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ARMY MEDICAL RESEARCH LABORATORY

FORT KNOX, KENTUCKY

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4 September 1952

A STUDY OF COMBINED THERMAL RADIATION BURN AND X-IRRADIATION EFFECTS ON MICE*

*Subtask under Effects of Irradiation, AMRL Project No. 6-59-08-013, Subtask, Early Effects of Ionizing Radiation.



MEDICAL RESEARCH AND DEVELOPMENT BOARD
OFFICE OF THE SURGEON GENERAL
DEPARTMENT OF THE ARMY

Report No. 94

A STUDY OF COMBINED THERMAL RADIATION BURN
AND X-IRRADIATION EFFECTS ON MICE*

by

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ABSTRACT

A STUDY OF COMBINED THERMAL RADIATION BURN AND X-IRRADIATION EFFECTS ON MICE

OBJECT

To study the effects of thermal radiation burns on the mortality rate of x-irradiated mice.

RESULTS AND CONCLUSIONS

Mice exposed to both total body x-irradiation (720 r) and thermal radiation burns (non-lethal) succumb more rapidly than animals receiving x-irradiation only. The mice were burned either immediately before or immediately after irradiation with the over-all result being approximately the same. The possible reasons for the earlier and higher mortality of the mice receiving both x-irradiation and non-lethal burns are discussed.

RECOMMENDATIONS

The type of study reported here should be extended by 1) determining oxygen consumption of the animals, 2) determining if factors responsible for the results are synergistic or additive, 3) combining graded sub-lethal irradiation with non-lethal thermal radiation burns, 4) applying thermal radiation burns at different times in relation to x-irradiation, 5) making histological studies of all tissue, and 6) evaluating changes in blood cell counts and blood protein content.

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A STUDY OF COMBINED THERMAL RADIATION BURN AND X-IRRADIATION EFFECTS ON MICE

I. INTRODUCTION

The possible damaging effects of combined x-irradiation and burns were discussed by Brooks (1) at the Symposium on Burns, 1950. He showed that the combination of contact burns and irradiation produced a higher mortality in dogs than did irradiation alone. However, little is known about the effects of thermal radiation burns in combination with x-irradiation injury. The similarity of theories on thermal burn injury and theories on irradiation injury offers certain speculations. Thermal burn injury is classified by Henriques into three groups as follows: (a) alterations in proteins, (b) alterations in metabolic processes, (c) alterations in the physical characteristics of cells.

The more common theories explaining tissue injury from x-irradiation are the following: (a) production of a toxic substance (Hercik 3; Hevesy, 4; Ellinger, 5), (b) changes in metabolic rates (Hevesy, 4; Evans, 6; Dowdy, 7; Crabtree, 8; Kimeldorf, 9), (c) changes in osmotic conditions and/or in permeability of the cellular membranes (Failla, 10; Zirkle, 11).

If any of the postulated theories for thermal burn injury and irradiation injury are valid, the combination of any two would tend to increase the damaging effects.

This report presents data on the mortality of hairless and normal mice exposed to non-lethal thermal radiation burns and total body x-irradiation.

II. EXPERIMENTAL

A. Methods and Procedures.

Forty-five hairless mice from a strain bred in this laboratory were used in preliminary experiments for studying the combined effects of thermal radiation burns (3.5 cal/cm^2) and total body x-irradiation (720 r).

The number of available hairless mice was not large enough to permit conclusive results thus the work had to be continued with Swiss Webster Albino mice.

Five hundred female mice (25 - 30 gm) were divided into five groups:

- Group A Burned before irradiation
- Group B Burned after irradiation
- Group C Irradiated only
- Group D Burned only
- Group E Control

Two days prior to burning a commercial depilatory preparation (Neet with Lanolin) was applied to the lower back and hind quarters of the animal. After approximately seven minutes the hair remover and hair were washed from the animal by warm tap water. Transmission measurements made by Hansen (12) on mouse skin one to three days after depilation showed that all signs of irritation to the skin attributable to the hair remover seemed to have disappeared. Skin reflection studies, using a self recording General Electric Spectrophotometer, made on hairless and depilated mice by Ascolese, White, Parr and Kuppenheim (13), twenty-four hours after depilation also showed no superficial differences.

Each animal to be burned was inserted into a water-cooled mouse container (diagrammatically illustrated in figure 1) and burned for 30 seconds in air, over the rump area. A six ampere carbon* arc was used at a distance of 4.5 cm.

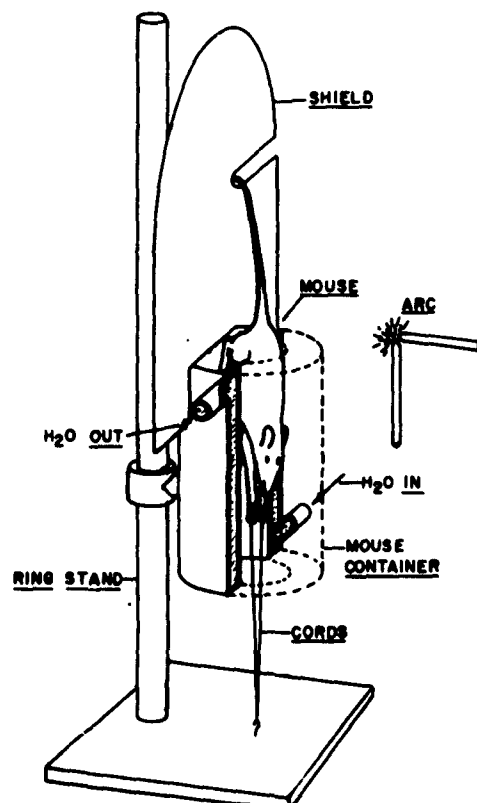


FIG. 1. DIAGRAM OF WATER-COOLED MOUSE CONTAINER

*Manufactured by National Carbon Company, Inc., Cleveland, Ohio
Micro projector cored carbons 6.4 mm x 6 in.

To assure a fairly constant burn area, the tail of the mouse was extended through a crevice in the protective shield where it could be held firmly. A cord was fastened to each of the forelegs and placed around a hook directly below the opening in the bottom of the container. Being firmly secured, the mouse was relatively immobile. The burned area covered approximately 10 to 15% of the total surface area (figure 2). The animal received, under the given conditions, approximately 3.5 cal/cm^2 . This value was obtained by simple calorimetric methods.

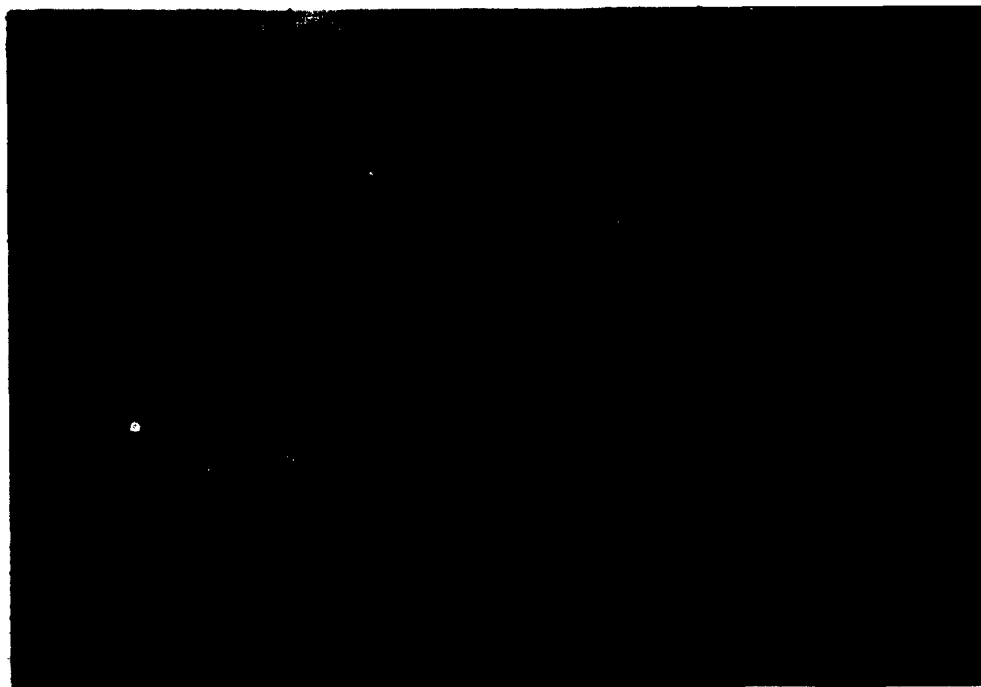


FIG. 2 HAIRLESS MOUSE BURNED FOR 30 SEC.

The x-irradiations were given to groups of ten animals, either immediately before or immediately after burning, using a Kelley-Koett deep therapy x-ray unit (operated at 200 KV, 6ma, inherent filtration 0.25 mm Cu, 1 mm Al plus 0.5 Cu added, 28 cm target distance, 48 r/min in air, a total of 720 r).

The animals were fed stock Purina Chow pellets, water ad lib, and observed for 30 days, the number of deaths being recorded every 24 hours.

B. Results

The results from the preliminary experiments using hairless mice indicate that animals exposed to 720 r x-irradiation and non-lethal burns showed a higher mortality than mice exposed to x-irradiation only.

The results using Swiss Webster Albino mice are presented in figure 3 and are in agreement with the preliminary data.

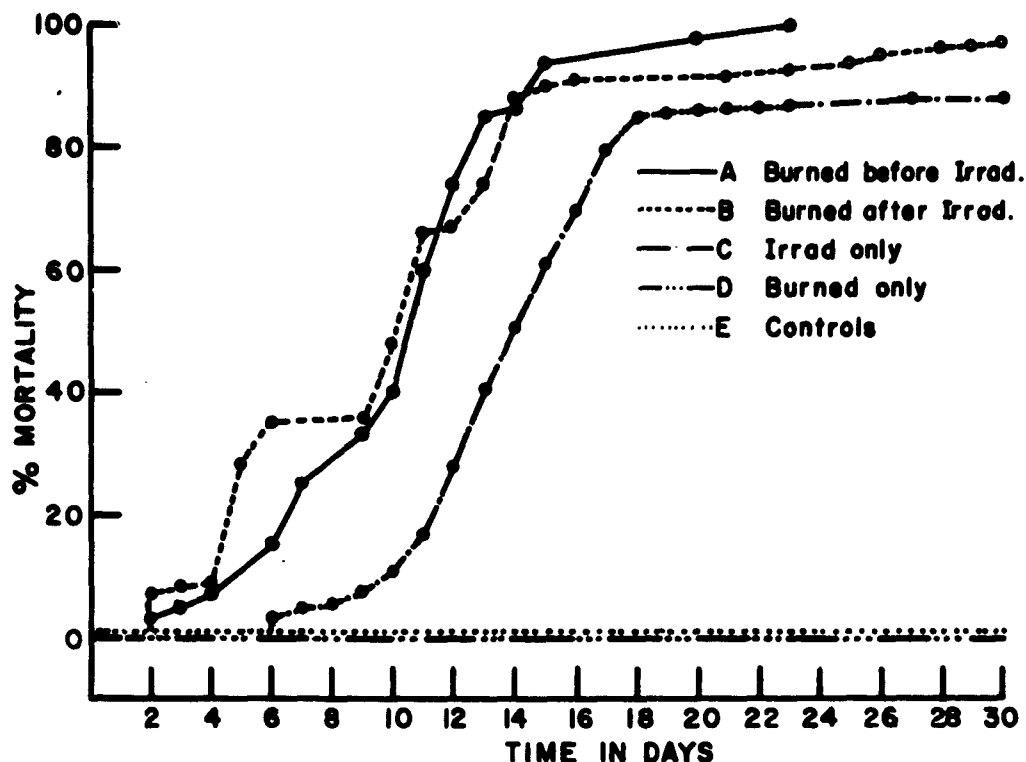


Fig. 3 - Mortality Rates

Mice exposed to total body Irrad. (720r) and thermal burns

Figure 3 shows a comparison of the mortality curves of the mice burned and then irradiated (A); the mice irradiated and then burned (B); and the mortality curves of the other groups (C, D, & E).

Earlier death occurred when irradiation was just preceded or immediately followed by a burn applied as previously described. The first death in the group of mice receiving both irradiation and burn occurred two days after treatment while the first death in the "irradiated only" group of animals occurred approximately six days after exposure to radiation. None of the "burned only" mice nor the control animals died during the observation period.

III. DISCUSSION

Burned and irradiated dogs autopsied by Brooks (1) revealed very little upon which to base a cause of death. However the greater mortality of this group, as compared to the control groups, indicated that

there was some factor responsible for this result. The present report on mice indicates the possible presence of some lethal factor produced by the combined application of non-lethal thermal radiation burns and x-rays.

In addition the present studies show that there is no difference in mortality rates when the animals were burned before irradiation or irradiated and then burned (see curves A & B, figure 3).

The earlier mortality of the burned and irradiated animals cannot be attributed to the effect of the burn alone or the irradiation alone. However, one may speculate on the possible reasons as to why a combination of burns and irradiation results in earlier death. The similarity of the theories explaining injuries thermally induced and injuries from x-irradiation may be pertinent.

The damage to proteins contributing to the maintenance of normal cell life that could be induced by minor thermal burns or x-irradiations are as follows: (a) inactivity of enzymes, (b) aberrations in the nucleus, (c) increased permeability of the nucleus and/or the cell wall, (d) denaturation of proteins. It is believed, according to Ellinger (5), that irradiation produces denaturation of the proteins which is similar to that seen in protein solutions irradiated in vitro. Protein solutions irradiated with ionizing radiation exhibited a coagulation, which, to the eye, appears the same as heat coagulation. There is, however, a definite difference, as emphasized by Ellinger. Heat coagulum by certain physicochemical processes can be 'redissolved', whereas coagulum from x-irradiation is in general an 'irreversible precipitate' (Ellinger, 5). The difference in behavior may be due to some protein substance present in the cell being altered by either the burn or the irradiation, but not by both. It may also be that different mechanisms are involved in the coagulation process in heat damage and in irradiation damage (Dessauer, 14).

It has been demonstrated that the irradiation of a protein leads to the formation of a toxic substance (Ellinger, 5). Such a substance may be responsible for the indirect effects discussed by Hevesy (4). The indirect effect is also thought to be present in burn injuries but to what extent is unknown.

Thermal burns may cause alterations in the metabolic processes. The heat causing the burn may upset the metabolism of the cell by altering its vital constituents. A resulting abnormal function may cause certain metabolites normally present to disappear and/or give rise to toxic substances causing cell death. The influence of body temperature on metabolic rate in radiation experiments is well known. Dubois, Doull, and Petersen (15) have shown that hibernating gophers, with a low body temperature, are more resistant to radiation injury. Others, (Evans, 6; U. S. Army Medical Bulletin, 16) have published similar findings.

The explanation of non-protein induced alterations in the physical characteristics of cells caused by thermal burns closely resembles the fluid flow theory of radiation damage (Failla, 10). These alterations may be attributed to a shift in the inter- and extra-cellular osmotic pressure, allowing the diffusion of a metabolite or some newly formed product through the cell membranes. This exchange of fluids may result in temporary injury, permanent injury or even in death of the cell.

Since there is such a close similarity between the damages produced by burns and damages produced by x-irradiation, the earlier death of the burned and irradiated mice may result from the additive effects of any of the previously postulated factors. It would be interesting to ascertain whether these damaging factors are synergistic, completely additive, or partially additive, as shown for radiation by Friedell (17), and Zirkle (18).

IV. CONCLUSIONS

Mice receiving a non-lethal thermal burn in addition to 720 r x-irradiation show an earlier and higher mortality than mice irradiated alone. From this one might infer that an animal receiving both the x-irradiation and the non-lethal thermal burn was undergoing a combination of severe stresses which produced a new, fatal stress. Thus, one might assume that the non-lethal burn produced a stress which amplified the stress of the x-irradiation to give the recorded results. According to the present theories on damages resulting from either thermal burns or x-irradiation, the explanation for this effect may be found on the cellular level.

The burn can be applied, either immediately before or immediately after irradiation, without changing the reported results. Considering this evidence, it is possible that the effects produced are solely the result of the combination of stresses, and not their sequence, if they occur within a relatively short time.

V. RECOMMENDATIONS

Studies should be extended to include the following:

1. Oxygen consumption studies on animals that have been irradiated and burned in order to determine metabolic relationships.
2. Determine whether or not factors responsible for the earlier mortality are synergistic or additive.
3. Combine graded sub-lethal irradiation with non-lethal burns.
4. Study the influence of thermal radiation burns when applied at different time intervals before and after irradiation.
5. Histological studies of all tissues to determine differences in cellular damage.
6. Evaluate changes in blood cell counts and blood protein content caused by irradiation and burns.

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