

The Influence of X-radiation on Mortality

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Following Thermal Flash Burns: The Site of Tissue Injury
as a Factor Determining the Type of Invading Bacteria

J. Douglas Reid, James W. Brooks, William T. Ham, and
Everett Idris Evans.*

Department of Bacteriology and the Surgical Research Laboratories,
Department of Surgery, Medical College of Virginia, Richmond.

Tissue injury due to atomic bomb explosion is caused by blast, thermal radiation, or ionizing radiation or by some combination of the three. The importance of each factor in producing injury or death is dependent upon the distance of the bomb victim from the point of the explosion, since the attenuation of ionizing and thermal radiation occurs at differing rates with increasing distance from the center of the blast.

Our particular concern in this study has been the intermediate zone, approximately 4200 to 7000 feet from the hypocenter of a 20 KT, nominal weapon. In this zone the victim is exposed to thermal injury and gamma irradiation which in combination appeared from the observations of Japanese medical scientists at the Hiroshima and Nagasaki disasters to produce a mortality higher than could be explained on the basis of either alone. The purpose of our studies is an attempt to

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* Deceased

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better understand the mechanism underlying this increased mortality.

In a group of animals studied previously (1) in which a deep second degree burn was produced by a thermostatically controlled burning iron and which involved 20 per cent of the animals' skin surface area, there was a mortality of 12 per cent. When a similar burn was complicated by 100r of total body x-irradiation, the mortality rate was increased to 73 per cent. Deaths in the combined injury group were definitely related to a beta hemolytic streptococcus septicemia.

Because the thermal contact burns produced in the earlier studies did not simulate flash burns produced by an atomic blast and in order to further study the effects of this combined type of injury, this present study was carried out. We are confining our report in this paper to those effects in which bacteria appear to play an important role.

METHODS

Animals: All animals used in the experiments were healthy, young, adult, male and female mongrel dogs. Prior to experiments they were kept under observation and studied for three weeks in order that only healthy dogs in optimum condition be used in the studies. Each animal was dewormed and given inoculations for distemper. Only those animals without skin lesions of any nature were used.

In our study 60 dogs were used. Twenty of these animals received flash burns only, 20 received x-irradiation only, and 20 received both flash burns and ionizing radiation.

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Both burns and x-ray exposures were carried out with the animals under anaesthesia.

Procedure for Burning: Immediately prior to burning, control blood studies, including blood volumes by the T₁₈₂₄ (Evans blue dye) technique (2), were done on each dog. Animals were deprived of water and food for 12 hours prior to the experiment in order that they might be in "basal" condition and to avoid death due to aspiration of vomitus while under anaesthesia. The anaesthetic agent used was sodium pentobarbitol (Nembutal) 25 mg. per kilogram of body weight I.V. Body surface areas were determined by using the Cowgill-Drabkin formula (3). Each burn placed on the dogs had an area of 20.7 sq. cm. An appropriate number of such burns was placed on each animal separated by an area of normal skin to equal 20 per cent of the animal's total skin surface area. The burns were administered by means of a 24-inch U. S. Army searchlight equipped with an ellipsoidal mirror (4). Each burn was produced by a thermal dose of 8.0 cal./cm.² delivered over a period of one second at constant thermal intensity. The resultant burn was deep second degree.

Irradiation Procedure: The roentgen ray apparatus used for whole body irradiations was a 1000 KVP resonant transformer, roentgen ray tube employing a tungsten target at right angles to the electron beam. Radiation factors were: 17r/min. as measured in air at 200 cm. F.S.D., h.v.l. in masonite 12 cm. at 200 cm. F.S.D. employing 1 mm. of Al. filter, whole body

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irradiation field. Doses in air and within masonite phantoms were measured with Victoreen Bakelite thimble chambers. Dogs were exposed at 200 cm. F.S.D. to 100r as measured in air. Each animal was exposed first on one side and then on the other, the usual "baking" technique. It required about 6.5 minutes to complete each exposure. In one-half of the animals tested the burns were given first; in the other half the irradiation was given first. No difference was noted in either group.

White Blood Counts: Peripheral white blood counts were done on each dog prior to any burn or x-irradiation. The average of such counts in each group of 20 dogs was considered to be the normal or control count for that group. Following flash burn and/or irradiation white blood cell counts, hereafter referred to as W.B.C., were done every other day until the termination of the experiment. In the animals receiving x-irradiation only, similar studies were carried out except for the omission of W.B.C. studies during the first eight hours following irradiation. White blood count studies were done every day that blood and/or wound cultures were taken.

Blood and Wound Cultures: In the preparation of media for culture of the blood, bacto-tryptose, dextrose agar, and broth media were used. The culture flasks were prepared essentially according to the methods described by Castaneda (5), the only modification being the addition of 0.1 per cent yeast extract

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to the medium.

The animals were prepared for bleeding by shaving the area of the femoral vein which was then scrubbed with a germicidal soap. This area was washed off with 70 per cent alcohol, followed by tincture of iodine and again by 70 per cent alcohol. The operating table was washed with soap and water after each operative procedure. Masks were worn by all personnel.

Blood specimens taken from the dogs in 5 ml. amounts were inoculated directly into the bottles of culture media. All cultures were incubated aerobically at 37° C and examined each day for evidence of growth. If no growth appeared in the culture flask at the end of five days incubation, the culture was discarded as negative. In previous experiments (1) duplicate cultures were run on each blood specimen, one of which was incubated under aerobic conditions and the other under anaerobic conditions. When it was found that the septicemia occurring in the animals was in all cases due to aerobic bacteria, the anaerobic cultures were discontinued.

Cultures of the wounds were taken with sterile cotton swabs which were streaked directly onto blood agar plates. The plates were examined after 24 and 48 hours incubation at 37° C. Representative colonies were picked from the plates to blood agar plates for further identification procedures.

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Bacteria isolated from the blood and burn wounds of the dogs were identified by the following procedures: Preliminary grouping of the streptococci was made into alpha hemolytic, beta hemolytic, and gamma types by their reaction on blood agar plates. The beta hemolytic streptococci were further subdivided serologically on the basis of their group carbohydrate C substance according to the method described by Lancefield. Alpha hemolytic and gamma types were not further identified as to species at this time but will be discussed in more detail in a later report. All micrococci (Staphylococci) isolated were identified as either Micrococcus pyogenes var. aureus or Micrococcus pyogenes var. albus on the basis of pigment formation.

RESULTS

Group 1 - Dogs with 20 per cent flash burns alone

The average W.B.C. count in this group of dogs prior to any thermal trauma was 12,200/cu. mm. During the first eight hours following production of a deep second degree burn by the "flash technique" and involving 20 per cent of the total skin surface area, there was a steady rise in the W.B.C./cu. mm. count from the control value of 12,200 to a peak of 24,100 at eight hours. During the next two weeks a gradual rise to the peak of 29,800 W.B.C./cu. mm. at 10 days was reached which corresponds closely with the peak incidence of positive blood cultures in this group (see Fig. 1).

This gradually declined to a normal control level of 12,400

(Insert Fig. 1)

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at 21 days postburn. The trend of this blood count is indicated in Fig. 2. (Insert Fig. 2)

Beginning with the third day postburn blood and wound cultures were taken. Of the 239 blood cultures, 24 per cent showed the presence of bacteria with gamma streptococci and Micrococcus pyogenes var. albus being the predominant isolates. The highest percentage of positive blood cultures occurred between the seventh and tenth days. Usually only one type of organism was isolated from each blood specimen although exceptions occurred as may be noted in Fig. 3. In only one instance was a beta hemolytic streptococcus isolated from the blood and this from an animal that subsequently died. One additional dog in this group died giving a fatality rate of 10 per cent. (Insert Fig. 3)

Since earlier studies with dogs exposed to thermal burns indicated that microorganisms invading the blood were with rare exceptions streptococci or micrococci and since we wished to obtain data on the source of these bacteria, the study of the wound flora was limited to the micrococci and streptococci. Other types of bacteria including the nonsporeforming rods were not uncommonly found in small numbers; but since they rarely appeared in our blood cultures, we did not concern ourselves with them in this study. Micrococci and/or streptococci were isolated from all 185 wound cultures taken. Micrococcus pyogenes var. albus with occasional Micrococcus pyogenes var. aureus was the predominant micrococcus isolated.

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Nearly every burn showed the presence of alpha hemolytic or gamma streptococci, and of particular interest was the high incidence (Fig. 4), 23.8 per cent of all burn cultures, showing beta hemolytic streptococci. ^(Insert Fig. 4) This is not surprising, however, in view of our findings with regard to the incidence of beta hemolytic streptococci in normal dogs. A survey of 25 normal healthy dogs in which the nose, throat, skin, rectum, and genitalia of each animal was cultured demonstrated that 36 per cent of the dogs carried this organism in one or more of the areas cultured. The predominant type as determined by Lancefield's method was the large colony G. Despite these findings it is of interest to note that this organism was isolated only once from the blood cultures.

Group 2 - Dogs with 20 per cent flash burns and 100r x-irradiation

The average W.B.C. count in the control dogs prior to any thermal or ionizing trauma was 12,700 W.B.C./cu. mm. The studies on this group of experimental animals were carried out at the same time intervals as those in the group with burns only. Essentially the same pattern of W.B.C. increase occurred during the first eight hours as in the latter mentioned group. In subsequent days the W.B.C. counts in all cases remained below the control level through the twenty-first day as indicated in Fig. 2.

Two hundred and twenty-one specimens of blood were cultured of which 47 or 21 per cent were positive for bacteria

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(Fig. 4). As noted in Fig. 3, the predominating organisms present in the blood were Micrococcus pyogenes var. albus, beta hemolytic streptococci, gamma streptococci, and Micrococcus pyogenes var. aureus in that order. Except in rare instances only one type of microorganism was isolated from a single specimen of blood. None of the cultures contained gram negative bacteria. The 32 per cent incidence of beta hemolytic streptococcus positive cultures either alone or associated with other microorganisms is particularly interesting. As noted in Fig. 4, they were isolated 15 times with 6 or 30 per cent of the animals being involved. Subsequently five animals died in this group, a fatality rate of 25 per cent. In four instances death was associated with a beta hemolytic streptococcus bacteremia. One animal had a combined infection with Clostridium perfringens, Micrococcus pyogenes var. albus, and gamma streptococci.

The predominant microorganisms found in the 159 wound cultures taken were Micrococcus pyogenes var. albus, gamma streptococci, beta hemolytic streptococci, and Micrococcus pyogenes var. aureus. Beta hemolytic streptococci associated with one or more of the above mentioned species were present in 53 per cent of the wound cultures. The similarity of the bacterial flora in the blood and in the wounds indicates to us that the latter tissue is the most likely source of the bacteremia. This is further borne out by the precipitin studies on representative strains of beta hemolytic strepto-

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cocci which indicated the similarity of the strains isolated from the blood and from the burn surface. Only G and L types were isolated from the blood of infected animals. Similarly G and L types were predominant in burns although C and D precipitin types were isolated occasionally.

Group 3 - Dogs with 100r x-irradiation only

One hundred and forty blood specimens were taken from six male and four female dogs over a period of three weeks as controls on our methods of bleeding and to determine the incidence of bacteremia in animals exposed to 100r x-irradiation only. As noted in Fig. 4, only two of the cultures were positive. In both instances the microorganism isolated was Micrococcus pyogenes var. albus.

DISCUSSION

A high incidence of positive blood cultures occurs both in dogs subjected to a second degree flash burn alone and in those exposed both to burn and sublethal x-irradiation. In control animals exposed to a similar degree of x-irradiation alone positive blood cultures are of rare occurrence. The predominant bacterial types isolated were streptococci and micrococci; gram negative bacteria were of extremely rare occurrence. This is in contrast to results reported by numerous other workers in which microorganisms of the family Enterobacteriaceae were the predominant isolates from animals

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exposed to midlethal or lethal x-irradiation. We attribute this absence of gram negative nonsporeforming rods in the blood of our animals to the use of an amount of x-irradiation insufficient to damage the intestinal mucosa to the point where bacterial invasion from this site could occur. Since the degree of x-irradiation used by us did not in itself predispose to bacteremia, it is apparent that the tissue damage due to burn was responsible for the high incidence of bacteremia in the two groups of dogs. Furthermore, the similarity of the bacterial flora in the burn wounds to the bacteria isolated from the blood supports this observation.

No apparent relationship exists between the leucocyte count and the incidence of positive blood cultures. Following burn alone there is a marked leucocytosis reaching its peak on the tenth day which also corresponds closely with the peak incidence of positive blood cultures in this group. The bacteremia in this group is a transient phenomenon involving bacteria from the wound site of relatively low virulence. Despite the fact that beta hemolytic streptococci were present in 25 per cent of the burns in this group, they rarely occurred in the blood stream. Either they were localized at the original site of injury or quickly disposed of once they had gained entrance to the blood. In contrast, following x-irradiation of the burned animals and subsequent to an initial peak incidence in leucocytes at eight hours

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postburn, there is a leucopenia which coincides with the greatest incidence of positive blood cultures. The incidence of positive blood cultures in this group is similar to that of the group in which burns alone were inflicted. In contrast, however, 32 per cent of the positive blood cultures showed the presence of beta hemolytic streptococci. Since typing studies demonstrated that these bacteria were similar to the beta hemolytic streptococci found in the burn wounds of our animals, it is apparent that damage to the immune mechanism by sublethal x-irradiation influences the type of microorganism that gains entrance to the blood stream allowing more virulent bacteria to enter from the wound site and produce a fatal septicemia.

SUMMARY

Experimental studies on dogs indicate that thermal flash burn and x-irradiation of such a degree that each in itself is relatively nonlethal does, in combination, produce an increased mortality in experimental animals.

A bacteremia due to streptococci and micrococci of relatively low virulence entering through the burn wound occurs in both the burned and burned plus x-irradiated animals. The 100r x-irradiation alone does not predispose the animals to a bacteremia. This bacteremia appears to be a transient phenomenon in the burned animals alone. However, in the burned and x-irradiated animals the depression of the body's defense mechanism by x-irradiation as evidenced in part by the leucopenia which occurs enables more virulent organisms

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to enter from the wound site and produce a fatal septicemia.

The site of the tissue injury and the local flora therein appear to determine the type of invading bacteria.

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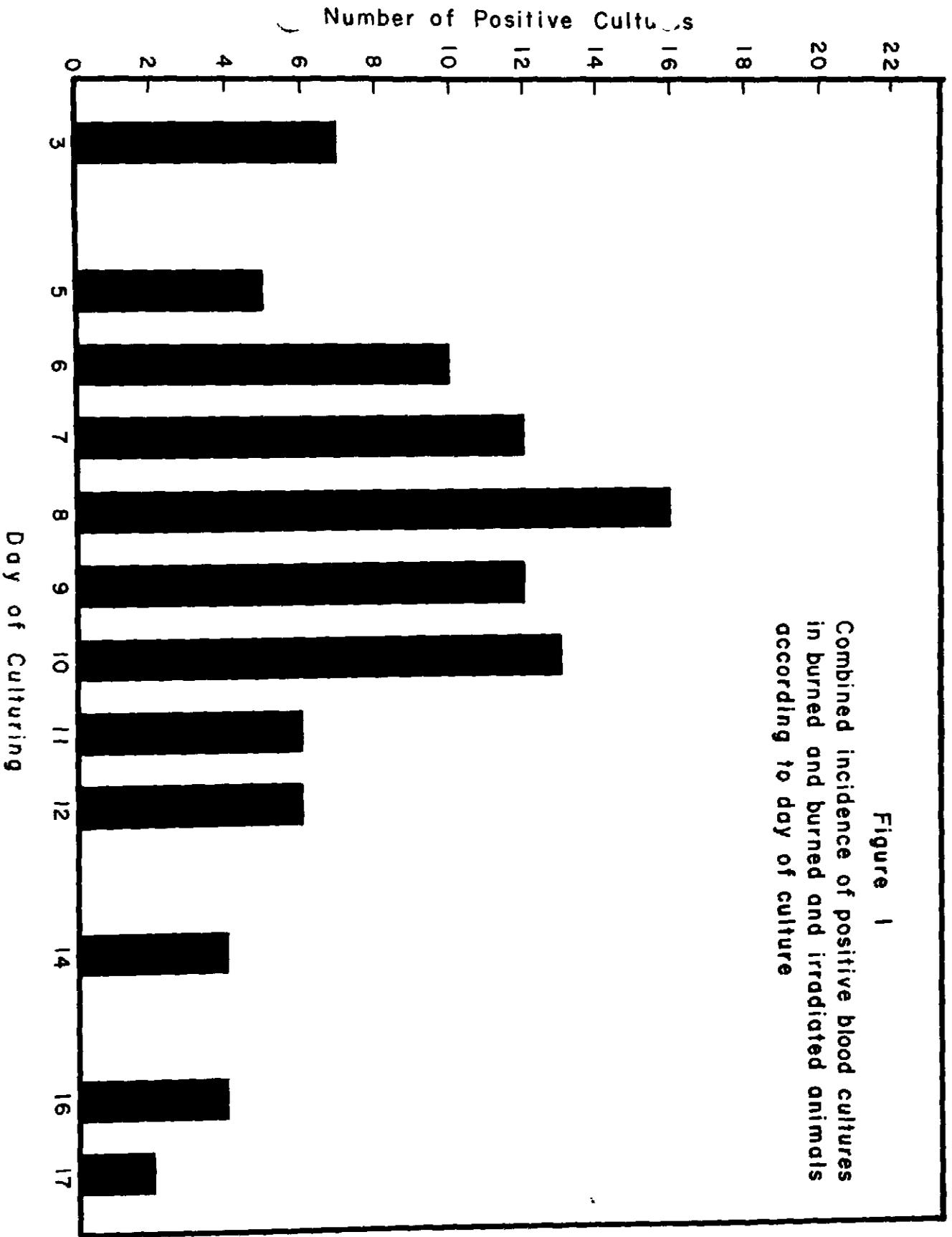


Figure 1
 Combined incidence of positive blood cultures
 in burned and irradiated animals
 according to day of culture

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W.B.C. Thousands/cu. mm.

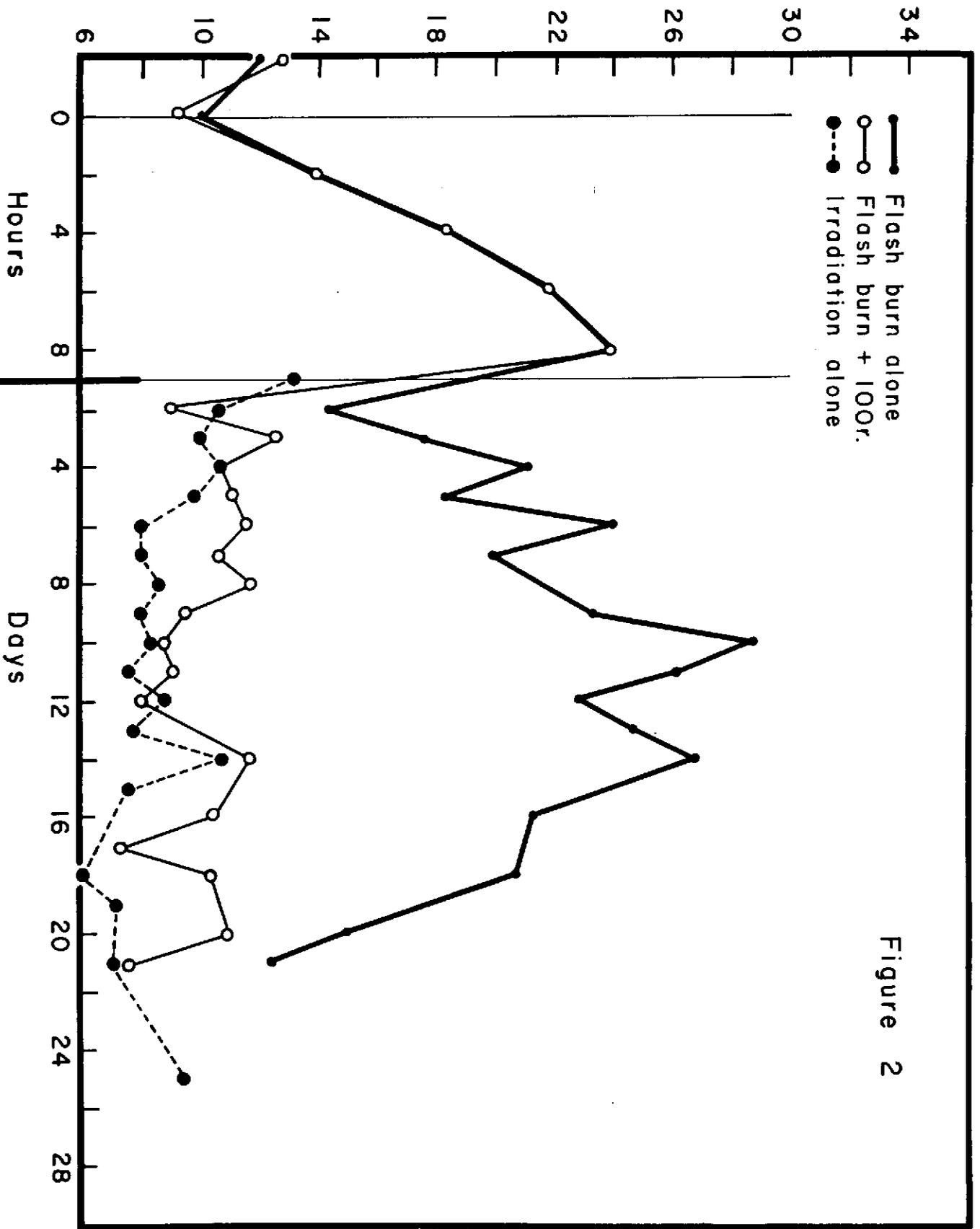


Figure 2

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Figure 3

Incidence of Occurrence of Various Types of Bacteria
in Positive Blood Cultures from Dogs Exposed to Flash Burns

Type Organism	Flash burn	Flash burn + 100r
gamma streptococci	23	8
M. albus	22	15
M. albus + gamma streptococci	8	1
M. aureus	3	6
beta streptococci	1	11
beta streptococci + M. albus	0	2
Proteus + gamma streptococci	1	0
beta + gamma streptococci	0	2
diphtheroid	1	0
Cl. perfringens	0	1
Sarcina lutea	0	1

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Figure 4

The Comparative Incidence of Positive Cultures and of Beta Hemolytic Streptococci in the Blood and Wounds of Dogs Exposed to Flash Burns and to Flash Burns and Irradiation

Culture source	Type wound	Number cultures	Number positive	Per cent	No. positive for beta strep.	Per cent
Blood	flash burns	239	57	23.8	1	1.7
	flash burns + 100r	221	47	21.26	15	31.9
	irradiated	140	2	1.42	0	0
Wounds	flash	185			47	25.4
	flash burns + 100r	159			85	53.4

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