Development of a Portable Blood Irradiator for Potential Clinical Uses

F. P. Hungate

December 1988

**Prepared for the U.S. Department of Energy
under Contract DE-AC06-76RLO 1830**

**Pacific Northwest Laboratory
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PACIFIC NORTHWEST LABORATORY
operated by
BATTELLE MEMORIAL INSTITUTE
for the
UNITED STATES DEPARTMENT OF ENERGY
under Contract DE-AC06-76RLO 1830

Printed in the United States of America
Available from
National Technical Information Service
United States Department of Commerce
5285 Port Royal Road
Springfield, Virginia 22161

NTIS Price Codes
Microfiche A01

Printed Copy

Pages	Price Codes
001-025	A02
026-050	A03
051-075	A04
076-100	A05
101-125	A06
126-150	A07
151-175	A08
176-200	A09
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226-250	A11
251-275	A12
276-300	A13

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SUMMARY

This document provides an account of the development of a fully portable blood irradiator and the evaluation of its safety and efficacy when implanted in goats, sheep, a baboon and dogs. The program was initiated because the control of lymphocyte populations by irradiation is a potential method for improving success in organ or tissue transplantation and for treating a variety of blood diseases.

Preliminary studies involved the use of a variety of radioactive source materials, including iron-55, iodine-125, thallium-204, and strontium-90. Later, we found that the beta-emitter thulium-170 has the appropriate energy, is relatively inexpensive, and can be encapsulated for safe use. Likewise, several structural materials were used, at various times, in our attempt to find one that is compatible with blood (nonthrombogenic), is not damaged by large doses of radiation, and is easily formed.

Among the models of irradiators tested, the wire coated with ^{238}Pu was of special interest, despite the fact that it was not appropriate for use in humans because the ^{238}Pu was not fully contained. This model was implanted as a transverse obstruction in the thoracic aorta of a dog for over a year without apparent damage to the vessel and with no evidence of thrombus formation. It was the source of approximately 1 Gy/day of alpha radiation that was delivered to the blood without producing detectable lymphocytopenia.

Failure of materials during the testing of an irradiator using strontium-90 led to the use of vitreous carbon as both the blood interface material and body of subsequent irradiator units. Thulium-170 was found to be entirely suitable as a source material because of its modest beta energy (1 MeV maximum), which enables it to effectively irradiate the blood while producing bremsstrahlung radiation of sufficiently low energy that it can be shielded with a reasonable mass (about 1 kg). Thulium has the special advantage that the stable isotope ^{169}Tm is used in initial construction stages. Only after the thulium is fully encapsulated is the ^{169}Tm activated by neutrons to the beta-emitter ^{170}Tm .

Irradiator design was guided by advice from consulting physicians and by the results of testing in animals. Animal testing showed the units to be easy and safe to use. Furthermore, their use resulted in rapidly diminished numbers of circulating lymphocytes, and an associated reduction of cells in lymphoid tissue. The cellular immune system was compromised, resulting in approximate doubling of the time for skin-graft retention and extension of kidney function in animals that received an unrelated kidney after excision of their own kidneys. In one dog with a pre-existing rough and scaly skin, diagnosed as an autoimmune disease, it appeared that the condition of the skin improved over the approximately 6 weeks of treatment with the blood irradiator.

Somewhat surprisingly, animals treated by blood irradiation did not appear to be more susceptible to infection even when treatment was prolonged and they were returned to the field (goats), or caged with other animals (dogs) on cessation of treatment.

Dosimetry data indicate that doses approaching 1 kGy to blood can be given without exceeding prescribed limits and, if shunts are placed on two limbs, either simultaneously or sequentially, doses approaching 2 kGy can be delivered. Further reduction of doses to skin may be achievable by other relatively simple techniques.

At this time no testing has been done on people. Thus, efficacy data may not fully reflect the clinical situation involving larger blood volumes. However, if doses delivered are sufficient to kill lymphocytes, efficacy may be similar. Our work with goats, which weigh about four times more than the dogs and have correspondingly greater blood volume, suggests that this may be the case.

It seems likely that preliminary clinical use will be in the area of treating blood diseases, since this will require no pretreatment such as that needed to suppress early graft rejection. Our observation that cell depletion in lymphoid tissue is associated with the use of blood irradiators suggests that some lymphoid diseases may be treatable by means of this technique.

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INTRODUCTION

The concept of irradiating circulating blood to reduce the numbers of circulating lymphocytes, thereby suppressing cellular immune reactions, has been amply described (Lajtha et al., 1962; Cronkite et al., 1962). The observation of Trowell (1952) that lymphocytes are sensitive to ionizing radiation and the later finding that lymphocytes are the initiators of cell-mediated immune reactions provided the necessary impetus for considering clinical applications. Members of this laboratory became interested in developing a portable irradiator when it was noted that a chronic lymphocytopenia developed following inhalation by dogs of the extremely insoluble plutonium oxide.

Essentially all of the work on blood irradiation at other laboratories used large, fixed radiation sources, with the patient's blood exteriorized by means of a shunt through a loop of tubing which passed through the irradiator (Cronkite, 1965; Persson et al., 1971; Weeke and Sorensen, 1971). Typically, the dose was administered in two to five treatments per week, using transit doses (i.e., the average dose to a unit of blood passing once through the irradiator) of 2 to 9 Gy. Although results of these studies indicated some efficacy, they were not sufficiently positive to warrant continuing use of blood irradiation as a routine procedure in organ transplants.

It is now known that relatively few circulating T-lymphocytes are needed to initiate rejection; furthermore, this sequence of stimulation by a foreign cell and the triggering of rejection occur in a time frame of less than 24 hours (Roser, 1976). Thus, one would expect that periodic irradiation would allow lymphocytes outside the circulatory system at the time of irradiation to return to circulation between irradiations and initiate rejection before a subsequent treatment is given. The question remains whether continuous irradiation sufficiently inhibits those lymphocytes circulating at the time of organ transplanting and immediately thereafter to suppress early rejection. There is no presumption that blood irradiation will provide chronic therapy, only that it can control early rejection with essentially no side effects deleterious to recovery of the transplanted organ's function. The work of Storb et al. (1969), using X rays to continuously irradiate the blood, showed suppression of early skin-graft rejection in baboons, thus encouraging further study of the concept of continuous irradiation of blood. X rays

were also used in a study of effects of blood irradiation using rats (Hagenbeek, 1977).

Many laboratories throughout the world have attempted to develop a fully portable blood irradiator capable of providing continuous irradiation. With only one known exception (Wolfe and Hume, 1967), all used ^{90}Sr - ^{90}Y as the energy source. It was finally concluded, by Weeke (1973), that a fully portable source was not possible because of the large mass of shielding required to absorb the bremsstrahlung radiation.

EXPLORATORY STUDIES

We have tested several types of irradiators at Pacific Northwest Laboratory (PNL; Table 1). Our studies were initiated using the concept of an irradiator implanted in an arterial blood flow, i.e., by replacing a segment of artery with the irradiator. The basic design had a thin tube interfacing with blood and was cast from epoxy. The radiation source was an annulus surrounded by tantalum shielding; the various parts were bonded together with epoxy (Figure 1). A sleeve of knitted dacron tubing served as a bridge between the device and the artery, permitting the artery to be sewn to the dacron. Initially, the source materials were ^{55}Fe and ^{125}I , both of which emit X rays that have energies low enough to be readily shielded. These materials and the tantalum shielding were potted in medical-grade silicone rubber. The devices were 6-8 mm in diameter, consistent with the dog's thoracic aorta, into which they were inserted, and were radiation-sterilized prior to surgery.

TABLE 1. Sequential Listing of Blood Irradiator Types Tested.

Radiation Source (maximum energy)	Blood Interface	Body of Irradiator	Location
^{55}Fe (6 KeV) photon	Epoxy, GBH ^a -coated	Epoxy + Tantalum, Dacron end pieces	Implant
^{125}I (35 KeV) photon	Dacron	Silastic-Saran™ Tantalum	Implant
^{204}Tl (0.76 MeV) β	Dacron	Silastic-Saran™ Tantalum	Implant
^{90}Sr ^{90}Y (0.55 MeV β 2.27 MeV β)	Silicone rubber	Epoxy Lead Silastic Dacron end pieces	A-V ^b shunt
^{170}Tm (1.0 MeV β)	Vitreous carbon	Vitreous carbon	A-V shunt

^aGraphite-Benzalkonium-Heparin

^bArteriovenous

The animals were sacrificed approximately 6 months after surgery. A significant ring thrombus was noted at the juncture of the device with the blood vessel in all five units tested (including two controls), but the unit which was coated with graphite-benzalkonium-heparin (GBH) was least affected. No lymphocytopenia was

observed in any of the animals having irradiators which used ^{55}Fe or ^{125}I and provided doses to blood calculated to be 0.005 Gy/day or less. We also observed movement of both ^{125}I and ^{55}Fe into blood when epoxy was the barrier material. No such movement was observed when Saran® film served as the barrier between source and blood.

FIGURE 1. Epoxy-Clad Blood Irradiator Modified at Implantation by Affixing Dacron Velour in the Ligation Groove with Silicone Rubber

In the absence of other isotopes that emit photons of suitable energy, the next unit was constructed with ^{204}Th . The energy of the 0.76-MeV beta radiation emitted was almost entirely absorbed in the first millimeter of blood adjacent to the wall of the device. The barrier between source material and blood was minimal. Two of these irradiators were implanted in dogs, one of which succumbed to embolic occlusion of the mesenteric artery a short time after surgery. (This is the only observed instance of thrombus migration and occlusion of vessels among the many animals and various species used.) The irradiator was recovered and used for dosimetry with both Fricke solution and thermoluminescent dosimeters (TLD). The ^{204}Th irradiator (Figure 2) delivered an initial dose of 0.1 Gy/day to blood but produced no detectable evidence of effects on levels of circulating lymphocytes or on retention of skin allografts, even after the irradiator was in place for a year.

FIGURE 2. Mark X Blood Irradiator, Using Beta Radiations from ^{204}Tl

Irradiation with Alpha Rays from Plutonium-238

To explore alternative types of radiation and other modes of delivering dose, we tested a new type of irradiator that required no shielding. It consisted of a 5-cm-long stainless steel wire, 0.25 mm in diameter, coated on 2.5 cm of its midsegment with 0.6 mCi of ^{238}Pu , which was covered by a thin layer of platinum. The ^{238}Pu was electrodeposited, and the platinum containment was sputter-deposited (Material Research Corp. Model 8620 rf-sputtering system) in two stages: an initial deposition of 7500 Å and a second layer of 3500 Å. There was no smearable alpha activity, and an alpha energy analysis showed no 5.5-MeV alpha-energy, as would be expected if some of the ^{238}Pu were not coated. The peak energy shifted to 4.82 MeV when the wire was coated with platinum.

The wire irradiator was implanted in the aorta of a dog by inserting the wire diagonally through the vessel and suturing it in place at both ends. A similar wire, not plated with ^{238}Pu , was implanted as a control in another dog. The surgeries

were successful in that both the irradiator and its control were implanted directly in the blood stream with no apparent deleterious effects. Some loss of alpha activity into blood, feces, and urine was observed initially, but levels in blood fell to near background within a few days. When this alpha-irradiator was removed after a year, during which no lymphocyte levels was observed, there was no evidence of thrombus or obstruction in the vicinity, and the control wire was equally free of obstruction by thrombus or deformation of the vessel wall (Figure 3) caused by the wire. The dose delivered to blood was determined by placing the wire in a measured volume of Fricke solution for a known period of time and determining the change in light absorption at 304 nm. The dose was calculated using a G value of 5.1. It appeared that the dose to blood was 1.1 Gy/day, although initial data had suggested that the dose would be only 0.4 Gy/day. In either case, the cumulative yearly dose was over 100 Gy (130 - 400).

FIGURE 3. Section of Thoracic Aorta Removed from Dog 828, Which Had a Wire Implanted in the Thoracic Aorta

These data substantiate the conclusion of Ragan et al. (1987) that lymphocytopenia induced by inhaled plutonium oxide is primarily caused by alpha

doses delivered to lymph passing through the tracheobronchial lymph nodes and not by irradiation of blood circulating through the lungs.

We also tested the beta-emitter ^{147}Pm as a radiation source but were unable to obtain sufficient doses to blood because of dose absorption in the barrier layer between the isotope and the blood. The other candidates, ^{125}I and ^{204}Th , were too costly and/or had specific activity too low to provide effective doses to blood. Nearly all attempts reported in the literature to develop portable blood irradiators made use of ^{90}Sr - ^{90}Y as source material. In all cases, the source material was distributed in such a way that the dose was delivered to blood over a significantly long portion of the shunt tubing through which the blood flowed. This resulted in shielding weights so great that the irradiator was not portable (Weeke, 1973).

A Strontium-90 Irradiator

We thought it possible to reduce the shielding mass by concentrating the source to deliver a very large dose to a very short segment of the column of shunted blood. In this way, the weight of shielding could be minimized. We contained 2 Ci of ^{90}Sr - ^{90}Y in a cylindrical zone 1 cm long within an approximately 2.5-cm-thick outer layer of Hevimet™ shielding. A layer of titanium, 0.025 mm thick, covered the ^{90}Sr - ^{90}Y (Figure 4). Silicone rubber tubing (2.6 mm ID, 3.7 mm OD) carried the blood from a teflon cannula inserted in the carotid artery, through the irradiator, to a cannula inserted in the jugular vein. The irradiator was constructed in two halves, held together by Allen screws (Figure 5), so that it was removable.

The dose to the dog's blood was measured by passing Fricke solution through the irradiator at a known flow rate and measuring the change in optical density. The transit dose was 0.4 Gy, assuming a flow rate of 100 ml/min. Total weight of the irradiator was almost 2 kg.

The ^{90}Sr - ^{90}Y irradiator was fastened around an arteriovenous (A-V) shunt, using medical-grade silicon rubber tubing. Continuity of blood flow was monitored for 8 hr. However, by 12 hr following the start of irradiation, flow had stopped because of thrombus formation. When the shunt was examined to re-establish flow, we found that the tubing had become brittle enough to crack and fracture, which was probably the cause of the clotting. Following this dose to blood (20-30 Gy), levels of

circulating lymphocytes fell and remained at approximately 2/3 of the pre-exposure level for about 6 weeks.

FIGURE 4. Schematic of the Mark XIV ^{90}Sr - ^{90}Y Irradiator (Units in Millimeters)

The experience with the ^{90}Sr - ^{90}Y irradiator was extremely valuable in that it clearly indicated a need for alternative structural and source materials if a portable unit was to be developed. The following conclusions resulted from the test:

1. The dose must be delivered from a localized source to minimize shielding mass.
2. The blood interface material must be extremely radiation-resistant.
3. The blood interface material must be nonthrombogenic.
4. The blood interface material must be sufficiently thin to permit delivery of a significant dose to blood and also be impermeable to the source material.

FIGURE 5. Photograph of the Mark XIV Irradiator Ready for Insertion of the Blood Transport Tube

5. The source must emit radiation sufficiently energetic to pass through a barrier and effectively irradiate blood. It must also have a high specific activity.
6. The shield for the source material must be portable.

Two fortunate circumstances occurred at this time. First, we became aware of thulium-170 as a potential radiation source that has a beta emission of appropriate energy and high specific activity. The second event was that one of the investigators, Roy Bunnell, was working with vitreous carbon (for another purpose). Thulim-170 emits beta radiation with a maximum energy of 1-MeV. It has a 130-day half-life and is readily produced from naturally occuring thulium oxide, which has a

neutron cross section of 125 barns and can therefore be converted by neutrons into the high-specific-activity ^{170}Tm . Our extensive experience with graphite indicated that vitreous carbon would be extremely radiation-resistant; independent studies showed that pyrolytic graphite was nonthrombogenic. In an afternoon's discussion we developed the basic concepts for the fully portable blood irradiator using ^{170}Tm as source material and vitreous carbon as the body and blood interface.

The first model tested in an animal had a pyrolytic graphite tube (2.8 mm ID, 3.3 mm OD) to carry the blood through the thulium/vitreous-carbon irradiator body (Figure 6). The irradiator had an inner containment-barrier layer, an adjacent layer of microencapsulated ^{170}Tm , and a thick outer layer of vitreous carbon (Figure 7). The silicone rubber tubing of the carotid-jugular shunt was slipped over the ends of the graphite blood tube. The transit dose rate, measured by Fricke dosimetry, was 0.16 Gy, assuming a blood flow rate of 100 ml/min.

FIGURE 6. Pyrolytic Inner Blood Tube, Vitreous Carbon- ^{170}Tm Irradiator and Shield Pieces

The irradiator was placed in the A-V shunt on a miniature goat and, in a few days, the numbers of circulating lymphocytes were depressed. Most importantly, there was no damage to either the pyrolytic graphite tube, nor were there changes in the appearance or loss of containment in the vitreous carbon portion which contained the ^{170}Tm . Furthermore, there was no apparent loss of containment of the thulium during the process of vitrification, which involved temperatures of 1000°C . Encapsulation of the ^{169}Tm in the irradiator unit before activation and cleanup of the unit after activation were relatively easy. Particularly attractive was the fact that all fabrication was done in a standard laboratory without concern for any radiation exposure during construction of the units.

FIGURE 7. Longitudinal Section Through the Wall of the Vitreous Carbon Body of A Thulium Blood Irradiator (250X), Showing the Various Layers

Vitreous Carbon - Thulium-170 (VC Tm) Irradiators

With these preliminary favorable data in hand, we evaluated alternatives to simplify and increase the efficiency of design and construction. Calculations indicated that eliminating the graphite tube and using vitreous carbon as the blood interface might increase the dose delivered to blood by a factor of 10. We tested the thrombogenicity of vitreous carbon tubes, prepared in a variety of ways and vitrified at temperatures up to 2500°C, by implanting them into A-V shunts and subsequently removing them to determine if clotting had occurred. These tests indicated that vitrification at 1000°C was adequate and that vitreous carbon is quite nonthrombogenic.

Forming the very thin blood interface layer proved to be difficult. Preliminary units in which this layer had been formed on a wax mandrel were irregular and often leaked small amounts of ^{170}Tm . It appeared that, even though it was extremely smooth, the wax left imperfections; there appeared to be no solution to this problem if we continued using a wax mold. Thus, a technique of spin-casting the inner tube was developed and has been used, with various refinements, ever since.

A three-step construction technique (Hungate et al., 1976; Bunnell et al., 1977) was developed: 1) to give the irradiator a smooth, thin, inner surface; 2) to encapsulate the $^{169}\text{Tm}_2\text{O}_3$; and 3) to obtain a cast, outer structural layer in which all of the unusable beta radiation would be absorbed. By absorbing the beta radiation in the low-Z carbon, secondary bremsstrahlung radiation was minimized.

Following construction, the irradiator was carbonized at 1000°C in a vacuum oven and, subsequently, the stable ^{169}Tm was converted to radioactive ^{170}Tm by placing the unit in a high neutron flux ($> 10^{14}$ neutrons/cm²). By using specially pure polyfurfuryl alcohol and Tm_2O_3 , radiations (other than those from ^{170}Tm and ^{171}Tm) were essentially zero. Units constructed in this way had the thulium macroencapsulated, with the thin blood interface layer on one side and the carbon body on the other. The thulium was also microencapsulated, with each individual granule of the thulium oxide completely surrounded by vitreous carbon. This was the result of suspending the granules of thulium oxide in a polyfurfuryl alcohol slurry, which was then carefully applied to the already cast blood barrier tube. This

slurry, in turn, was allowed to polymerize ("set up") before the outer body was cast around it. Leach tests using 6 N HCl showed that only small amounts of thulium were lost from containment when an irradiator unit was broken.

A special advantage of encasing the thulium in carbon was that all the machining and most of the other steps that required handling were done with nonradioactive ^{169}Tm . Following activation, only postirradiation cleanup, testing for leakage, placing the irradiator body in the shielding, and measuring the delivered dose (and, of course, implantation) were necessary. This greatly reduced the dose to personnel and simplified construction procedures. The vitreous carbon surrounding the ^{170}Tm source was thick enough to absorb the beta radiation of the ^{170}Tm and minimize conversion to high-energy bremsstrahlung. Despite this, as much as 1-2 kg of heavy metal shielding were required to reduce the secondary bremsstrahlung radiation to acceptable levels. The shielding was designed to protect the irradiator as well and to assist in holding the shunt hardware firmly in place during use.

Making the connection to the shunt hardware was a continuing problem. A simple slip-over type connection, such as that used with the initial pyrolytic graphite liner tube, was not satisfactory because of thrombus induction.

After conferring with potential physician users of the irradiators, a new connector design was developed using Teflon inserts fitted to slide into an enlarged section at each end of the vitreous carbon (VC) Tm units. The enlargement was drilled out to provide square shoulders, against which the Teflon connectors fitted (Figure 8). When these units were tested on animals we observed a strong tendency for clot formation at the butt joint between the Teflon connector and the irradiator. It appeared that the pulsatile flow tended to open up a slight space, and the resulting discontinuity induced clotting. We also observed radiation damage to the Teflon after 2 weeks of use. Therefore, a new design was developed, with integral extensions of the VC Tm irradiator sized to fit shunt connectors (Quinton Instruments) used at that time in dialysis of human patients. Figure 9 shows the irradiator ends and their connection to the Quinton connectors. The dimensions of the VC Tm units used in animal studies is shown in Figure 10.

FIGURE 8. Vitreous Carbon Thulium Blood Irradiator, Showing Teflon End Connectors

The profile of the radiation dose to the blood (Figure 11) was calculated using a modified Betblud code. Although there is an order-of-magnitude difference between the dose delivered to central and peripheral cells in each pass, it is doubtful that this is a significant disadvantage under conditions of chronic exposure. Marsaglia and Thomas (1965) have discussed the statistical nature of accumulated dose. Transit doses of individual irradiators were measured using Fricke solution flowing through the units at known flow rates. This, of course, gave the mean dose received by cells passing through the irradiator; doses to individual cells differed as much as 10-fold (Figure 11).

Initial testing of these VC Tm units was in pygmy goats weighing about 20 kg, in full-size goats weighing about 47 kg (Figure 12), in sheep weighing about 40 kg, and in one baboon. Maintaining patency in the shunt flow was always difficult

FIGURE 9. Vitreous Carbon Thulium Blood Irradiator, Showing Quick-Couple Attachments to Shunt Tubing

because excessive movement of the animal's head and neck caused irritation in the vessels due to movement of the cannula ends of the shunts. Flow was monitored at least twice daily with a doppler flow meter and, when flow stopped or nearly stopped, the shunt was opened and thrombus material aspirated--usually from the venous side. The irradiator was either replaced or immediately cleaned, resterilized with a liquid sterilant, such as 70% alcohol or benzalkonium, rinsed with sterile saline, and loaded with saline carrying about 500 units of heparin. The clean irradiator was then reinserted in the shunt, typically within 30 minutes, but in early studies more prolonged periods of interrupted radiation treatment were not uncommon. The tubing used to aspirate clots and remove obstructions was sterile; however, the process was conducive to the entry of bacteria. Yet even when clotting occurred repeatedly, septicemia arose only in occluded shunts left untended for a prolonged period. This lack of septicemia indicated that the humoral immune system was little affected. In some instances, treated animals that were no longer needed for this research were released to pasture (goats) or returned to their normal runs (dogs). There were no observed health problems in such animals even though their numbers of circulating lymphocytes did not return to normal for approximately 6 months after irradiation treatment ceased.

Animal response was initially monitored in terms of numbers of circulating lymphocytes and skin-graft rejection times. Because we were interested primarily in the numbers of T-lymphocytes, we considered ways to determine which population of lymphocytes was most depressed by irradiation. However, attempts to differentiate by size were not satisfactory. At that time we did not have antisera specific for either T- or B-cells, therefore we were forced to use total number of circulating lymphocytes. Typically, blood was drawn prior to treatment and two or three times weekly during the period of blood irradiation; routine hematology data were obtained on each sample. Samples were obtained by direct venipuncture or, occasionally, by opening the shunt and drawing blood directly into the blood tube. Clinical chemistry data were also obtained for blood samples drawn during early testing, but clinical chemistry was stopped when no significant changes were observed.

FIGURE 10. Dimensions (in.) of the Vitreous Carbon Thulium Blood Irradiator

Evaluation of VC Tm Units in Animals

Animals used to evaluate the irradiators were unrestrained, i.e., free to move about in pens or cages. These areas were systematically cleared of projecting hardware or other features likely to catch on the irradiator or the hardware used to support the irradiator. A simple cloth sling was used for the goats and sheep, but a much more complete protector was required for the dogs. The goats were routinely hobbled to prevent them from scratching, when they might catch a hoof in the shunt, thereby disconnecting it, which could, of course, lead to total loss of blood and death. The several kinds of harnesses used with the dogs were made as nearly "scratch-proof" as possible.

Typical lymphocyte responses to the VC Tm units are shown in Figure 13. It is clear that an immediate decline of circulating lymphocytes followed installation of

the irradiators. It is also evident that treatment was interrupted in many animals as a result of thrombus induction despite the use of a variety of clot suppressants such as heparin, aspirin and persantine. In all these cases, there was a concerted effort to remove any obstruction and restart flow. This required removal of the irradiator and, in some instances, the VC Tm unit required special treatment. For this or other reasons, the unit was removed and shunt flow was restarted, using a standard connector in place of the irradiator. This caused gaps in treatment, which are indicated in the figure only when the delay was in excess of 1 day. During the terminal period of treatment, flow was typically intermittent with prolonged periods of no treatment when stoppage occurred at night. Flow was usually monitored twice daily, using a Doppler flow meter with conducting jelly between the sensing unit and the silicone rubber shunt tubing.

The clotting problem appeared to be most severe at the venous vessel tip, although occasionally clots occurred at the arterial end. There was seldom evidence of thrombus induction within the shunt unless there was a poor fit of a connector and leakage occurred. Toward the end of each treatment period, clots had to be removed frequently, with some loss of blood each time. Aspirin and persantine were of little help as anticoagulants with the goats. Therefore, dicoumarol was tried, with the objective of maintaining activated clotting times at levels 2-3 times normal. To achieve this, extremely large doses of dicoumarol were used to overcome the vitamin K content of the feed, which was primarily alfalfa hay. This worked reasonably well if the animal's dietary intake remained constant. However, if feed intake decreased, a bleeding crisis developed so rapidly that vitamin K therapy to counteract a dicoumarol overdose was not always possible. Thus we discontinued using dicoumarol in goats and returned to injecting heparin twice daily.

Reciprocal full-thickness skin grafts on treated and control animals provided data of only limited usefulness because we were unable to satisfactorily quantify the rejection time. However, taking the data at face value, it appeared that irradiation of blood approximately doubled the time of skin retention. Biopsies suggested a lack of vascularization, but autografts were generally successful, i.e., "took," indicating that our technique was adequate. The difficulty in quantifying rejection time resulted, in many cases, from partial adhesion. It was rather difficult to distinguish between vascularized adhesion and the simple sticking of the graft to

the scab formed at the graft site. There was no improvement when split-thickness grafts were tested.

FIGURE 11. Relative Blood Dose Profile with 1/8-in. (0.32 cm)-Dia Tube

Testing in a Baboon

A blood irradiator unit was evaluated in a baboon in the laboratory of Dr. E. D. Thomas in Seattle, WA. Suppression of lymphocyte levels and prolonged skin allograft retention were similar to responses previously observed in goats.

FIGURE 12. Full-Size Goat Used to Test Blood Irradiator. Protective covering has been removed to show irradiator and externalized portion of arteriovenous shunt

An AV shunt was installed, at the Fred Hutchinson Cancer Center, on the left hind limb of a 12-kg baboon had previously been trained to restraint in a chair. The vasculature in this animal was exceptionally small, necessitating use of a No. 17 cannula in the femoral artery; the silicone rubber shunt tubing was 0.1-in. ID.

On the day following surgery, a VC Tm unit that delivered a transit dose of 0.26 Gy, assuming a flow rate of 100 ml/min of blood, was inserted in the shunt. Despite the restricted blood flow caused by the very small cannula and serious

incompatibilities between the 0.1-in. shunt tubing and the irradiator (0.125 in.), blood flow was maintained for 20 days. This was achieved by heparin therapy, daily removal of thrombi at points of flow turbulence, and insertion of a new shunt in the contralateral leg on the 16th day. Figure 14 shows lymphocyte levels during the test period.

FIGURE 13. Typical Goat Lymphocyte Response to Vitreous Carbon Thulium Blood Irradiator. Animal number and daily dose to blood are indicated on curves. Heavy portion of curve indicates time during which irradiator was in place

A skin allograft was applied on the 9th day following implantation of the irradiator. This graft was sloughed on day 31, 11 days following cessation of blood irradiation. The 22-day retention time was approximately twice that (10-12 days) observed in untreated animals.

FIGURE 14. Effect of Chronic Irradiation of Blood on Circulating Lymphocytes in a Baboon

As part of the safety procedures in this test, daily samples of urine-soaked wood chips were pooled and analyzed for radioactivity, as were daily blood samples. Neither showed evidence of ^{170}Tm , confirming the data previously obtained, which showed that the vitreous carbon barrier layer completely contained the radioactive thulium.

Evaluation in Dogs

Dogs were used for subsequent studies primarily because they are generally accepted in medical research as models for surgery as well as being useful for developing therapy for blood diseases. These dogs were obtained from the PNL beagle colony.

Protecting the irradiator shunt and its associated hardware is an acute problem in unrestrained dogs because they shake, scratch and bite anything which is irritating or catches their attention. A protective retainer was made from thermoplastic material used by physicians to form splints. Sheets of this material were cut and shaped to surround the dog's chest area, with holes for the forelegs to restrict movement of the unit (Figure 15). The leg area was padded with lambswool to minimize chafing. The front portion incorporated a metal half-cylinder to support the shielded irradiator, which was fastened to the half-cylinder by means of two machine screws. The screws were necessary to prevent shifting of the irradiator, especially when the dog attempted to dislodge the unit by shaking or scratching. The shunt tubing was also protected by attaching shaped pieces of the thermoplastic. Typically, these chest supports were changed about once a week, because wearing a particular unit for a longer time abraded the skin, particularly in the sternal area. Changing the unit also permitted washing the protector to remove hair, dried blood, etc.

To obtain supplemental data on skin allograft rejection times, 20 beagle dogs were paired, and reciprocal skin grafts were made between each pair of animals. The pairs were selected on the basis that they had no common ancestor in at least the two prior generations. Although pinpointing the specific day of graft loss continued to be difficult because of factors such as scab adhesion, the data showed a mean retention time of 10 days, with a range between 6 and 15 days. By comparison, similar grafts on four animals treated by blood irradiation were retained for an average of 25 days, with a range between 15 and 33 days. In other words, retention time in treated animals was about twice that observed in those untreated. These data are consistent with observations on goats and the one baboon treated, but the data from skin grafts were inconclusive because of the difficulty in judging rejection times.

FIGURE 15. Thermoplastic Retainer as Used on Beagle Dogs with the Irradiator as Part of the A-V Shunt

The longer retention times in treated animals can be explained by the reduced number of circulating lymphocytes (Figure 16); the data for this animal are typical. Data collected on this dog following blood irradiation showed that full recovery of numbers of circulating lymphocytes took about 6 months. Representative hematology data are included in the appendix.

FIGURE 16. Lymphocyte Response to Chronic Irradiation of Blood, Dog 1840, Female, Weighing 10 kg

As this phase of the study neared completion, it became evident that improvement was needed in the hardware used to hold the irradiator and protect it and the shunt tubing from damage.

The carotid artery and jugular vein are the most accessible and useful vessels for establishing a long-term shunt in animals. To minimize the length of tubing, we placed the irradiator and supporting hardware under the animal's head, but we considered the top of the shoulder as a possible alternative. The shoulder position has the potential advantage of easier access for the investigator and reduced accessibility to the dog. To support the units securely atop the shoulder we used a nylon mesh jacket, designed by A. Chatham, 5043 Oaknoll Avenue, Los Angeles, CA, for dogs used in research. The jackets are available in a variety of sizes and can

accommodate individual animals by adjusting the lacings. Those we used were small and fit the beagle dogs well.

To limit sideways shifting of the unit, the irradiator was attached to the mesh of the jacket by a series of linen or nylon ties through a thermoplastic strip in the shape of an inverted U. A protective cover of thermoplastic was also attached to the mesh of the jacket in such a way that it could be readily opened for access to the irradiator. The shunt tubing was tunneled beneath the skin on each side to the midlateral portion of the neck and was protected from that point to the irradiator by the thermoplastic cover over the irradiator.

Despite our attempts to adjust the lacings for a snug fit on the animal, the weight of the shielded irradiator unit (approximately 500 g) caused gradual shifting so that, on one side or the other, the shunt became stressed. This produced severe irritation by the Teflon vessel tips on the artery and vein, with consequent loss of shunt flow. None of our attempts to limit this lateral movement were successful. The shoulder position was therefore abandoned, and we continued for sometime to use the thermoplastic vests, slightly modified to further protect the shunt tubing and minimize chafing.

Toward the end of animal testing, the Chatham vests were modified by having a strip of nylon mesh zippered to the neck portion of the vest, so that the VC Tm units and shunt were protected and supported under the neck by the mesh. This modified vest appeared to be more comfortable for the dogs and gave better protection to the shunt hardware.

In collaboration with Dr. Tom Marchioro (Department of Surgery, University of Washington, School of Medicine, Seattle, WA), we then began a series of kidney transplant experiments in dogs. These experiments were conducted to provide direct data relevant to one of the potential applications and also provide a definite measure of the end point of functional immune suppression.

We had experienced difficulty in scheduling time in the reactor for activating the irradiators, which were previously activated one at a time. In order to provide adequate supplies of irradiators, a new design for simultaneously activating three irradiator units was developed (Figure 17) in 1981.

FIGURE 17. Graphite Carrier that Accommodates Three Vitreous Carbon Thulium Blood Irradiators During Activation by Neutrons

Because the graphite serves to carry heat from the site of activation to the jacket of the capsule, and thence to the circulating cooling water, a good fit of the unit in the graphite was essential. The capsules were sealed in an inert gas atmosphere so that potentially damaging oxygen was excluded. The seal, which was also of graphite, was tightened sufficiently to exclude the pressurized cooling water and retain the inert gas. A typical capsule design is shown in Figure 18. Such capsules are inserted into the reactor core area by a special tube designed for activation of materials. Activation times were typically 3 to 6 days, although longer times could have been used to produce units with more ^{170}Tm .

FIGURE 18. Shuttle in which Three Irradiator Bodies in a Graphite Holder are Sealed with Inert Gas; ^{169}Tm in Irradiator is then Activated to ^{170}Tm in a High Neutron Flux

While a stockpile of units was being developed and the new design for an activation capsule was prepared, we attempted to determine the extent of damage repair in blood systems exposed to radiation. This information is of interest because the irradiator treats only a small fraction of the blood at any one time. Therefore, frequency of exposure is dependent on the ratio between the flow through the irradiator and the total blood volume or cardiac output.

Repair of Radiation Damage as Measured by Response to Mitogens

The effect of irradiating at various dose rates was tested on dog blood exposed *in vitro* to gamma radiation from a Gammabeam 650 (AECL, Canada) irradiator loaded with cobalt-60. One set of blood samples was exposed to 19 Gy in 3 minutes; another, to 24 Gy given continuously (chronic) over 24 hours; a third set (control)

received no gamma radiation. The blood was held at ambient temperature and normal air atmosphere during the 24-hour exposure period. The mitogen concanavalin-A (Con-A) was added, in varying amounts, to the treated and control blood immediately following treatment and up to 48 hours after treatment. Response of the lymphocytes to the mitogen was measured by uptake of ^{125}I -labeled deoxyuridine (^{125}I -UDR) by the cells. Figure 19 shows that radiation reduced IUDR uptake both when the dose was acute and when it was given over a 24-hour period. The lack of difference in cell response to low and high dose rates suggests that repair is not a significant factor in the mitogen response and, by extension, may not be significant in the response to the intermittent radiation delivered to circulating blood. Holding the cells under ambient conditions for periods up to 48 hours did not affect their ability to respond to the mitogen.

FIGURE 19. Responses to Concanavalin-A in Irradiated and Unirradiated Dog Peripheral-Blood Lymphocytes

Survival of Kidney Allotransplants

In 1982 a study was initiated to evaluate the effect of irradiating blood with the VC Tm units on the persistence of kidney function following an allotransplant. The animal's kidneys were removed at the time of transplant, and persistence of function was measured in terms of survival time. The transplants were performed by Dr. Marchioro until our own team learned the technique. For each pair of animals, we usually activated three irradiators. The dogs were selected from the PNL colony and, prior to testing, all were typed for their dog lymphocyte antigens (DLA) by the Oncology Department, Fred Hutchinson Cancer Center, Seattle, WA. They were then paired so that the donor's type differed as much as possible from that of the host animal.

After obtaining at least one pretreatment blood sample for hematology, the treatment regimen was initiated by placing an A-V shunt (carotid artery to jugular vein) in two animals with disparate DLA types, and inserting an active irradiator in one and an inactive unit in the shunt of the other animal. A thermoplastic vest held the irradiator. Animals were individually caged. Blood flow was monitored at least twice a day; if flow stopped, the shunt was aspirated to remove clots, flow was re-established, and the irradiator was cleaned, sterilized and replaced in the shunt. This was repeated as needed until clots could not be removed and flow could no longer be re-established. Control animals were not routinely used when it became apparent that the data obtained did not warrant the expenditure of time in surgery.

The first animal tested (1841) received only 7 days of pretransplant irradiation and lived only 5 days longer than the control. Subsequently, animals received at least 10 days of VC Tm treatment prior to transplant so that numbers of circulating lymphocytes were minimal at the time the new kidney was introduced.

Figures 20 and 21 show typical responses of the lymphocytes and red cells to irradiation. (Additional hematology data are in the appendix.) The drop in red cell count near the end of the irradiation treatment is due mostly to direct blood loss during repeated attempts to remove thrombi from the shunt. The rise in red cells following removal of the irradiators in Dog 1769 illustrates this point. The prolonged radiation damage to lymphocytes is also reflected in all lymphoid tissues

(Figure 22), which show severe depletion in germinal centers following such a course of treatment with chronic blood irradiation.

FIGURE 20. Typical Lymphocyte and Red-Cell Response in Dogs with Kidney Allografts and Chronic Blood Irradiation

Dog 1873, whose lymphocyte response to irradiation is shown in Figure 21, presented an opportunity to observe the effect of treatment on an apparent autoimmune condition. Prior to surgery, the skin was observed to be extremely rough and atypical. The condition, which was normal for this animal, had been diagnosed as an autoimmune problem. Since lymphocytes are suspected of being mediators or initiators in such diseases, we were curious to observe whether the skin condition would respond to reduced numbers of lymphocytes. Although we had no pre-established criterion for evaluating a response, all observers noticed a remarkable improvement in the skin in the approximately 6 weeks of blood irradiation.

FIGURE 21. Response to Chronic Irradiation of Blood, Dog 1873, Female, Weighing 11 kg

At death (or sacrifice when death was imminent: BUN exceeded 200, urine output fell sharply, and emesis occurred), the kidney and typical lymphoid tissues were removed and preserved for histologic examination. Kidneys from untreated animals were uniformly enlarged and had an appearance typical of rejection. Some kidneys from treated animals were nearly normal in size and gross appearance; others were engorged, like those of untreated animals. Kidney sections of treated animals showed somewhat less cellular invasion than those of untreated animals (Figure 23). Representative tissue samples were sent to Dr. Aikawa (Professor of Pathology at Case Western Reserve University), identified only by reference numbers, so that the tissues could be examined without bias. When his reports were received, animal numbers were added to the report (Table 2). With some exceptions, the severity of the pathological lesions appears to be generally less in the kidneys from treated animals compared with those from untreated (control) animals. More detailed information as to specific cell types found needs to be

FIGURE 22. Effect of Chronic Irradiation of Blood on Cells in Lymph Nodes; A, Control; B, Treated

FIGURE 23. Histologic Appearance of a Transplanted Kidney from a Dog which Received Irradiation (A) and an Untreated Animal (B)

obtained in the future. No effects on liver or other nonlymphoid tissues were noted at the time of autopsy.

TABLE 2. Pathology Findings Provided by D. M. Aikawa for Tissues from Dogs some of which were treated by irradiating blood; all received a kidney allotransplant.

Tissue	Observations	Animal Number	Treatment Status
Kidney	Acute infarct--possibly hyperacute rejection	1692	Control
Kidney	Mononuclear cell infiltrates, particularly around arteries and glomeruli--possibly acute rejection	1888	Treated
Lymph Node	Normal	1888	Treated
Kidney	Hemorrhage and diffuse PMN ^a infiltrates	1888	Treated
Kidney	Massive infarct-possible hyperacute rejection	1820	Control
Lymph Node	Normal	1859	Control
Kidney	Massive mononuclear cell infiltrates; the vascular wall is infiltrated with mononuclear cells--possibly acute rejection	1873	Treated
Lymph Node	Normal	1873	Treated
Kidney	Hemorrhagic infarct--consistent with hyperacute rejection	1949	Control
Kidney	Vacuolization in the tubular epithelial cells, otherwise normal	1972	Treated
Kidney	Diffuse mononuclear cell infiltrates--consistent with acute rejection	1847	Treated
Kidney	Mononuclear cell infiltrates--consistent with acute rejection	1963	Control
Kidney	Vacuolization in the tubular epithelial cells, otherwise normal	1904	Treated

^aPolymorphonucleophils

The VC Tm treatment nearly doubled the mean post-transplant survival time (Figure 24) of treated animals (24 days) over that of untreated animals (15 days). It was more difficult to maintain flow through the shunts of treated than of untreated animals, but otherwise there was no evidence of deleterious effects in irradiated animals. The slightly greater problem in controlling infection in the treated animals appeared to be associated with contamination of blood when thrombi were removed.

FIGURE 24. Summary of Treatment and Survival History of Dogs with Kidney Allografts that were Treated with Portable Blood Irradiators

The protocol for the kidney transplants maximized the rejection process by using, in the first series of transplants, donors that had immunologic types quite different from those of the host animal. Because we were concerned that both donors and hosts being of the same breed and from the same breeding colony might prejudice the data toward a longer survival time, the last few tests used animals of a different breed as donors; this did not appear to shift the data significantly. Therefore, it seems reasonable to interpret the kidney transplant data as generally agreeing with the skin-graft data in showing an approximate doubling of retention time. In no case did we use any treatment other than the blood irradiator to extend graft retention times, i.e., to extend life.

Dosimetry for Personnel

To develop data about radiation doses for prospective users of the portable blood irradiator and to optimize shielding design, the model used for animal testing (shown schematically in Figure 25) was filled with liquid to simulate blood and placed on a styrofoam pad. Spectral measurements were obtained at designated angles and distances from the unit, and dose rates were measured with LiF TLD. The data obtained were used as input for a computer code which produced isodose curves (Figure 26) to identify areas that required additional shielding. From this information a new shielding design was developed (Figure 27). To test the new design, we constructed a shield from lead, although we planned ultimately to make the shields of tungsten because of its greater density. To verify measurements, we used a germanium detector with a beryllium window to identify spectral distributions and two thicknesses of TLD to obtain dose-rate data. We could then establish dose rates in air, using calibrated ionization-chamber electrometer equipment. Details of this study were presented at the American Association of Physicists in Medicine Annual Meeting in Seattle, WA, August 11-15, 1985.

With this lead shield and an irradiator containing 1.9 Ci of ^{170}Tm , a dose of 1.3 Gy/hr would be delivered to the blood of a person having a standard blood volume of 6.5 L. The ratio of the maximum dose to a patient's skin relative to the dose to blood is 8×10^{-4} . Therefore, a dose of 900 Gy delivered to blood is within the permissible total dose to a limb. Or, the unit can be moved from one limb to the other, essentially doubling the total dose to blood, yet remaining within the acceptable limits for skin dose.

FIGURE 25. Vitreous Carbon Thulium Blood Irradiator as Used for Dosimetry Study

FIGURE 26. Isodose Curves for Vitreous Carbon Thulium Blood Irradiator. Values are in units of $10 \mu\text{Gy}$ (mrad)/hr for an irradiator with an average dose rate to blood of $1.4 \text{ Gy}/100 \text{ ml}/\text{min}$

The ratio of dose to limb relative to dose to blood can be further reduced if tungsten is used for shield material. Additional distance between the device and the adjacent skin would also reduce the ratio, since dose to skin is nearly four times that received by bone when the unit is used on the arm. The leg would be an alternative place to wear the unit, but attachment of that much weight to the leg would pose a problem.

FIGURE 27. Optimized Shielding Design for Vitreous Carbon Thulium Blood Irradiator; Shield was made of Lead for Initial Verification Measurements. Dimensions in cm

The ratio of dose to limb relative to dose to torso can be further reduced if padding is used for shield material. Additional distance between the device and the adjustment would also reduce the dose to skin's nearby four times that received by bone with the unit used on the arm. The leg would be an alternative place to wear the unit, but attachment of that much weight to the leg would pose a problem.

FIGURE 2. Schematic diagram of a radiation shielding device for a limb. The device is designed to shield the limb from radiation. Dimensions are provided for the device and the limb. The device has a total width of 13.0 cm and a height of 10.5 cm. The limb has a diameter of 10.5 cm. The device is positioned around the limb, with a gap of 1.0 cm between the device and the limb. The device is labeled '1.0 cm' and '10.5 cm'.

DISCUSSION

The VC Tm units are safe to produce because they are constructed using nonradioactive materials and only after the thulium is encapsulated and all machining or other handling is completed are they made radioactive by neutron activation of the ^{169}Tm to ^{170}Tm . Their safety in use has been demonstrated in a series of tests on a wide variety of animals. Their safety relative to accidental breakage has been ensured by having the $^{170}\text{Tm}_2\text{O}_3$ microencapsulated as well as macroencapsulated in the vitreous carbon. If a unit should break, only the ^{170}Tm exposed to the point of fracture could escape containment. No instance of accidental fracture occurred in all of the animal studies.

The data from animals (lymphocyte response, skin-graft rejection times, kidney allograft survival times) clearly show potential for initial suppression of graft rejection. However, no data are yet available to indicate whether this would result in a higher success rate for long-term patency of grafts involving compatible immune systems. It does not appear likely that the VC Tm units will be useful in cases of unscheduled transplants, since a 10- to 14-day pretransplant treatment regimen is required. However, it could be quite useful when donors are related and when transplants can be scheduled in advance.

At this time it seems likely that the VC Tm units may be clinically tested on persons suffering from blood diseases who fail to respond to conventional chemotherapy. Certainly, this approach is preferable to whole-body radiation or to selective lymph-node irradiation, particularly if blood irradiation decreases number of lymphocytes in lymph nodes as it does in healthy animals. One can only speculate what other diseases can be appropriately treated by the VC Tm units.

The data obtained from the exposure of dog blood to alpha radiation from ^{238}Pu are relevant to the lymphocytopenia observed in dogs that have inhaled plutonium. Animals that have plutonium lung burdens resulting in annual doses of 8 Gy or more to lungs consistently show lymphocytopenia by the end of the first year after exposure (J. F. Park, personal communication). Since roughly 90% of the blood is not in the lung, the dose to total blood is about 10% of the dose to lungs. Thus, when the annual blood dose from the wire irradiator (100 Gy) is compared to the annual blood doses calculated for dogs that inhaled plutonium (at 1 Gy), it seems

clear that the dose to blood was not primarily responsible for the lymphocytopenia observed in animals that inhaled plutonium oxide.

The irradiation of blood with alpha-emitters does not appear to be feasible because of the difficulty of containment and the problem of delivering radiation doses to blood that are high enough to significantly suppress lymphocyte levels.

The concept for a fully portable blood irradiator has been established, and techniques for its production and that of the relevant hardware have been developed. Animal testing has established that the devices are safe to use and that the cellular immune system is suppressed. The exact mechanism of suppression, other than the killing of lymphocytes, has not been fully defined because of our inability to identify specific antigens in the animal systems used.

The data suggest that beneficial effects might be achieved in patients undergoing organ transplants if blood irradiation were used during scheduled operations, i.e., when the donor is a blood relative of the recipient.

Another potential area of application for the irradiator is for treating blood diseases. At this time it is not clear whether diseases such as chronic lymphocytic leukemia, lymphomas, etc., would benefit from blood irradiation. Most evidence suggests that such treatment is unlikely to lead to a cure, but that it might be supportive and especially useful as an adjunct to chemotherapy by reducing the population of target cells. It is my belief that clinical trials of this sort should be pursued as the next step.

It would be helpful to obtain animal data on the effect of chronic irradiation of blood to determine whether lymphocytes are mobilized from the diseased lymphatic tissues similarly to the way they are in the nondiseased animal. Such studies should also include the use of chemical agents reported to aid in such mobilization. These data would assist in guiding clinical trials.

ACKNOWLEDGMENT

This blood irradiator project has involved many people, the foremost being the co-inventors, L. R. Bunnell and W. F. Riemath. PNL veterinary surgeons who have worked on the project include: M. T. Karagianes, M. F. Gillis, S. E. Rowe, and R. E. Weller; the latter also served as co-project director over the last 3 years. E. D. Thomas, Fred Hutchinson Cancer Center, provided continuing advice and directed the baboon study; T. M. Marchioro, University of Washington, provided his expertise and skill in the kidney transplant phase of the work. M. Aikawa, Case Western Reserve University, Cleveland, OH, examined the kidney and lymph node tissues provided to him and reported his pathology findings. P. M. Roberson coordinated the dosimetry and shielding design work. Support personnel, particularly those working with the animals, provided continuing assistance, guidance, and advice; the well-being of the animals was always their principal concern.

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APPENDIX

TYPICAL HEMATOLOGY DATA FOR ANIMALS WITH IMPLANTED BLOOD IRRADIATOR

Appendix 1A. Dog 1840 – 1 Day Presurgery

FILE	SPECIES	ANIMAL NUMBER	SEX	DATE OF SAMPLE		
				MO	DAY	YR
1 ^A 2 ⁰ 3 ⁰ 4 ¹ 5		1 8 4 0 9	1 ^F 11	0 2	0 1	8 2 ⁶
TIME - 24 HR			FORM	SEC CODE	TAG	CARD NO.
17 C 7 0 C 20		21 0 3 1 23		24	25	26 27

CARD 02

(_____)

Appendix 1B. Dog 1840 – 10 Days Following Start of Blood Irradiation

HEMATOLOGY

FILE	SPECIES	ANIMAL NUMBER	SEX	DATE OF SAMPLE		
				MO	DAY	YR
1A	20014	511840	9102	102	112	810
TIME - 24 HR		FORM		SEC CODE	TAG	CARD NO.
170	1730	202103	123	24	25	2627

RESERVED FOR EMBOSSED PLATE IDENTIFICATION

A. IDENTIFICATION

1. EXPERIMENT NO.		(COL) 28	135
2. TREATMENT CODE			
3. AGE			
4. AGE DESIGNATION (CIRCLE ONE - COLUMN 40)			
- .TIME PRE-TREATMENT			
+ .TIME POST-TREATMENT			
BLANK - AGE SINCE BIRTH			
5. AGE UNITS		(CIRCLE ONE - COLUMN 41)	
H - HOURS	W - WEEKS	Y - YEARS	
D - DAYS	M - MONTHS		

B. STATUS

1 -ROUTINE	5 -PREGNANT
2 -SICK	6 -TERMINAL
3 -INJURED	(7) -OTHER (SEE COMMENTS)
4 -ESTRUS	

C. HEMATOLOGY LAB. NO.

ANIMAL NO. <u>1840</u>			
SPECIES <u>Dog</u>			
0 4 0	DAY	<u>2-12</u>	YEAR <u>82</u>
1 0 0	TEST	DILUTION FACTOR	CORRECTED VALUE
48 0 0 IF 90 9 RE-DILUTE	TNC $10^3/\text{mm}^3$		
50 0 5	RBC $10^6/\text{mm}^3$		
53 0 2	Hgb g/dl		
56 7 8	VPRC ml/100 ml		
59 0 7	MCV μ^3	_____	_____
62 0 3	MCH μg	_____	_____
65 0 1	MCHC %	_____	_____
OPERATOR <u>mp</u>		CALIBRATION:	
LAB NO. <u>2</u>		VEN ☐	CAP ☐

9. WBC X $10^3/\text{mm}^3$	(COL.)	66							
10. PLATELETS X $10^3/\text{mm}^3$	72								
11. RETIC. X $10^3/\text{mm}^3$	76								
12. SED RATE mm/Hr	78								

D. DIFFERENTIAL

FIRST COUNT	SECOND COUNT	1. NUMBER CELLS COUNTED	28
32	8.4 8.2	2. SEG. NEUTROPHILS	32
36	4 5	3. LYMPHOCYTES	36
40	8 7	4. MONOCYTES	40
44	3 5	5. EOSINOPHILS	44
48		6. BASOPHILS	48
52	1 0	7. BAND NEUTROPHILS	52
56	0 1	8. META-NEUTRO	56
60		9. MYELO-NEUTRO	60
64		10. IMM. LYMPH	64
68		11. IMM. MONO	68
72		12. NUCLEATED RBC	72
76		13. CELLS IDENTIFIED BELOW	76

OTHER HEMATOLOGY DATA

[illegible]

H. INVESTIGATOR COMMENTS

(BLOOD IRRADIATOR 10 DAYS POST SURGERY

I. LABORATORY COMMENTS

(

_____)

Appendix 1C. Dog 1840 - 29 Days Following Start of Blood Irradiation

FILE	SPECIES	ANIMAL NUMBER	SEX	DATE OF SAMPLE		
				MO	DAY	YR
1A ²	QJ.1 ⁴ ⁵	1.8.40 ⁶	7-	0.3	0.3	51 ¹⁰
TIME - 24 --			FOOT	SW CUB	TAG	CARD NO.
17	QJ.1 ⁴ ⁵	2.12.0	3	1	23	24
						25
						26

RESERVED FOR EMPOSSSED PLATE IDENTIFICATION

A. IDENTIFICATION

CARD 01

D. DIFFERENTIAL

PERCENT

ABSOLUTE $\times 10^{-3}$

CARD 02

1. EXPERIMENT NO.		(COL) 28	1, 3, 5
2. TREATMENT CODE			
3. AGE			
4. AGE DESIGNATION (CIRCLE ONE - COLUMN 40)			
- TIME PRE-TREATMENT			
+ TIME POST-TREATMENT			
BLANK - AGE SINCE BIRTH			
5. AGE UNITS		(CIRCLE ONE - COLUMN 41)	
H - HOURS	W - WEEKS	Y - YEARS	
D - DAYS	M - MONTHS		

B. STATUS

(CIRCLE ONE - COLUMN 42)

1 .ROUTINE 5 .PREGNANT
2 .SICK 6 .TERMINAL
3 .INJURED ⑦ .OTHER (SEE COMMENTS)
4 .ESTRUS

C. HEMATOLOGY LAB. NO.

(CCL.)
43

ANIMAL NO.	1840		
SPECIES	Dog		
	DAY	3.3	82 YEAR
	TEST	DILUTION FACTOR	CORRECTED VALUE
308 - 5000 RE-DILUTE	TNC 10 ⁶ /mm ³		
277	RBC 10 ⁶ /mm ³		
6.8	Hgb g/dl		
21.5	VPRC ml/100 ml		
76	MCV μ ³	_____	_____
24.4	MCH μg	_____	_____
31.8	MCHC g.	_____	_____
OPERATOR	MP		
LAB NO.	2	VEN ☐	CAP ☐

FIRST COUNT	SECOND COUNT	1. NUMBER CELLS COUNTED	26
32	85 88	2. SEG. NEUTROPHILS	32
36	2 1	3. LYMPHOCYTES	36
40	5 2	4. MONOCYTES	40
44	1 2	5. EOSINOPHILS	44
48		6. BASOPHILS	48
52	7 7	7. BAND NEUTROPHILS	52
56		8. META-NEUTRO	56
60		9. MYELO-NEUTRO	60
64		10. IMM. LYMPH	64
68		11. IMM. MONO	68
72		12. NUCLEATED RBC	72
76		13. CELLS IDENTIFIED BELOW	76

OTHER HEMATOLOGY DATA

[illegible]

H. INVESTIGATOR COMMENTS

(2400 / POST KIDNEY SURGEY)

1. LABORATORY COMMENTS

(

9. WBC X $10^3/\text{mm}^3$	(COL.)	
	68	
10. PLATELETS X $10^3/\text{mm}^3$	72	
11. RETIC X $10^3/\text{mm}^3$	76	
12. SED RATE mm HR	79	

Appendix 2A. Dog 1769 – 1 Day Presurgery

FILE	SPECIES	ANIMAL NUMBER	SEX	DATE OF SAMPLE		
				MO	DAY	YR
¹ A	² O.O.I	⁴ ⁵ 1769	¹¹ M	¹⁰ 02	¹⁶ 16	¹⁶ 82
TIME - 24 HR		FORM		¹⁸ SEC	TAG	CARD NO.
¹⁷		²⁰ ²¹ 03	²³ 1	²⁴	²⁵	²⁶

RESERVED FOR EMBOSSED PLATE IDENTIFICATION

A. IDENTIFICATION

CARD 1015

D. DIFFERENTIAL ☐ PERCENT ☐ ABSOLUTE $\times 10^{-3}$

CARD 02

1. EXPERIMENT NO.		(COL) 28	733
2. TREATMENT CODE			
3. AGE			
4. AGE DESIGNATION (CIRCLE ONE - COLUMN 40)			
☐ - TIME PRE-TREATMENT ☒ - TIME POST-TREATMENT BLANK - AGE SINCE BIRTH			
5. AGE UNITS		(CIRCLE ONE - COLUMN 41)	
H - HOURS	W - WEEKS	Y - YEARS	
D - DAYS	M - MONTHS		

E. STATUS

(CIRCLE ONE - COLUMN 42)

1 .ROUTINE	5 .PREGNANT
2 .SICK	6 .TERMINAL
3 .INJURED	7 .OTHER (SEE COMMENTS)
4 .ESTRUS	

C. HEMATOLOGY LAB. NO.

(COL.) 1/23

ANIMAL NO. <u>1769</u>			
SPECIES <u>Dog</u>			
	DAY	<u>2-16-52</u>	YEAR
	TEST	DILUTION FACTOR	CORRECTED VALUE
46	<u>13.1</u> IF 99.5 RE-DILUTE	TNC $10^2/\text{mm}^3$	
52	<u>6.27</u>	RBC $10^6/\text{mm}^3$	
53	<u>19.3</u>	Hgb g/dl	
56	<u>52.0</u>	VPRC ml/100 ml	
59	<u>63</u>	MCV μ^3	—
62	<u>23.3</u>	MCH μug	—
66	<u>37.1</u>	MCHC g/g	—
OPERATOR <u>nr</u>		CALIBRATION	
LAB NO <u>13</u>		VEN ☐	CAP ☐

D. DIFFERENTIAL

☐ PERCENT ☐ ABSOLUTE $\times 10^3$

CARD 02

FIRST COUNT	SECOND COUNT	1. NUMBER CELLS COUNTED	28
52	53	2. SEG. NEUTROPHILS	52
36	25	3. LYMPHOCYTES	36
40	3	4. MONOCYTES	40
49	1.6	5. EOSINOPHILS	44
48	2	6. BASOPHILS	48
52	2	7. BAND NEUTROPHILS	52
56		8. META-NEUTRO	56
60		9. MYELO-NEUTRO	60
64		10. IMM. LYMPH	64
68		11. IMM. MONO	68
72		12. NUCLEATED RBC	72
76		13. CELLS IDENTIFIED BELOW	76

OTHER HEMATOLOGY DATA

[illegible]

H. INVESTIGATOR COMMENTS

(PRE-SHUNT

1. LABORATORY COMMENTS

(

9. WBC $\times 10^3/\text{mm}^3$	(COL.)	68	
10. PLATELETS $\times 10^3/\text{mm}^3$		72	
11. RETIC. $\times 10^3/\text{mm}^3$		78	
12. SED RATE mm/Hr		79	

Appendix 2B. Dog 1769 - 14 Days Following Start of Blood Irradiation

RESERVED FOR EMBOSSED PLATE IDENTIFICATION

CARD 02

**Appendix 2C. Dog 1769 – 31 Days Following Start of Blood Irradiation, 11 Days
After Kidney Transplant and 3 Days After the Dog Had Pulled the
Shunt Loose; Entire Shunt was Therefore Removed**

FILE	SPECIES	ANIMAL NUMBER	SEX	DATE OF SAMPLE		
1A	201014	511769	15M	1103	119	82
TIME - 24 HR		FORM	SEC CODE	TAG	CARD NO.	
1709100		2021031	2324	25	26	27

RESERVED FOR EMBOSSED PLATE IDENTIFICATION

A. IDENTIFICATION

1. EXPERIMENT NO.	(COL) 28	1.35
2. TREATMENT CODE		
3. AGE		
4. AGE DESIGNATION (CIRCLE ONE - COLUMN 40)		
- TIME PRE-TREATMENT		
+ TIME POST-TREATMENT		
BLANK - AGE SINCE BIRTH		
5. AGE UNITS (CIRCLE ONE - COLUMN 41)		
H - HOURS	W - WEEKS	Y - YEARS
D - DAYS	M - MONTHS	

B. STATUS

(CIRCLE ONE - COLUMN 42)

1 - ROUTINE	5 - PREGNANT
2 - SICK	6 - TERMINAL
3 - INJURED	7 - OTHER (SEE COMMENTS)
4 - ESTRUS	

C. HEMATOLOGY LAB. NO.

(COL) 43 2

ANIMAL NO. 1769			
SPECIES Dog			
U	DAY	3-19	82 YEAR
C	TEST	DILUTION FACTOR	CORRECTED VALUE
46	TNC		
IF 99.9 PE-DILUTE			
50	RBC		
10 ⁶ /mm ³			
53	Hgb		
g/dl			
56	VPRC		
ml/100 ml			
59	MCV		
μ ³			
62	MCH		
μg			
65	MCHC		
%			
OPERATOR		CALIBRATION:	
LAB NO. 2		VEN ☐ CAP ☐	

D. DIFFERENTIAL

☐ PERCENT ☐ ABSOLUTE X 10³

CARD 02

FIRST COUNT	SECOND COUNT	1. NUMBER CELLS COUNTED	26
32	8.9	8.8	32
36	0	1	36
40	1	2	40
44	2	2	44
48			48
52	8	7	52
56	1	0	56
60			60
64			64
68			68
72			72
76			76

OTHER HEMATOLOGY DATA

TYPE OF DATA	CODE	VALUE
		()
		()
		()
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		()
		()
		()

H. INVESTIGATOR COMMENTS

(
)

I. LABORATORY COMMENTS

(
)

9. WBC X 10 ³ /mm ³	(.COL.) 68	
10. PLATELETS X 10 ³ /mm ³	72	
11. RETIC. X 10 ³ /mm ³	76	
12. SED RATE mm/HR	78	

Appendix 2D. Dog 1769 – Euthanized

Appendix 3A. Dog 1873 – 2 Days Presurgery

FILE	SPECIES	ANIMAL NUMBER	SEX	DATE OF SAMPLE		
				MO	DAY	YR
1 ^A 2 ⁰ 3 ⁰ 4 ¹ 5 ⁰		1 8 7 3	10 ^A 11 ^F	0 5	2 5	8 2
TIME - 24 HR		FORM	SEC CODE	TAG	CARD NO.	
17 ⁰ 18 ⁷ 19 ³ 20 ⁰	21 ⁰ 22 ³ 23 ¹		24	25	26	27

RESERVED FOR EMBOSSED PLATE IDENTIFICATION

A. IDENTIFICATION

CARD 01

D. DIFFERENTIAL

PERCENT

ABSOLUTE $\times 10^3$

CARD 02

1. EXPERIMENT NO.		(COL) 28	135
2. TREATMENT CODE			
3. AGE			
4. AGE DESIGNATION (CIRCLE ONE - COLUMN 40)			
☐ - .TIME PRE-TREATMENT ☒ + .TIME POST-TREATMENT ☐ BLANK - AGE SINCE BIRTH			
5. AGE UNITS		(CIRCLE ONE - COLUMN 41)	
H - HOURS	W - WEEKS	Y - YEARS	
D - DAYS	M - MONTHS		

B. STATUS

(CIRCLE ONE - COLUMN 42)

1 -ROUTINE	5 -PREGNANT
2 -SICK	6 -TERMINAL
3 -INJURED	7 -OTHER (SEE COMMENTS)
4 -ESTRUS	

C. HEMATOLOGY LAB. NO.

(COL)
43

132

ANIMAL NO.		1873	
SPECIES		Dog	

	DAY	5.25	52 YEAR
	TEST	DILUTION FACTOR	CORRECTED VALUE
46	TNC		
IF 99.9 RE-DILUTE			
50	RBC		
	$10^6/\text{mm}^3$		
53	Hgb		
	g/dl		
56	VPRC		
	ml/100 ml		
55	MCV		
	μ^3		
52	MCH		
	μg		
55	MCHC		
	%		
OPERATOR		MP	
LAB NO		32	
		VEN ☐	CAP ☐

FIRST COUNT	SECOND COUNT	1. NUMBER CELLS COUNTED	26
32 63	60	2. SEG. NEUTROPHILS	32
36 28	28	3. LYMPHOCYTES	36
40 3	7	4. MONOCYTES	40
44 5	3	5. EOSINOPHILS	44
48 1	0	6. BASOPHILS	48
52 0	2	7. BAND NEUTROPHILS	52
56		8. META-NEUTRO	56
60		9. MYELO-NEUTRO	60
64		10. IMM. LYMPH	64
68		11. IMM. MONO	68
72		12. NUCLEATED RBC	72
76		13. CELLS IDENTIFIED BELOW	76

OTHER HEMATOLOGY DATA

[illegible]

H. INVESTIGATOR COMMENTS

(CBE AND BUREAU)

1. LABORATORY COMMENTS

(

9. WBC X $10^3/\text{mm}^3$	(COL.) 68	
10. PLATELETS X $10^3/\text{mm}^3$	72	
11. RETIC. X $10^3/\text{mm}^3$	76	
12. SED RATE mm/HR	79	

Appendix 3B. Dog 1873 - 8 Days Following Start of Blood Irradiation

FILE	SPECIES	ANIMAL NUMBER	SEX	DATE OF SAMPLE		
				MO	DAY	YR
1A	20,0,1	45 1,8,7,3	10F	110,6	0,4	8,2
TIME - 24 HR		FORM	SEC CODE	TAG	CARD NO.	
17	0,7,3,0	2C 27 0,3	1	23	24	25 26 27

RESERVED FOR EMBOSSED PLATE IDENTIFICATION

A. IDENTIFICATION

1. EXPERIMENT NO.		(COL) 28	1,3,5
2. TREATMENT CODE			
3. AGE			
4. AGE DESIGNATION (CIRCLE ONE - COLUMN 40)			
— .TIME PRE-TREATMENT			
+ .TIME POST-TREATMENT			
BLANK - AGE SINCE BIRTH			
5. AGE UNITS		(CIRCLE ONE - COLUMN 41)	
H - HOURS	W - WEEKS	Y - YEARS	
D - DAYS	M - MONTHS		

B. STATUS

1 -ROUTINE	5 -PREGNANT
2 -SICK	6 -TERMINAL
3 -INJURED	7 -OTHER (SEE COMMENTS)
4 -ESTRUS	

C. HEMATOLOGY LAB. NO.

ANIMAL NO. <u>1873</u>		43	
SPECIES <u>Dog</u>			
		DAY	6-4 82 YEAR
		TEST	DILUTION FACTOR CORRECTED VALUE
46	2 1	TNC	
IF 99.9 RE-DILUTE		10 ³ /mm ³	
50	4 0	RBC	
		10 ⁶ /mm ³	
53	5 3	Hgb	
		g/dl	
56	1 3	VPRC	
		ml/100 ml	
59	6 5	MCV	
		μ^3	
62	4 0	MCH	
		$\mu\mu\text{g}$	
65	7 1	MCHC	
		%	
OPERATOR <u>MP</u>		CALIBRATION	
LAB NO <u>12</u>		VEN ☐ CAP ☐	

9. WBC X $10^3/\text{mm}^3$	(COL.)	68					
10. PLATELETS X $10^3/\text{mm}^3$		72					
11. RETIC. X $10^3/\text{mm}^3$		76					
12. SED RATE mm/HR		79					

D. DIFFERENTIAL

FIRST COUNT	SECOND COUNT	1. NUMBER CELLS COUNTED	28
32	80 8.2	2. SEG. NEUTROPHILS	32
36	8 6	3. LYMPHOCYTES	36
40	9 8	4. MONOCYTES	40
44	1 1	5. EOSINOPHILS	44
48		6. BASOPHILS	48
52	2 3	7. BAND NEUTROPHILS	52
56		8. META-NEUTRO	56
60		9. MYELO-NEUTRO	60
64		10. IMM. LYMPH	64
68		11. IMM. MONO	68
72	1 1	12. NUCLEATED RBC	72
76		13. CELLS IDENTIFIED BELOW	76

OTHER HEMATOLOGY DATA

[illegible]

H. INVESTIGATOR COMMENTS

1 CBC and Bun

1. LABORATORY COMMENTS

(

_____)

Appendix 3C. Dog 1873 - 21 Days Following Start of Blood Irradiation and
6 Days After Kidney Transplant

FILE	SPECIES	ANIMAL NUMBER	SEX	DATE OF SAMPLE		
1	2	3	4	MO	DAY	YR
1	A	2001	1	10	17	82
TIME - 24 HR		FORM	SEC CODE	TAG	CARD NO.	
17	0	8	0	0	20	21
21	0	3	1	23	24	25
26				27		

RESERVED FOR EMBOSSED PLATE IDENTIFICATION

A. IDENTIFICATION

1. EXPERIMENT NO.	(COL) 28	1.35
2. TREATMENT CODE		
3. AGE		
4. AGE DESIGNATION (CIRCLE ONE - COLUMN 40)		
- TIME PRE-TREATMENT		
+ TIME POST-TREATMENT		
BLANK - AGE SINCE BIRTH		
5. AGE UNITS (CIRCLE ONE - COLUMN 41)		
H - HOURS	W - WEEKS	Y - YEARS
D - DAYS	M - MONTHS	

B. STATUS

(CIRCLE ONE - COLUMN 42)

1. ROUTINE	5. PREGNANT
2. SICK	6. TERMINAL
3. INJURED	7. OTHER (SEE COMMENTS)
4. ESTRUS	

C. HEMATOLOGY LAB. NO.

(COL) 43

ANIMAL NO. 1973			
SPECIES DGC			
	DAY		YEAR
1	2	3	4
46	7	1	
IF 99 RE-DILUTE			
50	7	2	
53	3	4	
56	5	7	
59	6	7	
62	4	7	
65	6	7	
OPERATOR		CALIBRATION	
LAB NO		VEN ☐ CAP ☐	

D. DIFFERENTIAL

☐ PERCENT ☐ ABSOLUTE X 10³

CARD 02

FIRST COUNT	SECOND COUNT	1. NUMBER CELLS COUNTED	28
34	8.6	2. SEG. NEUTROPHILS	32
38	3	3. LYMPHOCYTES	36
40	1	4. MONOCYTES	40
44	1	5. EOSINOPHILS	44
48	1	6. BASOPHILS	48
52		7. BAND NEUTROPHILS	52
56		8. META-NEUTRO	56
60		9. MYELO-NEUTRO	60
64		10. IMM. LYMPH	64
68		11. IMM. MONO	68
72	2	12. NUCLEATED RBC	72
76	1	13. CELLS IDENTIFIED BELOW	76

OTHER HEMATOLOGY DATA

TYPE OF DATA	CODE	VALUE
Chem # 852	BUN	94
Protarombin Time		
animal 04		1.8
Control 05		12.6
Activated Thromboplastin time		
animal 06		20.2
Control 07		33.7

H. INVESTIGATOR COMMENTS

CBC, BUN, platelets
PT, PTT

I. LABORATORY COMMENTS

9. WBC X 10 ³ /mm ³	(COL) 68	
10. PLATELETS X 10 ³ /mm ³	72	5.48
11. RETIC. X 10 ³ /mm ³	76	
12. SED RATE mm/HR	79	

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