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Role of Infection in the Delayed Deaths of Mice Following Extensive Burn Injury. (26978)

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This study is concerned with the role of infection in the delayed deaths of mice following thermal burns; it does not deal with the problem of shock encountered during the first 2 days following injury. Extensive studies with experimental animals exposed to irradiation or to traumatic shock indicate that a reduction in natural resistance to infection follows this type of injury(1-5). In patients with extensive burn injury, the initial traumatic shock may be followed later by shock accompanying bacterial invasion and generalized "burn decompensation"(6-9); although the injured tissues may suffer localized infection within the first 4 days, invasion of the deeper tissues seldom occurs before the 5th post-burn day(10,11).

The delayed mortality in mice following extensive burn injury has long been investigated in our laboratories(12,13). This mortality occurs during the first 3 weeks after injury in animals surviving the initial period of traumatic shock. Therapy with 0.9% NaCl solution, given subcutaneously or intraperitoneally in amounts equivalent to 15% of body weight, produces over 85% survival at the end of the 2nd day. The mortality rate rises rapidly during the first 10 days but may not reach its maximum (approximately 75%) until the 3rd week of injury. The toes, tails and skin of the burned area become gangrenous and may slough off. Food consumption decreases and the animals become emaciated. No significant changes have been observed in body temperature, in the circulatory system (hematocrit, bleeding volume and plasma proteins), or in blood NPN levels. None of the histopathological changes observed in lungs, liver, spleen, adrenals, pancreas and kidneys of burned mice have been adequate to explain the deaths(14). Our studies indicate that infection is an important factor in the cause of delayed deaths of burned mice. Moreover, Rosenthal(14) and Millican and coworkers(15,16) have demonstrated that some chemotherapeutic agents are effective in promoting the survival of these burned mice.

Methods. Female albino mice (NIH or GP strains), weighing 16-22 g were employed. Burn trauma was produced by standardized procedures previously described(12,13). Anesthetized animals were dipped to the axilla in water at 70°C and held there for 8 seconds. This degree of injury is rapidly fatal to over

85% of the mice in 24 to 48 hours if they remain untreated and so 0.9% NaCl solution was given either intraperitoneally or subcutaneously in amounts equivalent to 15% of body weight. They were housed in glass jars in groups of 5 on dried sugar cane bedding.

In 3 experiments a total of 97 mice were available for observations on delayed deaths. To assess the role of infection, cultures were made of the various tissues of each of 43 of these mice selected at random and sacrificed at varying intervals after injury. Nineteen animals in Series 1 were killed on the 4th to 7th post-burn days; 10 in Series 2 were sacrificed on the 3rd and 4th post-burn days; and 14 in Series 3 on the 2nd, 3rd, 5th and 9th post-burn days. In Series 1 and 2 the mice were killed as soon as jar mates began to die but before they became moribund; in Series 3 although more than half of the deaths occurred after the 9th day, 11 of the mice were sacrificed on or before the 5th day. A 4th group of 10 supposedly normal mice was selected at random from the large colony and sacrificed as controls on our techniques of sampling tissues. Since no deaths were occurring among the stock mice and since no bacteria usually associated with the more common endemic infections in such laboratory colonies had been identified in a study of bacteria isolated from 33 or more burned mice just before this investigation was begun, it was not considered essential to culture the organs of a large number of stock animals.

The burned mice, especially those in Series 1 and 2, were usually too sick to move about the jars readily and consequently the denuded, necrotic surfaces of the burned tissues and gangrenous extremities were constantly subjected to contamination by fecal droppings that collected rapidly in the bedding. Their fur was visibly soiled.

Relatively large pieces of tissues were examined. Samples of muscle about 1 g in weight were cut from an area adjacent to an injured surface in each thigh of all burned mice and from a similar area in the control mice. The thoracic cavities were opened under aseptic conditions first and the exposed tissues sampled. Then the peritoneal cavities were entered. The peritoneal fluid was cultured as soon as the cavity was opened. Relatively large samples of the organs were removed and placed directly in broth: the whole heart was divided and half placed in each of 2 tubes, 1 lobe of a lung, no less than half the spleen, a piece of liver equal in size or larger than the sample of spleen, and 1 kidney. Any capsular material surrounding the tissues was severed in several places with sterile scissors.

All materials were cultured in about 10 ml of neopeptone broth in tubes. In addition, the hearts (blood and tissue) of most of the mice and also thigh muscles from each leg of all mice were cultured in deep dextrose brain medium or Robertson's ground meat medium under vaseline seals so that adequate anaerobiosis would be provided for isolation of obligate anaerobes. All cultures were observed for the growth of organisms and each was plated on blood agar as well as on infusion agar after 24 and 48 hours incubation at 37°C. Positive cultures were studied in appropriate media for further identification. Since these studies were made before some of the more refined methods for controlling the overgrowth of spreaders were developed and found so effective, they were not available for use.

Results. Bacteria were isolated from the heart, lungs, liver, spleen and/or kidneys of 40 of the 43 burned mice; 3 of the mice of Series 3, which reacted less severely to the burn than those in the other 2 series, were apparently more resistant to penetration of bacteria from infected foci. Twenty-eight of the 40 had positive heart cultures. These and other findings are shown in Table I. It is probable that the tissues which harbored several types of bacteria were actually infected with only one or 2 strains; the others were no doubt present in extremely small numbers, and not playing a major role in the infection. The striking observation was the finding of bacteria in most of the tissues of each injured mouse; the heart cultures, with 70% positive, contained organisms less often than those of the other tissues, and the lung cultures, with 88% positive, were the ones that most frequently showed growth. This high incidence of bacteria in the lung tissues may have been due to the presence of latent infection in the lungs of these animals at time of burn injury.

It appears that bacteria found ample opportunity for colonization on the damaged sur-

TABLE I. Incidence of Bacteria in Cultures of Tissues of Burned Mice and of Mice Used as Controls on Sampling Techniques.

Mice	Tissues cultured	Cultures not made	Negative cultures	Total	Results of culturing: Positive cultures 1 Genus	2 Genera	3 Genera	4 Genera	5 Genera
43 Burned	Heart	0	15	28	19 (7)[1]	9	0	0	0
	Lung	2	5	36	25 (8)[1]	8	2	1	0
	Liver	1	9	33	22 (12)[1]	8	3	0	0
	Spleen	2	12	29	22 (12)[1]	6	1	0	0
	Kidney	2	11	30	23 (13)[1]	5	2	0	0
	Combination of Organs from ea. Mouse	0	3	40	15 (8)[1]	13	6	4	2
10 Controls on Sampling Tech.	Heart	1	9	0	0	0	0	0	0
	Lung	1	8	1	1[2]	0	0	0	0
	Liver	1	9	0	0	0	0	0	0
	Spleen	0	8	2	2[3]	0	0	0	0
	Kidney	0	7	3	3[4]	0	0	0	0
	Combination of Organs from ea. Mouse	0	4	6	6	0	0	0	0

1. Numbers in parenthesis indicate number of cultures included in each group which contained spreaders and which consequently may have contained additional species that were not detected in subcultures.
2. Aerobic Gram-positive spore-forming rod.
3. *Proteus* (1), *E. Coli* (1).
4. Anaerobic Gram-positive rods (2), diphtheroid (1).

faces, were successful in extending their growth to adjacent tissues and eventually accomplished generalized invasion. Bacteria were isolated from cultures of thigh muscles of 44 of 47 burned mice; the negative cultures were from mice in Series 3. In general, the species isolated from the thigh muscles were the same as those predominating in the cultures of heart, lungs, liver, spleen and kidneys of 30 of 35 burned mice for which data were comparable.

In studies of this type the questions of extensive terminal invasion from the respiratory and gastro-intestinal tracts of moribund animals, and of gross contamination during handling of specimens must be considered. Since every effort was made to select mice that were not moribund and since approximately 75% of the cultures of peritoneal fluid were sterile, it has been assumed that in most instances the isolations reflect a reduction in the natural resistance to invasion of organisms multiplying in burned surfaces or from latent foci in deeper tissues. While the cultures of muscle tissue from control animals indicate that some contamination did occur during the taking of the samples, it was limited. It appears that the possibility of skin contaminants may enter into any interpretation of the results of cultures of muscle tissue, but is of much less significance in evaluation of cultures of internal organs.

The organisms most frequently isolated from the internal organs of the burned mice belong to the following genera: *Staphylococcus, Streptococcus, Pasteurella, Proteus* and *Escherichia*. Their frequency of occurrence varied from 15 to 24% of the isolations. Diphtheroids, *Aerobacter cloacae* and *Pseudomonas aeruginosa* were demonstrable in less than 10% of the isolations.

It is of interest that strains of *Pasteurella* were isolated from 16 of the 43 mice; they were isolated from heart blood 11 times, from the lungs 9 times and from the kidneys twice. Overgrowth by other organisms probably interfered with more frequent isolation. These strains had the morphological and biochemical

characteristics of the pneumotropic strains which were isolated from several colonies of mice, including some from the Nat. Inst. Health, and described by Jawetz(17). This organism was found to exist as a latent infection in the respiratory tract of mice and appeared to be widespread in mouse colonies (18).

All of the streptococci appeared to belong to the enterococcus group; of the 15 isolations, 10 were *alpha* hemolytic and 5 were *beta* hemolytic strains. While no serological grouping was done, the biochemical characteristics of all strains were sufficiently similar to those of *Streptococcus faecalis liquefaciens* or *Streptococcus zymogenes* for them to be tentatively classified with these species.

Discussion. It would appear from the results of the experimental work that generalized bacterial invasion of the tissues may occur during the first 10 days following extensive burn injury in mice. Systemic infection must have been the cause of the delayed deaths of many of the mice. The physiological state of the host and the biochemical nature of the burned surfaces were, no doubt, as important determinants(19) in the spread of infection as the virulence of the organisms or any reduction in the effectiveness of the natural cellular and humoral defense mechanisms. Non-specific factors such as nutritional inadequacies(20) and environmental humidity(21) probably played a role. While the predominating organisms demonstrable in the tissues were ones ordinarily considered of low invasiveness and virulence, it is well established that bacteria undergo rapid and profound changes in cultural characteristics and pathogenic properties as a result of physicochemical factors in their environment. Goshi, Cluff, Johnson and Conti(22) have found that induction of infection of the burned skin of rabbits within 3 days after injury required only $\frac{1}{10}$ the dose of coagulase-positive staphylococci necessary to produce infection in normal rabbit skin. Blood cultures and autopsy examination of these animals did not show bacteremia or metastatic infection, but the burned area in the rabbits was minimal in comparison with that in the injured mice in our series; the effect of the burn was localized to the site of injury and the host defenses continued to operate. They(23) also found that in rabbits immunity to infection by pathogenic staphylococci in necrotic burns could be induced by vaccination and this immunity was passively transferable with rabbit antiserum. These findings are in agreement with those of Rosenthal and Millican (14,15,16) who have reported that chloramphenicol therapy is partially effective in protecting against delayed deaths, and that human gamma globulin as well as the serum of animals convalescing from burn injury possess protective activity against deaths(14,15). The data add additional support for the role of infection in the cause of deaths in the burned mice.

There is some question as to whether the mouse may be more susceptible to the establishment of generalized infection following thermal injury than are other animals, due to the less effective bacteriacidal properties of the serum toward both Gram-positive and Gram-negative organisms(24,25). Muschel and Muto(26) as well as Kornfeld, Hammond and Miller(27) have shown that bactericidal antibody, such as that found in other animals,. is present in the normal mouse and lack of bactericidal action may be attributed to a lack of complementary activity(26,28). The incidence of bacteremia is not such a prominent phenomenon in dogs, rabbits and guinea-pigs following irradiation as it is in mice(29,30). Reports suggest that while sublethal radiation has been found to activate subclinical or latent infections in mice, the findings are not as definite in rats. However, in humans with extensive burns, there is a rising incidence of deaths from septicemia due to *Pseudomonas aeruginosa* or to *Staphylococcus aureus*, (7,8,9) and frequently visceral lesions are present(31).

Summary. Studies have been described in which generalized infection appeared to be an important factor in the delayed deaths in mice during the 3 weeks following extensive burn injury. Bacteria of low virulence and invasiveness apparently spread both from previously localized foci of latent infection and from areas of colonization on damaged surfaces.

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Correlation between Heat-Resistance of Polioviruses and Other Genetic Markers.* (26979)

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The study of the genetic markers of poliovirus has assumed added importance as a result of the wide use of attenuated viruses as vaccine. The association of certain tissue culture markers with the presence or absence of neurovirulence and the stability of these traits has been investigated intensively. The present study deals with the resistance of polioviruses to heating at 50°C and correlation of this property with 2 other widely used markers, efficiency of plating at low bicarbonate concentrations(1) and capacity to multiply at 40°C(2).

Materials and methods. Viruses. Sixteen strains representing the 3 immunologic types of poliovirus were studied. Three strains (SI, SII, SIII) were provided by Dr. Joel Warren and 10 strains were obtained through the courtesy of Dr. J. L. Melnick (PII, PIII, MIA, MIB, MIC, MID, MIIIA, MIIIB, MIIIC, MIIID). The other 3 strains (Mah, MEF-1, Sau) were carried in this laboratory.

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