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# Protection by Vaccination Against *Pseudomonas* Infection After Thermal Injury

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Active immunization is effective in the prophylaxis of *Pseudomonas* septicemia in burned mice. Vaccines were prepared from bacterial cells and growth medium of Verder's 10 different O serological types of *Pseudomonas aeruginosa* strains, as well as from *Escherichia coli* and *Proteus mirabilis*. Mice given a tail burn could be significantly protected against a local *Pseudomonas* challenge by both specific and, to a lesser extent, by nonspecific *Pseudomonas* vaccines prepared either from bacterial cells or from the medium in which they were grown. The vaccine was effective when administered prior to or after thermal trauma. After a more extensive rump burn, the protective effect of a specific vaccine given after thermal injury was significant only when the challenge was postponed until 4 days postburn; the level of protection was less than in the mice with smaller burns.

Since the most prevalent cause of death in man after thermal injury is now gram-negative septicemia, and especially *Pseudomonas* septicemia (7, 10, 15), we have been studying means by which to combat these infections in burned animals. Recent progress has been made in the prevention of overwhelming infection in burned patients by the application of topical chemotherapeutic agents, such as mafenide hydrochloride, silver nitrate, and gentamicin (7, 14, 17). However, most of these agents have defects in that they may cause serious electrolyte disturbances, pulmonary complications, allergic responses, and other toxic manifestations (7, 14). Most important of all, some patients who have been treated with these agents still die as a result of generalized infections, probably due to the emergence of resistant bacteria. The problem of resistance may become even more serious with the passage of time.

We have approached the problem of infection by studying active immunization in burned mice as a means of preventing death due to *Pseudomonas* septicemia. Surprisingly little has been published about the prophylaxis of *Pseudomonas* infections by vaccination in humans (2, 4-6) or experimental animals (3, 13, 20). Previously, we reported that burned mice were protected against an intraperitoneal *P. aeruginosa* challenge by vaccinations of killed *P. aeruginosa* organisms administered before or after the burn (13). The present report extends that study but differs in that (i) we investigated the effect of vaccination of mice with burns of increasing severity; (ii) we challenged burned mice locally, rather than intraperitoneally, to simulate the pathogenesis of human infection; (iii) we studied the effect of specific, as well as nonspecific, vaccines in burned mice challenged with *Pseudomonas*; and (iv) we prepared vaccines from both bacterial cells and from the medium in which they were grown.

Our results demonstrate that specific, as well as nonspecific, vaccination is a useful prophylactic agent in burned mice challenged with *P. aeruginosa*.

## MATERIALS AND METHODS

*Animals.* Conventional, female (18 to 20 g) National Institutes of Health stock or general purpose albino, Swiss-Webster mice were used in all of these experiments.

*Types of thermal trauma studied.* All mice were anesthetized with diethyl ether prior to the following burns of increasing severity: (i) tail burn, the tail was immersed in water at 70 C for 5 sec and then dabbed dry (16); and (ii) rump burn, the mouse was immersed in water to the level slightly above the genitalia at 70 C for 3 or 5 sec and then dabbed dry.

After the burn, groups of 10 animals were placed in a plastic shoe box-type cage (29 × 18 cm). Mice were allowed Purina laboratory chow and water ad libitum.

The tail burn without challenge caused no 21-day mortality. The same burn with 0.1 ml of vaccine given afterward, but again without challenge, produced less than a 1% 21-day mortality. The rump burn without

challenge caused a 13% 21-day mortality and a 10% mortality when the mice were given 0.1 ml of vaccine afterward.

*Preparation of Pseudomonas challenge.* Mice were challenged with suspensions of *P. aeruginosa* of the 180 strain (ATCC 19660), the O:8 strain (isolated from mouse drinking water bottles), or the Mills (O:4) strain. Brain heart broth was inoculated with the strain studied and incubated for 18 hr at 37 C with shaking. The cultures were centrifuged for 10 min in a Sorvall centrifuge, and the supernatant fluid was discarded. The cells were diluted with 0.85% NaCl to an optical density at 660 nm ($OD_{660}$) of 0.66 to 0.69 in a Coleman Junior spectrophotometer (containing approximately $2 \times 10^8$ microorganisms per ml). A $10^{-1}$ dilution was made of the suspension of organisms, which was then used to challenge the mice locally.

*Challenge technique.* The burned area, either tail or rump, was dipped into the $10^{-1}$ dilution of *Pseudomonas* organisms, and the mouse was then placed directly into its cage.

*Preparation of vaccines.* Vaccines were made with all of the different strains of *P. aeruginosa* listed in Table 1. The O antigenic types of each of the different strains were obtained originally from Elizabeth Verder of the National Institutes of Health (18). In addition, vaccines were prepared from stool culture isolates of *Escherichia coli* and *Proteus mirabilis* of normal conventional mice.

Each organism was inoculated into brain heart broth and incubated at 37 C for 18 hr. The *Pseudomonas* cultures were shaken, while the others were not. The cells were separated from the supernatant liquid by centrifugation in a Sorvall centrifuge for 10 min. The supernatant fluid was recentrifuged at a higher speed for 0.5 hr and was sterilized by passing through a membrane filter (Millipore Corp., Bedford, Mass.). A 1-ml amount of Merthiolate solution 1:1,000 (Eli Lilly & Co., Indianapolis, Ind.) was added to 9 ml of sterile supernatant vaccine and was stored at 4 C.

The cells separated above were washed twice with 0.85% NaCl and diluted with saline to an $OD_{660}$ of 0.77. The suspension of cells in a closed container was then placed in a boiling-water bath for 3 hr. Afterward, a sample of the cell vaccine was checked for sterility. To the remainder, 1 ml of Merthiolate was added to each 9 ml of cell vaccine stored at 4 C.

In some of the experiments, the vaccines were given 3 and 2 days prior to burning, while in others they were given immediately after burning or at various time intervals thereafter. In all cases, 0.1 ml of the vaccine was injected subcutaneously at the indicated times.

*Bioassay of the effect of vaccines.* Mortality was recorded daily for 21 days as the test for efficacy of therapy. Autopsies were performed on all dead animals during the first 21 days postburn and on all survivors at 21 days.

*Statistical analysis.* The probability of 0.05 ($P = 0.05$) was chosen as the level of statistical significance. A difference is judged as statistically significant only when the calculated chi-square equaled or exceeded the tabulated chi-square at a probability of 0.05 with 1 degree of freedom.

## RESULTS

*Vaccine administered prior to thermal trauma: cross protection experiments with tail burn.* Table

TABLE 1. *Protection of burned mice challenged locally with P. aeruginosa 180[a] by specific and nonspecific cell vaccines[b]*

| Cell vaccine group | O antigenic type | No. of mice | Days postburn[c] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 14 | 21 |
| Control, 0.85% NaCl[d] | — | 99 | 1 | 5 | 31 | 51 | 54 | 62 | 73 | 82 | 82 |
| *P. aeruginosa* 180 | 1a, 1c, 1d | 80 | 0 | 0 | 0 | 0 | 1 | 3 | 3 | 3 | 3 |
| *P. aeruginosa* 2243 | 1a, 1b, 1c | 40 | 0 | 0 | 3 | 8 | 10 | 13 | 13 | 25 | 25 |
| *P. aeruginosa* Lawson | 1a, 1e | 40 | 0 | 0 | 0 | 0 | 0 | 8 | 10 | 15 | 15 |
| *P. aeruginosa* 1369 | 2 | 40 | 0 | 0 | 8 | 8 | 8 | 15 | 28 | 40 | 40 |
| *P. aeruginosa* 2108 | 3 | 40 | 0 | 0 | 5 | 10 | 13 | 20 | 25 | 40 | 42 |
| *P. aeruginosa* Mills | 4 | 40 | 0 | 0 | 3 | 8 | 13 | 23 | 28 | 50 | 53 |
| *P. aeruginosa* 1M | 5 | 40 | 5 | 5 | 5 | 10 | 10 | 15 | 23 | 25 | 25 |
| *P. aeruginosa* 58F | 6 | 40 | 0 | 0 | 0 | 5 | 10 | 15 | 20 | 38 | 40 |
| *P. aeruginosa* 2915 | 7a, 7c | 40 | 0 | 0 | 5 | 10 | 15 | 20 | 25 | 33 | 38 |
| *P. aeruginosa* T488 | 8 | 40 | 0 | 0 | 3 | 3 | 8 | 10 | 15 | 33 | 38 |
| *P. aeruginosa* T6370 | 9 | 40 | 0 | 0 | 3 | 5 | 5 | 10 | 30 | 48 | 48 |
| *P. aeruginosa* G2312 | 10, 1a | 40 | 0 | 0 | 0 | 5 | 5 | 5 | 13 | 15 | 15 |
| *E. coli* | — | 40 | 0 | 0 | 8 | 13 | 15 | 23 | 30 | 48 | 48 |
| *Proteus mirabilis* | — | 30 | 0 | 0 | 0 | 10 | 17 | 30 | 33 | 40 | 40 |

[a] Mice were challenged by dipping burned tail in a suspension of *Pseudomonas* organisms 5 hr after thermal injury.

[b] Vaccines were prepared from boiled cells and were injected subcutaneously (0.1 ml) 3 and 2 days prior to a tail burn.

[c] Results given as percentage of cumulative mortality.

[d] Control mice received 0.1 ml of saline subcutaneously on the same schedule.

1 lists the 21-day mortality rates of burned mice previously immunized with vaccines and challenged locally with *P. aeruginosa* 180. The *Pseudomonas* challenge produced a 73% mortality at 7 days postburn and an 82% mortality at 14 and 21 days postburn in the control group. Administration of a specific vaccine (*P. aeruginosa* 180) prior to burning resulted in the lowest mortality of 3% at 7, 14, and 21 days postburn. Nevertheless, vaccines prepared from all 10 O antigenic types of Verder significantly protected ($P < 0.01$) burned mice against a *P. aeruginosa* 180 challenge with 21-day mortalities varying between 15 and 53%. A similar nonspecific protective effect was observed with vaccines made from cells of *E. coli* and *Proteus mirabilis*.

Table 2 gives the results of the same type of experiment, but in this case using vaccines prepared from the medium in which the bacteria had been growing. The 21-day mortality rate of these controls, which received brain heart broth, was 71%. As with the vaccines prepared from cells, the supernatant vaccines prepared from the specific organism (*P. aeruginosa* 180) showed the lowest mortality of 4% at 21 days, but the supernatant vaccine from all other O antigenic types also produced a significant lowering of mortality, varying between 8 and 43% ($P < 0.001$). Again, *E. coli* and *Proteus mirabilis* supernatant vaccines showed a significant nonspecific protective effect ($P < 0.05$).

In many cases, the vaccine prepared from the medium produced a lower mortality than the vaccine prepared from the cells, but the difference between the two did not show statistical significance ($P > 0.05$). The good protective effect observed with the vaccine prepared from the medium suggests that antigens or other products responsible for protection are excreted into the medium.

In a separate experiment, on the other hand, when the *P. aeruginosa* 180 and *P. aeruginosa* O:8 vaccines were injected at intervals of 16, 13, 10, and 7 days prior to the tail burn, rather than 3 and 2 days prior, only the specific vaccine significantly protected against the *P. aeruginosa* 180 challenge at 4 hr postburn.

*Cross protection experiments with a rump burn.* In the case of challenge with an extremely virulent organism such as *P. aeruginosa* 180 5 hr after the burn, prior injection of specific vaccines prepared either from cells or medium did not significantly protect a burned mouse when the area of the burn was increased from the tail to rump burn.

However, challenged with less-virulent *Pseudomonas* organisms, specific and nonspecific vaccines protected against the more severe burn.

TABLE 2. *Protection of burned mice challenged locally with P. aeruginosa 180[a] by specific and nonspecific supernatant vaccines*

| Supernatant vaccine[b] group | O antigenic type | No. of mice | Days postburn[c] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 14 | 21 |
| Control, brain heart broth[d] | — | 99 | 1 | 4 | 25 | 37 | 45 | 54 | 59 | 71 | 71 |
| *P. aeruginosa* 180 | 1a, 1c, 1d | 80 | 0 | 0 | 0 | 1 | 3 | 3 | 3 | 4 | 4 |
| *P. aeruginosa* 2243 | 1a, 1b, 1c | 40 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 8 | 8 |
| *P. aeruginosa* Lawson | 1a, 1e | 40 | 0 | 0 | 13 | 20 | 28 | 30 | 35 | 38 | 43 |
| *P. aeruginosa* 1369 | 2 | 40 | 0 | 0 | 0 | 5 | 5 | 10 | 15 | 23 | 25 |
| *P. aeruginosa* 2108 | 3 | 39 | 0 | 0 | 3 | 5 | 5 | 8 | 21 | 28 | 28 |
| *P. aeruginosa* Mills | 4 | 39 | 0 | 0 | 3 | 3 | 3 | 3 | 5 | 10 | 10 |
| *P. aeruginosa* 1M | 5 | 40 | 0 | 0 | 3 | 10 | 10 | 20 | 20 | 30 | 35 |
| *P. aeruginosa* 58F | 6 | 40 | 0 | 0 | 3 | 5 | 8 | 8 | 15 | 30 | 30 |
| *P. aeruginosa* 2915 | 7a, 7c | 40 | 0 | 0 | 5 | 5 | 5 | 8 | 10 | 23 | 25 |
| *P. aeruginosa* T488 | 8 | 40 | 0 | 0 | 0 | 0 | 0 | 3 | 8 | 18 | 18 |
| *P. aeruginosa* T6370 | 9 | 40 | 0 | 3 | 5 | 5 | 5 | 13 | 13 | 20 | 25 |
| *P. aeruginosa* G2312 | 10, 1a | 40 | 0 | 0 | 5 | 8 | 8 | 8 | 10 | 18 | 18 |
| *E. coli* | — | 40 | 0 | 0 | 8 | 10 | 13 | 33 | 38 | 45 | 50 |
| *Proteus mirabilis* | — | 40 | 0 | 0 | 3 | 13 | 18 | 30 | 38 | 45 | 45 |

[a] Mice were challenged by dipping burned tail in a suspension of *Pseudomonas* organisms 5 hr after thermal injury.

[b] Vaccines were prepared from the medium and were injected subcutaneously (0.1 ml) 3 and 2 days prior to a tail burn.

[c] Results given as percentage of cumulative mortality.

[d] Control mice received 0.1 ml of a membrane-filtered brain heart broth subcutaneously on the same schedule.

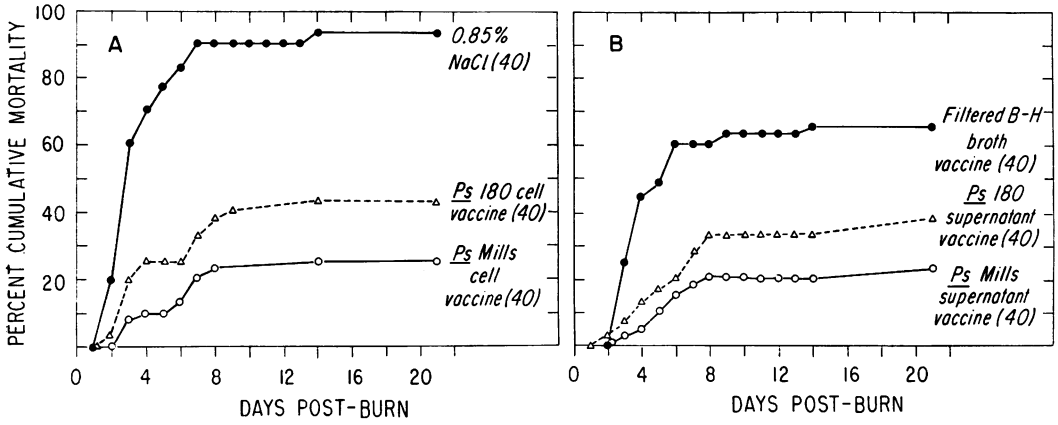


FIG. 1. *Effect of vaccine on mortality of burned mice challenged locally with P. aeruginosa, Mills strain. Animals received 0.1 ml of vaccine, 0.85% NaCl, or filtered brain heart broth subcutaneously 3 and 2 days prior to thermal trauma. Mice were given a rump burn at 70 C for 5 sec. After burning (5 hr), the rump area was dipped in a $10^{-1}$ dilution of a standard suspension of P. aeruginosa, Mills strain. In A, the animals were given a vaccine prepared from cells; in B, from the supernatant medium. The numbers in parentheses indicate the total number of mice studied in three separate experiments.*

Figure 1A demonstrates the significant protection afforded by a specific *P. aeruginosa* Mills cell vaccine, as well as by the nonspecific *P. aeruginosa* 180 cell vaccine, in burned mice given a *Pseudomonas* Mills challenge. The 21-day mortality for the saline controls was 93%; for the *P. aeruginosa* Mills cell vaccine, 25%; and for the *P. aeruginosa* 180 cell vaccine, 43%. In the same kind of experiment, but with supernatant vaccine instead (Fig. 1B), the 21-day mortality for the medium controls was 65%; for the *P. aeruginosa* Mills supernatant vaccine, 23%; and for the *P. aeruginosa* 180 supernatant vaccine, 38%.

In each of these cases, the specific vaccine gave the lowest mortality, but the nonspecific vaccine also showed a significant difference ($P < 0.05$) compared with the control. Filtered brain heart broth with Merthiolate also gave partial protection ($P < 0.01$) against the *Pseudomonas* challenge when compared with the saline controls.

Figure 2 demonstrates cross protection of vaccines prepared from cells and medium in mice challenged with *P. aeruginosa* strain O:8. In Fig. 2A, significant protection ($P < 0.05$) over the control (✱2) was afforded by both the specific undiluted *P. aeruginosa* O:8 cell vaccine (✱5) and the nonspecific undiluted *P. aeruginosa* 180 cell vaccine (✱4) and even by the $10^{-8}$ diluted cell vaccine of *P. aeruginosa* O:8 (✱3). On the other hand, the nonspecific $10^{-8}$ diluted *P. aeruginosa* 180 (vaccine (#1) showed no protection.

In Fig. 2B, the specific supernatant vaccine (✱4) showed good protection after 21 days ($P < 0.001$), whereas the nonspecific *P aeruginosa* 180 supernatant vaccine (✱2) lowered the mortality from 48% to 32% during the same time period ($P > 0.05$). Boiling the supernatant O:8 vaccine (✱3) did not significantly change the good protection by the unboiled vaccine. Filtered brain heart broth with Merthiolate (✱1) also gave partial protection ($P > 0.05$) against the *Pseudomonas* challenge when compared with the saline control (✱2, Fig. 2A).

On the other hand, if the *P. aeruginosa* 180 and *P. aeruginosa* O:8 vaccines were injected at intervals of 16, 13, 10, and 7 days prior to the rump burn, rather than 3 and 2 days prior, only the specific vaccine significantly protected against the *P. aeruginosa* O:8 challenge 4 hr postburn.

*Vaccine administration after thermal trauma.* Figure 3 shows the results of a *P. aeruginosa* 180 cell vaccine given immediately or 5 hr after burning and challenge with *P. aeruginosa* 180 at 5, 24, 48, and 72 hr postburn. There was significant protection by vaccine (administered immediately or at 5 hr postburn) when the challenge was given at 24 and 48 hr postburn ($P < 0.01$). When the challenge was given 5 hr postburn, the vaccines demonstrated no significant protection. Challenged at 24 hr, the vaccines lowered 21-day mortality from 80% to 38 to 43%; challenged at 48 hr, the vaccines decreased 21-day mortality from 60% to 7 to 9%; challenged at 72 hr, the control mortality was too low to show any effect by the vaccines.

When the burned animals were treated with a nonspecific *Pseudomonas* vaccine after the tail burn, the results were not quite as effective as those with the specific vaccine. Animals given *P. aeruginosa* 2915, *P. aeruginosa* Lawson, and *P.*

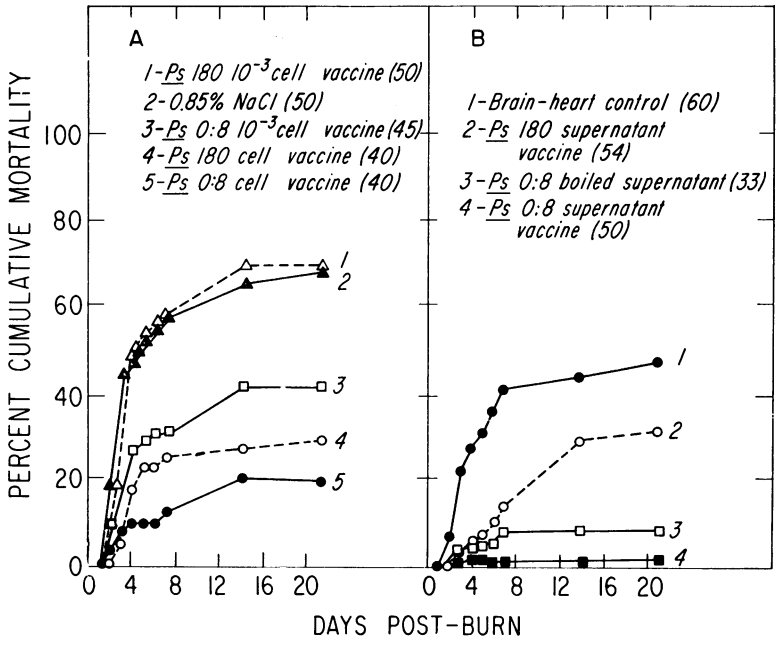


FIG. 2. *Effect of vaccine on mortality of burned mice challenged locally with P. aeruginosa, strain O:8. Animals received 0.1 ml of vaccine, 0.85% NaCl, or brain heart broth subcutaneously 3 and 2 days prior to thermal trauma. Mice were given a rump burn at 70 C for 3 sec. After burning (5 hr), the rump area was dipped in a $10^{-1}$ dilution of a standard suspension of P. aeruginosa, strain O:8. In A, the animals were given a vaccine prepared from cells; in B, from the supernatant medium. The numbers in parentheses represent the total number of mice studied in four separate experiments.*

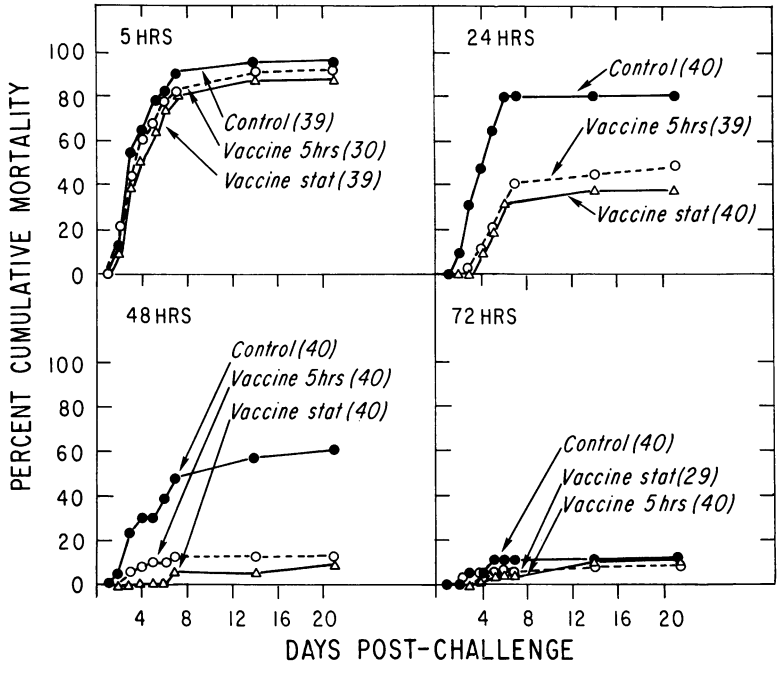


FIG. 3. *Effect on mortality of burned mice given a specific vaccine after a minimal burn and challenged with P. aeruginosa 180. Animals were given a tail burn at 70 C for 5 sec. The control group received 0.1 ml of 0.85% NaCl immediately postburn; vaccine groups received 0.1 ml of P. aeruginosa 180 vaccine immediately or 5 hr postburn. Animals were challenged with P. aeruginosa 180 at 5, 24, 48, and 72 hr postburn. Numbers in parentheses indicate the total number of mice in four separate experiments; stat indicates immediately.*

*aeruginosa* 1M cell vaccines after a tail burn and challenged with *P. aeruginosa* 180 showed decreased 21-day mortalities when challenged at 48 and 72 hr postburn (Table 3).

On the other hand, when the burned animals were treated with an *E. coli* cell vaccine after the tail burn, there was no significant protection observed on challenge at 5, 24, 48, or 72 hr after challenge with *P. aeruginosa* 180.

When the severity of the burn was increased from the tail to the rump burn, the specific vaccine given after the burn was not as effective as after the tail burn. When the challenge was given 5, 24, 48, and 72 hr postburn, the specific vaccine showed no significant lowering of 21-day mortality. Nevertheless, when the challenge was given 96 hr after the burn, the specific *P. aeruginosa* 180 cell vaccine given immediately or 5 hr after the burn lowered the 21-day mortality from 95% to 62 to 67% (Fig. 4). These differences show statistical significance ($P < 0.01$).

Additional experiments, in which a second or third injection of 0.1 ml of *P. aeruginosa* 180 cell vaccine was administered 24 and 48 hr after the burn, showed no improvement on the results of only one injection of vaccine after the burn.

TABLE 3. *Protection of burned mice challenged locally with P. aeruginosa 180 by nonspecific Pseudomonas vaccines*[a]

| Vaccine | Mortality at 21-days after challenge at | | |
|---|---|---|---|
| | 24 hr | 48 hr | 72 hr |
| | % | % | % |
| *P. aeruginosa* 2915 (O:7a, 7c) | | | |
| Control | 73 | 35 | 10 |
| Vaccine, immediately | 65 | 18 | 20 |
| Vaccine, 5 hr | 63 | 20 | 5 |
| *P. aeruginosa* Lawson (O1:a, 1e) | | | |
| Control | 78 | 48 | 33 |
| Vaccine, immediately | 75 | 33 | 18 |
| Vaccine, 5 hr | 75 | 33 | 13 |
| *P. aeruginosa* 1M (O:5) | | | |
| Control | 75 | 55 | 43 |
| Vaccine, immediately | 68 | 25[b] | 15[b] |
| Vaccine, 5 hr | 55 | 23[b] | 8[b] |

[a] Animals were given a tail burn at 70 C for 5 sec. Each vaccine group consisted of 40 mice. Vaccines were prepared from cells and were injected subcutaneously (0.1 ml) immediately or 5 hr after burn. Control mice received 0.1 ml of 0.85% NaCl subcutaneously immediately after the burn. Mice were challenged by dipping the burned tail in a standard suspension of *Pseudomonas* organisms at the time intervals indicated above.

[b] $P < 0.02$.

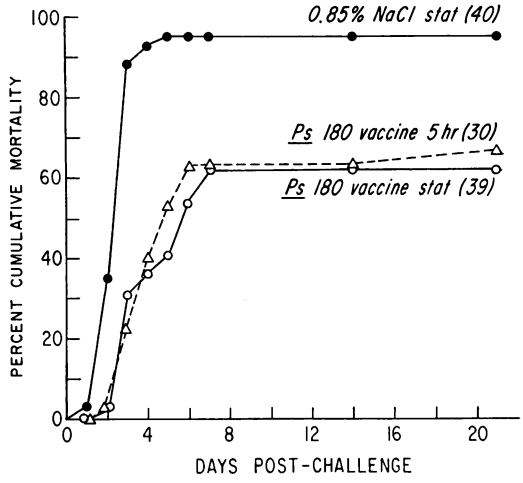


FIG. 4. *Effect on mortality of burned mice given a specific vaccine after a severe burn and challenged with P. aeruginosa 180. Animals were given a rump burn at 70 C for 3 sec. The control group received 0.1 ml of 0.85% NaCl immediately postburn; vaccine groups received 0.1 ml of P. aeruginosa 180 vaccine immediately or 5 hr postburn. All animals were challenged with P. aeruginosa 180 at 4 days postburn. Numbers in parentheses indicate the total number of mice in four separate experiments; stat indicates immediately.*

*Autopsy findings.* Macroscopic autopsy findings of animals that died after *Pseudomonas* challenge were the same as reported previously (13). At 21 days, the survivors were autopsied, and the percentage of mice with renal abscesses was compiled in mice given the tail burn and vaccinated afterward. When a specific vaccine derived from *Pseudomonas* cells or supernatant fluid was given, the vaccinated animals showed a reduced percentage of renal abscesses compared to controls not vaccinated when challenged at 24, 48, and 72 hr after the burn. When a nonspecific *Pseudomonas* cell vaccine was given, the vaccinated mice also showed a reduced percentage of renal abscesses, but only when challenged at 48 and 72 hr postburn.

## DISCUSSION

The results of these experiments show that active immunization is effective in the prophylaxis of *Pseudomonas* septicemia in burned mice. The efficacy of vaccination depends upon the severity of the burn, the specificity of the vaccine, the virulence of the challenging organism, and the time of challenge in relation to the burn injury.

Generally, a vaccine is most effective when it is specific, when the burn is not severe, when the challenging microorganism is not too virulent, and when there is a time lag between burn injury and challenge.

The results of these experiments agree with those published by us earlier with an intraperitoneal challenge (13). In a different experimental model, Walker et al. (20) found that active immunization with some strains of *Pseudomonas* protected rats in which the undersurface of an autograft was seeded with *Pseudomonas*. The only other study in which vaccines were administered prophylactically in burned patients was published by Feller (2). In a small group of 24 burned patients, he observed that a vaccine prepared from a single strain of *P. aeruginosa* administered after thermal injury decreased the incidence of septicemia due to *Pseudomonas* from 51% to 29%; however, the control and vaccine groups were not compared during the same time period. He had even better success with the combined use of vaccine and human hyperimmune serum administered prophylactically in diminishing deaths and septicemia due to *Pseudomonas*; however, here again numbers were small, and comparison was made with nonsimultaneous controls. In conditions other than burns, there have been isolated reports in the literature in which patients with generalized *Pseudomonas* infections were treated with vaccines prepared from organisms isolated from wounds and organs. Some authors had success (5, 6), while others had none (4).

These experiments also raise the question as to the mode of action of vaccination. Is the protective action due to antibody formation, to stimulation of the reticuloendothelial system, or to some unknown factor? In a previous report, we demonstrated that the primary and secondary antibody response to sheep erythrocytes was normal after burns of various degrees of severity in mice (12). Other investigators have found similar results with other antigens (1, 8, 9). We observed that antibody levels began to rise at 3 days and reached a high plateau at 4 to 5 days after antigenic stimulation. Two arguments in favor of antibody protection by vaccination are (i) specific vaccines were always more effective than nonspecific vaccines; and (ii) protection could be demonstrated in the more severe rump burn only when the challenge was given 4 days postburn, a time at which the antibody levels would be at their peak. Against the antibody argument in favor of a nonspecific protective effect on the reticuloendothelial system are: first, vaccine injected soon after the burn protected mice with the less severe tail burn when the challenge was given as early as 24 hr postburn. From the sheep erythrocyte studies, antibody levels were not sufficient at that time to protect against the challenge; second, vaccines prepared from *E. coli* and *Proteus mirabilis* were almost as effective as the nonspecific vaccines prepared from various O antigenic strains of *P. aeruginosa* after the tail burn. It seems unlikely that all gram-negative bacteria have a common antigen against antibodies to the virulent *P. aeruginosa* 180 to account for their effectiveness; and, third, vaccination schedules started 2 weeks prior to the burn, rather than 3 days prior, significantly protected only in the case of the animals given the specific vaccine. Antibody levels should be high after 2 weeks of immunization. Since these antibodies did not protect in the case of the nonspecific vaccine immunization, the protective action of the vaccine injected only 3 and 2 days prior to thermal trauma could have occurred by stimulation of the reticuloendothelial system. Of course, it is entirely probable that vaccines may act by a combination of these as well as other mechanisms.

Although burned mice do not usually die with *Pseudomonas* infections (11, 19), the models described by Rosenthal (16) and us have been useful in therapeutic studies of *Pseudomonas* infections in burned mice. The protection provided by specific and nonspecific vaccines in burned mice challenged with *P. aeruginosa* suggests that the use of vaccines might be valuable as part of the therapy in the prevention of septicemia and death due to *Pseudomonas* in human burned patients. Since the encouraging results reported by Feller (2) with a monovalent vaccine alone are not entirely conclusive, further controlled prophylactic studies, perhaps with a polyvalent vaccine, should be undertaken in burned patients.

ACKNOWLEDGMENTS

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