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Effects of Thermal Burn and X-Irradiation on Early Mortality.* (31026)

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Thermal burn applied immediately before X-irradiation significantly increased mortality during the time period characteristic of radiation injury to the gastrointestinal tract(1). The increased mortality was attributed to an inhibitory effect of toxic materials produced at the site of the burn on recovery of the intestinal epithelium from radiation damage. If this were so, the degree of inhibition would be expected to reach a maximum some time after the burn, then decrease. This possibility was tested by a study of early mortality in rats when the interval between thermal burn and X-irradiation was varied. If the synergism between X-ray and thermal burn was due to a nonspecific inhibition of cell proliferation, then it would be expected to occur in bone marrow and be reflected by changes in the formed elements of the blood. This possibility was also studied.

Materials and methods. Mortality studies. Two series of rats were exposed to thermal burn and/or X-irradiation as previously described(1). The first consisted of 6 groups containing 10 animals each. One group received 700 R of X-irradiation alone while another received a standard one-minute burn followed immediately by irradiation. The 4 remaining groups received the burn at 6-96-hour intervals prior to irradiation. The second series was a replication of the first except that the burn was administered at different times after irradiation.

Peripheral blood studies. Four groups of 10 rats each were used. The first was exposed to a one-minute burn and the second to 700 R of X-irradiation. The third group received the burn followed immediately by 700 R of X-irradiation, while the fourth received the burn 96 hours after the irradiation. The cells in the bloods from each group were counted at 4, 7, 10, 14 and 21 days after injury. Bloods samples, taken by cardiac puncture, from at least 5 animals from each group were examined at each time period.

Erythrocyte studies. The erythrocytes in blood diluted ($1:10^5$) in Eagle's solution were counted and a distribution of cell volumes determined employing a Coulter Counter (Model B with plotter). The distribution represented a division of erythrocyte volumes into 25 distinct class intervals, called channels, representing a range of cell volumes from 6 to 160 cubic microns. An analysis of the operation of the Coulter Counter has been published(2).

Leukocyte studies. The leukocytes in blood diluted 1:500 were counted after the erythrocytes had been lysed by Cetavlon(3).

Results. Mortality. Administering the thermal burn at times up to 96 hours before X-irradiation increased mortality in the first 8 days postirradiation, the time period characteristic of gastrointestinal injury (Fig. 1). Mortality was maximum when the burn was administered at 72 hours prior to irradiation. When the burn was administered at times up to 96 hours after irradiation, fewer animals died from gastrointestinal injury but there was an increased mortality in the time period characteristic of bone marrow injury.

Erythrocytes. The frequency distribution of the cell volumes in a group of randomly

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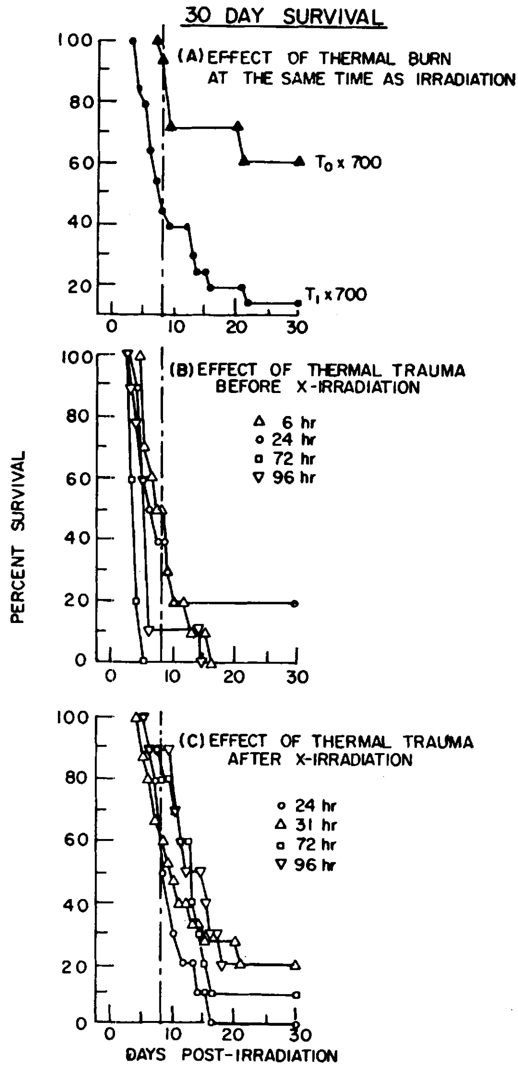

FIG. 1. Effect of varying the time between administration of thermal burn and x-irradiation. Vertical line has been drawn at 8 days postirradiation. Animals dying before 8 days had symptoms of severe intestinal injury. Animals dying after 8 days were severely anemic.

selected rats prior to any experimental treatment extended from channels 7 to 16 and showed a maximum at channel 11, which corresponded to 70.4 cubic microns (Fig. 2a). The changes in the erythrocyte count following the various treatments are recorded in Table I.

After thermal burn alone, the erythrocyte

TABLE I. Erythrocyte Counts (Million Cells per Cubic mm) at Different Times Following Various Treatments.

Day post-irradiation	T_1X_0	T_0X_{700}	T_1X_{700}	$T_1X_{700}(96)$
4	6.93	7.75	6.60	6.50
7	6.83	6.81	5.53	5.87
10	6.38	4.21	3.92	2.92
14	7.49	3.59	4.67	2.30
21	6.23	5.01	4.79	3.50
Nontreated control, 7.77×10^6 cells per mm^3				

Each value represents the mean of 5 animals. T indicates thermal burn and the subscript, 1 or 0, indicates duration of the burn in min. X indicates irradiation and the subscript, 700 or 0 indicates the dose in rads. The (96) indicates that in this group the burn was administered 96 hr after irradiation.

count decreased to a minimal value at day 10, increased to the control level by day 14, then decreased again until day 21. No change

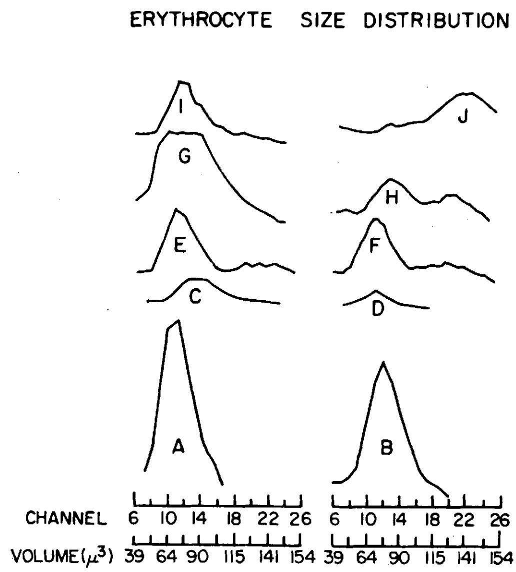

FIG. 2. Erythrocyte volume distribution after exposure to x-irradiation and/or thermal burn. Erythrocyte count for the sample is given in brackets. a) Control (7.77 million/mm^3). b) 10 days after a 1-minute burn (6.33 million/mm^3). c) 21 days after 700 R of x-irradiation (2.37 million/mm^3). d) 21 days after 700 R of x-irradiation (0.74 million/mm^3). e) 21 days after 700 R of x-irradiation (4.68 million/mm^3). f) 21 days after 700 R of x-irradiation (4.39 million/mm^3). g) 21 days after a 1-minute burn and 700 R of x-irradiation administered together (6.55 million/mm^3). h) 21 days after a 1-minute burn and 700 R of x-irradiation administered together (3.73 million/mm^3). i) 21 days after exposure to a 1-minute burn and 700 R of x-irradiation administered together (2.72 million/mm^3). j) 21 days after exposure to a 1-minute burn administered 96 hr after 700 R of x-irradiation (3.99 million/mm^3).

in frequency distribution of the erythrocyte volumes was noted (Fig. 2b).

After irradiation alone, no change in either the erythrocyte number or the volume distribution was found prior to day 4. By day 7 there was a 10% decrease in the erythrocyte count. By day 10, the erythrocyte count had fallen to less than 50% of the pretreatment value. The count remained at this level through day 14 with a return toward the preexposure value by day 21. At this time, the frequency distribution of the erythrocyte volumes showed a second population of cells, which were larger in volume than normal. This second population was found only in animals which had erythrocyte counts higher than the day 14 level. Thus, the animals fell into two groups: one of which did not show the second population and which also had a low count (Fig. 2c, d) and another which showed the second population and had an erythrocyte count much elevated above the day 14 level (Fig. 2e, f).

When the burn was administered immediately prior to irradiation, the erythrocyte count at 10 days was decreased further than occurred with irradiation alone. At day 14, the count had begun to recover and both groups showed a similar count by day 21. At this time, a second population of erythrocyte volumes was found. In the animal which showed the greatest recovery of erythrocyte count toward the control level, the two populations were found to be merged into a single population of cells having a broad range of volumes (Fig. 2g).

When the thermal burn was administered 96 hours after the X-irradiation, the erythrocyte count was observed to be minimal by day 14. This value was below that of the group which had received the burn with the irradiation. At day 21 the second population was observed as in the other irradiated groups and in some cases practically all of the erythrocytes were located in this second (larger cell volume) population (Fig. 2j).

Leukocytes. The changes in leukocyte count are recorded in Table II. In the animals which received only the thermal burn, the leukocyte count was found to be decreased by the fourth day. However, by day 7, the count had increased to 20% above the control level. The count then returned to the control level by day 10 and remained so for the duration of the experiment.

TABLE II. Leukocyte Counts (Cells per Cubic mm) at Different Times Following Various Treatments.

Day post-irradiation	T_1X_0	T_0X_{700}	T_1X_{700}	$T_1X_{700}(96)$
4	11626	750	715	<50
7	18297	54	612	1463
10	14859	1154	<50	366
14	16867	1153	4176	3681
21	14929	10534	10777	11649
Nontreated control, 15077 cells per mm^3				

See footnote to Table I.

The leukocyte count in the animals which received only the irradiation decreased to essentially zero by the seventh day. The time sequence of leukocyte recovery was found to parallel that of the erythrocytes.

When the burn was administered with the irradiation, the leukocyte count followed a course parallel to that found in the animals which had received only the irradiation, except that the numbers of cells were much higher.

When the thermal burn was delayed to 96 hours after the irradiation, the leukocyte count was found to closely parallel those changes observed in the group which received the burn and X-irradiation at the same time.

Discussion. Mortality. The increase in mortality in the time period characteristic of gastrointestinal injury when thermal burn preceded irradiation, indicates that the burn may either potentiate the radiation injury or delay the recovery. This effect was most pronounced when the burn was administered between 72 to 96 hours preirradiation. When the thermal burn was administered after the irradiation, the early mortality was less as the time interval between the two injuries was increased. There was no increase in mortality in the period associated with death from gastrointestinal injury (over that found with X-irradiation alone) when the burn was administered at 96 hours after irradiation. However, an increase in mortality did occur in the time period characteristic of bone marrow injury. The observations suggest that the synergism between burn and irradiation takes the form of a nonspecific inhibitory action on proliferating tissues.

Erythrocytes. The initial decrease in erythrocyte count following thermal burn may reflect direct destruction of these cells(4). The further decrease through day 10 suggests an imbalance between maturation and loss of the erythrocytes, due in part to the action of the burn "toxin"(5) and factors associated with the increased extravascular excursions of the erythrocytes(6).

The lack of an early change in the erythrocyte count after X-irradiation has been previously recorded(7) and is believed due to the long life of these cells. By day 10 there was a decrease in cell number which remained at 50% of control through day 14, probably due to damage of the erythropoietic stem cells. This response has been associated with the aplastic bone marrow found at this time (8).

There was a trend toward the return of erythrocyte numbers to the pre-exposure value by day 21 after irradiation. The second population of erythrocytes observed at this time was characterized by cells having a larger volume than the control population. Stohlman and his colleagues(9,10,11) have observed that a severe stress to the hematopoietic system of the rat may be followed by a release into the peripheral circulation of a population of "young" reticulocytes having much larger volumes than normal reticulocytes.

In the group which received both the thermal burn and the X-irradiation, the greater initial decrease in the erythrocyte count was due in part to the additive effect of the irradiation and thermal damage to circulating erythrocytes. The higher count found by day 14 compared to the group which received only the irradiation may have been influenced by the fact that there were early fatalities and thus the animals sampled at day 14 represented a selected population. Administration of the burn at the time of irradiation had only a slight effect on the recovery as measured by the cell counts, an observation consistent with the data of other workers (12).

The erythrocyte changes in the group which received the burn 96 hours after the irradiation indicated an extended inhibition of hematopoietic activity. At 14 days following irradiation, the erythrocyte count was minimum. In the case of the group which had received the burn at the same time as the irradiation, a partial recovery was found in the blood cell count by day 14. Even by day 21, the former group showed an erythrocyte count approximately 20% lower than in the latter group though, at this time, this group represented a selected population.

Leukocytes. Following thermal burn the early decrease in the peripheral blood leukocyte count (day 4) was probably due to a migration of these elements into the injured area. This acute inflammatory response has been well authenticated. The increase in cell numbers which was found at day 8 after the burn may have been influenced by a leukocyte-stimulating factor, leukotaxine, presumably elaborated from cells after injury(13). The response of the leukocytes found in the irradiated group was typical of the manner in which these cellular elements are affected by radiation(14).

Summary. Administration of thermal burn at times up to 96 hours before X-irradiation increases mortality in the time period characteristic of radiation injury to the gastrointestinal tract. The maximum effect was observed at 72 hours. Administration of the burn at times up to 96 hours after X-irradiation decreased mortality due to injury of the gastrointestinal tract relative to these receiving the simultaneous injuries but increased mortality in the bone marrow phase of the radiation syndrome. When the thermal burn was administered at 96 hours after X-irradiation there appeared to be an inhibition of recovery in the bone marrow as shown by the reduced cell counts in their peripheral blood. The possibility that the synergism between thermal burn and X-irradiation is due to a protraction of radiation induced inhibition of cell renewal systems is suggested.

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Use of I^{125}-Labeling in Radioimmunoassays.* (31027)

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Since the classical work of Yalow and Berson(1), radioimmunoassays for insulin and human growth hormone (HGH) have become almost routine procedures in many laboratories. Iodination of these protein hormones with NaI^{131} by the method of Greenwood, Hunter and Glover(2) has yielded labeled hormones of high specific activity with minimal loss in immunologic competence. The use of I^{131} with a half-life of 8 days has the disadvantage of necessitating frequent iodinations of these hormones to provide an adequate number of disintegrations per minute for their use in the chromatoelectrophoretic method of radioimmunoassay. To obviate this problem, an isotope of longer half-life is desirable. Therefore, I^{125} with a half-life of 56 days has been extensively evaluated in our laboratory and is now being used exclusively for radioimmunoassays using the double antibody technique(3,4). While specific activities of I^{125}-labeled hormones must be less than I^{131}-labeled hormones to prevent damage to these proteins(5), preparations with specific activities of 50-100 millicuries per milligram have proven to be very acceptable. When

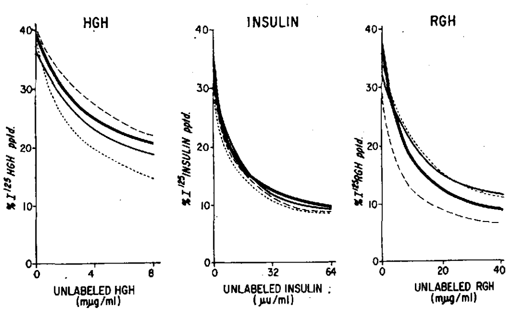

FIG. 1. Radioimmunoassay standard curves using I^{125}-labeled hormones. The stability of I^{125}-labeled hormones is demonstrated by the similarity of individual standard curves using the same iodinated preparations over a 4-month period in the HGH, insulin, and RGH radioimmunoassays. ——— 1st month, ——— 2nd month, — — — 3rd month, -------- 4th month.

frozen in aliquots containing 30% bovine serum albumin in 0.07 *M* barbital buffer (pH 8.6), I^{125}-HGH, I^{125}-insulin, and I^{125}-rat growth hormone (RGH) have been found to be stable over a period of 4 months (Fig. 1). In the double antibody radioimmunoassay technique, the total radioactivity in each assay tube as well as the bound fraction is counted. For this method, therefore, labeled hormones of lower specific activity are quite satisfactory, in contrast to the needs of the

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