The Regenerative Ability of Hemopoietic Tissue Following Lethal X-Irradiation in Dogs

By Victor Perman, Eugene P. Cronkite, Victor P. Bond and Dale K. Sorensen

The successful treatment of the hemopoietic phase of the acute irradiation syndrome in dogs following doses of radiation that are in the range of LD_{100/30} dose has been reported by Sorensen et al.\textsuperscript{1} and Bagdasarov et al.\textsuperscript{2} The present study is concerned with the ability of hemopoietic tissue to regenerate spontaneously following doses of radiation in excess of the LD_{100/30} day dose. The gastrointestinal and cerebral phases that occur with higher doses of radiation than were used in this study, along with the hemopoietic phase, constitute phases of the acute radiation syndrome.\textsuperscript{3,4}

The hemopoietic syndrome, which in mammals is produced by doses of radiation in the range of LD_{50/30} is characterized by severe bone marrow depression with non-survivors succumbing in the second or third week in most species. Leukopenia and thrombopenia are associated with infection and hemorrhage as causes of death in the hemopoietic phase.\textsuperscript{5}

The use of antibiotics in controlling infections and reducing the mortality rate in irradiated mice, rats and dogs has been studied extensively.\textsuperscript{6-8}

Several studies on dogs\textsuperscript{9-13} indicate that antibiotics singly or in combination appeared to be somewhat effective in reducing the mortality in the LD_{50} range. The use of antibiotics alone or in combination with stored whole blood was ineffective in reducing the mortality when the dose approached the LD_{100} range.

The studies of Allen et al.\textsuperscript{13} indicate that the use of small quantities of fresh whole blood, when given according to a predetermined schedule, was ineffective in reducing the mortality rate in dogs receiving graded doses of whole body radiation.

Several investigators have shown that the principal defect in postirradiation hemorrhage may be thrombopenia.\textsuperscript{15-20} These studies have shown that infusion of fresh intact platelets (not lyophilized or disintegrated platelets) would correct postirradiation hemorrhage.

Sorensen et al.\textsuperscript{1} reported on the use of several antibiotics in conjunction with fresh whole blood transfusions, supportive therapy and good nursing care as being effective in reducing the mortality rate of whole body x-irradiated dogs with doses near the LD_{100/30} dose. Bagdasarov et al.\textsuperscript{2} have shown...
HEMOPOIETIC REGENERATION FOLLOWING X-IRRADIATION

that large quantities of fresh intact platelets given on a predetermined schedule with daily administration of penicillin was effective in reducing the mortality in the LD_{100} dose range. Hager et al. have shown that whole blood and irradiated blood given with antibiotics on a predetermined schedule was effective in treating three dogs receiving lethal total body irradiation.

The present study was undertaken to determine the dose of radiation beyond which spontaneous regeneration of hemopoietic tissue would not take place, as manifested by quantification of peripheral blood elements, provided that the major causes of death during this phase could be alleviated. No attempts to evaluate methods of treatment were made. Daily clinical and laboratory findings dictated the course of treatment.

MATERIALS AND METHODS

Methods used were similar to those employed previously. Of a total of 40 mongrel dogs used (weight 32 to 53 pounds, average 39.5), 10 were assigned to each of four irradiation dose groups: I, II, III, IV. A 250 KVP x-ray machine was used, and radiation factors were the same as those reported previously. For an exposure dose of 100 r as reported here, the tissue dose along a central axis depth dose curve was approximately constant and equal to approximately 75 r or 72 rads. Five dogs in each irradiated group served as controls, and blood studies included hematocrit, total white blood cell counts, platelet counts and differential cell counts performed daily. Necropsy was performed on all animals that did not survive.

Treatment consisted of the use of antibiotics to control infections as manifested by a rise in body temperature starting approximately from the 8th to 13th day after irradiation, and fresh platelets. In general oxytetracycline (60 mg. per pound of body weight) was given initially in divided daily doses at 12-hour intervals. A portion of the total dose (10 to 15 mg. per pound of body weight) was administered intravenously in isotonic saline or 5 per cent dextrose solutions and the remainder given orally. When there was failure to control infection as manifested by a rise in body temperature, aqueous penicillin G (100,000 units per pound group I, or 50,000 units per pound, groups II, III, IV) and dihydrostreptomycin (120 mg. per pound in group I or 60 mg. per pound in groups II, III, and IV) was administered intramuscularly and subcutaneously in divided doses twice daily at 12-hour intervals. With one exception, additional antibiotics were required in dogs with prolonged survival and ultimate recovery. Tetracycline (60 mg. per pound of body weight) was administered twice daily at 12-hour intervals. In animals that recovered, the administration of antibiotics could usually by discontinued after a normal body temperature was maintained for three to four days and the white blood cell count was over 1,000 per cu. mm.

In group I, platelet replacement therapy was in the form of whole blood transfusions from a donor colony. Blood was collected from the femoral artery and given as described previously. From 20 to 35 per cent of the recipient’s calculated blood volume was given in a single transfusion as required. Platelet rich plasma from 50 ml. blood, prepared as described previously, was used in addition in groups II, III and IV. Platelet rich plasma or blood transfusions were given when the blood platelet level was 5,000 per cu. mm. or less, usually on approximately the 10th to 12th day postirradiation. Subsequent transfusions were given when the platelet count dropped or massive bleeding was observed. Additional fluid therapy (isotonic saline or 5 per cent dextrose) was usually necessary during the period when inappetence occurred. Inappetence was of major importance during the critical period (10th day until clinical recovery), and the diet was varied to entice animals to eat.

RESULTS

Control groups. All dogs in the control groups died. The mean survival times and ranges are given in table 1.
Table 1.—Summary of Dose Response Data on Control and Treated Dogs (30 Days)*

<table>
<thead>
<tr>
<th>Group</th>
<th>Control Dogs</th>
<th>Treated Dogs</th>
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<td></td>
<td>Dose (r)</td>
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<td>Died/Total</td>
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<td>I</td>
<td>420</td>
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<td>II</td>
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<tr>
<td>IV</td>
<td>550</td>
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*Included in this table are the data reported by Sorensen et al.1 which were accumulated under identical experimental conditions.

All the dogs in the control groups recovered rapidly from the pentobarbital sodium anesthesia after irradiation without ill effects. From the first to the third postirradiation day, dogs in all groups refused to eat part or all of their food. Diarrhea was commonly observed from the second to the seventh (gastrointestinal phase) postirradiation day. The onset of fever was noted to occur in all dogs between the 7th and 13th (hemopoietic phase) day with a mean onset on the 10.5 day for group I, 10.5 day for group II, 10.0 day for group III, and 8.0 day for group IV. The duration of the febrile period was from 1 to 6 days with a mean of 2.5 days. Rectal temperatures of 104.5 F. to 107 F. were found. Clinical findings observed during this period are similar to those reported by other investigators.1,2,4 Petechial to ecchymotic hemorrhages of the visible mucous membranes were commonly seen. Ulcerated lesions on the buccal and gingival surfaces and tonsils occurred frequently. Cellulitis with edema and hemorrhage was commonly observed to occur about the head or one or more of the extremities.

Terminal anorexia, vomiting and diarrhea with variable amounts of blood in the feces occurred in many dogs.

Hematologic findings. The changes in the total leukocytes, platelets and hematocrit levels are shown in figure 1. They are similar to those reported by other investigators for this dose range.5

The mean hematocrit values for all groups ranged from 43 to 47 volumes per cent prior to irradiation and 37 to 42 volumes per cent on the 9th day postirradiation. Thereafter, the hematocrit drop was related to the length of survival of the animal, with the greatest drop occurring in animals that survived the longest period of time.

The mean platelet level for all control dogs was approximately 350,000 per cu. mm. prior to irradiation. This level was essentially maintained for the first five postirradiation days. In all groups, the first significant drop in platelets occurred between the 5th and 7th postirradiation day, with the mean values of all groups being 0 to 10,000 platelets per cu. mm. on the 10th postirradiation day.

The mean leukocyte value for all control dogs was 11,400 per cu. mm. prior to irradiation. In all groups, a 40 per cent drop in total leukocytes occurred 24 hours postirradiation and the mean leukocyte counts of all groups fell to 1,000 per cu. mm. or less by the 7th day. Between the 7th and 9th days, the
HEMOPOIETIC REGENERATION FOLLOWING X-IRRADIATION

mean leukocyte values ranged from 100 to 1,000 per cu. mm. depending on the magnitude of the dose.

The gross and histopathologic studies of the control dogs reveal findings similar to those reported by other investigators for this dose range.4,24 Dogs which died early (9th to 11th day) appeared to have more massive and severe infections with edema and hemorrhage at the site of inflammation. The dogs surviving until the 13th to 19th day had similar lesions although usually less pronounced; however, the hemorrhages observed were more generalized, particularly hemorrhages involving the gastrointestinal tract.

Therapy group I. In group I, three of five treated dogs recovered and were followed in this study for 80 days. Two dogs died, one on the 25th and the other on the 23rd postirradiation day. The clinical findings in the initial 10 days were similar to the control group. The hematologic findings for treated dogs in that part of the postirradiation period prior to treatment were essentially the same as the control dogs.1

The onset of fever in group I dogs occurred between the 9th and 12th days,
at which time the dogs were placed on either oxytetracycline or penicillin and dihydrostreptomycin. The first antibiotic was effective in controlling infections from four to six days, at which time they were placed on either penicillin and dihydrostreptomycin or oxytetracycline for five to seven days. When an additional antibiotic was required, tetracycline and/or chlorotetracycline was given. In this group, antibiotics were given for a 12 to 23 day period in dogs that recovered.

Fresh whole blood transfusions were given when the platelet count approached zero and had remained there for one to three days. From two to nine transfusions of approximately 20 to 40 per cent of the recipient's blood volume were given at one to five day intervals to maintain circulating platelets necessary to control hemorrhage. Platelet counts as high as 70,000 were attained 24 hours after transfusions, which fell to zero level in one to five days. The last transfusion required in surviving dogs was given on the 19th, 30th and 34th postirradiation day, after which time production of new platelets was apparently sufficient to control hemorrhage.

Polycythemia, hematocrits 66 and 67 vols. per cent subsequent to blood transfusions, developed terminally in two dogs. Both dogs refused to eat and vomited frequently during the terminal three to four days, while on penicillin and dihydrostreptomycin.

The administration of large volumes of fresh whole blood (20 to 35 per cent of recipient's blood volume) was usually associated with a reaction characterized by restlessness, urticaria, hyperpnea, tenesmus and defecation or urination. These reactions and polycythemia prompted the use of platelet rich plasma in subsequent groups. No transfusion reactions were encountered with the platelet rich plasma if the transfused volume was less than approximately 15 per cent of the recipient's blood volume.

The onset of regeneration in group I dogs as manifested by peripheral blood counts began on the 19th, 30th and 32nd postirradiation days. Figure 2 shows clinical and blood findings on a representative treated dog in this group.

Therapy group II. Four of five dogs receiving 460 r of total body irradiation made complete recoveries and were followed for 80 days in this study. One dog died on the 30th postirradiation day.

The clinical course in group II treated dogs is similar to the group II control dogs. The hematologic findings in the initial 10-day postirradiation period are essentially the same as the control group, figure 1.

Antibiotic therapy was started on one dog on the 7th day and on four dogs on the 8th day. Terramycin, penicillin, and dihydrostreptomycin, chloromycetin and tetracycline were used in that order. One dog required only two antibiotics while four antibiotics were used on the remaining dogs. The length of the antibiotic therapy period in surviving dogs ranged from 16 to 22 days.

From three to six transfusions of platelet rich plasma or blood transfusions were required to maintain effective platelet levels to control hemorrhage during the critical period from the 10th to the 28th day.

All dogs in the group received supportive therapy in the form of isotonic saline or 5 per cent glucose solutions as indicated by their clinical appear-
Fig. 2.—Peripheral blood values as a function of time on a representative recovered treated dog in group I. Also depicted are the periods of chemotherapy and the daily body temperature.
Fig. 3.—Peripheral blood values as a function of time on a representative recovered treated dog in group II. Also depicted are the periods of chemotherapy and the daily body temperature.
HEMOPOIETIC REGENERATION FOLLOWING X-IRRADIATION

Fig. 4.—Peripheral blood values as a function of time on a treated dog in group II which succumbed on the 30th postirradiation day.

ance. Forced feeding to encourage eating and diet variation was practiced in all dogs in this group. Clinical and blood findings on dogs in this group are shown in figures 3 and 4.

Therapy group III. All five treated dogs in group III died between the 21st and 27th day. The course of treatment was similar to that for group II. A comparison of the hematologic data with the control group for the 10-day postirradiation period is shown in figure 1.

Therapy group IV. Four of five treated dogs in group IV died between the 10th and 18th postirradiation day. The treatment for this group was similar to groups II and III.

One dog (fig. 5) showed signs of regeneration on the 36th day postirradiation. At that time, the white blood cell count started to rise slowly. From the 36th day to the 54th day the white blood count fluctuated between 1500 and 3500 cells per cu. mm. A bone marrow biopsy from the wing of the ilium revealed that the marrow was of low cellularity with active myelopoiesis and erythropoiesis. The white blood cell count started to drop about the 53rd day and reached zero level on the 63rd day. A terminal rise in temperature reoccurred on the 61st day, and could not be controlled. The dog expired on the 63rd day. Necropsy revealed a few scattered petechial hemorrhages on the visceral surfaces. The lymph nodes were small and slightly hemorrhagic. Microscopic sections of bone marrow, lymph nodes and spleen revealed marked hypoplasia.

DISCUSSION

The results of these studies support the findings of Sorensen et al.¹ and Bagdasarov et al.,² that the treatment of the hemopoietic phase of the acute
Fig. 5.—Peripheral blood values as a function of time on a treated irradiated dog in group IV which succumbed on the 63rd post-irradiation day. Also depicted are the periods of chemotherapy and the daily body temperature.
HEMOPOIETIC REGENERATION FOLLOWING X-IRRADIATION

radiation syndrome is feasible at the LD,00 dose. Successful treatment with complete hemopoietic regeneration was possible in the present studies with doses of radiation up to 460 r.

The question arises as to why complete hemopoietic regeneration failed to occur at higher doses of radiation. A possible explanation is that the dose of radiation was sufficiently great to destroy the pool of primitive cells necessary for regeneration. This explanation is not completely satisfactory since evidence of partial regeneration at the 550 r dose (fig. 5) is shown. Puck\textsuperscript{25} has shown with HeLa cell cultures that the regenerative capacity (survival curve) appears to fall off exponentially with the dose of x-radiation used. Similar results with marrow cells in mouse transplantation studies have been shown by McCullock and Till.\textsuperscript{26} In the present study on dogs with regard to hemopoietic recovery following lethal irradiation, the onset and rate of regeneration appears to be radiation dose dependent. The time of onset and the rate of regeneration in marrow transfusion experiments is dependent on the number of marrow cells transfused.\textsuperscript{27} On this basis, it would appear that the regenerative capacity is dependent on the number of primitive cells surviving or transfused. This is further indicated in the time relationship of the marrow transplant to irradiation day. In such studies in rodents,\textsuperscript{27} a sufficiently large dose of marrow transfused up to day 4 made no change in the survival rate after irradiation.

If the number of surviving primitive cells and thus, in principle, the regenerative capacity is dose dependent over a wide range, then maintenance of the dog by replacement and other therapy for a sufficiently long period of time should allow regeneration to take place even at high dose levels. This appears to be the case (fig. 5) at the 550 r dose levels. Regeneration is first noted by a rise in the leukocyte number and the appearance of granulocytes in the differential smears on the 34th post irradiation day. It would seem reasonable, with dogs that died, that had survival been of sufficient duration (35 days) regeneration would have been possible. In other studies,\textsuperscript{21} survival in dogs with therapy at higher dose levels was reported; however, the radiation was given at a lower dose rate, with resulting reduced biological effectiveness.

Apparently the animals could not be maintained sufficiently long for regeneration to take place, particularly in groups III and IV, and a number of factors may have contributed to death during this period. The intestinal phase of the acute irradiation syndrome was apparent at all dose levels; however, this did not appear to be of sufficient severity to alter greatly the clinical course of the hemopoietic phase in this study. The length of survival of control dogs (table 1) was dose related. Death was associated with acute inflammatory edema and hemorrhage, typical of the hemopoietic phase.

The control of hemorrhage in treated dogs was possible with either fresh whole blood or platelet rich plasma. The large transfusions of fresh whole blood (group I) gave rise to polycythemia. Further, transfusion reactions with whole blood were encountered when the transfused volume exceeded 15 per cent of the recipient's blood volume. Bliss\textsuperscript{28} has shown that this reaction is produced consistently with large amounts (20 ml./Kg. of body weight) of non-
autologous plasma, apparently mediated through the release of histamine. In subsequent groups, platelet rich plasma was used. The platelet levels observed were comparable to platelet levels obtained with the original quantity of whole blood while the transfused volume was less than one-half. Transfused platelet enriched plasma appeared to be as effective as fresh whole blood in controlling hemorrhage. Necropsy examination of treated dogs revealed a minimal amount of gross hemorrhage. It would appear from this observation that hemorrhage was not a significant factor in the cause of death during this period.

Supportive therapy and good nursing care was necessary during the critical period from the onset of therapy until the onset of hemopoietic regeneration. Each treated animal was managed as a separate clinical case. Inappetence appeared to be a distinct problem in all dogs that succumbed. Supportive fluid therapy was necessary during such prolonged periods. In all recovered dogs, no long periods (3–4 days) of complete inappetence occurred. In those cases in which complete inappetence occurred, the animal usually died without evidence of hemopoietic regeneration. Dog 520 (fig. 4) had complete inappetence on the 10th postirradiation day, which continued until the death of the animal on the 30th day.

The control of infection with antibiotics was a major problem in this study. The effectiveness of antibiotics early in the course of the treatment period was satisfactory, for periods of 3–6 days in most dogs. It was apparent that the effectiveness of the antibiotics was greater when parenteral routes were employed for at least a portion of the total dose, particularly when vomiting was associated with the oral administration of the antibiotic. Subsequent antibiotics were somewhat less effective in controlling infection in groups III and IV, during the terminal stages. The development of antibiotic resistant organisms during this intensive therapy period is extremely likely and was thought to be a major factor contributing to death in these groups. This was particularly true after the first antibiotic became ineffective in controlling infection. In these animals, the ineffectiveness of the second and third antibiotics used, where in early experiments they were effective, suggests the development of antibiotic resistant organisms. In groups I and II, however, it is likely that the onset of regeneration (20th to 32nd day) was a significant factor in controlling infections in dogs that recovered.

The question of possible marrow repopulation by transfused peripheral blood cells with a proliferative potential as being responsible for the hemopoietic regeneration is raised. DNA synthesizing cells are found normally in the peripheral blood and under some conditions radiation protection has been obtained with blood. The radiation dose dependence of the onset and rate of hemopoietic regeneration does not support this concept. Further, Bagdasarov has shown that packed platelets and antibiotic therapy were effective in prolonging the survival time for a sufficient period to allow for spontaneous hemopoietic regeneration at slightly lower doses of radiation.

In relationship to homologous transplantation of bone marrow, these studies indicate that an immunologic response is possible at the dose levels employed in this study. Significantly higher doses may be required to abolish this re-
HEMOPOIETIC REGENERATION FOLLOWING X-IRRADIATION

sponse completely, as has already been shown in other studies. It must be remembered that immunologically competent cells are present in the blood, and can lead to homograft rejection.

CONCLUSIONS

1. Successful treatment of the hemopoietic phase of the acute irradiation syndrome in dogs at the LD_{100} (400 r) dose is possible on an individual case basis with good nursing care.
2. The onset and rate of regeneration is related to the radiation dose. Partial regeneration with subsequent death occurs with a 550 r dose.
3. The control of infection during the critical period prior to regeneration appeared to be the limiting factor in successful treatment at the dose levels used in this study.

SUMMARIO IN INTERLINGUA

1. Le tractamento successose del phase hematopoietic del syndrome de ir- radiation acute in canes al dose de DL_{100} (400 r) es possibile in casos individual per meticulose attention valetudinari.
2. Le inception e le rapiditate del regeneration es relationate con le dose de radiation. Regeneration partial con morte subsequente occurre con un dose de 550 r.
3. Le prevention de infection durante le periodo critic ante le regeneration esseva apparentemente le factor decisive pro un tractamento successose al nivelllos de dosage empleate in iste studio.

REFERENCES


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