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ACUTE RADIATION EFFECTS ON MAN REVEALED BY UNEXPECTED EXPOSURES*

SVEN-ÅGE KILLMANN,[1] EUGENE P. CRONKITE,[1] VICTOR P. BOND[1] & THEODOR M. FLIEDNER[1]

By tradition—and perhaps for simplicity—the acute radiation syndrome is subdivided into the central nervous system syndrome, the gastrointestinal syndrome, and the haemopoietic syndrome. Only the last of these will be considered here.

It is the purpose of this paper to review some laboratory procedures that have recently been proposed as biological dosimeters of acute radiation injury. The development of biological dosimeters is important not only from a prognostic point of view, but also because they may be of help in clarifying the possible indications for bone marrow transplantation in the treatment of radiation injury. A conservative therapeutic programme which has been used successfully in irradiated dogs will also be discussed.

Among the recently proposed biological dosimeters of radiation injury are the urinary excretion of β-aminoisobutyric acid (BAIBA) (Rubini et al., 1959) and the mitotic index of the bone marrow (Fliedner et al., 1959).

Excretion of β-Aminoisobutyric Acid

BAIBA is an amino-acid which has been known for about 10 years. Its origin and excretion under normal and pathological conditions have been reviewed recently (Killmann et al., 1961 b). The sources of BAIBA and the pathways leading to its formation have been extensively studied by Fink et al. (1952, 1956). Their work indicates the existence of the following pathway: thymine→dihydrothymine→β-ureidoisobutyric acid→BAIBA. BAIBA arises not only from thymine but also from thymidine and from DNA. Many other pyrimidines have been investigated but none has been found which is metabolized to BAIBA. In swine, valine may be a precursor of

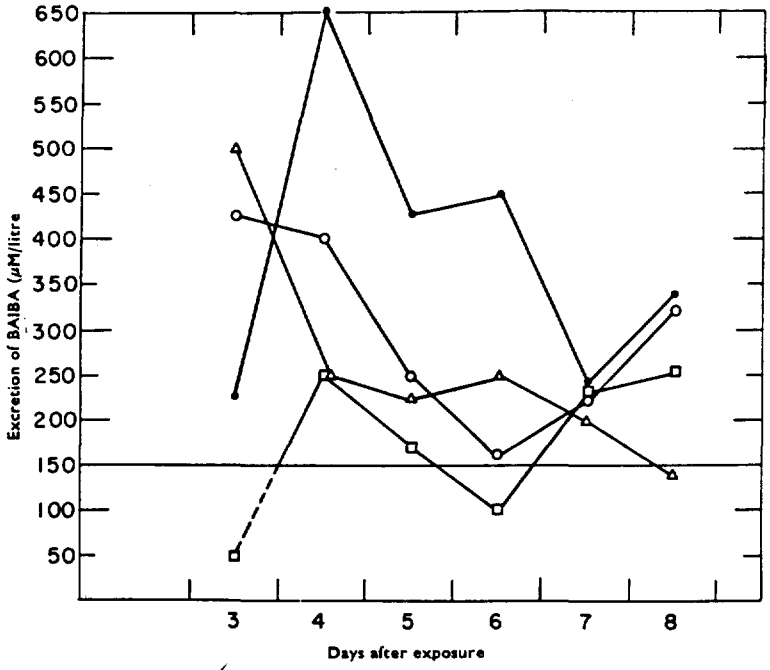
* Research supported by the United States Atomic Energy Commission, Washington, D.C., USA.
[1] Medical Research Center, Brookhaven National Laboratory, Upton, Long Island, New York, USA.

BAIBA, but evidence for a similar pathway in man is lacking. In man, BAIBA appears to be a specific catabolite of thymine-containing compounds.

Following substantial exposure to ionizing radiation with ensuing cell destruction and inhibition of DNA-synthesis one might expect an increased excretion of BAIBA in the urine. A study of this question was undertaken by Rubini et al. (1959) in the 8 operators who were exposed in the criticality accident at Oak Ridge in June 1958 (Fig. 1). Urine specimens were

FIG. 1

URINARY EXCRETION OF BAIBA IN VICTIMS OF THE Y-12 ACCIDENT, OAK RIDGE, JUNE 1958



Upper normal limit of BAIBA excretion with method used = 150 μM/litre

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Reproduced, by permission, from Rubini et al. (1959)

received from the 3rd to 8th day after exposure. In 3 patients who received less than 100 rad no excess urinary excretion of BAIBA was detectable. The remaining 5 patients with higher exposures (236-365 rad) excreted BAIBA in amounts larger than normal, with a maximum during the first two days on which specimens were available, that is day 3 and day 4. The excretion of BAIBA was roughly dependent on the dose.

In this study, the output of BAIBA was estimated by means of two-dimensional paper chromatography. Subsequent studies of the same urine

specimens by Kretchmar (1959) with the more precise but time-consuming technique of column chromatography confirmed the results of Rubini et al. The source of the excess BAIBA excreted by the heavily irradiated is not clear. It is possible that it originates from the breakdown of DNA derived from destroyed cell nuclei, but it is equally possible that thymine-containing molecules which were destined for DNA-synthesis are shunted into a catabolic pathway as a result of radiation-induced inhibition of DNA-synthesis. This problem can be approached experimentally. Whatever the mechanism of increased BAIBA excretion after irradiation, however, it should be realized that excess BAIBA excretion is far from being specific for radiation injury. Increased urinary BAIBA output has been reported in bacterial infections, in chronic leukaemia, in liver disease, and following surgery and short periods of starvation. The last-mentioned finding may be pertinent since irradiated patients may have severe nausea and vomiting producing a short period of relative starvation. Some persons normally have a high BAIBA excretion; this is an hereditary trait. A number of studies in different laboratories and with differing techniques (reviewed by Killmann et al., 1961 b) indicate that in a Caucasoid population, 10-15% normally excrete more than 400 μM of BAIBA per 24 hours. In other races this percentage is even higher. The high BAIBA excretion in these so-called "normal high excretors" appears to be constant with time.

From what has been said it will be obvious that data on BAIBA excretion following acute exposure to ionizing radiation must be evaluated with considerable caution. Low values during the first week after exposure probably indicate that the exposure has been low and that the prognosis is good, but more data are needed. In contrast, elevated values do not necessarily indicate heavy exposure, since high excretion may have many causes other than radiation injury. In such cases pre-exposure determinations would be of considerable help.

In summary, determinations of urinary BAIBA excretion are potentially useful as a biological dosimeter of radiation.[1] Much experimental work on the constancy and dose-dependence of BAIBA excretion is needed, however, before the importance of this parameter can be appraised.

Mitotic Index

The bone marrow aplasia that is caused by radiation is due to cell destruction and mitotic inhibition. The proliferative activity of a tissue is often estimated by the mitotic index (I_m), i.e., the fraction of all cells which are in mitosis at any time:

[1] During the meeting, Dr Hempelmann mentioned that BAIBA-excretion had been increased in the fatally irradiated individual at Los Alamos, December 31, 1958, and Dr Andrews referred to studies by Dr Kretchmar in Oak Ridge which showed a massive increase in BAIBA output in leukaemic patients after therapeutic whole-body irradiation.

$$I_m = \frac{N_m}{N}$$

where N_m = number of mitotic cells in sample and N = number of all cells in sample.

In the Y-12 accident at Oak Ridge, Fliedner et al. (1959) followed the mitotic index of the bone marrow after exposure. Bone marrow particles were squashed, stained by Feulgen's method, and the number of mitotic figures among 3000-5000 cells enumerated. It should be noted that with the method used, N represents all bone marrow cells irrespective of cell type and maturation level. The reason for this is that in Feulgen-stained preparations exact cytological classification is not possible; nevertheless Feulgen stain was used because it allows a more accurate recognition of mitotic figures than do ordinary bone marrow stains.

In normal males the average mitotic index determined in this way is 8.8 per 1000 nucleated bone marrow cells. The Oak Ridge patients were first studied on the 4th day after the accident. At this time there was a very

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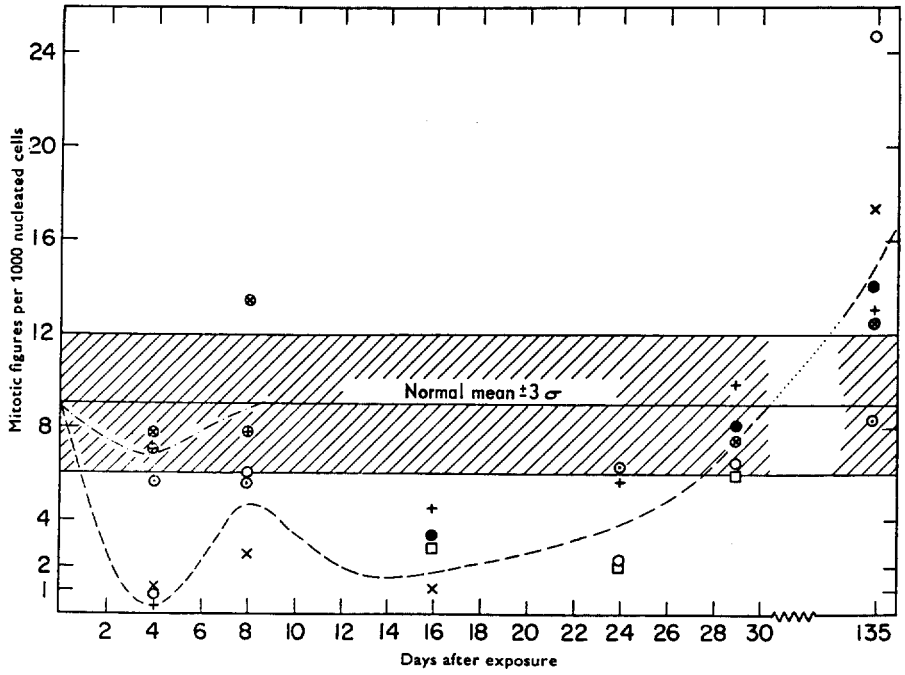
FIG. 2

MITOTIC INDEX OF THE BONE MARROW IN VICTIMS OF THE Y-12 ACCIDENT



Reproduced, by permission of S. Karger, Basel/New York from Fliedner et al. (1959).

marked reduction in the mitotic index of the heavily irradiated victims (236-365 rad) (Fig. 2). There was a significant difference between this group and the 3 workers who were exposed to less than 100 rad: in the latter group the mitotic index was essentially within normal limits; in the high-exposure group, the mitotic index remained low for about 3 weeks, but 4 weeks after exposure was again within normal limits.

The explanation of the low mitotic index of the bone marrow after radiation injury is complex. The mitotic index of the marrow as defined by Fliedner et al. will depend on several factors:

1. Mitotic time: other things being equal, any increase in mitotic time will increase the mitotic index, and any decrease in mitotic time will lower the mitotic index.

2. The relative numbers of red and white cell precursors in the marrow at any time: in normal marrows, red cell precursors contribute about 2/3 of all mitoses, whereas neutrophil precursors—in spite of their larger numbers —only account for somewhat less than 1/3 of all mitoses in the marrow (Killmann et al., 1961 a).

3. The relative number of cells that have the capacity to divide and the relative number that have matured and lost this capacity. The mitotic index decreases after radiation injury because cells are prevented from going into mitosis and because there is a decrease in the relative number of red cell precursors which contribute the bulk of the mitoses (Brucer, 1959). On the other hand, the prolonged mitotic time and the disappearance from the marrow of cells incapable of mitosis (e.g., segmented neutrophils) would tend to increase the mitotic index. With these multiple variables it is virutally impossible to predict what would happen to the mitotic index after irradiation of such a mixed cell population as is found in the bone marrow. The data collected by Fliedner et al. clearly demonstrate, however, that the mitotic index falls: with doses of the order of 236-365 rad, mitoses were virtually absent on the 4th day. In spite of the theoretical objections referred to, we believe that the mitotic index will be useful as a biological dosimeter for doses up to approximately 250-350 rad. The chances are that the course will be uneventful if the mitotic index on the 4th day is essentially normal. A severe depression of the mitotic index to around 0.1 % on day 4 indicates an exposure to 250-350 rad *or more*. Thus, a mitotic index of 0.1 % on day 4 is compatible with survival—as in the Y-12 cases—but does not necessarily guarantee a favourable prognosis.

Reliable determinations of the mitotic index require some experience on the part of the investigator, but specialized equipment is not necessary. Serial mitotic indices may well prove to be helpful in evaluating the exposure of irradiated human beings. It is important to note in this context that these counts can be completed within approximately 12 hours after the marrow has been aspirated.

Other Parameters

Other parameters that may eventually become of prognostic value are the number of circulating DNA-synthesizing cells in the blood and the DNA-synthesizing capacity of bone marrow cells.

DNA-synthesizing cells are present in very small numbers, about 5-10 per mm^3, in the blood of normal man (Bond et al., 1959). This capacity for DNA-synthesis can be demonstrated by in vitro incubation of the peripheral blood with the specific DNA-precursor, tritiated thymidine (^{3}HTDR). The cells in the blood yielding positive autoradiographs with ^{3}HTDR are mononuclear cells which are not easily classified morphologically. The victims of the Y-12 accident at Oak Ridge were studied by this technique

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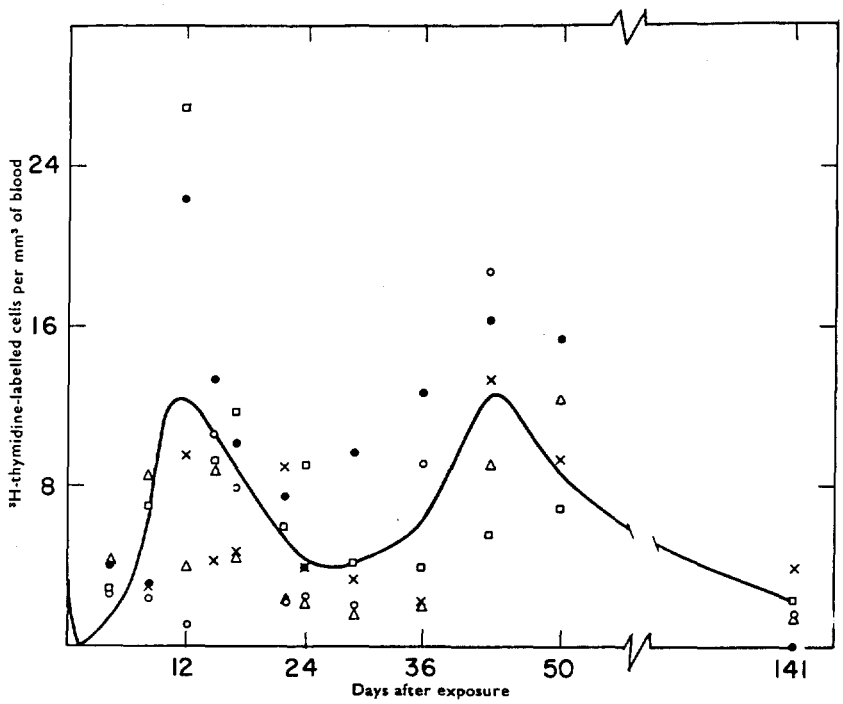
FIG. 3

CHANGES IN THE NUMBER OF DNA-SYNTHESIZING MONONUCLEAR CELLS IN THE PERIPHERAL BLOOD OF VICTIMS OF THE Y-12 ACCIDENT



Estimated total exposures:

- • 365 rad
- △ 270 rad
- ○ 339 rad
- × 327 rad
- □ 236 rad

Reproduced, by permission, from Cronkite & Bond (1960)

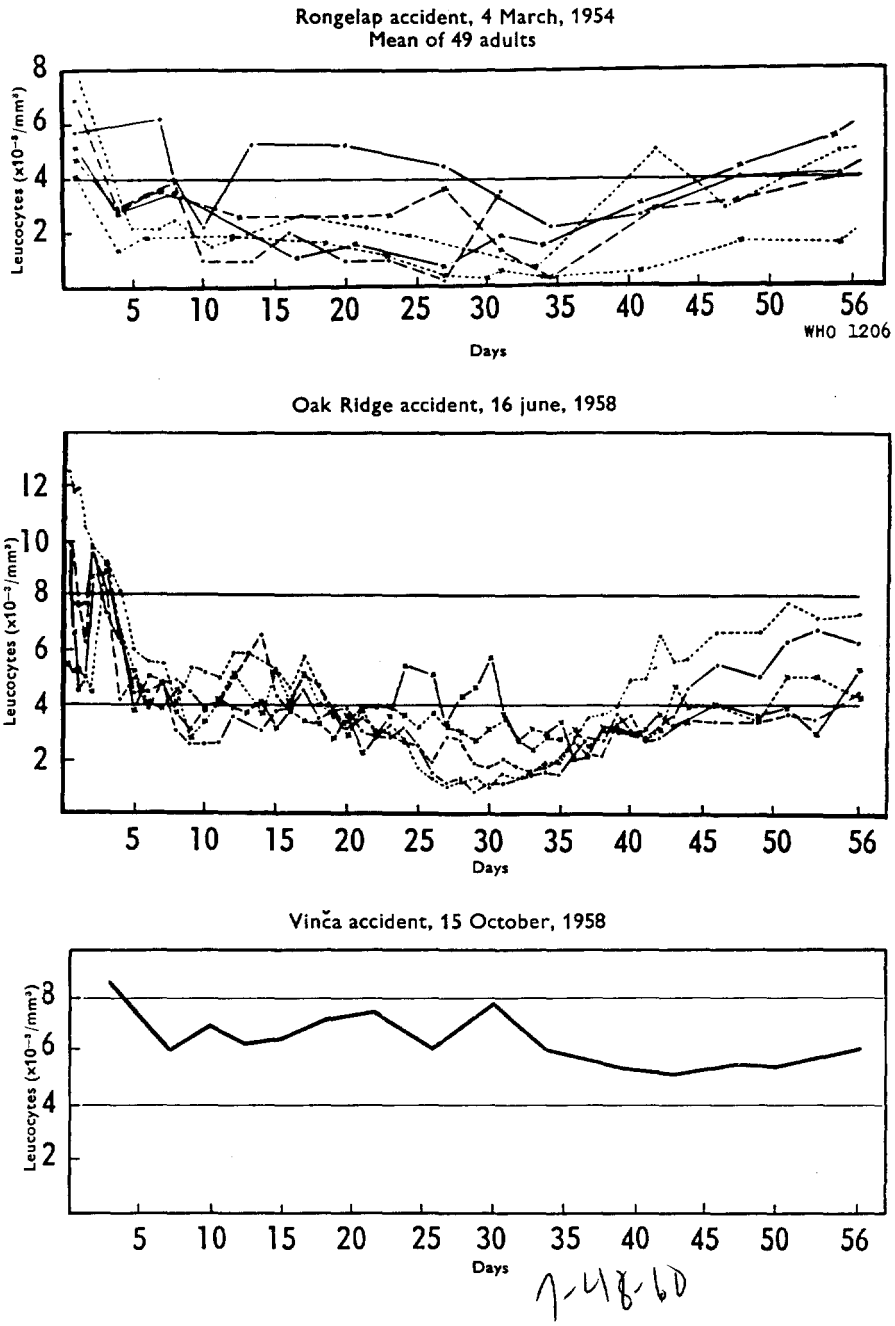
by Bond et al. (1961). Shortly after exposure the number of DNA-synthesizing cells decreased; it then rose to above normal, reaching a peak around days 8-10, after which it fell again and rose to a second peak above normal around days 35-40 (Fig. 3). Similar studies were also made in irradiated dogs; immediately after exposure there was a sharp drop in the number of DNA-synthesizing cells, followed by an increase. This increase was much more pronounced in dogs that had been partially shielded or had received only unilateral irradiation than in dogs exposed to uniform radiation. The function of these cells is not clear; the only thing we know about them is that they synthesize DNA, which is a prerequisite for cell replication. This should be reason enough to study these cells further, since the survival of patients suffering from radiation injury is dependent upon cell regeneration.

A more direct approach is to estimate the capacity of the remaining bone marrow cells to synthesize DNA. This can be done by determining the percentage of a cell line that is labelled after in vitro incubation with ^{3}HTDR. Fliedner et al. (unpublished observation) have recently used this technique to study changes in rat bone marrow during the first few hours after exposure to 500-1500 r. Both in the red cell and the white cell precursors an initial rise in labelling percentage was noted, rapidly followed by a marked decline Studies over longer time intervals are clearly needed. It would be reasonable to expect that cell regeneration would be preceded by a return to the normal labelling percentage, or even a rise above it. With a sufficient amount of ^{3}HTDR of high specific activity and a fast film emulsion, such thymidine uptake tests should be ready for evaluation after a few days' exposure and might thus become clinically useful if the above supposition should prove to be correct.

The laboratory tests that have been discussed so far all require special skills or equipment. In addition, all the studies described need to be extended and substantiated before they can be considered of practical value in prognosis. As yet the most useful laboratory procedure for evaluating the status of irradiated individuals is serial blood counts. Blood counts are indispensable in the clinical management of such cases and give some idea of later developments at a relatively early stage. The magnitude of the initial lymphocyte depression is an indication of the severity of exposure. In dogs the rapidity of the decrease in total white count correlates well with the lethality (Cronkite & Bond, 1960). A similar relationship exists in man. Leucocyte counts around 500 per mm^{3} were found as early as one day after the atomic bomb explosions in Japan in persons who were close to the hypocentre (Le Roy, 1950). It will also be apparent from Fig. 4 that the white cell counts of the Yugoslav patients fell more rapidly and more severely than those of the patients injured in the Y-12 accident who received a smaller dose. Quantitative comparisons are difficult to make, however, because the counts were made by different laboratories.

FIG. 4

COMPARISON OF CHANGES IN WHITE CELL COUNTS IN VICTIMS OF 3 RADIATION ACCIDENTS



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Recently, Thoma & Wald (1959) have tried to make a broader comparison of the blood counts in persons accidentally exposed to radiation. They attempted to reduce various parameters, such as the white cell count, platelet count, haematocrit, etc., to a common denominator by assigning "score values" to deviations from the normal. In this way, they arrived at "profiles of injury", on the basis of which five groups at different exposure levels (less than 150, 240-365, 400-600, 640-1350, and 9200 rad) could be differentiated quite well within 6 days after exposure. Even if only the cumulative scores for total white count are plotted, by day 6 patients who received more than 400 rad can be reasonably well distinguished from those who received less.

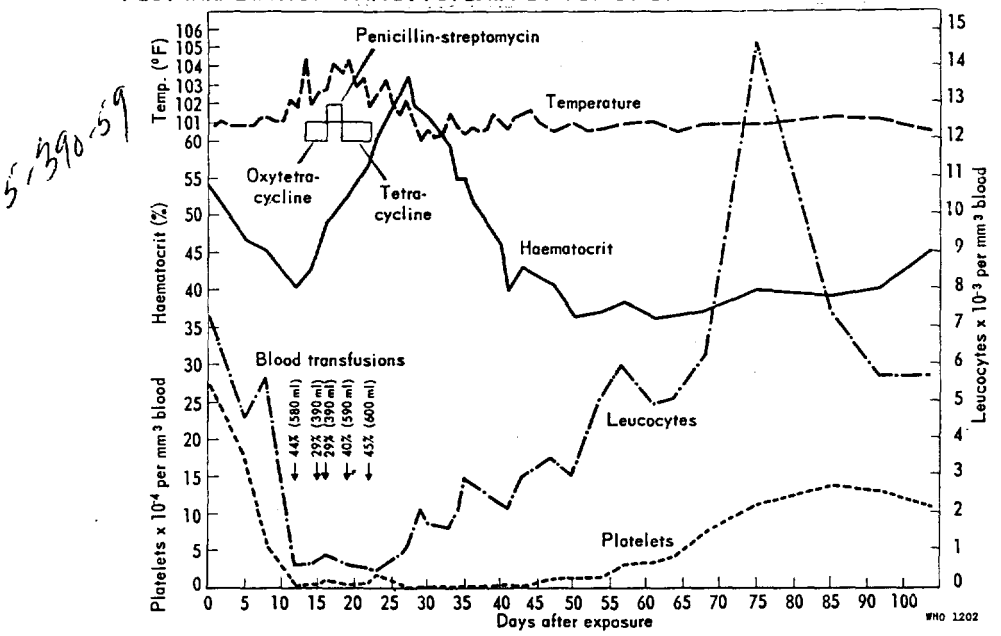
Treatment of the Haemopoietic Syndrome

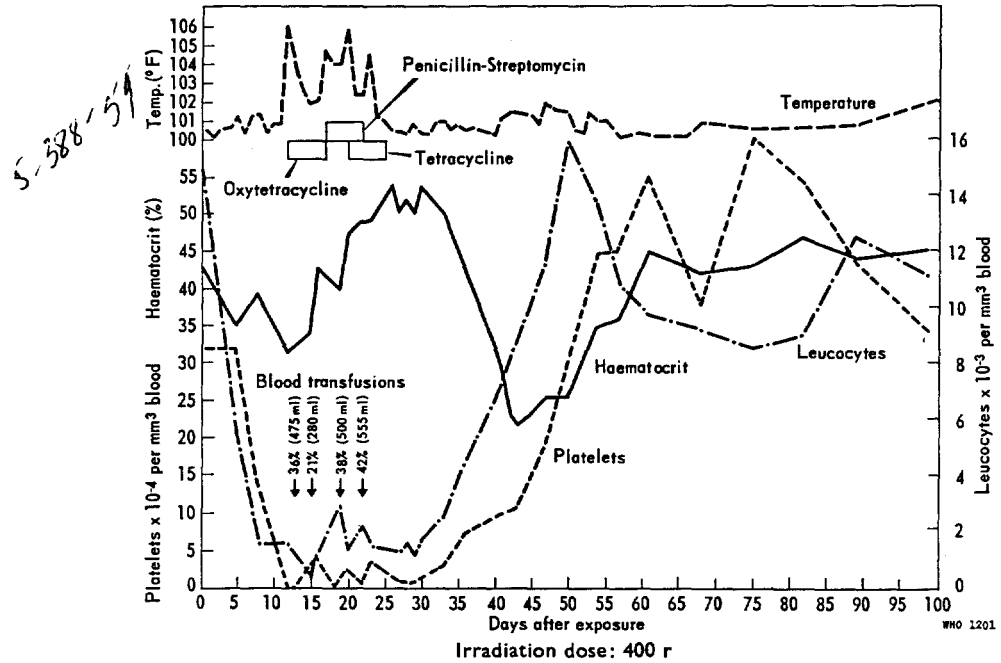
The treatment of a patient with the haemopoietic syndrome after exposure to ionizing radiation presents the same problems as the management of any other patient with pancytopenia. However, the irradiated patient presents the additional challenge that the aplastic state of the bone marrow may be reversible. Thus, if the patient can be carried through the critical period, he will recover, in contrast to many cases of idiopathic or drug-induced bone marrow aplasia.

Sorensen et al. (1960) have studied the efficacy of a flexible and individually adjusted conventional replacement therapy in dogs subjected to whole-body irradiation. Twenty mongrel dogs ranging in weight from 23 lb. to 59 lb. were used. The animals had been dewormed, vaccinated against canine distemper and infectious canine hepatitis, and held for observation for at least six weeks before irradiation. Animals were divided into pairs on the basis of similarity in size, weight, age and breed. Of each pair, one dog was placed in the control group and one in the therapy group and both were irradiated on the same day, using a 250 kVp X-ray therapy unit. The dose rate was approximately 27 r per minute and the total dose for each animal was 400 r, measured as the dose in air at the proximal skin surface in the centre of the field. Phantom measurements showed that the midline tissue dose was about 75% of the air dose at the proximal skin surface. All animals were given a physical examination daily and blood counts were done at regular intervals. Therapy in the treatment group consisted of antibiotics, transfusions and fluids.

Antibiotic therapy with oxytetracycline was initiated as soon as an animal developed fever, and was continued after the fever had subsided. When in spite of this treatment the temperature again went up, usually 3-5 days later, the animals were put on penicillin and streptomycin. When this was no longer effective in controlling the infection, the dogs were switched to tetracycline. Erythromycin was also used occasionally. The doses were significantly higher than ordinarily used in clinical medicine.

FIG. 5
TREATMENT CHARTS OF 2 DOGS CARRIED THROUGH SEVERE POST-IRRADIATION PANCYTOPENIA BY FUNCTIONAL REPLACEMENT THERAPY





Irradiation dose: 400 r

EFFICACY OF FUNCTIONAL
REPLACEMENT THERAPY IN DOGS AT VARIOUS DOSE LEVELS 5-411-59

Dose-response data (30 days)					
Control group			Treated group		
Dose (r)	Died/total	Mean survival time (days)	Dose (r)	Died/total	Mean survival time (days)
400	9/10	16.2	400	2/10	22
420	5/5	13.4	420	2/5	24.9
460	5/5	14.0	460	1/5	30
500	5/5	12.2	500	5/5	23.4
550	5/5	10.8	550	4/5	17.8

Blood transfusions were given mainly for keeping the platelet count above the critical level. Transfusions were usually started when the platelet count had been 5000 or less for 24 hours. Blood was collected from donor dogs in a solution of EDTA disodium salt [1] and immediately transfused. Large transfusions were used to raise the haematocrit level and to minimize the probability of repetitive transfusion producing homologous sensitization to platelets. During the critical period, transfusions were given about every 72 hours, depending on the platelet count. If the haematocrit was high enough platelet-rich plasma was given. Fluids were given when necessary to combat dehydration.

The control dogs developed fever 7-17 days after exposure, with a mean of 11 days. Six of these dogs had cellulitis. Bleeding occurred on the 10th to 14 th day. Nine of the control animals died; the mean survival time was 16.2 days with a range from 12 to 22 days. Necropsies of these dogs showed haemorrhage and infection.

In the therapy group 8 of 10 dogs recovered. One dog died from infection on day 16 and one on day 26. Up to the time when treatment was initiated, the dogs in the therapy group showed a similar clinical picture to those in the control group. Two representative treatment charts are shown in Fig. 5. They demonstrate the effectiveness of antibiotics in bringing the fever under control and also the necessity of changing from one antibiotic to another because of "escape" of the infection.

Transfusions of fresh blood were started on day 12 or day 13 and resulted in significant rises in the platelet count. The half-time of the circulating platelets was less than 24 hours, however, and transfusions had to be repeated frequently, about every 48-72 hours. Two to five large

[1] Disodium dihydrogen ethylenediaminetetra-acetate dihydrate.

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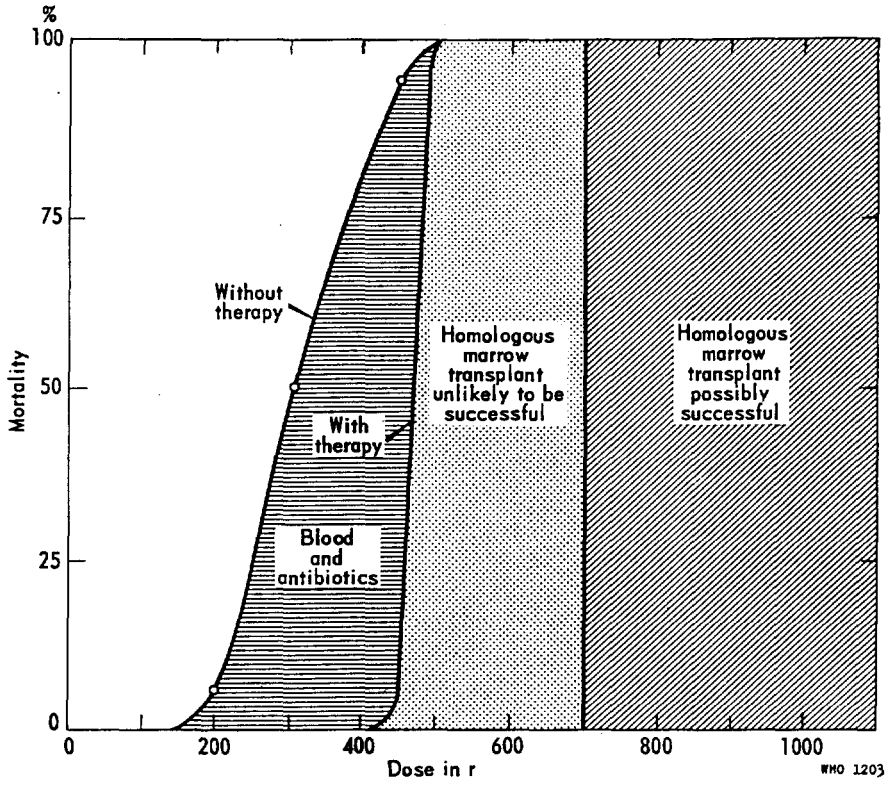
POSSIBLE EFFECTS OF VARIOUS TYPES OF THERAPY ON THE DOSE-MORTALITY CURVE IN DOGS EXPOSED TO X-RAYS



Reproduced, by permission, from Sorensen et al. (1960)

transfusions were required to fill the platelet production gap. The last transfusion was usually given on day 22, 23 or 24. The difference in survival—1 out of 10 dogs in the control group *versus* 8 out of 10 in the treatment group—clearly indicates the efficacy of functional replacement therapy at the dose level investigated. Similar treatment programmes have been tried at higher levels of exposure, but so far no significant benefit has been observed when the dose was 500 r or more (see table on preceding page).

The effects of functional replacement therapy are summarized in Fig. 6. In essence the treatment employed shifts the LD_{50} of dogs from approximately 275 r to about 450 r. At the same time the dose-mortality curve becomes steeper than in untreated animals. Functional replacement therapy does not seem to be of particular value beyond 500 r. Cell transplantation, on the other hand, seems to be successful only at higher doses

(Thomas et al., 1959). This leaves us with an unsatisfactory therapeutic vacuum between the highest dose level at which replacement therapy is effective and the lowest exposure that depresses the immune response sufficiently to allow a homologous graft to take. A similar gap may perhaps exist in man.

It is difficult to estimate the dose range in human beings where functional replacement therapy would be necessary and helpful. The inhabitants of the Marshall Islands who received about 175 r did not require therapy. Some victims of the Oak Ridge accident received brief treatment with antibiotics (Brucer, 1959) and some of them were close to needing platelet transfusions. In general, replacement therapy will be needed for exposures above the level of the minimal lethal dose, which is probably in the vicinity of 250 r. Obviously, the frequency with which this treatment will be needed will increase with the dose. The upper limit of exposure at which this therapy will be successful in man is not known. The Yugoslav accident (Jammet et al., 1959; Mathé et al., 1959) might have shed some light on this question, but since bone marrow transplants were also administered interpretation of the regeneration and survival data is difficult. Establishment of this upper limit is particularly important since it would also delineate when an attempt at bone marrow transplantation would be justified. Homologous bone marrow transplantation in man may not be an innocuous procedure and should be employed only after serious consideration. At the present time attempts to transplant homologous bone marrow are justified, we believe, in the presence of severe pancytopenia and when chances that replacement therapy alone would be sufficient are small. This would probably mean patients who had survived the gastrointestinal syndrome, who had vomited severely for more than 48 hours after exposure, who at 24 hours had less than 500 lymphocytes per mm^3, or who had a rapidly declining white cell count. Frequent bone marrow aspirations may be helpful in making this difficult decision. It may be worth emphasizing again that bone marrow transplantation is not the only treatment available in acute radiation injury. If it is permissible to extrapolate to man the findings in dogs reviewed above, it may be expected that a conservative therapeutic replacement programme would be life-saving in many cases, particularly in the lower lethal dose range. This programme can be summarized as follows:

1. Large doses of antibiotics when signs of infection are present; the choice of antibiotics should preferably be based on repeated cultures and sensitivity tests.

2. In case of haemorrhage or immediate danger of bleeding, transfusions of fresh blood or platelet-rich plasma (within four hours after collection, preferably less), using siliconized equipment and plastic bags.

3. Protection of the patient against exogenous stresses, particularly infection.

4. Good general medical care and close supervision by personnel trained in treating patients with pancytopenia.

5. No prophylactic use of antibiotics because this may result in unnecessary development of resistant bacteria.

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