THE COMBINED EFFECTS OF THERMAL BURNS AND WHOLE-BODY X IRRADIATION. II. ANEMIA*

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INTRODUCTION

In a previous paper[2] it was shown that the burn area required to kill 50 per cent of rats exposed to radiant thermal energy is reduced by non-lethal radiation doses. Mortality was increased in the first 48 hours as well as in the succeeding period, especially at eight to 15 days. Brooks, *et al.*,[6] have observed an increased mortality in dogs given thermal burns and exposed to ionizing radiation, and they believe that the number of burn casualties at Hiroshima is partially referable to the combined trauma of ionizing radiation and burns. In both mice[11] and pigs[4] irradiation superimposed upon thermal damage produces an increased mortality. Although this increased mortality associated with the combined injury is now well documented, the altered physiology resulting from such trauma has not been clearly defined. Infection has been shown to play a major role in the combined injury in swine, as streptomycin markedly reduces the mortality in such animals given 400 r and a 15 per cent burn. Histamine levels[5] and the white counts follow very closely the values observed in the unburned pig irradiated with 400 r, and apparently cannot account for the increased mortality observed in the combined injury.

The present study was undertaken as part of a survey of the pathologic physiology of the rat exposed to ionizing radiation and radiant thermal energy in an attempt to anticipate the medical problems posed by such injuries. The purpose of this portion of the investigation was to delineate the severity and the extent of the anemia accompanying the combined injury of thermal and ionizing radiation in the rat, and to compare the anemia with that produced by the single trauma. Concomitant studies of the gross and microscopic pathology,[3] the coagulation and platelet abnormalities,[8] and the alterations in erythrocyte fragility[1] were made on the same rats.

The blood volume, hematocrit, red cell number, hemoglobin concentration, and reticulocyte population were measured in burned and irradiated rats and in three combinations of these injuries. Calculations of the circulating red cell volume and plasma volume were made, and the severity of the anemia evaluated by correlation of the values obtained.

METHODS

Female rats of the Sprague-Dawley strain, weighing initially between 140 and 180 Gm. and approximately 6 weeks old, from the laboratory colony, were used in all experiments. They were housed in individual wire cages and fed Purina Chow and water *ad lib.* The animals were divided into seven experimental groups as follows: Group I—control (C); Group II—500 r total body X irradiation (X_5); Group III—100 r total body X irradiation (X_1); Group IV—four strip burns plus 100 r (B_4X_1); Group V—two strip burns plus 500 r (B_2X_5); Group VI—two

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The opinions or assertions contained herein are those of the writers and are not to be construed as official or reflecting the views of the Navy Department or the Naval Establishment at large.

TABLE I. *Effect of X Irradiation, Burning, or a Combination of Both on Body Weight of Rats.*

	Ratio of Final to Initial Body Weight by Groups[a] (Per Cent)						
Days	C	X_1	X_5	B_2X_5	B_4X_1	B_4	B_2
1	100	102	100	103	104	104	104
3	104	103	93	93	103	110	104
5	108	105	92	93	107	106	107
8	114	115	104	103	107	109	112
11	119	120	108	99	115	114	115
15	122	123	115	98	120	119	119
21	128	127	129	116	122	121	120
30	134	139	127	118	127	129	128

[a] C = control; X_1 = 100 r total body roentgen irradiation; X_5 = 500 r total body roentgen irradiation; B_2 = 15 per cent burn; B_4 = 25 per cent burn; B_2X_5 = 15 per cent burn and 500 r total body roentgen irradiation; B_4X_1 = 25 per cent burn and 100 r total body roentgen irradiation.

strip burns (B_2); and Group VII—four strip burns (B_4). Hereafter the animals of a given group will be referred to frequently by the designation in parenthesis (C, X_5, B_4X_1, etc.).

All animals were clipped and depilated, using a commercial strontium sulfide depilatory according to the method of Kuhl, *et al.*[10] Approximately 72 hours following depilation the animals were anesthetized with pentobarbital sodium (4.5 mg./100 Gm. subcutaneously) and were treated according to the experimental procedure. Control rats were depilated and anesthetized only.

The four groups exposed to X irradiation were radiated with a 250 KVP Westinghouse therapy unit operating at 15 ma., with 0.5 mm. Cu and 1 mm. Al filter at a skin to target distance of 40 in. and with a dosage rate of 23 r/min., HVL 1.5 mm. Cu. Dose rates on a particular day were calculated from several measurements in air made with a Victoreen chamber.

The four groups which were burned were treated by positioning the anesthetized rats on a rotary turntable behind asbestos insulated rectangular apertures measuring 2.5 by 12 cm. by means of an elastic cloth sling. A Mitchell-Nelson carbon arc background projector focused to a usable spot 3.5 cm. in diameter was the energy source. The turntable rotated past the focused beam at a speed of one revolution every 42 seconds. Such treatment yields an exposure time of approximately 0.4 second with a total energy delivery of 9 cal./sq. cm. as measured by a black body radiometer. The visible strip burn resulting from a single exposure was slightly overlapped for each succeeding exposure to produce a contiguous burn. The two strip burns (B_2 and B_2X_5) gave mean burn areas which were 15 per cent of total body area, and the four strip burns (B_4 and B_4X_1) gave mean burn areas of 25 per cent of total body area. Histological examination revealed that these burns are essentially equivalent to the characteristic white burn described by Sheline, *et al.*[12] The burns were applied to the back and sides of the animals, and did not interfere with excretory functions nor seriously impair ambulation. Animals in the B_4X_1 and B_2X_5 groups were first given total body X irradiation and burned approximately 20 to 60 minutes later.

The rats were assigned to individual groups immediately after depilation by a random number table. The selection of a particular rat from a particular experimental group for determinations on any given observation day was made by selecting the lowest numbered surviving member of that group. A single animal from each group was used for determinations performed on the following days after injury: Days one, three, five, eight, 11, 15, 21 and 30.

The experiment was run in six replications, each replication starting at three-week intervals so that at each post-injury day in each experimental group there were six animals, with certain exceptions where the number was reduced to four or five.

Blood volume was measured using P^{32}-labeled cells. Blood was drawn from donor rats of the same approximate age into heparinized syringes and incubated with 10 microcuries of P^{32} for an hour at 37° C., stored overnight in the refrigerator, and then washed three times with a 3-to-1 saline-plasma mixture. Final suspension of the

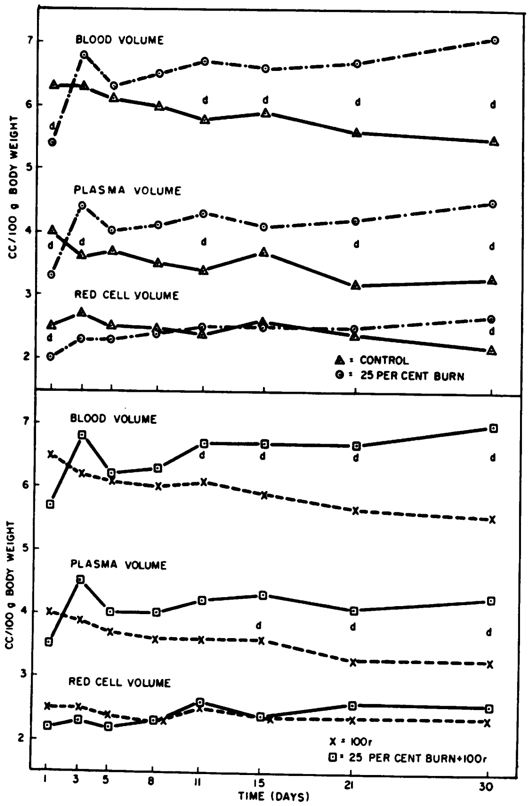

FIG. 1. Mean blood, plasma, and red cell volumes after a 25 per cent burn and exposure to 100 roentgens of roentgen irradiation. The letter d between points indicates a statistically significant separation of the treatment groups (5 per cent level).[13]

cells was made in rat serum so that the hematocrit was approximately 30, and 0.2 ml. of this suspension was injected into the surgically exposed jugular vein of the recipient rat. Five minutes were allowed for intravascular mixing, after which the heart was exposed and blood was drawn into a dry siliconized syringe and then ejected into a heparinized flask. Whole blood was plated, air dried, and counted to an accuracy of 1 per cent with an end window G-M tube using an automatic sample changer. Plasma from the blood injected was routinely checked for radioactivity. Blood volume was determined from the dilution of the injected cells, and erythrocyte and plasma volumes were calculated from the hematocrit of cardiac blood. The hematocrit was determined in duplicate in standard bore capillary tubes plugged with mercury and sealed with hot beeswax, then spun for 30 minutes at 3000 RPM in an International size 2 refrigerated centrifuge at 5 to 10° C. Packed red cell and plasma columns were measured with a millimeter scale.

Hemoglobin was determined by the method of Evelyn and Malloy,[9] and red cell counts were made using Levy hemocytometer chambers and Thoma pipettes. Reticulocytes were stained with brilliant cresyl blue and counted as a wet preparation. Two drops of dye (½ saturated solution of dye in 0.9 per cent NaCl) were added to one drop of blood in a spot plate, mixed, and after five minutes 1000 cells were counted wet under a cover slip. This procedure was found to be more satisfactory for rat erythrocytes than many of the standard methods. Calculation of the number of reticulocytes per cu. mm. was made by multiplying the per cent reticulocytes by the red count.

STATISTICAL ANALYSIS

Analysis of the seven groups on each of the eight measurement days was made simultaneously, using the method of Tukey.[13] This method separated statistically different groups at the 5 per cent level and permitted intergroup comparison on a given observation day.

RESULTS

Twenty-four hours following the burn the B_4 rats had a decreased blood volume; in such rats the 25 per cent burn caused an 18 per cent reduction in the mean blood volume, a 20 per cent reduction in the mean red cell volume, and a 17 per cent reduction in the plasma volume, as shown in Figure 1. However, recovery occurred rapidly and the blood volume was little different from the controls by Day 3. Both 15 and 25 per cent burns caused an increased blood volume by Day 11, and this was maintained over the 30-day period studied. The plasma volume

TABLE II. *Blood, Plasma, and Red Cell Volume in Rats Given a Maximal Sublethal Dose of Irradiation (475 r) and a 25 Per Cent Body Area Burn.*

Volume	Treatment[a]	4 Hrs.	1 Day	2 Days	4 Days	7 Days	9 Days	11 Days
		Time after Treatment						
Blood Volume (cc./100g)	C	5.8[b]	6.0	6.4	6.1	6.2	5.7	5.9
	B_4	3.9*[c]	4.6*	6.1	5.8	6.2	6.2*	6.3
	X_{475}	6.1	6.1	6.5	6.2	5.8	5.7	5.4*
	B_4X_{475}	3.6*	4.6*	5.8	6.2	5.5	5.6	5.9
Plasma Volume (cc./100g)	C	3.2	3.4	3.6	3.5	3.5	3.3	3.4
	B_4	1.7*	2.6*	3.8	3.5	3.6	3.6	3.6
	X_{475}	3.5	3.6	3.7	3.7	3.6	3.6	3.6
	B_4X_{475}	1.5*	2.6*	3.6	4.2*	3.8	4.1*	4.3*
Red Cell Volume (cc./100g)	C	2.6	2.6	2.8	2.6	2.7	2.4	2.5
	B_4	2.2*	2.0*	2.3*	2.3*	2.6	2.6	2.7
	X_{475}	2.6	2.5	2.8	2.5	2.2†	2.1†	1.8*
	B_4X_{475}	2.1*	2.0*	2.2*	2.0*	1.7*	1.5*	1.6*

(a) C = control, B_4 = 25 per cent burn, X_{475} = 475 roentgens of total body roentgen irradiation, B_4X_{475} = 25 per cent burn and 475 roentgens of total body roentgen irradiation.

(b) All groups have six rats except Day seven which has four rats.

(c) Singly asterisked values are statistically different from non-asterisked values in any cell. The dagger defines a second statistically different group (5 per cent level).

was elevated by Day 3 but wide variation in individual values indicates that the mean is not an accurate reflection of any single value. The increment in blood volume seen at Day 11 occurred chiefly in the plasma volume, as the red cell volume was not significantly altered from the control value after the first day, and the mean was actually below the control mean until Day 11. Untreated controls undergo a gradual reduction in blood volume from 6.3 to 5.5 cc. per 100 Gm. body weight over the interval studied. Consequently, the differences between the blood volume of the control and burned rats considered on the basis of final weight becomes greater with time. The change in blood volume was essentially due to a gradual reduction in the plasma volume, as a change in the red cell volume was not seen except on the 30th day.

Rats irradiated with 100 r have only small changes in blood, plasma, and red cell volumes (Fig. 1). However, the fall in red cell volume observed in rats given 500 r was striking, as shown in Figure 2. On Day 15 the red cell mass was reduced to 46 per cent on the control level, although the plasma volume per unit weight was unaltered. These factors resulted in a decline in the blood volume from 6.6 cc. per 100 Gm. on Day 1 to 5.3 cc. per 100 Gm. on Day 11 (Fig. 2). The reduced blood volume is accentuated by the gain in body weight which begins at Day 8, as shown in Table I.

When the 25 per cent burn is superimposed on 100 r of irradiation (B_4X_1), the volume changes observed in the B_4 rats appear (Fig. 1). The increased blood and plasma volume after the third day was comparable in magnitude to the changes observed in the B_4 rats. Similarly, the red cell volume of the B_4X_1 group was reduced by the burn and, despite a temporary depression in reticulocyte production, recover at approximately the same rate as the B_4 group (Fig. 1).

The B_2X_5 rats made no appreciable weight gains until the 21st day, although the X_5 rats began to gain weight on Day 8 (Table 1). The combined injury series had a more rapid fall in the red cell volume than the X_5 series. Despite the greater loss of red cells over the first 11 days, the combined injury rats were able to maintain a more normal blood volume than the X_5 series. This was accomplished by an expansion of the plasma volume, which exceeded that observed in either the B_2 or X_5 series. From

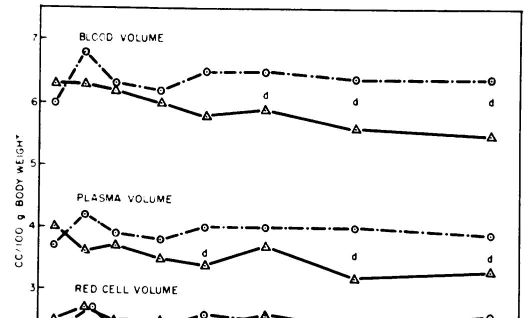

FIG. 2. Mean blood, plasma, and red cell volumes after a 15 per cent burn and exposure to 500 roentgens of roentgen irradiation. The letter d between points indicates a statistically significant separation of the treatment groups (5 per cent level).[13] The letters dd indicate a significant separation from the control value.

the statistical analysis employed it could be demonstrated that the B_2X_5 series had red cell volumes significantly below the X_5 group. On Days 8 and 11 the difference was greater than the volume of erythrocytes destroyed during the burn episode. Thus on Days 1, 3, 5, 8 and 11 the mean red cell volume per 100 Gm. body weight of the X_5 series exceeded the B_2X_5 series by 0.2, 0.1, 0.3, 0.4 and 0.6 cc., respectively (Fig. 2).

In the fatally injured rats given 475 r and a 25 per cent burn the mean red cell volumes are different from those of the irradiated rats by the amount of erythrocytes destroyed concomitantly with the burn as shown in Table II. The blood volume of both combined and irradiated rats in this experiment confirmed the reduction in blood volume observed in the more severely irradiated rats. Similarly, the increase in plasma volume of the combined series exceeded that of the irradiated group. Clinically the hemorrhagic defect in the B_4X_{475} series is not as pronounced as that observed in the B_2X_5 series.

In burned animals the peripheral blood measurements (hemoglobin concentration, hematocrit, and red cell count) were lowered by the burn at Day 1 (Table III). The B_2 and B_4 rats showed a rapid rise in reticulocytes from the control level, which reached a maximum on Days 5 to 8 and was maintained through Day 30 despite a normal red cell volume attained on Day 5 (Table IV). Although the red cell volume was normal, the increased plasma volume of the burned rats caused a reduction in the hematocrit, hemoglobin and red cell count of the B_4 series (Table III).

Irradiated rats given 100 r had an insignificant fall in the red cell volume (Fig. 1). However, reticulocytes fell to a low level on Day 3, but had returned to normal by Day 5 (Table IV). The depressed erythrocyte production is not of sufficient duration to significantly alter the other erythrocyte values measured (Table II).

Irradiation with 500 r produced a pronounced fall in the hemoglobin concentration, hematocrit, red cell count and red cell volume (Fig. 3). This is detectable on Day 8, and reaches a maximum on Day 15. At this time a loss in the mean red cell volume of 1.6 cc. per 100 Gm. had occurred, with a 56 per cent reduction in total circulating hemoglobin. The return of the red cell volume to normal was concomitant with a sharp rise in reticulocyte count initially seen on Day 15 in two X_5 rats (23 and 20 x 10^4 cells per cu. mm.) and in two B_2X_5 rats (21 and 17x10^4 cells per cu. mm.). The reticulocytosis was indicative of the regeneration of ery-

TABLE III. *Hemoglobin, Hematocrit and Red Cell Count after 100 r and a 25 Per Cent Burn.*

		Days after Treatment								
	Type	1	3	5	8	11	15	21	30	Av. S. D.
Hct. (%)	C	40	43	40	41	41	41	43	41	±2.3
	X_1	39	38	39	39	40	40	43	42	±1.4
	B_4	38	35	37	38	36	37	36*	36	±2.4
	B_4X_1	38	34*	35	37	38	36	38*	38	±2.7
Hb. (g / 100 cc)	C	14.8	15.8	15.5	15.7	14.3	15.1	16.9	16.0	±1.3
	X_1	14.8	15.0	14.6	15.5	14.6	14.6	15.3	15.8	±1.3
	B_4	14.2	13.3*	14.6	14.0	12.7	12.8	13.6*	12.2*	±1.4
	B_4X_1	15.0	12.6*	13.1*	13.8	13.2	13.0	13.1*	13.9	±1.5
R. B. C. (million / cu mm)	C	5.77	5.92	5.91	6.30	6.41	6.02	6.58	5.92	±0.64
	X_1	5.72	5.87	6.26	5.46	6.07	5.86	6.04	6.70	±0.57
	B_4	4.97	5.50	5.91	5.51	5.29	4.93	4.82*	5.05	±0.76
	B_4X_1	5.64	4.37*	4.42*	5.51	5.34	4.43*	4.69*	5.22	±0.68

C = control, X_1 = 100 roentgens total body irradiation, B_4 = 25% body area burn, B_4X_1 = 25% body area burn and 100 roentgens total body irradiation.
* = statistically different groups (5% level).

throcytes in the interval following Day 15, as seen in the increased red cell volume, hemoglobin, and hematocrit values observed on Day 30 (Figs. 2 and 3). In contrast, the prolonged reticulocytosis of the B_2 and B_4 series was not accompanied by an elevation of the red cell volume.

Rats subjected to the combined insult of a 25 per cent burn and 100 r had red cell values which closely followed the pattern established in the B_4 rats. On Day 3 the B_4X_1 group had a significant reduction from control levels in the hematocrit, red count, and hemoglobin concentration with a small reduction in red cell volume. Similarly, low values were observed on Day 5, although not demonstrable statistically (Table II). The reticulocyte count of the B_4X_1 rats displayed the initial fall seen in the X_1 group at 3 days, after which it rose rapidly (Table IV). The difference in hemoglobin, hematocrit and red cell volume between the B_4 and B_4X_1 groups is not significant, although during this period the combined injury group is consistently below the burned rats. The latter findings suggest that 100 r produces a defect in red cell production which is potentiated by the additional erythropoietic load, but as the depressed function is of short duration in comparison to the half life of the red cell, the demonstration of such an effect is difficult with the number of animals used here.

In contrast to the B_4X_1 series, the B_2X_5 rats approximate the irradiation pattern more closely than the burn response. Although the severity of the reduction in red cell volume did not exceed the maximum seen in the X_5 group, it developed more rapidly (Fig. 2). In the B_2X_5 rats the hematocrit, hemoglobin, and red cell count were significantly below the values found for all other groups at Day 8, and remained below control levels through Day 21 (Fig. 3). The hemoglobin, hematocrit, and red cell count of this combined injury group was lowest from Days 11 through 15, and statistically below the values of the X_5 group on Days 8 and 11. Reticulocytosis in B_2X_5 and X_5 groups did not appear until Day 15, but in the interval between the 15th and 30th days 62 per cent of the initial red cell volume was regenerated, representing 0.65 Gm. of hemoglobin per 100 Gm. of body weight. The reticulocytosis was more pronounced in the B_2X_5 than the X_5 series, and at Day 21 the mean values for red cell volume, hemoglobin and hematocrit exceeded those observed in the irradiated animals (Fig. 3). The higher red cell volume of the B_2X_5 series is statistically different from the X_5 on Day 21; but, in general, variability of the

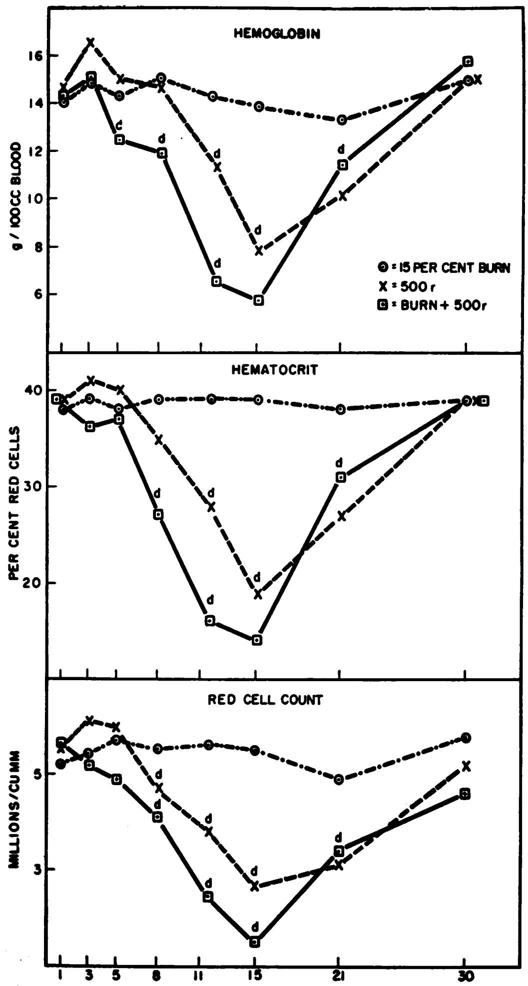

Fig. 3. Hemoglobin, hematocrit, and red cell count after exposure to 500 roentgens of roentgen irradiation and a 15 per cent burn. The letter d indicates a statistically significant separation of the treatment groups and also indicates a statistically significant difference from the control level (5 per cent level).[13]

values excludes any comparison of the recovery rate from the mean values of the hemoglobin concentration, red count, or hematocrit.

DISCUSSION

The characteristic response of the rat to a 25 per cent burn is an initial fall in blood, plasma and red cell volume, followed by a rapid recovery of the red cell volume and a prolonged elevation of the blood and plasma volume, with a concomitant reticulocytosis. A radiation dosage of 100 r produces only a transient depression in the reticulocyte count, whereas 500 r reduces the blood volume, red cell volume, and other erythrocyte dependent values for a period of about 15 days. The reduced rate of erythrocyte production plus the loss of red cells due to the hemorrhagic defect causes a severe anemia in the higher radiation dosage.

Only three combinations of thermal burns and irradiation have been studied in this investigation—a moderate amount of irradiation and a large burn (B_4X_1); a large dose of irradiation in combination with a moderate burn (B_2X_5); and a large dose of irradiation with a large burn (B_4X_{475}). The burn response obscures the irradiation effects when a large burn is superimposed on a small dose of irradiation. The variables studied in this report show the transient fall in reticulocytes on Day 3 to be an indication of the exposure to ionizing radiation in small doses, although by the fifth day this is replaced by a reticulocytosis.

Rats given 500 r and a 15 per cent burn display hematopoietic changes closely related to that observed with 500 r alone. The time course of recovery is similar and, although the anemia is only slightly more severe in the combined traumata, it develops more rapidly. For example, one B_2X_5 rat had a severe anemia on Day 5, although a generalized reduction in this group did not appear until the eighth day. The more rapidly developing anemia which occurs in the B_2X_5 group cannot be entirely related to the initial loss of red cells due to the burn. The difference between the combined injury animals and the irradiated only animals is most pronounced on Day 11. At this time the combined injury animals have reached the low ebb of the anemia, comparable to that seen in the irradiated group at 15 days. In contrast, results from the severely burned rats given a maximal sublethal dose of radiation

TABLE IV. *Number of Reticulocytes in Irradiated and Burned Rats.*

Days After Burn		Reticulocytes after Treatment[a][b] (No. x 10^4/cu mm.)						
		C	B_2	X_5	B_2X_5	X_1	B_4	B_4X_1
1	Mean	15.5	13.3	6.5	7.0	5.0	13.9	8.5
	S. D.	8.6	6.4	3.8	2.4	3.4	4.5	4.3
3	Mean	15.0	25.2†[c]	0.6	0.0	1.7	22.6*	3.6
	S. D.	4.2	11.1	0.7	0.0	1.6	5.2	4.1
5	Mean	15.4	20.0	1.0	0.3	10.6	34.5*	25.0*
	S. D.	6.2	7.6	1.2	0.3	6.9	13.6	4.2
8	Mean	17.2	25.2	1.1*	2.1*	16.9	37.4*	44.3*
	S. D.	4.8	8.2	0.9	1.7	3.4	14.3	13.5
11	Mean	12.0	20.3	4.3	5.1	19.2	26.7*	33.4*
	S. D.	3.7	8.4	3.7	3.7	4.7	9.1	18.4
15	Mean	11.0	16.5	10.0	8.9	12.8	27.4*	39.7*
	S. D.	4.8	12.0	10.1	9.5	7.2	6.1	33.1
21	Mean	11.3	15.2	53.6†	86.0*	13.9	31.9*	37.4*
	S. D.	5.4	3.6	35.2	39.0*	6.9	18.0	11.9
30	Mean	12.2	20.5	17.5	15.1	10.5	31.7*	14.7
	S. D.	4.5	6.0	11.1	8.6	3.0	10.3	11.4

(a) C = control; B_2 = 15 per cent body area burn; B_4 = 25 per cent body area burn; X_1 = 100 roentgens total body roentgen irradiation; X_5 = 500 roentgens total body roentgen irradiation; B_2X_5 = 15 per cent body area burn and 500 roentgens total body roentgen irradiation; B_4X_1 = 25 per cent body area burn and 100 roentgens total body roentgen irradiation.

(b) Six animals per group

(c) Singly asterisked values are statistically different from non-asterisked values in any cell. The dagger defines a second statistically different group (5 per cent level).

(475 r) show no alteration in the rate of red cell loss between the burned and irradiated controls. The difference between the combined injury and irradiated groups given 500 r may be due to the increased number of survivors in the irradiated group. However, it is impossible to rule out the fact that the lethality of the radiation may be the factor causing the increased red cell destruction.

It has already been noted that ionizing radiation markedly increases the percentage mortality in the burned rat.[2] From our data it is seen that the combined traumata in the B_2X_5 rats produces an anemia which reaches a level comparable with that observed in the irradiated group. Such values represent extreme depletion of the circulating red cell volume and appear in the B_2X_5 group at least four days before the irradiated group. Both in the B_2X_5 and the B_4X_{475} groups the mechanical fragility test indicates an abnormal fragility of the red cell at this time.[1] Whether the loss of red cells is due to the initial insult of the burn or is compounded by undefined changes during the course of the burn is not answered by these studies. It is probable, however, that the increased loss of red cells is a factor in the elevated death rate observed in the combined irradiated and burned rats. However, the experimental method using serial sacrifice precludes correlation of the anemia and the death of the particular rat measured. Indeed, such a study is inherently a study of the survivors.

The reticulocyte counts on the 15th day indicate that the recovery process has begun in two combined injury and two irradiated rats. However, the range on Day 21 makes any comparison of recovery rates difficult, although all the variables measured tend to indicate that the B_2X_5 rats recover more rapidly than the irradiated animals.

Rats given a 25 per cent burn and a maximal sublethal dose of irradiation (475 r) almost invariably die before 15 days. Such rats show a progressive reduction in the red cell volume during the 11-day period measured. The initial reduction in the red cell volume is not different from that observed in the burned controls, and the fall in the red cell volume over the period measured is parallel to that seen in the rats exposed to 475 r only. A compensatory increase in the plasma volume maintains the blood volume at near normal levels throughout the entire period. The deaths at seven to 15 days occur at the same time as the reduced red cell volumes, but none of the variables studied correlate with the deaths observed during the first 48 hours in the combined animals.

In contrast to the anemia produced by a large irradiation dose (500 r), the 25 per cent burn does not cause a severe anemia in the rat. Two significant findings indicate that this may be related to a stimulation of the erythropoietic tissue. First, spleen weight measurements made in the burned rat show a marked increase in weight, and histologically the spleen displays numerous hematopoietic centers by the seventh day.[3] Second, the reticulocyte counts are twice normal, and maintained for a period of three weeks following a 25 per cent body area burn. The capacity of the rat for red cell production is prodigious, as has been shown by Davis, *et al.*,[7] who demonstrated that the normal rat can regenerate one-half his red cell volume in seven days, following a 50 to 60 per cent hemorrhage by acute bleeding. They note reticulocyte counts during the interval similar to those maintained for 30 days by the more severely burned rats.

In view of the evidence indicating intense hematopoietic activity over a prolonged time in the burned rat, the normal erythrocyte values during this interval were surprising. Apparently a continuing stimulus to erythrocyte production is present which is not related to the volume of erythrocytes present. When the reticulocytosis is used as a criterion of increased red cell production one would conclude that the burned rat is producing red cells at a very rapid rate, even though the red cell volume is essentially unchanged during this interval. Consequently, this evidence must indicate an increased rate of erythrocyte destruction in the burned rat.

SUMMARY

A comparison of the effects of 25 per cent and 15 per cent body area burns, and 100, 475 and 500 roentgens of total body roentgen irradiation with the effects of three combinations of these on blood volume, red cell volume, hematocrit, red cell count, hemoglobin and reticulocyte population has been made in rats.

Both 15 per cent and 25 per cent body area burns produced an increased blood and plasma volume over the 30-day period studied. Although red cell volume was not appreciably altered after the fifth day, a slight reduction in hematocrit, hemoglobin and red count was observed throughout this period. The sustained reticulocytosis of the burned rat, with concurrent normal red cell volumes indicates an accelerated destruction of erythrocytes during the post-burn period.

The exposure to 100 r of ionizing radiation produced only a transient depression of the reticulocyte level. However, 500 r caused a severe anemia, with a concomitant fall of all the measured values for 15 days. Such a combined injury produced an anemia which developed more rapidly than that caused by 500 r alone. The anemia of the combined injury series was detectable on Day 8, and on Day 11 was equal to the maximum observed in the irradiated animals on Day 15. A discussion of the contribution of such factors to the increased death rate seen in combined injury is given.

The erythrocyte values in rats given a 25 per cent burn and 100 r more closely approximated the values of the burned than of the 100 r irradiated rats. Of the measured values the reticulocyte count proved to be the only useful indicator of exposure to ionizing radiation in this low dosage. Even the fall in this variable was obscured by the fifth day in the reticulocytosis characteristic of the burned rat.

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