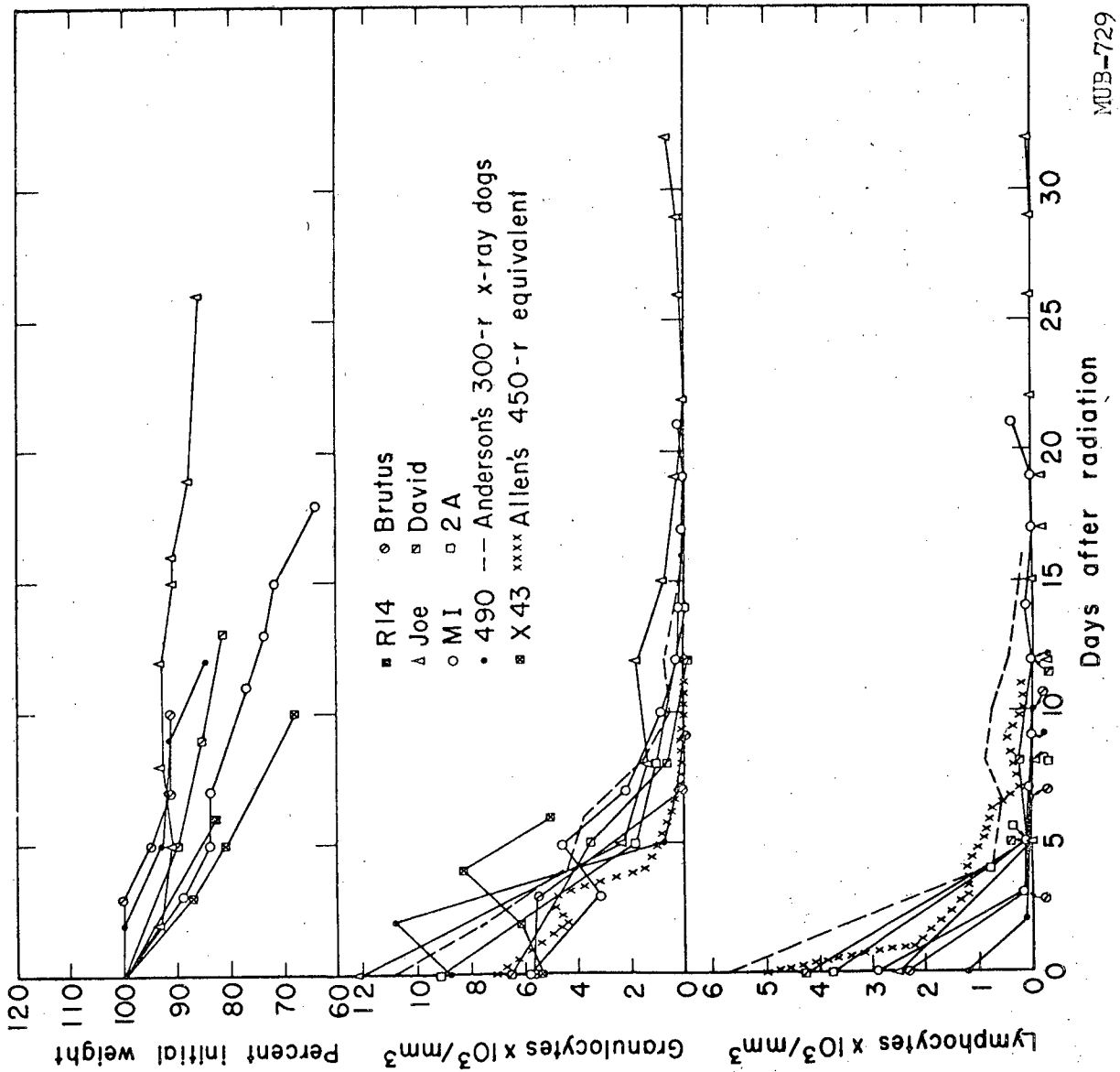
Fig. 11. Changes in weight, granulocytes, and lymphocytes in lethally irradiated dogs dying after the tenth postirradiation day.
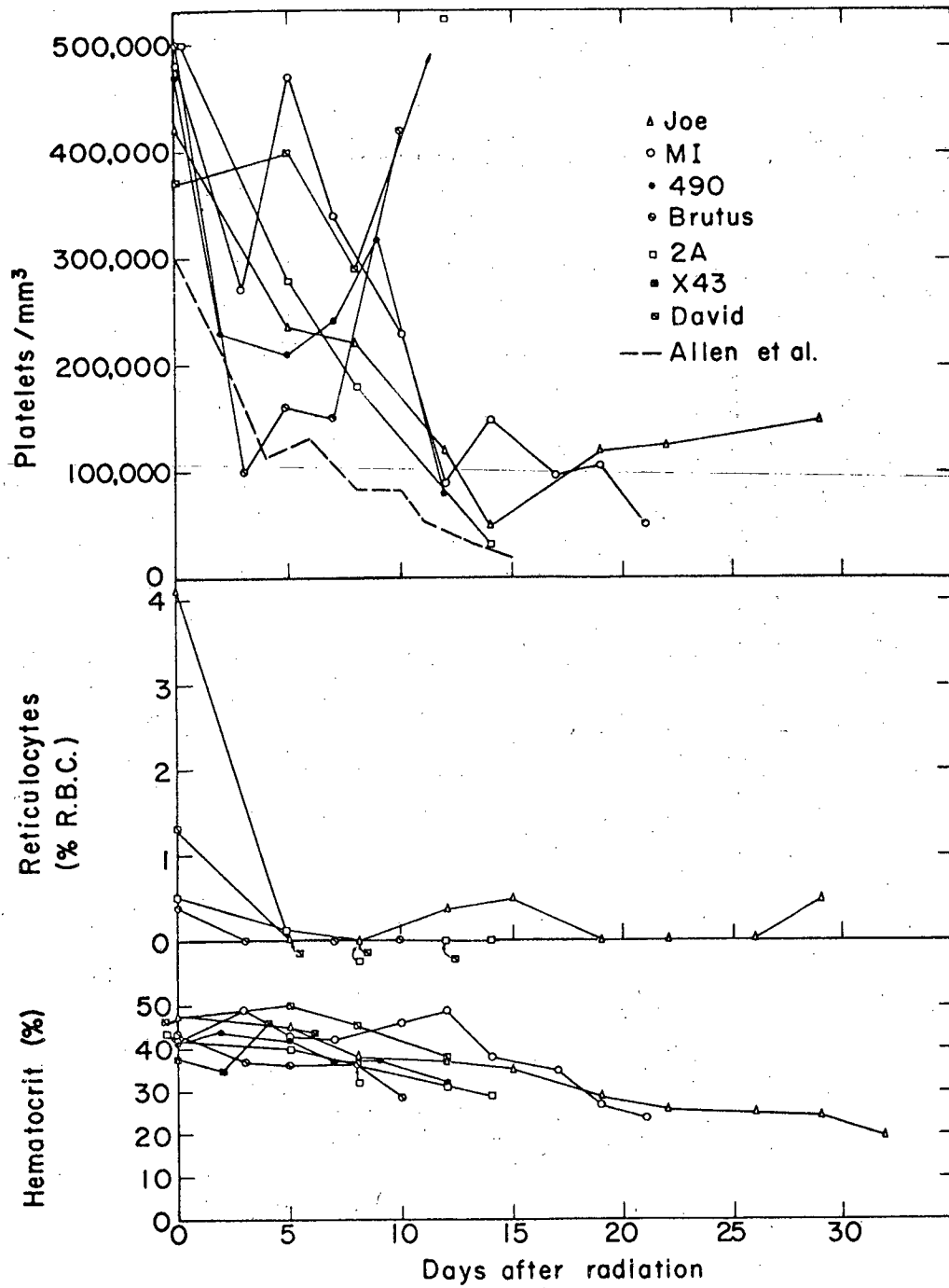
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Fig. 12. Changes in platelets, reticulocytes, and hematocrit in lethally
irradiated dogs dying after the tenth postirradiation day.

without other symptoms during the first week after
irradiation. Towards the end of the first week and beginning
of the second week the animals became hypoactive and anorexic,
showed continuing weight loss, and frequently had emesis and
bloody diarrhea. Six of the animals died between the 11th
and 14th day. One lived for 24 days and one for 61 days
after the irradiation. Dog Joe, the 61-day survivor, presents
an interesting clinical study. During the first 2 weeks his
clinical course was similar to those of the other members
of this series. However, despite having less than 500
granulocytes from the 19th to the 29th day, and almost no
lymphocytes in his peripheral blood during this time, the
animal survived and on about the 30th day after irradiation
began to show hematopoietic recovery. Between the 30th
and 35th day there was a marked rise in peripheral granulocytes,
reticulocytes, and platelets. The lymphocytes, however,
did not return and even on the 50th post irradiation day
no lymphocytes were seen in the peripheral smear. Shortly
after the 30th day the condition of the animal improved and
by the 40th day he appeared relatively normal though quite
thin. Antibiotics were discontinued on the 46th day.
During the next 2 weeks the animal's condition slowly
deteriorated, and he died on the 61st day. Post mortem
histology showed no signs of regeneration either in lymph nodes
or spleen. Gross and microscopic pathology indicated that

bronchopneumonia and interstitial pneumonitis were probably the causes of death. Figure 13 depicts the serial hematologic findings in this dog. This experiment demonstrates the profound damage to lymphatic structures associated with the use of Y^{90}-DTPA. This marked damage to lymphatic structures was seen in all of the other dogs in this series except for Dog M-1, who died on the 24th day. On the 21st day this animal showed some reappearance of lymphocytes in the peripheral blood and had a count of 338 lymphcytes/mm^3 of blood at that time. At post mortem study Dog M-1 did show good evidence of regeneration of lymphocytes in lymph nodes and spleen. The most characteristic gross post mortem findings in the dogs in this series were hemorrhagic pneumonias and gastrointestinal bleeding associated with submucosal petechiae in the pyloric end of the stomach and in the colon. These findings correlate with the increased concentration of Y^{90}-DTPA in these sites described in the preceding section. In distinction from the dogs in the sublethal series, each of the dogs in this lethal group showed a steady weight loss beginning immediately after the irradiating procedure (see Fig. 11).

The hematologic data of these dogs depicted in Fig. 12 follow the same pattern as that in the sublethal control series previously presented. The data of Allen's 25 mongrel dogs given 450 r of 200-kv X-rays and a series of 20 beagle dogs succumbing to 300 r total body radiation from 250-kv X-ray
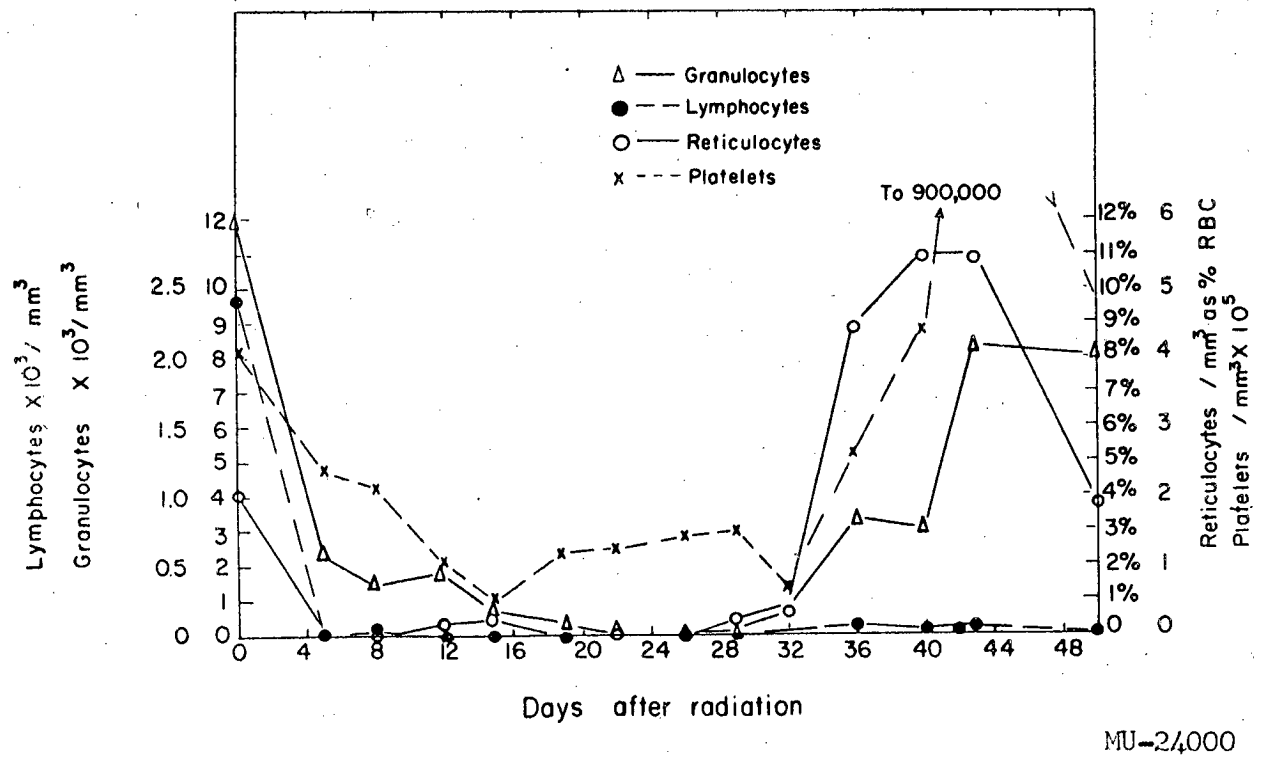
Fig. 13. Hematologic changes in Dog Joe following Y^{90} DTPA irradiation.

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machines (32, 70) are also plotted for comparison. It
is apparent that all the dogs in this series had much more
profound lymphopenias than that seen in either the 300-r
or the 450-r X-ray dogs. In connection with this severe
lymphopenia it is of interest to note that a subcutaneous
implant of homologous skin was found to be viable and
showed marked proliferation in dog Brutus at the time of his
death 11 days after the subcutaneous skin inoculation. The
granulocytes, on the other hand, generally dropped at a rate
comparable to that seen in dogs dying from a 300-r X-ray
exposure. The reticulocytes showed an early severe depression
but the platelets seemed much less affected than either the
granulocytes or reticulocytes. In general there was a much
slower drop in platelets than granulocytes, and the absolute
platelet levels never became profoundly depressed. Two
dogs (2-A and M-1) showed a preterminal drop in platelet levels
below 50,000/mm^3. This relative absence of severe thrombo-
cytopenia in the face of the fatal hemorrhagic diathesis
present is quite significant. Many publications have
stressed thrombocytopenia as the etiologic agent in post-
irradiation bleeding. However, as early as 1948 Allen et al.
(70) pointed out that the manifestations of abnormal bleeding
frequently appear before severe thrombocyte depressions are
seen following radiation exposure. Indeed, the role of
pure thrombocytopenia as the cause for abnormal bleeding in many
other conditions has been questioned by Craddock et al. (73)

and Winchell et al. (74). It was therefore of interest
when we noted that all the animals in this series had the
peculiar intravascular polysaccharide described by
Anderson (75). This material appears to be specific
following radiation and is found in greatest abundance in
the small vessels of the kidneys, liver, and heart. Its
possible role in the production of postirradiation bleeding
has been described (75, 76). The finding of this interesting
substance in the tissues of our dogs dying from the acute
radiation syndrome stimulated further investigation of this
material. (This work does not relate directly to the body
of this paper but is described in Appendix B.)

Table VI summarizes the clinical course and pathologic
findings in dogs given supralethal radiation and dying in
less than 6 days. Figure 14 represents the patterns of
weight and hematologic changes in these animals.

The clinical course of the animals in this series was
characterized by hypoactivity and anorexia appearing
immediately after the radiation procedure. Emesis and bloody
diarrhea appeared soon afterwards. As can be seen from the
rapid weight loss, these animals became severely dehydrated
owing primarily to their pernicious vomiting, and died in
acute fluid and electrolyte imbalance. Dogs Blue, No. 82,
and No. 234655 had hemoconcentration, as evidence by an
increase in venous hematocrit. Dog M-8 had evidence of a

Table VI. Summary of lethally irradiated dogs surviving less than 6 days

Dog number	Dose administered Total mC	Dose administered mC/lb body wt	Estimated dose to lymph nodes (rads)	Clinical history	Time of death after radiation (days)	Gross pathology	Histological pathology
M 8	346.0	15.73	<659[a]	Hypoactive, anorexic, emesis from first day.	3-1/2	Markedly dehydrated, purpuric areas in lung. Evidence of pulmonary infarct in right lung.	Hypoplasia and hemorrhage in spleen. Liver shows focal edema, necrosis, and RBC in sinusoids. Focal hemorrhagic pneumonitis. Bone marrow shows severe hypoplasia; kidney shows chronic glomerulonephrites, focal petechiae.
82	329.8	16.09	674	Lethargic, anorexic from day 1. Bloody diarrhea and emesis from day 3.	5	Markedly dehydrated. Petechiae in trachea, lungs, gastric and colonic mucosa.	Lumph nodes and spleen markedly hyperemic, appear to be about 30% of normal cellularity. Lungs congested, inflamatory infiltrate, much hemosiderin.
Blue	379.0	21.0	880	Lethargic, hypoactive; emesis from day 2. Bloody mucoid diarrhea day 4.	4	Markedly dehydrated. Lymph nodes moderately hemorrhagic, bone marrow gelatinous. Petechiae in bladder.	
234655	535.9	22.6	947	Hypoactive, anorexic, emesis from day 2. Bloody diarrhea day 4.	4	Markedly dehydrated. Coalescent petechiae in distal ileum and colon. Petechiae in trachea, bronchioles. Blood clots in bladder.	Lymph nodes hypoplastic, lungs show hemorrhage and congestion. Liver shows patchy areas of parenchymal necrosis. Mucosal necrosis in large intestine.
F 19			b	Hypoactive, anorexic; emesis from day 1.	4-1/2	Markedly dehydrated. Hemorrhagic lymph nodes. Petechiae in G-I tract from stomach to colon becoming confluent distally. Petechiae in kidneys, hematuria.	Spleen hypoplastic with an apparent 20% of normal cellularity Colon and small bowel show possible partial slough of mucosa. Lung shows hemorrhagic pneumonia.

a) Urine recycling stopped after 4 hr and dose to lymph nodes less than the calculated dose of 659 rads.
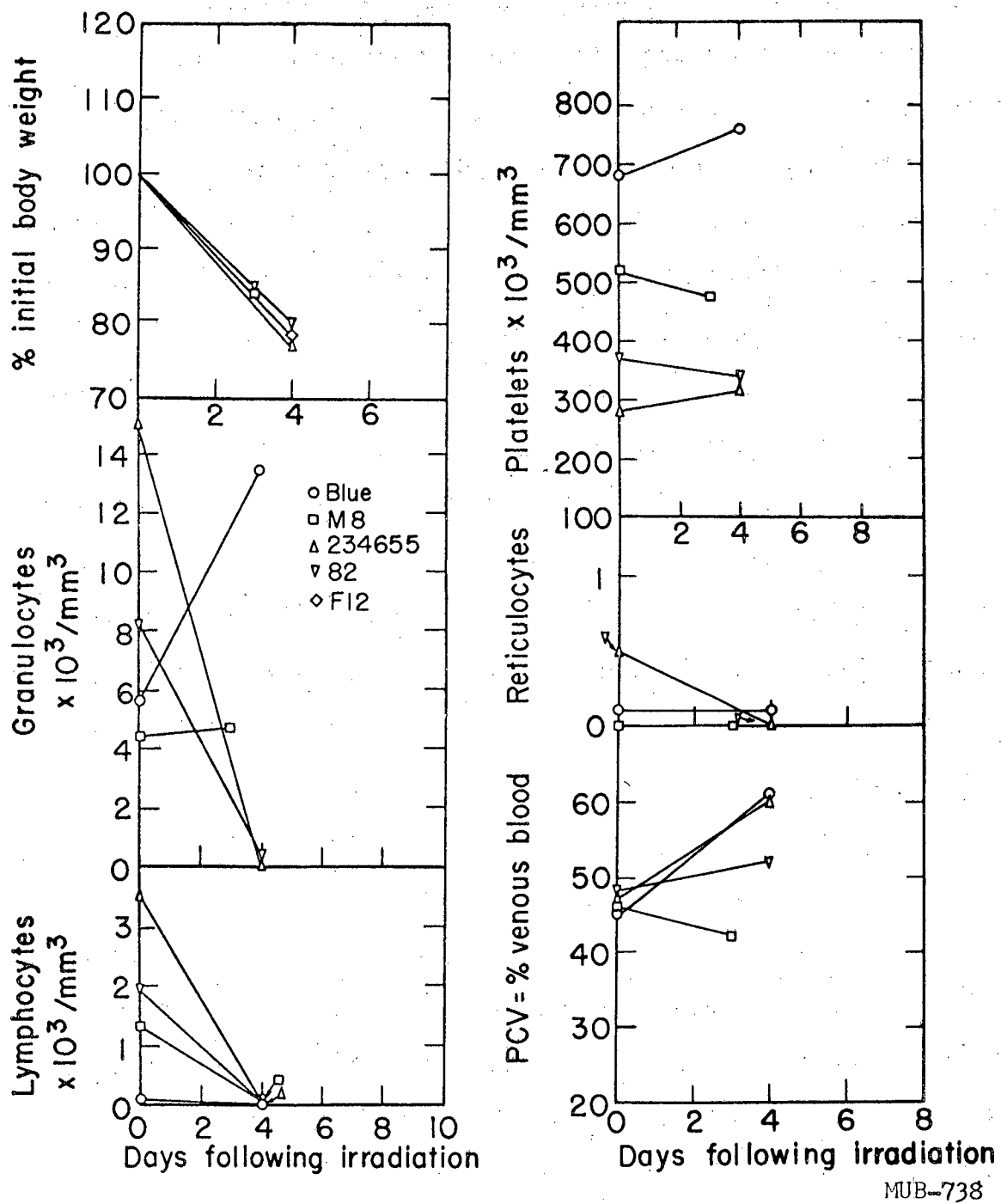b) Accident in urine recycling after 2-3/4 hr precluded dosimetry.

Fig. 14. Changes in body weight and hematologic parameters in lethally irradiated dogs surviving for less than six days.

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pulmonary infarct in his right lung and his death probably reflects one of the hazards of the urine-intravenous recycling procedure rather than a radiation effect.

The remaining dogs in this series seem to define a reasonable upper limit of tolerance to the irradiation procedure. Dogs Blue and No. 234655 both received more than 20 mC Y^{90} per lb body weight. Dog 2-4, in the "lethal-greater-than-10-days" series previously described, received 19.24 mC Y^{90}/lb and survived to die of a hematopoietic-type death on the 14th day. Yet Dog No. 82 in this present series received only 16.09 mC/lb and suffered the same fate of acute dehydration as the other dogs in the series who received higher doses. In general, however, it appears that the early acute syndrome of pernicious vomiting, with death in less than 5 days of acute dehydration and electrolyte imbalance, is seen when doses in excess of 20 mC/lb are administered.

Several factors may modify this upper limit of tolerance to the Y^{90}-DTPA irradiating procedure. First it would appear that the sustained high blood-urea levels induced in these animals to promote diuresis during the radiating procedure may significantly contribute to the dehydration, anorexia, and emesis seen immediately following this procedure (77). At present it appears that the upper level of tolerance to radiation when this method is used may be significantly extended merely by replacing the urea infusion by a water and saline infusion to promote diuresis. Secondly, it is possible that careful post-

irradiation fluid and electrolyte therapy may carry the dog over this early acute period so that he may survive to succumb to a "hematopoietic failure" type of death.

Post mortem examination of the animals in this "death -in-less-than-six days-series" uniformly showed marked dehydration, as well as petechiae and purpura mainly localized in the lungs, GI tract and bladder. Histologically their lymph nodes were hemorrhagic, and both their lymph nodes and spleen were hypoplastic. The degree of lymphatic hypoplasia seen was not as severe as that seen in dogs given lower dosages of radiation and dying more than a week later (see previous series). The colonic mucosa was generally the most affected portion of the gastrointestinal tract, and here partial mucosal slough was common.

The liver in Dogs M-8 and No. 234655 showed focal areas of parenchymal necrosis. Microscopic examination of the lungs confirmed the gross finding of hemorrhagic pneumonia.

The hematologic data are sparse because of the short survival. The lymphocytes uniformly dropped to near zero levels on the 4th day. The peripheral granulocytes were below $500/mm^3$ in dogs No. 234655 and No. 82 by the 4th day, but M-8 and Dog Blue actually showed increases in the concentration of peripheral granulocytes at this time. No significant changes of platelet concentration were seen by the 4th day. The packed cell volume on dogs Blue, No. 234655 and No. 82 rose by the 4th day, indicating hemoconcentration.

It is significant that despite the high level of radiation exposure suffered by the kidneys, no marked elevations in the blood creatinine were seen in any of the animals, either acutely in any of the series of dogs described or chronically in the long-term survivors in the sublethal group. The relative radioresistance of the kidney to radiation exposure is well known. Recently, however, there have been several studies on the late appearance of hypertensive disease and nephrosclerosis in animals whose kidneys had received significant acute radiation exposures. In man it would appear that nephrosclerosis is an inconstant finding after renal irradiation occurring after the kidney is exposed to more than 2300 r (61, 62, 63, 64, 65, 66, 67, 68). The only dogs in this series who received the equivalent of more than 2300 r to the kidneys were all well in the lethal range, hence no long-term survivors have been studied with respect to the incidence of this condition.

4. Discussion

Radiation of dogs by using Y^{90}-DTPA as described in the previous section shows a high degree of selectivity in depressing lymphatic elements, observed in both the lymph nodes, spleen, and peripheral blood. Specific lymphocytic depression can be obtained by using appropriate doses of Y^{90}-DTPA without notably depressing peripheral granulocytes, platelets, or reticulocytes. This lymphocytic depression

appears to be more profound and of longer duration than that obtained by LD 60/30 of 250 kv X-rays in comparable dogs. One dog was described in which essentially no peripheral lymphocytes were seen in the peripheral blood as late as the 50th day following irradiation.

The postirradiation clinical course of the animals is dosage-dependent. Dogs receiving less than 5 mC/lb generally show no signs of abnormality other than selective lymphopenia. One animal has been given two sublethal radiation exposures by this procedure and has now survived greater than 15 months after the first exposure in good health. Animals given doses of approximately 8 to 15 mC/lb die generally late in the second or early in the third week of a "hematopoietic failure" type of syndrome and show severe damage to lymphatic structures at post mortem examination. Dogs given more than 20 mC/lb generally develop pernicious vomiting and die in fluid and electrolyte imbalance within 5 days. Dogs treated in the dosage range of 15 to 20 mC/lb may show either the hematopoietic-type of death or the acute-dehydration-type death. The syndrome of acute fluid and electrolyte imbalance seen at the higher dosage levels may be modified by deleting urea from the diuresis-induction regimen and by careful fluid and electrolyte therapy after irradiation.

It would appear from comparison of the hematologic data of dogs irradiated with conventional 250-kv X-ray machines and those radiated with Y^{90}-DTPA that the dosage in rads delivered to lymph nodes by using Y^{90}-DTPA is roughly equal in effect to more than twice its value (expressed in roentgens) delivered by the 250-kv X-rays.

F. Autologous and Homologous Bone Marrow Transplantation Studies in Dogs Irradiated by Y^{90}-DTPA Urine-Recycling Technique

1. Introduction

The radiation dose delivered to lymph nodes and bone marrow, the changes in the peripheral blood picture, and the macroscopic and microscopic changes at post mortem examination in the animals described in the previous section all suggest that the animals irradiated with Y^{90}-DTPA died after the first 10 days as a result of hematopoietic and lymphopoietic failure. It was important to establish that this was indeed the case; that is, that the death of the animals was due to damage to the hematopoietic and lymphopoietic systems rather than damage to other vital organs. This point could be demonstrated by saving the lives of lethally irradiated (Y^{90}-DTPA) dogs by the use of bone marrow transplants. Should bone marrow transplants be successful in saving the lives of the lethally irradiated dogs, the bone

marrow would be established as the critically damaged tissue when this irradiation procedure is used. Since it is known that cells obtained from bone marrow are capable of repopulating lymphatic structures, life-saving success of marrow transplants would also reflect the importance of damage to lymphatic structures with Y^{90}-DTPA.

Autologous bone marrow transplants -- the use of the animals' own bone marrow -- was chosen as the test of the above outlined hypothesis. Use of the animals' own bone marrow bypasses the complex homograft problems encountered with the use of foreign (homologous) bone marrow.

Since the primary goal in developing the Y^{90}-DTPA irradiating procedure was to prepare a large animal for tissue homografting, successful bone marrow homografts also had to be attempted to complete the evaluation of this procedure. Since the work described in previous sections demonstrated a selective depression of lymphocytes -- the cells generally associated with the homograft rejection response -- this suggested that bone marrow homografting could be accomplished successfully. This section of the work attempts to evaluate the use of bone marrow autografting and homografting in clarifying the above-mentioned issues.

2. Materials and Methods

The type of animal used, the irradiation procedure, and the postirradiation care are identical with those

mentioned in Part III E of this report. All the irradiated animals were males, and all the homologous bone marrow donors were unrelated females, except in one case as noted in the text. In the autologous bone marrow series just prior to irradiation, multiple bone marrow aspirations were performed by using Bierman bone marrow needles. The bone marrow was aspirated in siliconized syringes containing small amounts of Connaught heparin (free from preservatives). The bone marrow was placed in cooled heparinized Hanks' solution in a 300 ml Fenwall plastic transfer pack (100 to 110 ml Hanks' solution plus 50 mg Connaught heparin). The following bone marrow taps were performed: three taps in femur (proximal and distal epiphyseal end, mid-shaft), two in iliac crests (posterior-superior and posterior-inferior iliac prominences), one in scapula (just superior to the glenoid labrum), two in the humerus (proximal and distal epiphyseal ends). A total of between 50 and 100 ml of bone marrow and blood was aspirated. Nucleated cell counts were performed on the final bone marrow mixture and the counts were corrected for maximum contamination by circulating white blood cells. The bone marrow in heparinized Hanks' solution was stored at 4 to 6° C in a commercial refrigerator. No attempt was made to remove particulate matter other than by passing the bone marrow through a commercial blood administration filter at the time of its administration. In some experiments bone

marrow was nfused intravenously 2 hr after cessation of urine recycling. In all others it was injected intra-arterially 18 to 24 hr after the termination of the urine recycling. Bone marrow was infused intra-arterially in certain animals so as to bypass possible removal of infused cells by the capillary bed of the lung, a process known to occur under certain circumstances (57). In the homograft series of animals the bone marrow was infused as noted in each individual case.

The animals who received the bone-marrow by the intra-arterial route were anesthesized with sodium pentabarbital, and an incision was performed over the femoral vessels. The femoral artery was isolated and a Rochester plastic needle was inserted throughout its full length, the plastic tip coming to lie in close approximation to the abdominal aortic bifurcation. The bone marrow was then infused intra-arterially and the perforated femoral artery was ligated to avoid excess bleeding. The animals receiving the bone marrow infusion intravenously were not anesthesized. Blood was drawn (two or three times a week for the studies mentioned in the previous section of this report.

3. Experimental Results

a. Autologous Bone Marrow Transplantations

Table VII summarizes the clinical and pathologic pictures

seen in dogs irradiated by using Y^{90}-DTPA, given autologous
bone marrow, and surviving for more than 1 month. Figures 15
and 16 summarize the body weight and hematologic changes
in these animals. The first two dogs, W-8 and Blackie, were
irradiated in the sublethal range, as discussed in the
previous section. Both had uneventful clinical courses. It
is of interest that both developed characteristic lympho-
penias that persisted beyond the 30th day of observation
similar to that seen in the sublethally irradiated untreated
dogs described previously.

The next five dogs on the table received dosages of
radiation well within the lethal range, and had uneventful
clinical courses for the first few weeks following radiation.
Dog BE appeared normal for the first several months, but
4 months after irradiation became sick and died. The
etiology of this illness was undetermined, but it was felt
that he succumbed to one of the canine distempers endemic in
our dog population. Dog Fatty became anorexic and hypoactive
during the 16th and 30th day. After the 30th day his
appetite returned and his condition was rapidly improving
when he was accidentally killed on the 33rd day by an
intravenous injection of an antibiotic suspension. Dog W-5
appeared normal throughout the observation period and at present
is living and well. Dog W-4 had mild diarrhea at the end of
the first week, which subsided, and the animal was well until
late in the 6th week when he manifested signs of gastrointestinal
bleeding and died on the 43rd day. Dog 14-D developed signs of

Table VII. Summary of dogs given autologous bone marrow transplants after irradiation and surviving more than 1 month

Dog number	Dose administered Total mC	mC/lb body wt	Estimated dose to lymph nodes (rads)	Clinical history	Survival status	Gross pathology	Histologic pathology	Nucleated bone marrow cells administered
W-8	305.4	12.91	141.3[c]	Appeared normal throughout observation period except for sporadic mild diarrhea after 6th day.	Living >3 mo	---	---	2.77×10^9
Blackie	141.6	5.89	248	Normal throughout observation period. Renal homograft was attempted 2nd day but was technical failure.	Living >6 mo	---	---	6.06×10^8
B. E.	204.9	10.78	452.0	Appeared normal during first few months after radiation, but a few weeks before demise animal became thin, hypoactive, and anorexic. Blood creatinine taken preterminally was slightly elevated.	Died at 4 mo	Animal emaciated, tissues unremarkable except for thinning of the renal cortex (cortex medullary ratio of 1:3 or 1:4).	(Post mortem autolysis precluded examination.)	2.28×10^8
Fatty[a]	358.7	11.57	485.0	Normal to 16th day, anorexic and hypoactive 16-30 day. Appetite returned and animal improving from 30-33 day. On 33rd day animal accidently given pulmonary embolism with antibiotic suspension.	Died 33rd day, of pulmonary embolism	Lymph nodes fibrotic. Antibiotic suspension seen in small vessels of lungs.	Lymph nodes and spleen showed normal cellularity. Bone marrow normally cellular. Kidney grossly normal but area of microabscess seen.	7.60×10^8
W-5	440	14.92	597.2[c]	Appeared normal throughout observation period.	Living >3 mo	---	---	4.55×10^9
W-4	339.0	12.33	773.8[c]	Mild diarrhea 5th-8th days, then appeared normal until preterminally, when melena was seen.	Died 43rd day	Lymph nodes fibrotic. About 300 cc of blood in stomach with small amounts distally in G-I tract. No definite gastric ulcer seen.	Lymph nodes and spleen show 30 to 40% repopulation, hemosiderin and much collagenous fiber present. Stomach shows necrotic slough of distal mucosa -- only base of gastric glands being viable. Bone marrow moderately hypoplastic.	3.82×10^8
14-D	384.6	18.68	78	Normal until 18th day when anorexia, drooling, emesis and weight loss appeared. Began to improve after 30th day. By end of 2nd month was back to normal. At present hair sparse, but normal in all other details.	Living >9 mo	---	---	1.64×10^9
491[b]	--	--	--	Renal graft attempted 4th day, technically a failure. Animal healed surgical wound uneventfully and had an unremarkable, normal recovery.	Living >7 mo	---	---	4.09×10^8

a) In dogs Fatty and 14-D the bone marrow transplant was administered intravenously, 2 hr after cessation of urine recycling.
b) Spill during urine recycling precludes dosimetry estimations.
c) Dose to lymph nodes estimated on basis of direct measurement of the average Y^{90} DTPA in the plasma during recycling. The low plasma levels in dog W-8 probably reflect inadequate urine recycling.

% initial body weight

120
110
100
90
80
70

Granulocytes x 10³/mm³

20
18
16
14
12
10
8
6
4
2
0

20,604

o 14D   ◇ BE
□ Fatty   ● W8
▲ RE   ■ W4
▼ 491   ● Blackie
■ W5   ●—● (Anderson) 300 r x-rays (no autologous
                   bone marrow given)

8mos

Lymphocytes x 10³/mm³

6
5
4
3
2
1
0

8mos

0   5   10   15   20   25   30   35   40   60 90 120 150 180

Days after radiation

MUB-731
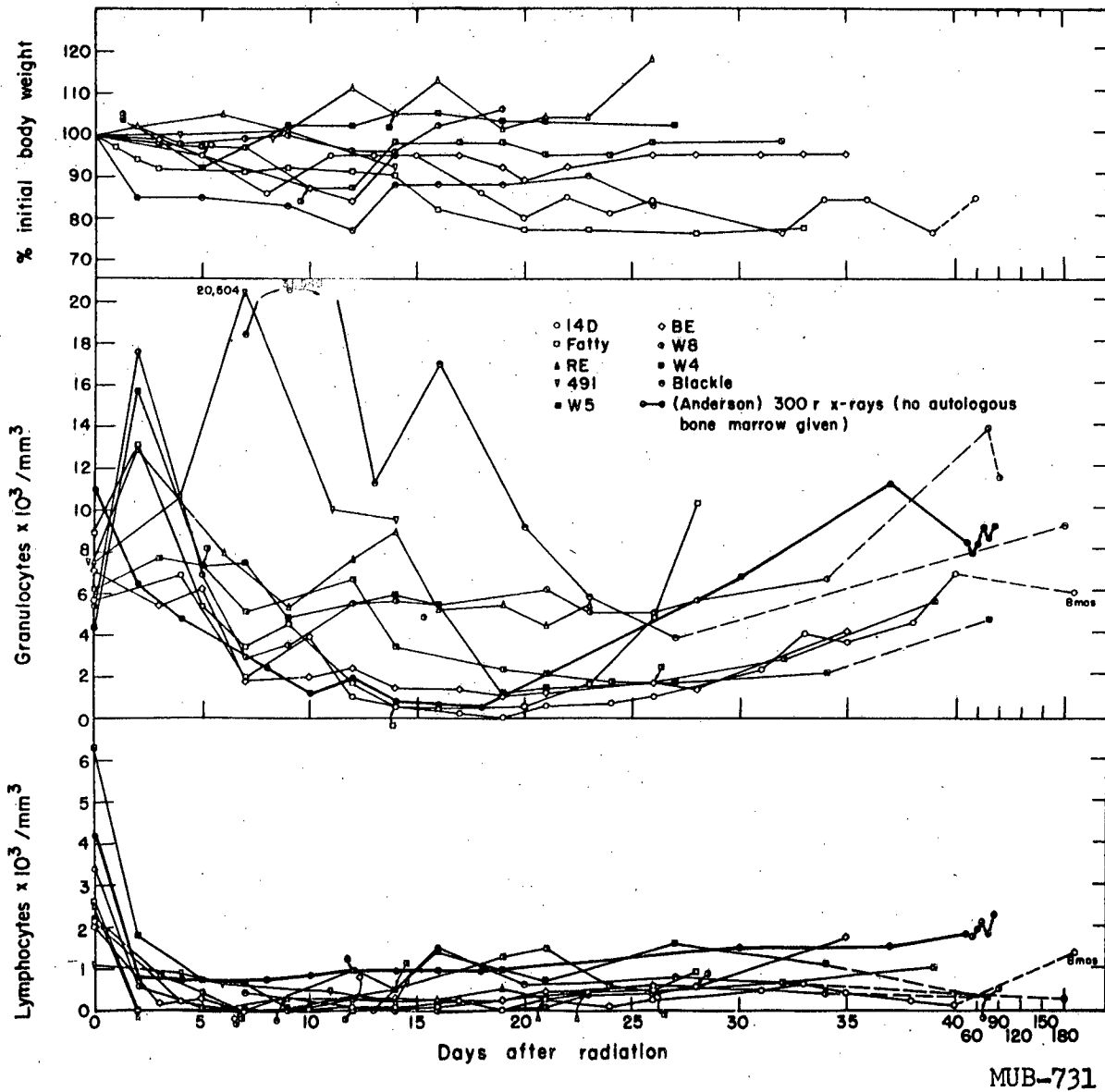
Fig. 2   Fig. 15.   Changes in body weight, granulocytes, and lymphocytes in irradiated
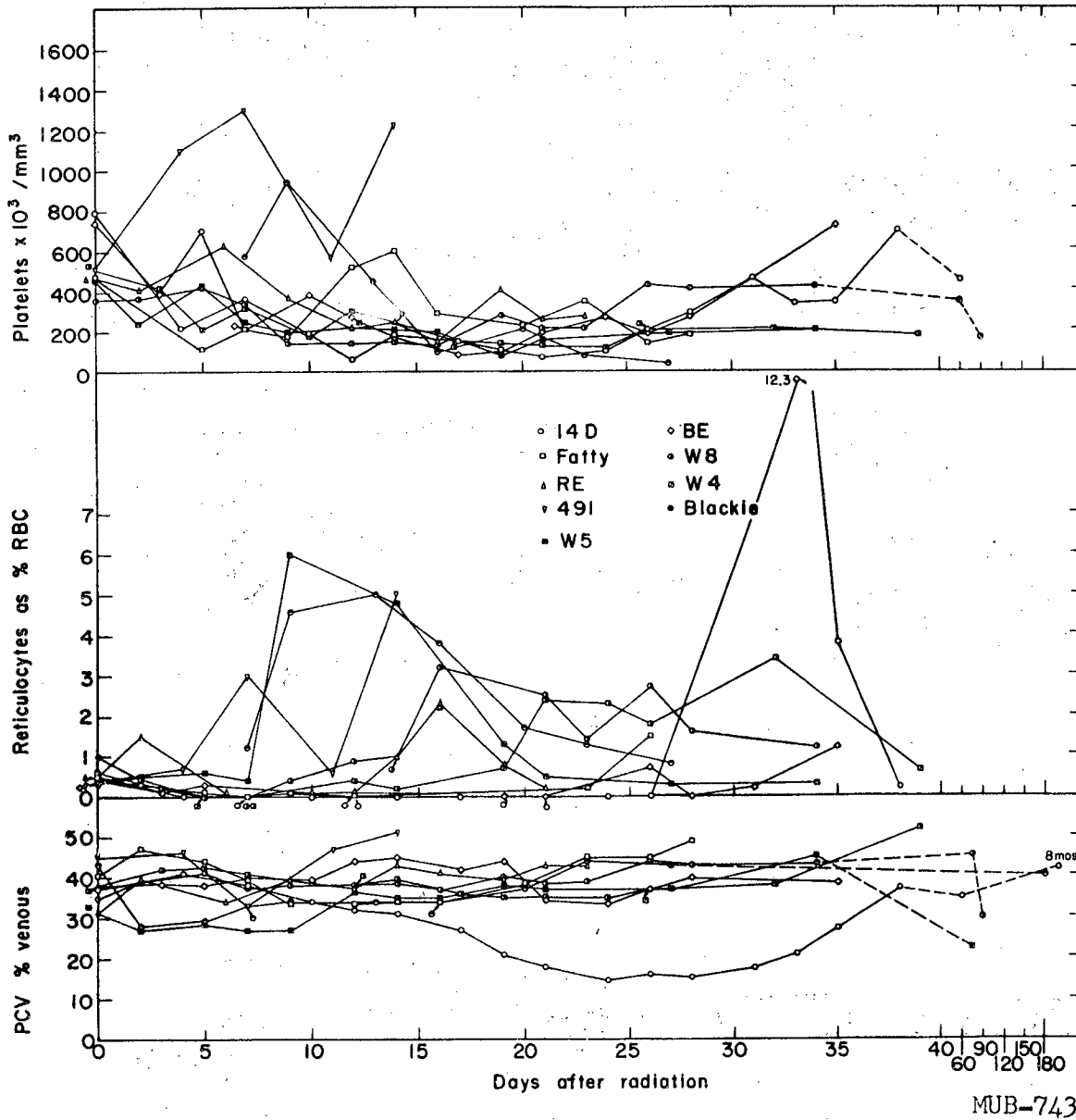dogs receiving autologous bone marrow.

Fig. 16. Changes in platelets, reticulocytes, and packed cell volume
in irradiated dogs receiving autologous bone marrow.

gastrointestinal damage in the third week. These signs
gradually subsided and the animal is living and except
for sparse body hair is well at the time of this writing.

In general the clinical course of these animals given
lethal radiation plus autologous bone marrow was benign as
compared with the clinical course of the animals given
comparable radiation exposures without bone marrow treatment.
This difference made itself apparent during the first
few days after the administration of the bone marrow, before
one would expect significant growth of the cells of the
graft to influence the animal's clinical behavior. Such
vague clinical findings receive some objective support from
the relative stability of body weight following the radiation
in the large majority of dogs in the series (Fig. 15) in
comparison with the lethally irradiated dogs not receiving
bone marrow autografts (Fig. 11). This clinical behavior
suggests some tonic effect of infused autologous bone
marrow beyond its ability to proliferate and repopulate
depleted marrow cavities (30).

Two dogs in this series (BE and W-4) demonstrate that
although good clinical recovery occurred in these animals
following autologous bone marrow infusions, they probably
could not be considered perfectly normal. Thus BE seemed
to succumb to an illness which the other dogs in the colony
similarly exposed were able to resist; dog W-4 died more
than 6 weeks after the radiation exposure of gastrointestinal

bleeding, in addition, Dog 14-D, although in excellent general health, shows marked sparcity of body hair at the present time.

The last dog in this series, Number 491, had an accident during recycling period precluding dosimetry estimations. His clinical course was quite uneventful, and at present appears quite normal over 7 months after the radiation exposure.

The histologic pathology of dog Fatty confirmed the hematologic findings of return of hematopoietic function. His bone marrow as well as the lymph nodes and spleen showed normal cellularity. It is interesting that these lymphatic structures show a normal histologic picture when the last blood count performed before this animal's death revealed that the absolute lymphocyte count remained below $1000/mm^3$. Post mortem histology on W-4 revealed damage to the gastric mucosa and only moderate repopulation of lymphatic structures. The relationship of these findings to either canine diseases or late radiation effects is unclear.

In reviewing the hematologic finding on this group of animals (Fig. 16) it is remarkable to find that all animals showed a postirradiation lymphopenia which persisted despite the administration of autologous bone marrow. Even 6 weeks to 3 months following the irradiation dogs W-4, 14-D, W-5, and W-8 had peripheral lymphocyte counts significantly below that

found in the dogs of Anerson that survived 300 r from 250-kv
X-rays without bone marrow treatment. However, if we compare
the lymphocyte levels postirradiation seen in this series with
those obtained in the lethal untreated group described in the
previous section (Fig. 12) we see that the dogs treated with
autologous marrow generally have a larger number of
circulating lymphocytes than the dogs receiving no autologous
marrow and comparable amounts of Y^{90}-DTPA radiation. Dogs
lethally irradiated with Y^{90}-DTPA have usually less than
100 lymphocytes per mm^3 of peripheral blood from 5 days
postirradiation until the time of their death. The autologous-
marrow-treated animals receiving their marrow 24 hr after
the irradiation procedure usually have several hundred
lymphocytes per mm^3 of peripheral blood after the 9th or 10th
postirradiation day. Thus, although autologous bone marrow
given more than 24 hours after irradiation with Y^{90}-DTPA does
not restore peripheral lymphocytes to normal levels, it does
increase the peripheral lymphocyte number above that seen in
the control animals not transfused with marrow.

Alpen and Baum (78) and Mannick et al. (79) have
reported that autologous bone marrow is capable of rapidly
restoring lymphopoiesis in the X-irradiated dog. The
limited return of circulating lymphocytes in the animals
receiving Y^{90}-DTPA irradiation followed by autologous bone
marrow in distinction to the results obtained in X-irradiated-

autologous-marrow treated dogs probably reflects the more severe damage sustained by lymphatic tissues when Y^{90}-DTPA is used for irradiation.

The return of circulating granulocytes in the irradiated dogs given autologous bone marrow in this series was slower than the response obtained in autologous-marrow-treated X-irradiated dogs (78, 79). Dogs No. 14-D and Fatty, both of which were given autologous bone marrow intravenously 2 hr after the cessation of urine recycling, had the greatest depression of granulocytes and the slowest return to normal of any of the dogs in this series. Indeed, the rate of peripheral granulocyte recovery is not even as rapid as that seen in the LD_{60-30} X-ray survivors of Andersen. It would therefore appear that the capacity of autologous bone marrow to save the lives of these two lethally irradiated dogs was not associated with the bone marrow's ability to raise peripheral granulocyte levels. The remaining dogs in this series all of whom received intra-arterial autologous marrow infusion 18 to 24 hr after the cessation of urine recycling generally had peripheral granulocytes that maintained themselves above levels of 1000 cells per mm^3 of blood.

In general there was a gradual decrease in platelet counts, reaching its lowest point at about the 20th day. However, profound thrombocytopenias were not seen, and only a few platelet values are noted below 100,000/mm^3.

The alterations in the reticulocyte counts reflect womewhat the pattern of changes seen in the circulating granulocyte counts. Dogs No. 14-D and Fatty, who received their marrow infusion intravenously 2 hours after cessation of recycling, had essentially no reticulocytes in the peripheral blood smear until after the 20th day. The other dogs in the series show variable early return of the reticulocytes, indicating erythropoiesis probably as a result of earlier proliferation of the marrow autograft.

The packed cell volume showed no remarkable change in the members of this series except for Dog No. 14-D, who showed a progressive slow drop reaching a minimum about the 28th day. It is noteworthy that this dog showed the most prolonged depression of reticulocytes as well. After the 30th day the packed cell volume began to rise, following reticulocytosis which rose to 12.3% on the 33rd day.

Table VIII summarizes the clinical course and pathological results on three dogs given supralethal radiation followed by autologous bone marrow transplants, and dying in less than 1 month. Figure 17 summarizes body-weight and hematologic changes in these animals. It can be seen that the dogs in this group generally received higher doses of radiation than did the animals in the autologous-marrow-treated survivors just described. The first animal in this series, No. 4-G, received the marrow autograft 2 hr after the termination of urine recycling. He died of a hemorrhagic

Table VIII. Summary of dogs given autologous bone marrow after radiation and surviving less than 1 month

Dog Number	Dose administered Total mc	mc/lb body wt	Estimated dose to lymph nodes (rads)	Clinical history	Survival status	Gross pathology	Histologic pathology	Nucleated bone marrow cells administered
4-G [a]	389.5	18.55	777	Hypoactive, anorexic from first day, emesis and mucoid diarrhea from 2nd day. Began to show improvement (more active, started eating, etc.) on day 7. On 11th day passed tarry stools and had bloody emesis, and died with G-I bleeding on 12th day.	Died 12th day	Some submucosa petechiae in bronchioles and stomach. Marked subserosal hemorrhage around ileocecal and appendiceal areas. Digested blood in stomach and tarry material in lower duodenum -- no definite ulcer in G-I tract seen. Mesenteric lymph nodes hemorrhagic.	Lymph nodes and spleen showed moderate cellularity with 40 to 50% of normal cellularity. Bone marrow showed early regeneration. Colon showed marked submucosal hemorrhage but mucosa was intact.	----
X-50	366.3	19.8	830	Known to have suffered small pulmonary embolae during urine recycling from clotted blood in tubing. Hypoactive after radiation but ate and drank well. Developed large hematoma on 2nd day in area of femoral artery cut-down. Anorexic, dehydrated, and hypoactive on 3rd day, dead on 6th day -- markedly dehydrated.	Died 6th day	--------	--------	3.00×10^9
Chain	---	---	1467 [b]	Remained normal in appearance except for slow weight loss until 17th day, when animal became anorexic. On 19th day was hypoactive, coughing, anorexic, and vomited. Died on 20th day.	Died 20th day	Diffuse hemorrhagic pneumonia, submucosal petechiae in pyloric end of stomach and in colon. Mesenteric lymph nodes enlarged and markedly hemorrhagic.	Lymph nodes and spleen quite cellular (70 to 100% of normal), germinal follicles present. Hyperemea, edema, and congestion in lungs.	4.01×10^8

a) Bone marrow given intravenously 2 hr after cessation of urine recycling.
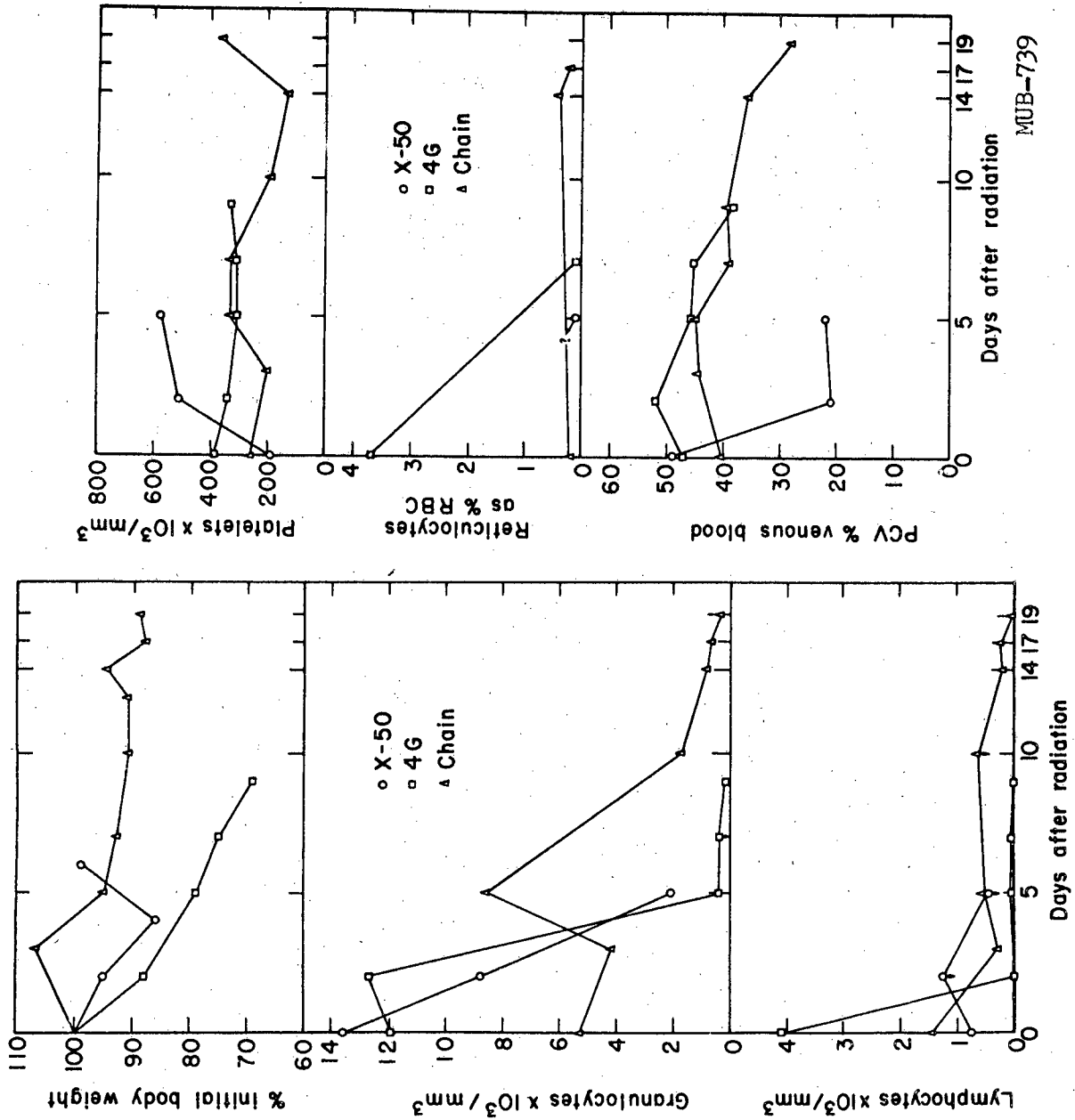b) Dosimetry estimation on basis of actual blood level of Y^{90} during recycling procedure.

Fig. 17. Changes in body weight and hematologic parameters in
irradiated dogs receiving autologous bone marrow and surviving
less than 1 month.

diathesis on the 12th day, and histologically showed
approximately 40 to 50% cellularity of lymph nodes and spleen.
The bone marrow showed signs of early regeneration. However,
despite this apparent regenerative activity in lymphopoietic
and hematopoietic structures, this animal continued to show
severe depressions of lymphocytes, granulocytes, and
reticulocytes until the time of his death. Since none of the
dogs in the control series of untreated animals given lethal
amounts of Y^{90}-DTPA (see Table V in the preceding section) and
dying around the 12th day showed any signs of regeneration
of lymphatic and hematopoietic structures, it appears that
the autografted marrow cells were responsible for the cellu-
larity seen in the lymph nodes and bone marrow of this dog.

Dog X-50 accidentally suffered pulmonary embolae
during the recycling procedure from clotted blood in the
intravenous recycling unit tubing. In addition he lost
an estimated several hundred ml of blood in the area of the
femoral arterial cannulation following the bone marrow
infusion. He became anorexic, dehydrated, and hypoactive
and died on the 6th day.

Dog Chain received the highest radiation dose of any
dog in the autologous-marrow-treated series. He remained
normal in appearance until the 17th day, when he became
anorexic. On the day prior to his death he was noted to be
coughing heavily, was hypoactive, and showed evidence of emesis.

He died on the 20th day of a hemorrhagic pneumonia. At the
time of his death the lymph nodes and spleen showed almost
normal cellularity. His circulating blood did not reflect
this return of histologic lymphopoiesis, and preterminally
he had a lymphocyte count which was less than 100 cells/mm^3
of blood.

The results obtained from the dogs in these series
establish that the administration of bone marrow autografts
can prevent the death of dogs given lethal irradiation with
Y^{90}-DTPA. The autologous marrow appears to promote the
return of circulating granulocytes, reticulocytes, and
platelets, and to some degree lymphocytes. Three dogs were
given autologous bone marrow intravenously 2 hr after the
cessation of recycling, at a time when significant amounts
of isotope still remained in the body. Two of these dogs
survived, and the pattern of their peripheral blood elements
suggested autogenous return. However, the third animal
(4-G) died on the 12th day and showed recovery of cellularity
in lymphatic and hematopoietic structures, although peripheral
granulcoytes, reticulocytes and lymphocytes were markedly
depressed at the time of his death. It would thus appear
that the autologous bone marrow administered intravenously
2 hr after the cessation of urine recycling is capable of
histologically repopulating lymphatic and hematopoietic
structures. However, the peripheral evidence of returning

lymphopoiesis and hematopoiesis is delayed to a greater
extent than when the marrow is given more than 24 hours after
the radiation procedure. These results also indicate that
these infused marrow cells are quite radiosensitive, since
cells infused 2 hr after recycling are exposed to only
approximately 12.5% of the total radiation dose delivered in
a given case. It is possible that radiation damage inflicted
prior to the administration of the marrow cells could have
influenced the survival of these transfused cells.

It appears advantageous to wait at least 24 hr after
the radiation procedure before administering bone marrow if
one desires a proliferation of the graft cells that is evident
in circulating blood elements.

It has been previously shown that autologous bone marrow
is capable of promoting the return of lymphocytes to the
peripheral circulation to normal levels in dogs subjected to
lethal X-radiation (78, 79). That this is not the case if
Y^{90}-DTPA irradiation is used may be related to the greater
efficacy of our technique in destroying lymphatic structures.
Since probably less than 1% of the total radiation dose is
delivered within 24 hours following radiation it appears
doubtful that the residual radiation delivered to the graft
cells when they are administered 24 hr after the cessation
of urine recycling would significantly affect the proliferation
of these cells.

In addition to the dogs listed in the above series, one dog (No. RE) was given an estimated 514 rads to the lymph nodes, and then received 100 ml of his own whole blood collected just prior to irradiation in a manner identical to that used in the collection of autologous bone marrow. His clinical and hematological course was similar to the lethally irradiated dogs in this series who received autologous marrow. That fresh whole blood would have the ability to repopulate hematopoietic and lymphopoietic tissues is not unexpected, since such appears to be the case in mice (35, 80), and recently suggested evidence to this effect has also been observed in dogs (81). This dog, of course is merely a single observation, and further studies are required to evaluate the use of fresh autologous blood in treating lethal radiation injury.

b. Homologous Bone Marrow Transplantations

Table IX summarizes the clinical and pathological results obtained on a series of animals receiving radiation with Y^{90}DTPA followed by homologous bone marrow transplantation. Figures 18 and 19 summarize the body weight and hematologic changes on these animals. The first two animals on Table IX (No. W-3 and No. 202) received radiation dosages generally in the transition zone between sublethal and lethal ranges as determined in the previous section. Both these animals

Table IX. Summary of irradiated dogs given homologous bone marrow

Dog number	Dose administered Total mc	mc/lb body wt	Estimated dose to lymph nodes (rads)	Clinical history	Survival status	Gross pathology	Histologic pathology	Nucleated bone marrow cells administered
W-3[a]	500	20.0	201.9[b]	Remained normal throughout observation period except for excessive hair loss. 7 wk after radiation homotransplants of kidney and skin were attempted from female bone marrow donor. Kidney functioned well for 7 days. Skin sloughed in approximately 10 days.	Living >3 mo	Transplanted kidney, surgically removed on 7th day, was enlarged 2 times normal size, entirely hemorrhagic, and friable, with gross architecture destroyed.	Microscopic architecture of transplanted kidney entirely destroyed. All that remains is necrotic debris and blood elements.	2.98×10^9 (given intravenously 3 days after radiation)
202	86.3	6.61	277	Somewhat hypoactive after 14th day. Hair growth poor. Given no special care after 25th day and at present is normal in appearance.	Living >7 mo	----	----	--- (given intra-arterially 24 hr after radiation)
R-12	342.4	14.27	643.0[b]	Normal until 17th day, following which he became increasingly hypoactive and anorexic. Mucoid bloody diarrhea after 22nd day. Given i-v fluids and blood after 21st day. Forced oral feeding attempted 33-35th days followed by emesis -- died 35th day.	Died 35th day	Mucosa in distal small bowel and colon thin and reddened. Marked hair desquamation. Lymph nodes small and pale. Spleen grossly normal. Adrenal cortical hyperplasia.	Lymph nodes and spleen quite cellular. Centers of germinal follicles necrotic and edema under capsule. Skin markedly atrophic with epidermis about 2 cell layers thick, marked loss of rete pegs. Bone marrow very cellular. Islets of Langerhans markedly prominent in pancreas.	2.19×10^9 (given i-v 5 days after radiation)
W-7	578.0	24.08	973.0[b]	Normal until 17th day, following which he became hypoactive and anorexic. Developed purulent conjunctivitis on 22nd day. Emesis from 24th day.	Died 26th day	Old submucosal petechiae in stomach, areas of mucosal erosion near pylorus. Blood in lumen of small bowel and colon. Marked adrenal hyperplasia.	Lymph nodes and spleen have approximately 35% of normal cellularity, germinal centers present. Bone marrow shows focal areas of active hematopoiesis. Gastric mucosa shows complete disruption of architecture in crypts, mucosa very thin.	2.89×10^9 (given i-v 2 days after radiation)
493	---	---	1142[b]	Intra-arterial bone marrow was given without filtration. One day after infusion animal unable to move hind limbs; embolization and infarction from bone marrow and blood clots suspected. Animal slowly improved but became anorexic and hypoactive on 10th day. On 12th day had marked bloody diarrhea and was in shock.	Died 12th day	Markedly dehydrated. Petechiae in stomach. Colon filled with bloody material, mucosa hemorrhagic. Area of cecum had marked purpura of serosal surface. All mesenteric lymph nodes markedly enlarged and hemorrhagic.	Lymph nodes and spleen showed good cellularity, germinal centers present. Bone marrow showed good cellularity. Large amounts of submucosal hemorrhage in colon.	(Given intra-arterially 2 days after radiation -- donor was sister)

a) Urine recycling faulty; estimated dosimetry to lymph nodes on the basis of Y^{90} levels in blood during recycling.
b) Estimated dose to lymph nodes on the basis of levels of Y^{90} in blood during recycling.
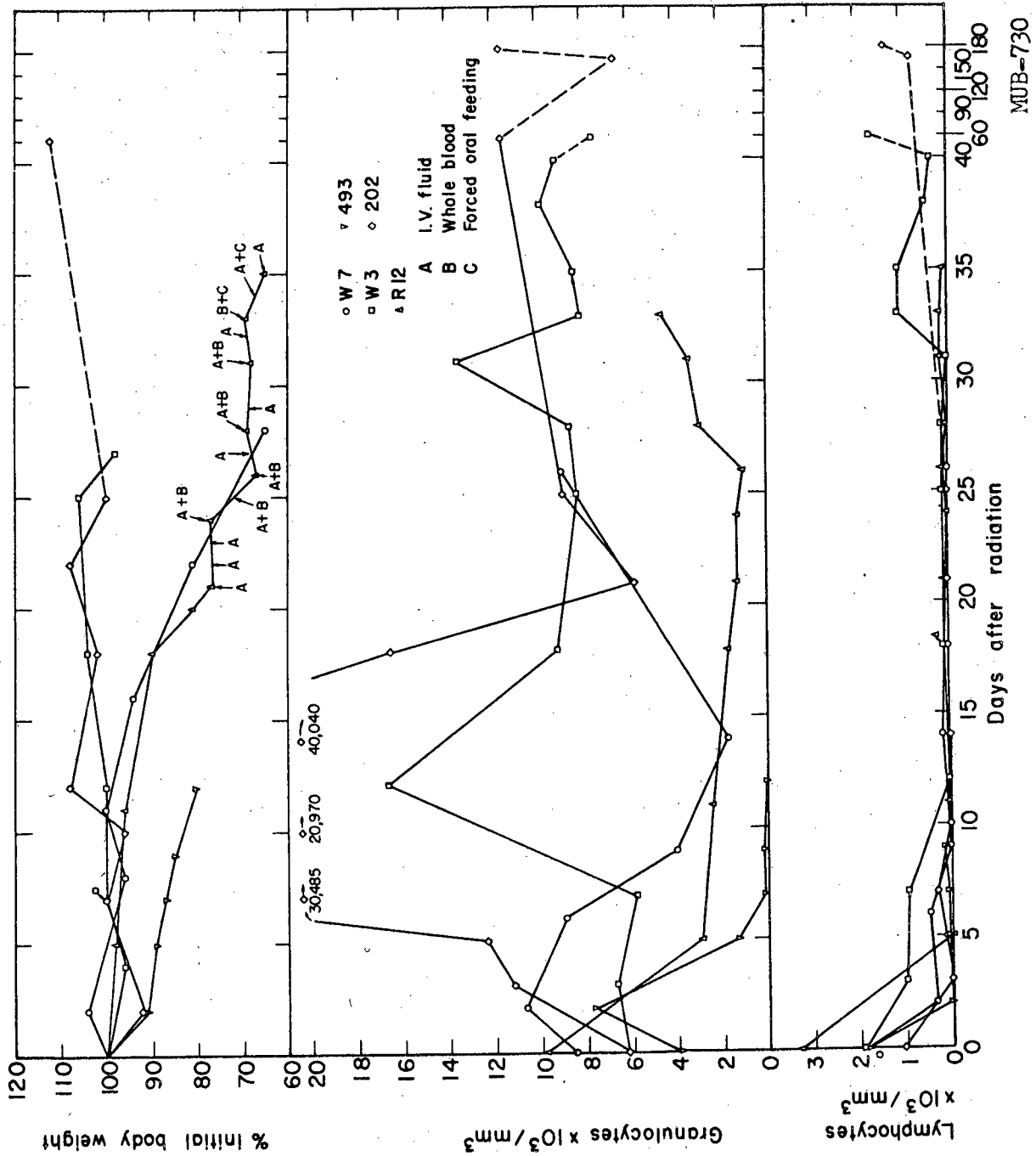
Fig. 18. Changes in body weight, granulocytes, and lymphocytes in irradiated dogs receiving homologous bone marrow.
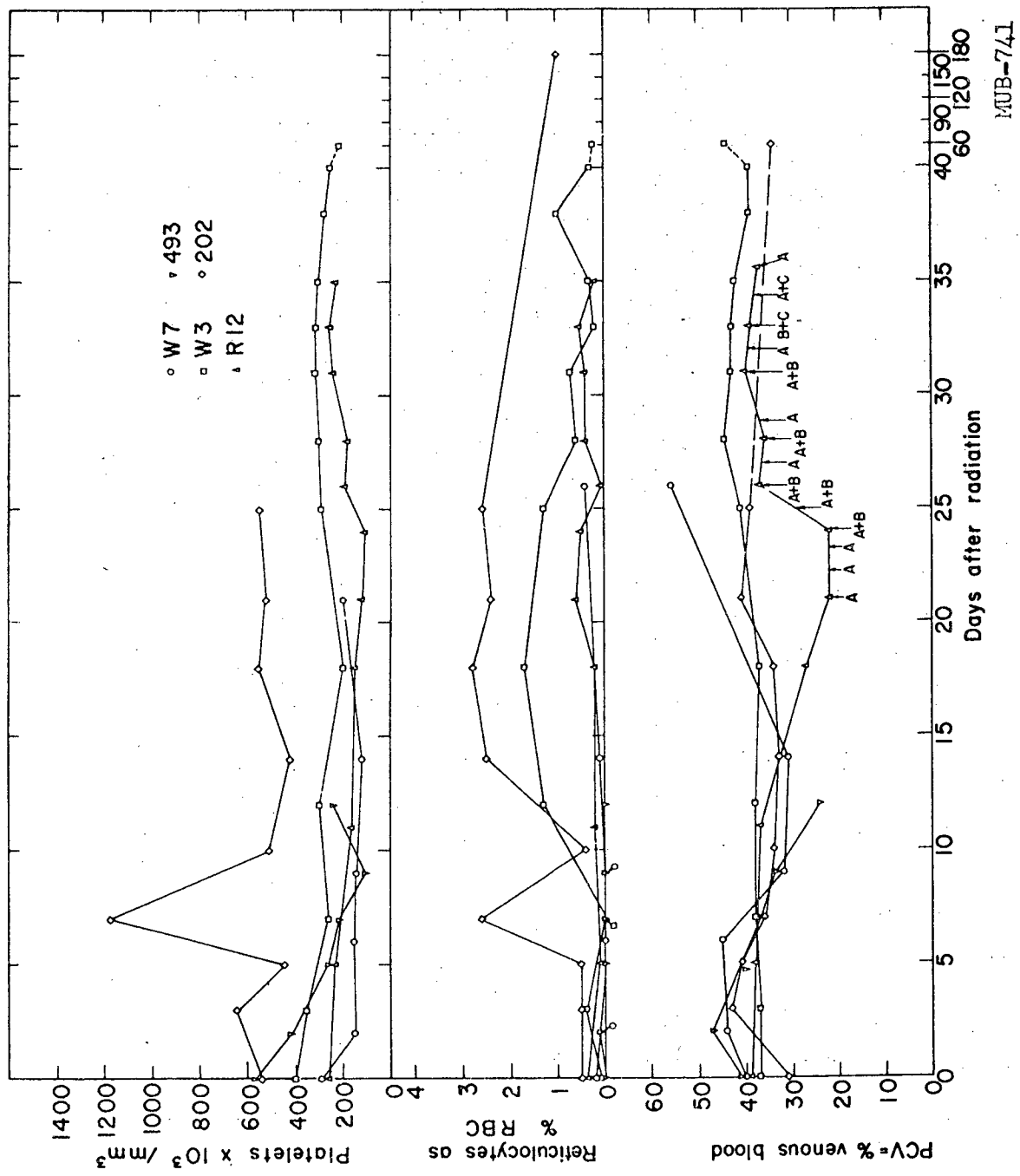
Fig. 19.   Changes in platelets, reticulocytes, and packed cell volume in irradiated dogs receiving homologous bone marrow.

clinically were well except for excessive hair loss and hypoactivity appearing after the third week and continuing until the sixth to eighth week, when they became more active and hair loss diminished. Both are still living more than 3 and 7 months, respectively, postirradiation. Seven weeks following irradiation No. W-3 was given a renal and skin homotransplant from his female donor. The kidney functioned well for 7 days and then suddenly ceased to produce urine. The kidney was removed; it was found that it was enlarged two times its normal size, it was purpuric, the gross architecture was distorted, and there was no evidence of blood flowing through the parenchyma. Histologically the renal parenchyma was almost completely destroyed and was replaced by extravasated blood. The full-thickness skin graft appeared necrotic at approximately 10 days. Although it is possible that both the renal and skin grafts represented late technical failures, it is probable that they were immunologically rejected by the host.

The next two dogs in this series (No. R-12 and No. W-7) received quite high lethal doses of radiation. Both appeared normal until the seventeenth day, following which they became hypoactive and anorexic. Dog No. R-12 developed signs of a mucoid, bloody diarrhea on the 22nd day. He was given intravenous fluids and blood from his unrelated female bone marrow donor after the 21st day. Forced oral feedings

on the 33rd to 35th days were not tolerated, and he died on
the 35th day. Gross post mortem examination was not
particularly remarkable. Histologic examination revealed
cellular lymph nodes, spleen, and bone marrow. However,
the centers of the germinal follicles in both the lymph
nodes and spleen contained eosinophilic debris and cells with
large pale nuclei. Areas of edema were noted under the
capsule of the spleen. The skin was markedly atrophic,
with the epidermis no more than two cell layers thick.
Marked loss of the rete pegs was noted. The islets of
Langerhans were markedly prominent in the pancreas, and
the adrenal cortex was hypertrophic. The histologic changes
noted in the lymph nodes and spleen are quite similar to
the changes believed by Vos and De Vries (25) to be
pathegnomonic of secondary disease.

Dog No. W-7 received a very high dose of radiation
which should have placed him in the lethal-in-less-than-
6-days group of dogs who died of acute fluid and electrolyte
imbalance soon after the radiation exposure (see previous
section). However, as has been mentioned, he appeared quite
normal until the 17th day. He subsequently became hypoactive
and anorexic and died on the 26th day. He was not given any
supportive care other than the routine antibiotic regimen
used in all animals following radiation. Gross post mortem
examination revealed that the immediate cause of death was

gastrointestinal bleeding. Marked adrenal hyperplasia was present. Histological examination revealed approximately 35% of normal cellularity in the lymph nodes and spleen with focal areas of active hematopoiesis in the bone marrow. Gastric mucosa showed disruption of normal architecture.

Dog No. 493 received the highest radiation dosage of any dog of this series, a dose which in the control group should have been lethal in less than 6 days. The autologous bone marrow was taken from a female sibling and was administered intra-arterially without filtration. On the day after the marrow infusion, the animal was unable to move its hind limbs, and embolization with bone marrow particles and blood clots and subsequent infarction was suspected. The animal slowly improved but became hypoactive and anorexic on the 10th day. On the 12th day he developed a severe bloody diarrhea and died. Gross post mortem examination revealed marked dehydration, with gastrointestinal bleeding as the immediate cause of death. Histologically, the lymph nodes, spleen, and bone marrow showed good cellularity.

The body-weight changes of these animals depicted in Fig. 18 showed three main patterns. Dogs No. W-3 and No. 202, who received radiation dosages estimated to be generally in the transition zone between sublethal and lethal ranges,

showed no weight changes during the observation period.
Dogs No. R-12 and No. W-7, receiving high lethal radiation
doses, generally maintained their weight until the 17th day,
after which they showed progressive weight loss. Dog No. 493,
who received the highest dose, showed an early weight loss soon
after radiation.

The hematologic changes in these dogs (Fig. 19) also
reflected the heterogeneity of the members of this group.
The lymphocytes in all the members of this group were
markedly depressed for the first month to levels lower
than that seen in the animals treated with autologous-marrow
and to levels similar to those in untreated lethally radiated
dogs. Dog No. W-3, the animal in this series who received the
lowest radiation dose, showed a return of lymphocytes on the
33rd day. Dog No. 202 also showed a slow return of peripheral
lymphocytes after the 1st month. Dogs No. W-7, No. R-12,
and No. 493 all had severe lymphopenias at the time of their
death. Homologous marrow appears to possess less ability to
restore peripheral lymphocyte levels than does autologous
marrow. This seems to be the experience of other investigators
using external X-ray and gamm-ray sources (78, 79, 81).

In both of the high sublethally irradiated dogs (No. 202
and No. W-3) there was a marked increase of circulating
granulocytes 4 days after the administration of the marrow
homograft. In dog No. 202 granulocytes rose from pre-

irradiation levels of slightly more than 6000 cells/mm^3 to 40,000 cells/mm^3. This increase lasted about 16 days before granulocyte levels returned to baseline values. The circulating granulocytes in Dog No. W-3 rose to about 16,500 cells/mm^3 from a baseline level of a little more than 6,000 cells/mm^3. This increase lasted approximately 11 days. However, sublethally irradiated dogs not given bone marrow also tended to show a rise in peripheral granulocyte levels at this time period after irradiation, but usually of smaller magnitude (see Fig. 10 in previous section).

Dog No. R-12 showed an early rapid drop in circulating granulocytes, then a very slow drop to the 26th day, followed by a rise. Dog No. W-7 had a granulocyte drop reaching a low point on the 14th postirradiation day (11 days after marrow infusion), following which there was a rise reaching normal levels at the time of the animal's death. Both these dogs had significantly higher peripheral granulocytes at all times after radiation than did comparably irradiated untreated dogs in the control groups. At no time did their peripheral granulocyte count drop below 1000 cells/mm^3.

Dog No. 493 had a rapid and severe drop of peripheral granulocytes which persisted to the time of his death on the 12th day. However, as has been mentioned previously, his marrow showed good cellularity at post mortem examination.

The platelets of the dogs in this series showed no remarkable change other than a sharp rise in peripheral platelet number in Dog No. 202, 4 to 6 days after administration of the marrow homograft.

The reticulocyte levels of these dogs resembled the general behavior of the granulocytes previously discussed. The high sublethally radiated dogs No. W-3 and No. 202 had elevations of their reticulocyte count above their baseline levels 4 to 9 days after administration of the autologous marrow transplant. Again, the sublethally irradiated un-treated controls showed a similar phenomenon (Fig. 10 in previous section). Six to 12 days after the autologous marrow was administered, the lethally irradiated dogs No. R-12 and No. W-7 showed a return of reticulocytes to the peripheral circulation, but this return was not brisk, as in the dogs treated with autologous bone marrow (Fig. 16). Dog No. 493 showed no return of reticulocytes at the time of his death on the 12th day postirradiation and 10th day post-marrow-transfusion.

The changes in packed cell volume in these animals were not particularly remarkable. Dog No. 493 showed a steady drop in packed cell volume after the 2nd postirradiation day, with a marked drop between the 7th and 10th days after the administration of the homograft marrow. Dog R-12 also showed a drop in PCV, manifest after the 9th day following

the marrow homograft. These changes may possibly reflect graft antibodies attacking recipient red cells, but this is a moot question.

Since Porter originally reported the feasibility of using the sex chromatin marker in female granulocytes originally described by Davidson as a means of following proliferation of homotransplanted marrow in dogs (82, 83), investigators have used this marker as a critical criterion of the success of marrow homografts in dogs (6, 9, 26, 81). We also have attempted to use this technique to follow the progress of homografted marrow in our animals. Figure 20 shows the pattern of appearance of the "clubbed" neutrophils in the marrow-homografted dogs in this series. Significant increase of the peripheral club count (greater than 1% of all granulo-cytes present) appeared to occur between the 7th and 12th days following marrow grafting. After the 1st month the dogs scored had between 1 and 2% clubs. Prior to irradiation, three of the dogs (No. W-7, No. W-3, and No. R-12) had 0% clubs in their peripheral blood, and one (No. 202) had 0.38% clubbed forms. The clubbed forms in the female donors to these dogs ranged from 2.3 to 5.5%. It therefore appeared that all four of the dogs surviving the 2nd postirradiation week had a "take" of bone marrow from their unrelated female donors. It appeared significant that the dogs irradiated in high sublethal range (No. W-3 and No. 202) appeared to have the best takes as determined by the club counts. However,
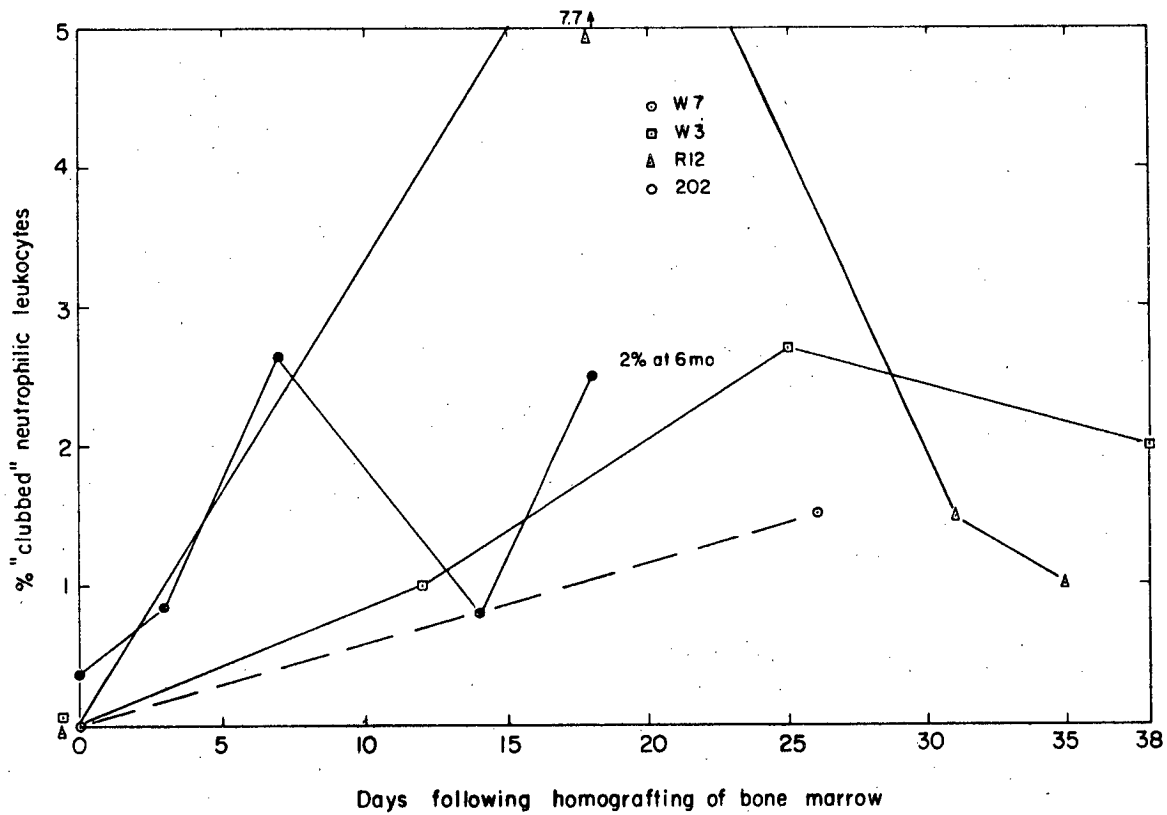
Fig. 20.   Pattern of appearance of "clubbed" granulocytes in irradiated
           dogs receiving homologous bone marrow.

it was soon found that dogs in the sublethally irradiated untreated control group also had between 1 and 3% of clubs in their granulocytes following irradiation. This latter finding was, of course, quite disconcerting. If the forms in the peripheral blood that we were calling "clubs" were the same forms that other authors have used to establish homologous bone-marrow takes, then perhaps some critical re-evaluation is in order.

The club forms that were seen both in these marrow-homografted dogs and in the sublethal nongrafted animals had the typical drumstick shape and terminal position. They did not have chromatin strands connecting them with other than one nuclear lobe. However, in general they were smaller than those described by Porter (82) or those usually seen in female dogs. They did conform sufficiently closely to the usual description of a "club" as to offer a problem in distinguishing them from true clubs (assuming that there is a real morphologic difference between these forms and true clubs). Figure 21 shows the appearance of clubs seen in a female beagle, a male following homologous marrow transplantation from a female, and an untreated male following sublethal radiation. Until further work enables us to distinguish true female leukocytes in male hosts and whether the radiation procedure of itself will result in an increase of club forms, we feel that, in our hands, club counts are of dubious value. It was important, therefore, for us to

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Fig. 21. Appearance of "clubbed" granulocytes in (left)
sublethally irradiated male dog not given female
bone marrow; (center) lethally irradiated male dog
given female bone marrow; (right) untreated female
dog. (times 4330 diameters magnification)

determine if the long-term survivors (No. W-3 and No. 202)
given radiation in the high sublethal range were true
chimeras by means other than following the appearance of
"clubbed" neutrophils in these male dogs. Dog No. W-3
was given a kidney and skin homograft from his female donor
7 weeks after the irradiation procedure. The kidney
functioned very well for 7 days and then abruptly ceased
functioning. Neither gross nor histologic examination could
determine with certainty whether the kidney was immunologically
rejected or suffered thrombosis of either its artery or vein.
The full-thickness skin graft sloughed at the end of 10 days.
Although the losses of both the kidney and the skin graft
might conceivably be due to technical failure, it seems
probable that they were immunologically rejected.

4. Discussion

The administration of autologous bone marrow is capable
of saving the lives of dogs lethally irradiated with Y^{90}-DTPA.
Histologically, lymphatic and hematopoietic structures are
rapidly repopulated after the use of marrow autografts,
but the appearance of the corresponding formed elements in
the peripheral blood is delayed. This latter phenomenon is
also seen to some extent in X-irradiated animals given marrow
grafts (84). Clinically, it appears that the administration
of marrow autografts has a tonic effect on the experimental

animal beyond its cell-proliferative effect, because im-
provement in the animal's clinical condition is seen within
24 hours following the graft and prior to any significant cell
proliferation. Dr. Ashikawa recently reported on the
mortality-reduction effect of postirradiation administration
of parenteral lipids in mice (85). Since bone marrow invariably
contains lipid material, it is conceivable that the marrow-
cavity lipids act in a similar fashion to the more simple
lipids used by Dr. Ashikawa. (A pilot experiment done in
collaboration with Dr. Ashikawa, using dog marrow lipid in
midlethally irradiated mice, yielded suggestive but inconclusive
results on this point.) It would appear from the results
obtained that transplanted marrow cells are quite radio-
sensitive and better results are obtained if the marrow is
administered a day or more after the radiation procedure to
allow for fuller clearance of the Y^{90}-DTPA from the body of
the experimental animal. Animals described in this section
were given bone marrow infusion intravenously or intra-
arterially. Although it appears that the arterial route is
an acceptable mode of marrow administration, its value
relative to the intravenous route cannot be judged on the
basis of such a small heterogenous series.

In the small series of homologous marrow transplantations
here described, there is evidence that homologous tissue
transplantation is possible following irradiation with

Y^{90}-DTPA. In two lethally irradiated dogs given homologous marrow (No. W-7 and No. R-12) the evidence for a "take" of the marrow cells can be summarized with the following points:

(a) Prolonged postirradiation survival of the animal beyond that seen in the untreated control group.

(b) Histological evidence of early repopulation of lymph nodes, spleen, and bone marrow.

(c) Maintenance of peripheral granulocyte levels significantly above those seen in untreated control groups.

(d) Finding of donor female markers on leukocytes (clubs) -- this evidence is controversial for reasons given above.

(e) Development of clinical signs suggestive of secondary disease which were not seen in autologous-marrow-treated dogs, e.g., anorexia, weight loss, diarrhea and emesis, appearing after the 17th postirradiation day.

(f) The appearance of histologic changes thought to be characteristic of secondary disease.
The third lethally irradiated dog died too early for most of the above criteria to be evaluated; however, histologically, definite evidence of early repopulation of lymphatic and hematopoietic structures were seen, indicating a successful take in this animal as well.

In the two dogs irradiated in the high sublethal range the evidence for a successful homologous marrow take is

meager. Clinical symptoms appearing several weeks after the marrow homograft and clearing after the second month, could be interpreted as representing a mild secondary disease. Appropriate appearance of clubbed neutrophils was seen in the peripheral blood, but the value of this evidence is dubious. In one of these dogs (No. W-3) the chimeric state, if it ever existed, probably did not persist after the 7th postirradiation week. It would be an attractive prospect to think that sublethally irradiated dogs would tolerate a bone marrow homograft, just as some sublethally radiated humans have tolerated renal homografts (86, 87). However, more concrete ways of evaluating the chimeric state must be used than merely following the appearance of clubbed neutrophils.

The homologous transplantation studies presented here are preliminary in nature; a great deal of further study is necessary to define the adequacy of the Y^{90}-DTPA procedure in paving the way for tissue homografts. The minimum dose that would allow for a successful take, the maximum radiation dose at which homologous marrow transplantation would prevent death of the animal, and the optimal dose range for successful homografting are problems which remain to be evaluated. It appears that better markers of the homograft than clubbing of neutrophils should be used. The final criterion for

successful achievement of the chimeric state should be the ability of the recipient animal to accept tissue grafts such as kidney and skin from the donor.

Experiments now in progress are beginning to evaluate the above outlined parameters. Encouraging results are being obtained in animals receiving treatment in the low-lethal-dose range (8 mC per lb of body weight) given homologous bone marrow and subsequently evaluated by means of kidney and skin homografts.

5. Conclusions

1. Administration of bone marrow autografts is capable of saving the lives of dogs given lethal radiation with Y^{90}-DTPA.

2. Successful bone marrow homografts can be achieved by using the Y^{90}-DTPA procedure.

3. Following the administration of autologous or homologous bone marrow, evidence of an early return of hematopoiesis and lymphatopoiesis is seen histologically and in the circulating blood.

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Dr. A. C. Andersen collaborated with me on both the histologic studies and the studies on the IVS material. In addition Dr. Andersen graciously donated experimental animals and equipment during the early experimental work when they were crucially needed. My indebtedness to him is as much for his friendship as for his collaboration.

Miss Patricia Garbutt and the technicians of the Donner Clinic on frequent occasions performed needed hematologic studies during the course of this work. To them I feel a debt of sincere gratitude.

Dr. John Schooley prepared the rats for sampling of thoracic duct lymph, and certainly this particular experiment would not have been performed without his aid.

Finally, I wish to acknowledge those who made my coming to the Donner Laboratory possible. Fellowships from the National Foundation and later from the National Institutes of Health sustained me during my years at Berkeley.

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APPENDIX A

Comparative Dosage in Mouse and Dog Bone Marrow on Exposure to 250-kv X-Ray Beams (Small-Cavity Effects, Cortical Bone Absorption Effect)

Bone, because of its relatively high electron density, absorbs more radiant energy than does a comparable volume of soft tissue at an equivalent depth in the body (88). The high flux of secondary photoelectrons produced in bone can increase the energy absorbed in the small cavities surrounded by the bone to a significant degree if these small cavities have dimensions comparable to the range of the photoelectrons. Spiers (33), using monoenergetic X-rays (lambda = 0.13A) characteristics of those produced by the 200- to 250-kv X-ray machines, demonstrated that this effect was quite significant in cavities of the size of those occupied by osteocytes and the Haversian canals. Subsequently, this finding has been widely used to explain the damage to viable bone after radiation doses that had previously been estimated to be nonlethal to osteocytes (89).

Epp, Woodard, and Weiss (90) recently reported bone marrow dosimetry calculations, taking into account the samll-cavity effect, on mice exposed to 250-kv and Co^{60} photons. Their primary interest was to see whether or not differences in the small-cavity effect for photons of various energies could be used to explain possible RBE differences between Co^{60} γ rays

and 250-kv X-rays. However, their data lend themselves to the problem of contrasting differences in dosimetry between mouse and large-mammal bone marrow due to the small-cavity effect.

For 250-kv X-rays, the average energy absorption in the total marrow of mouse, takinginto account the small-cavity effect, is 98.6 ergs/cm^3/r (90). If we take approximately 2 mm or greater as the size of the average bone marrow cavity in dogs and other large mammals, we have the average energy absorption in the bone marrow cavity when exposed to 250-kv X-rays of 90.8 to 91.7 ergs/cm^3/r (90). Given the same exposure to 250-kv X-rays, the mouse bone marrow then receives between 6.9 and 7.8 ergs/cm^3/r, or 7.5% to 8.6% more radiation than does the bone marrow of large mammals, such as the dog. In addition, the increased cortical bone thickness surrounding the marrow cavities of large mammals such as the dog causes appreciable attenuation of the photon beam. This is not the case with the thin-walled bony cortex of mice (approximately 40 to 400 micra). The cortical bone overlying the femur of a dog is of the order of 3 to 4 mm in thickness (as roughly measured in several dogs). If we take photon of λ = 0.13A (considered by Spiers to be characteristic of those produced by 250-kv X-rays) and the absorption constants of bone for 250-kv X-rays of λ = 0.349 (33), then the attenuation of a 250-kv X-ray beam in passing through 3 mm of bone

is approximately 10%. Mouse bone cortex is much less than 1/2 mm thick. The maximum attenuation is therefore less than 1%. We may say, then, that with respect to cortical attenuation the bone marrow in the dog femur receives approximately less than 91% of the radiation received by mouse bone marrow at comparable tissue depths on exposure to the same 250-kv X-ray beam.

Combining the estimates made by considering the small-cavity effect and the cortical-bone-attenuation effect, we have an estimate that 16% (approximately 9% + approximately 8%) less radiation is absorbed in the marrow cavities of large animals such as dogs than in the marrow cavities of mice. Of course, in the many homografting experiments done in mice, in which less energetic photons than those of the 250-kv X-ray machine were used, the small-cavity effect is even greater than calculated, and the preferential irradiation of bone marrow is much more marked.

APPENDIX B

Studies on the Intravascular Polysaccharide Associated

with Total-Body Irradiation: Occurrence and Significance with

Systemic and Gastrointestinal Beta Radiation

In 1958, A. C. Andersen described an interesting material
in the vasculature of dogs receiving supralethal total-body
X-irradiation (75). This substance was found mainly in the
small vessels of the kidney and heart but often was present
in the arterioles and capillaries of the liver, meninges, and
gastrointestinal tract. The lumina of many of these vessels
appeared completely occluded by globules of this material.
The surrounding areas often contained extravascular red
cells and fibrin, indicating that hemorrhage into tissue
may be intimately associated with the presence of large
globules of this material in small vessels.

Andersen characterized this intravascular material by
histochemical techniques and concluded that it probably was
a protein-polysaccharide complex; he named it IVS (intra-
vascular polysaccharide). He studied tissues of other
mammals (swine, guinea pigs, rats, mice, hamsters, monkeys,
and rabbits) dying from the acute radiation syndrome and
found the IVS material present (76). Animals dying of causes
other than radiation damage did not show IVS in their tissues.
It would appear that the complex solubilities of this IVS in
the fixing solutions used in routine histologic procedures as

well as the difficulty in distinguishing it on H and E sections have in the past kept many other investigators from observing this material in the tissues of irradiated animals.

A clue to the origin of this material appeared when Andersen locally X-irradiated segments of exteriorized small bowel in the dog and later noted the appearance of the IVS in the meninges of the brain (91). This remarkable abscopal effect indicated that irradiation of the GI tract was a critical factor in the production of this IVS.

The following study was undertaken primarily to evaluate the role of irradiating the gastrointestinal mucosa _in situ_ in the development of IVS.

Using systemically administered Y^{90} chelated with DTPA for producing supralethal total-body β irradiation, we have noted the presence of large quantities of this IVS in the appropriate organs. Y^{90} given orally is not appreciably absorbed from the gastrointestinal tract, and, being a pure β emitter, irradiates the gastrointestinal mucosa locally (92). It was felt that comparison of the ability of total-body β radiation (whole body including gastrointestinal mucosa) with that of orally administered Y^{90} (gastrointestinal mucosa irradiation only) to produce the IVS of Andersen would give insight into the role of the gastrointestinal mucosa in the production of this material.

1. <u>Materials and Methods</u>

Forty-two purebred beagles between the ages of 6 months and 2 years were divided into systemically and orally treated groups. Systemically Y^{90}-treated dogs were given the material as the DTPA chelate, as described in the body of this paper. The Y^{90} chloride (carrier-free) was administered orally to the beagles in 250 g of mixed beef and dog rations after the beagles had been fasted for 24-hr. All dogs in the total-body β-radiation experiments were allowed to die from irradiation damage. Those in the gut β-irradiating experiments were sacrificed on the 7th day. Post mortem examinations were performed and the tissues were placed in Bouin's solution. The tissues were embedded either in celloidan or paraffin, sectioned, and then stained with H and E, Pollak's trichrome, and methylene blue. In order to estimate the dosage in the gut-irradiation experiment, five dogs were given barium sulfate with 2 to 4 mC of carrier-free Y^{90} chloride added, and then serial X-rays of the abdomen were performed to determine the progress of the bolus of barium through the gastrointestinal tract. From this, the time of contact of the barium sulfate bolus with various portions of the gastrointestinal tract could be estimated. In this series of experiments, castor oil was given by mouth and enemas were performed when the barium sulfate was noted to be in the colon. This was done to

minimize the time of passage of the barium sulfate through the large bowel. All IVS estimations were done on the basis of 100 power magnification surveys of 1.5×2.0 cm sections of heart, lung and kidneys stained with Pollak's trichrome.

2. Experimental Results

a. Dosimetry calculations

There are many variables involved in the dosimetry of orally administered isotopes. At best, one can hope for only a "barn door" type of estimation. The following assumptions were made in arriving at our particular estimation of the dose received in the dogs given Y^{90} Cl_3 by mouth in this series:

(a) The 250 g of food given orally remained as a bolus of approximately 250 g throughout the passage through the gastrointestinal tract.

(b) The density of the meat and food in this bolus was approximately equal to the density of water.

(c) The Y^{90} was dispersed equally throughout all parts of the food bolus, and this dispersion did not change during passage through the gastrointestinal tract.

(d) There was no selective absorption of Y^{90} on the mucosa, and the Y^{90} remained intralumenal at all times.

(e) The radiation flux at the surface of the bolus was one-half the equilibrium flux within the bolus.

In Fig. B-1, dx represents the slice in the middle of the
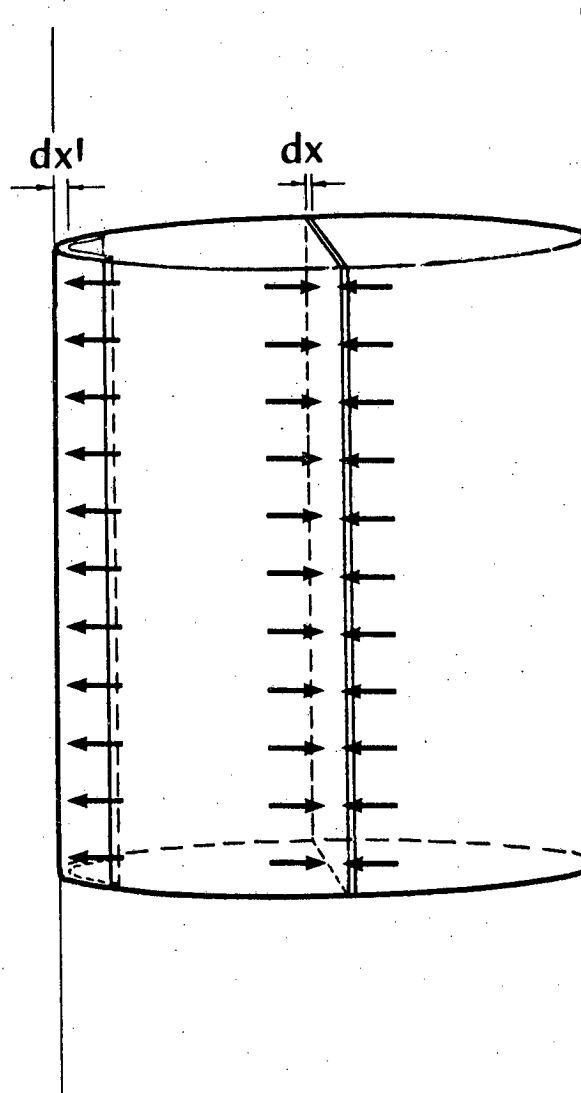
MU-24254

Fig. B-1. Idealized representation of food bolus containing Y^{90}
in the gut lumen.

bolus in which equilibrium has occurred; that is, the number of β particles giving up their energy within this volume is equivalent to the number of β particles originating within this volume. The dx' represents a thin slice at the surface of the bolus. In general, it can be said that in dx the β particles enter the slice from both sides; however, with the thin slice dx' lying on the surface, β particles can enter it from only one side. We can then roughly say that the flux of particles passing through the slice dx' at the surface of the bolus is approximately 1/2 the flux of particles passing through dx. If we consider a slice, dx, having a volume of 1 ml and being in equilibrium with the surrounding solution of the bolus, it can be said that the energy of the β particles emitted from this volume per minute (and also the energy given up by the β particles in this volume during their passage through it) can be given by the equation

$$E = \frac{\text{Vol. of dx}}{\text{Vol. of bolus}} \times \text{number of mC in bolus} \times \text{dpm/mC}$$

$$\times \text{av. energy per disintegration.}$$

For the case where the volume of the slice dx is 1 cc, the volume of the bolus 250 cc, the bolus contains 1 mC Y^{90}, the average energy of the Y^{90} β taken as 0.9 Mev and expressing the energy in rads we obtain the following:

$$(1/250) \times (2.22 \times 10^9 \text{ dpm/mC}) \times .9 \text{ Mev}) \times (1.6 \times 10^{-6} \text{ ergs/Mev}) \times (10^{-2} \text{ rad/erg}).$$

This is approximately equal to 0.128 rad/min/mC given up in the volume dx. The amount of energy given up in the volume of the thin surface slice dx' is one half of this amount or approximately 0.064 rad/min/mC. Table B-I lists the average time of contact of the bolus with the mucosa in various portions of the gastrointestinal tract. These figures are the averages of estimates obtained from five separate dogs. If the value (in rads/min/mC) given up to the volume dx' is multiplied by the time the bolus stays in contact with the mucosa of various portions of the GI tract, the dose values per mC administered are obtained (3rd column of Table B-I). To determine the total radiation exposure of various portions of the GI tract in a given dog, one simply multiplies the number given in column 3 of this table by the number of mC of Y^{90} given the dog. These dosimetry estimations are by necessity rough approximations, but are quite helpful in orienting us with respect to the level of Y^{90} required to produce effects that are evident clinically and histologically. Note that since the time of passage through the large bowel was measured after castor oil and enemas were given the control series of dogs receiving barium sulfate, the time of passage through the large bowel was necessarily a minimal one. The time the bolus spent in the large bowel of the dogs subsequently given large doses of Y^{90} was a variable which was uncontrolled;

Table B-I. Dose to various portions of G-I mucosa after oral administration of Y^{90} in 250-g bolus

Organ	Average time bolus was in contact with mucosa[a] (hr)	Estimated dose per mC Y^{90} administered (rad/mC)
Stomach	2.2	8.45
Small intestine	2.5	9.60
Large intestine	>12.5	48

[a]Times obtained from evaluation of serial X-rays in five dogs given $BaSO_4$.

therefore, the estimate of dosage to the large bowel

represents a minimum, and the dosage probably received by

the large bowel was greater than the calculated value.

b. <u>Systemic β-Irradiation Using Y^{90}-DTPA</u>

Table B-II summarizes the data obtained from a series

of dogs given Y^{90}-DTPA systemically by the method described

in the body of this paper. The estimated radiation doses in

rads are listed for the gastric mucosa, ileum and colon.

The radiation dose to the other tissues of the body can be

found by comparing the gastrointestinal dose given on this

table with the tissue distribution and dosimetry data given

in Table II of this report. None of the animals were

sacrificed, all died as a result of irradiation or spontaneously

from other causes. The degree of petechial hemorrhage was

estimated at the gross post mortem examination on the basis

of the number of petechial or purpuric lesions present in

the gastrointestinal mucosa, pleura, lungs, heart or kidneys.

The preterminal platelet counts listed are the last platelet

count performed prior to the death of the animal. In practically

all instances this was one to three days prior to the animals'

death. The quantity of IVS present was evaluated independently

by two observers. If only one or two particles of IVS material

were noted in all of the sections examined then the animal was

scored as 1+. If IVS could readily be recognized in the tissues

Table B-II. IVS survey following systemic administration of Y^{90}-DTPA

Dog number	Dose (rads)			Date of death (time after irradiation)	Petechial hemorrhage (at death) (0-4+)	Preterminal platelet level (No/mm³)	IVS (0-4+)
	Gastric mucosa	Ileum	Colon				
2-A	831	545	763	14 days	++++	38,000	++++
M-I	859	532	744	24 days	++	45,000	++++
David	619	383	536	12 days	++++	600,000	++++
Chain	1603	993	1389	14 days	+++	140,000	+++
4-G	849	526	736	12 days	+++	330,000	+++
R-14	<1109	<687	<961	12 days	++++	161,000	++
234655	1035	641	897	4 days	++	315,000	++
82	737	456	638	7 days	+	340,000	++
M-8	<720	<446	<624	4 days	+	475,000	++
Brutus	408	253	353	12 days	++++	420,000	++
W-7	1063	659	921	26 days	--	200,000	+
203	213	132	185	2 months	--	--	+(H&E)
F-19	--	--	--	5 days	++	400,000	±
W-4	845	524	732	2 months	--	174,000	--

Table B-II -- continued

Joe	650	403	563	2 months	--	--	475,000
Fatty	550	328	459	1 month	--	--	200,000

upon initial examination but a large fraction of the small vessels were free of this material then the animal was scored as 2+. If most of the small vessels observed in the tissue contained IVS then the animal was scored as 3+ or 4+ depending on the amount of the material. An animal was considered to have unequivocally significant quantities of IVS if scored 2+ or greater.

The animals listed on the upper portion of Table B-II all had 2+ to 4+ IVS in their tissues. All of these animals had petechial hemorrhages and none of them had severe thromobytopenia preterminally. Five of these animals died between the 12th and 14th days after irradiation. Of the remaining animals, three died between 4 and 7 days and one died at 24 days following irradiation.

On the lower portion of Table B-II are listed the animals having 0 to 1+ IVS in their tissues at post mortem examination. Only one of these, Dog No. F-19, had petechial hemorrhages. This dog had just trace quantities of IVS in the tissue sections that were examined. All other animals had neither petechial hemorrhages nor significant amounts of IVS and had survived 26 days or more after irradiation.

The clinical course, hematological and pathological findings on these dogs are presented elsewhere in the body of this report.

These results in dogs irradiated using Y^{90}-DTPA show that: significant quantities of IVS can be found as early

as 4 days and as late as 24 days after irradiation; petechial
hemorrhage was present in all cases where IVS was present in
2+ quanty or greater; the petechiae could not be the result
of thrombocytopenia.

The distribution of the IVS in the tissues of these
dogs given Y^{90}-DTPA was exactly similar to that seen in the
X-irradiated dogs reported by Andersen (75, 76, 91).

The kidney was the organ that contained by far the
largest amount of the IVS material (see Fig. B-2). The heart
often showed the IVS material in the small blood vessels in
the myocardium. The liver was another frequent site for
finding the IVS material, particularly in those animals dying
soon after irradiation -- that is, within 7 days. The IVS
was found in many other materials, very frequently in the
gastrointestinal tract, submucosally, and within the small
blood vessels supplying the villi.

It was noteworthy that, in frequent association with
intravascular polysaccharide plugs occluding the entire
lumen of a small vessel, red blood cells and fibrin were
noted extravasated in the tissues around the occluded blood
vessels. This suggested that the IVS was embolic and caused
an infarct, with loss of vascular integrity, in the small
vessel which it is seen plugging. Certain gross character-
istics of this IVS material can be inferred from considering
histologic pictures, such as Figure B-3, in which a particle
of IVS is seen straddling the bifurcation of a vessel. Such
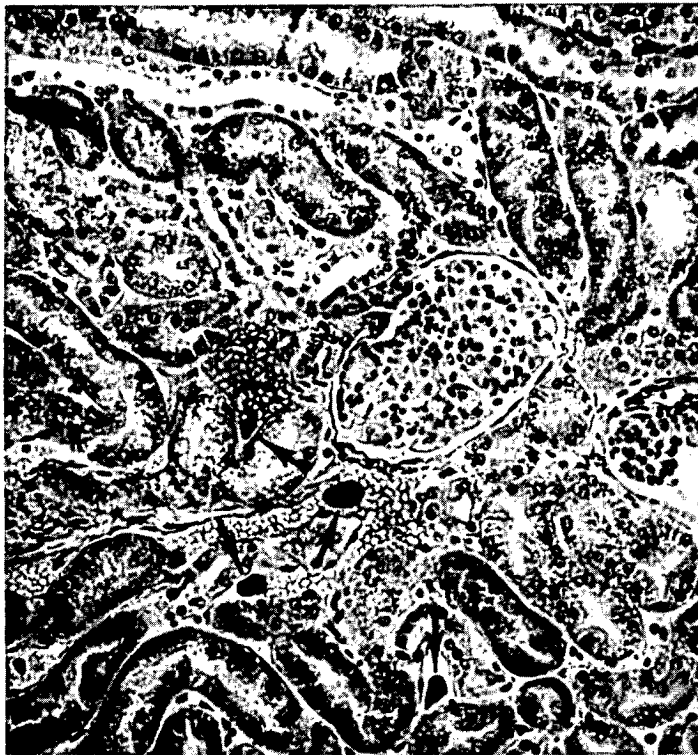
ZN-2991

Fig. B-2.   IVS seen in vessels in kidney of dog #2A (above)
            and dog #234655 (below).
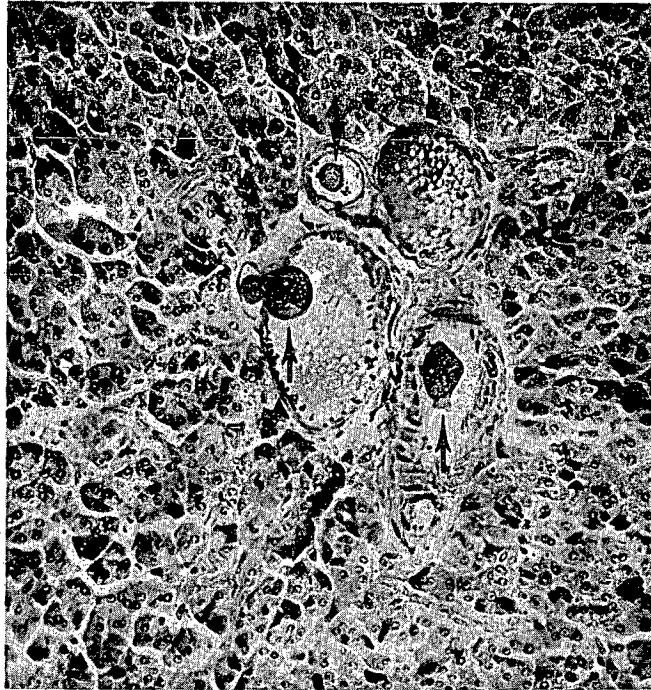
ZN-2988

Fib. B-3. IVS seen straddling bifurcation of pancreatic blood
vessel in dog. #2A

histologic demonstrations seem to indicate that the material is at least somewhat plastic and can be molded to the shape of the vessel in which it finds itself, much like a drop of fat or a drop of oil in aqueous solution.

Table B-III summarizes the results obtained from the gut-irradiation studies using orally administered $Y^{90}Cl_3$. Animals (3 to 6 kilos) receiving 29 mC or more showed depression, anorexia and diarrhea beginning the second to third day following the feeding of $Y^{90}Cl_3$. The symptoms progressed and all animals were sacrificed on the 7th day after the administration of the radioisotope. Blood values remained within the normal range throughout the experiment. Gross examination at sacrifice revealed no lesions in dogs receiving less than 29 mC $Y^{90}Cl_3$, but those receiving more than this dose generally showed pulmonary congestion, hyperemia of the liver, mucosal hemorrhages in the distal small intestine, and denudation of the colonic mucosa. Dog No. 1-C also had multiple pulmonary petechiae. These lesions were confirmed histologically. None of these animals had significant amounts of IVS in their tissues.

It is of relevant interest that similar dogs given only 1 to 9 mC of Sr^{90} as a $Sr^{90}-Y^{90}$ equilibrium mixture (activity of Y^{90} equal to that of Sr^{90}) by mouth uniformly died between the 6th and 7th days following its administration (93). These dogs given 1 mC or more of $Sr^{90}-Y^{90}$ showed

Table B-III. IVS survey following oral administration of $Y^{90}Cl_3$

Dog no.	mC $Y^{90}Cl_3$ administered	Dose (rads to mucosa)			Petechial hemorrhage (at sacrifice 7 days after irradiation (0-4+)	IVS (0-4+)
		Stomach	Small bowel	Large bowel		
5-F	2.0	17	19	96	--	--
5-G	2.0	17	19	96	--	--
33-A	3.7	31	36	178	--	--
22-E	3.7	31	36	178	--	--
5-E	4.0	34	38	192	--	--
5-H	4.0	34	38	192	--	--
8C3	7.3	62	70	350	--	--
D-24	7.3	62	70	350	--	--
F-I	14.6	123	140	701	--	--
22-F	14.6	123	140	701	--	--
1-D	21.0	177	202	1008	--	--
97-B	21.0	177	202	1008	--	--
B-24	29.3	248	281	1406	±	±
C-24	29.3	248	281	1406	±	--
1-C	37.0	313	355	1776	+	--
M-8	58.5	494	562	2808	±	+
20-B	58.5	494	562	2808	±	--
12-G	87.8	742	843	4214	±	--

Symptoms -- Progressive increase in diarrhea with increasing dose. Dose above 29 mC showed anorexia, listlessness and bloody diarrhea 2 to 3 days prior to sacrifice. Histopathology: Severe necrosis of colon mucosa in dogs receiving over 30 mC $Y^{90}Cl_3$.

complete denudation of the intestinal mucosa from the mid-jejunum distally including the ileum and colon. Extensive hemorrhagic lesions were also noted in the lungs, spleen, lymph nodes and marrow cavities and IVS was found within all organs examined.

The discrepancy between both the gross pathologic and histologic lesions seen in the dogs receiving 2 to 87.8 mC $Y^{90}Cl_3$ orally, reported here, and that seen in the dogs receiving 1 to 9 mC Sr^{90}-Y^{90}, previously reported, may possibly be explained by the metabolic differences between divalent yttrium and trivalent strontium. 5 to 60% of orally administered strontium-90 is absorbed systemically as compared to less than 0.05% absorption of orally administered yttrium-91 (92). While the yttrium remains intraluminal and simply passes over the mucosal surface, the strontium passes through the gastrointestinal mucosa to be absorbed systemically. It is not unreasonable to assume that the process of absorption of the strontium prolongs the contact and proximity of the pure beta-radioactivity with the gastrointestinal mucosa thereby greatly increasing its radiation dose. Following its absorption and prior to its eventual accumulation in bone, the Sr^{90} may irradiate structures far removed from the gastrointestinal tract.

3. Discussion

The pattern of radiation damage as well as the dose-response is different for Sr^{90}-Y^{90} equilibrium mixtures versus Y^{90} alone. The damage due to 29 to 88 mC Y^{90} given orally is to a large extent limited to the mucosa of the large bowel; petechial hemorrhages are not characteristically seen in structures other than the GI tract and IVS is not found. Oral administration of 1 to 9 mC Sr^{90} as Sr^{90}-Y^{90} equilibrium mixtures produces systemic damage mimicking total body X-irradiation. In this dose range Sr^{90}-Y^{90} will produce mucosal denudation of the small bowel associated with widespread petechial hemorrhages and IVS.

The results obtained from the oral administration of large doses of $Y^{90}Cl_3$ establish that destruction of colonic mucosa, per se, is not associated with the appearance of significant amounts of IVS. Other experiments (91), have established that destruction of small bowel mucosa by 1100 to 1700 rad of X-irradiation does produce petechial hemorrhages in non-irradiated organs (kidney) associated with IVS but not associated with thrombocytopenia. Destruction of similar segments of small bowel mucosa by manual trauma or the intraluminal injection of boiling water in non-irradiated animals resulted in death of the animals in 3 to 5 days without associated petechial hemorrhages or IVS in other organs. Irradiation of the thorax or liver alone with 1100 rad of X-irradiation also did not produce widespread petechial

hemorrhages or IVS in the dog (91). Dogs given high levels
of total body irradiation by fast neutrons (285 rad), 250-kv
X-rays (300 r to 350 r) (91), or Y^{90} β-irradiation (Table B-II
of this report), develop petechial hemorrhages and IVS.

It appears from these observations that there is a strong
association between widespread petechial hemorrhage following
irradiation and the presence of IVS; that thrombocytopenia is
not present in many instances where postirradiation petechiae
are observed and therefore thrombocytopenia cannot be the
cause of the hemorrhagic diathesis in these instances; that
radiation destruction of small bowel mucosa with X-rays is
associated with the presence of IVS while the destruction of
colonic mucosa with beta radiation is not associated with the
presence of IVS.

4. Conclusions

1. Evidence is presented that following systemic Y^{90}-
DTPA irradiation in the dog petechial hemorrhage is associated
with the presence of IVS rather than the presence of thrombo-
cytopenia.

2. A method for selective β-irradiation of the mucosa
of the gastrointestinal tract with $Y^{90}Cl_3$ has been presented.

3. Significant quantities of IVS are not present after
destruction of colonic mucosa by β-irradiation but as has
previously been demonstrated, is present after comparable
destruction of small bowel mucosa.

4. The radiation pathology seen after oral $Y^{90}Cl_3$ administration differs from that seen with oral administration of $Sr^{90}-Y^{90}$ equilibrium mixtures and comparable local intestinal mucosal damage occurs at a much higher dose range when using Y^{90} alone as compared to $Sr^{90}-Y^{90}$.

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