A Procedure for Measurement of Wound Healing, with Special Reference to Burns. (31484)

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Studies have been currently performed(1) on the production of *Pseudomonas aeruginosa* septicemia in mice by local immersion of the burned tail in a culture of these organisms. A technic was also developed for the local application of therapy in the tail by encasing it in a section of rubber tubing which is fastened to the skin at the base of the tail. With these procedures it is possible by measuring the viable tail length to determine the response of the tail to injury, the effect of bacterial or other challenge, and the influence of various therapeutic agents upon wound healing.

Methods. A strain (NIH) of albino mice of 18 to 22 g weight were used. The burn was produced by immersion of the tail of the etherized animal in water at 70°C. For purposes of producing infection by local challenge, 5 second immersion was used, but for other purposes the extent of injury can be varied by adjusting the temperature and the time of exposure.

For the application of local treatment to the tail, sections of soft rubber tubing were cut slightly longer than the tail (8 to 10 cm). The proximal end of the tubing was cut at a 45 degree angle, and the pointed tip was extended over the base of the tail where it was fastened to the loose skin of the back by means of two Michael clips. The distal end of the tube was closed by insertion of a small cork (size 00). For mice under 25 g weight, soft latex tubing of 3/16 inch inside diameter and wall thickness of 1/32 inch was employed. The tubes must be large enough to accommodate the swollen tail, or strangulation at the base may occur.

A few days after the burn the line of demarcation of viable and nonviable tail is sufficiently sharp to obtain fairly accurate measurements with a small flexible ruler. This is accomplished on the ventral surface, employing the distal rim of the anus as a base.

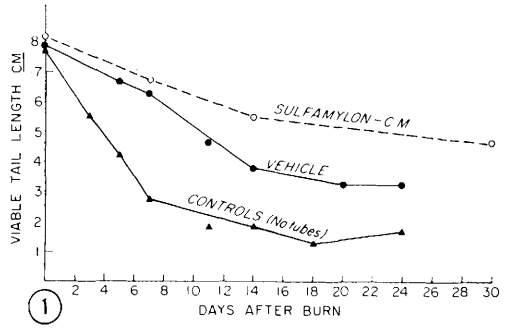
Most of the experiments with *P. aeruginosa* were done with strain 180. This strain was sufficiently virulent to produce death in 93% of 158 mice in 21 days, when the burned tail was immersed in an 18 hour broth culture adjusted in a Coleman spectrophotometer to an optical density of 0.7 at 660 mμ and further diluted with equal parts of 0.85% sodium chloride. The challenge was given within a few hours after the burn(1).

Local therapy can be applied by injecting the material into the distal open end of the tubing with a syringe. It can be repeated as desired, but in the study carried out on the assay of drugs against pseudomonas infections we have used one injection in a hydrophylic cream base, given at 6 hours after the challenge.

The tubes frequently came off after the first week. They can be easily replaced, but in the above study(1) this was not done, since it was shown that systemic bacterial invasion, as indicated by mortality, does not occur when the challenge is delayed for a week after the burn. In this report local therapy was applied at 6 hours after the pseudomonas challenge, by filling the tubes with desired concentrations of drugs suspended in a vanishing cream base. Measurements of tail length were made after 7 to 10 days, when the tubes were either removed or became unfastened.

Experiments were performed with groups of 10 mice, and the viable tail length of each group was averaged at various intervals after the burn.

Results. Burn without pseudomonas challenge. Immersion of the tail for 5 seconds in water at 70°C results in progressive loss of viability so that in animals without challenge and without tubes only 37% of the tail was viable after 1 week and 15% after 2 weeks (Fig. 1). Important factors in loss of viability are desiccation and contamination with surface bacteria, for enclosure in a tube with the application of an inert hydrophylic cream vehicle increased the viable tail to 80% of its original length 1 week after the burning and



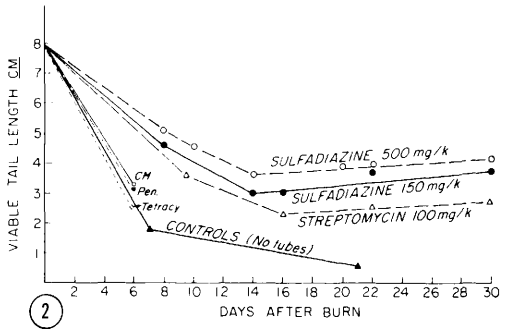


FIG. 1. Tail Burn. Local Therapy. No Challenge. In the top curve 8.5% sulfamylon or 0.25% chloramphenicol (C.M.) in a hydrophylic cream were applied in the tubes covering the burned trails. In the middle curve the cream alone was applied. Viable tail lengths in cm; average tail lengths in 2 goups (10 mice each) combined in each curve.

FIG. 2. Tail Burn with local pseudomonas challenge. Systemic Therapy. No tubes on tails. CM = chloramphenicol, 150 mg/kg (10 mice) Pen = penicillin g, 200 mg/kg (10 mice); Tetracy = tetracycline, 150 mg/kg (10 mice); sulfadiazine (20 mice each curve), all administered orally. Streptomycin (20 mice) subcutaneously. Control curve a composite of 34 mice.

to 49% at 2 weeks. Incorporation in the cream of effective antibacterial agents (chloramphenicol 0.25% or sulfamylon (homosulfanilamide acetate) 8.5%) further increased the viable tail length to 70% at 2 weeks and 58% one month after the burn. These results indicate the advantages of the "closed" system for treating burns, and of local antibacterial therapy for suppressing surface infection. Slight increases in tail length were frequently observed in groups of animals surviving beyond 2 weeks. This is probably an expression of growth of the surviving tail, since a general increase in body size occured during this period.

Burn with pseudomonas challenge. Immersion of the burned tail in a 1 to 1 dilution of pseudomonas culture 2 hours after the burn accelerated the loss of viability: so that in the control animals only 22% of the original tail length was viable after 1 week, 15% at 2 weeks, and 7.5% after 3 weeks (Fig. 2). Since most of the mice with tail burns, challenged with pseudomonas strain 180, succumb during the first week in the absence of treatment, the tail lengths after 1 week were obtained from mice in which the challenge was delayed for 3 or 4 days after the burn; under these conditions the total mortality was 38 and 20% respectively, and tail lengths were obtained on the survivors.

Effect of local therapy. Recent studies on burned animals and humans have demonstrated that homosulfanilamide (sulfamylon) applied locally in a cream base(2) and local application of 0.5% silver nitrate(3) can materially lower the mortality from pseudomonas infections. We have found several other drugs capable of protecting mice from local pseudomonas challenge when they are applied in appropriate concentrations in the tubes enclosing the burned tail.

Among the drugs giving 90 to 100% survival to mice challenged locally with pseudomonas strain 180 were sulfamylon 8.5%, chloramphenicol 0.25%, sulfadiazine 0.5%, streptomycin 0.5 to 1%, gentamycin 0.5%, and neomycin 0.4%, all applied to the burned tail in a hydrophylic cream base(1). The relative effectiveness of these drugs applied locally 4 hours after challenge on viable tail length will be reported later. However, preliminary observations revealed a marked effect from all of them the average viable tail computed from separate observations on these 6 drugs was 70% of its original length 1 week after the burn, 59% after 2 weeks and 45% after 30 days. Thus a degree of protection was obtained approaching that of chloramphenicol and sulfamylon applied to the burned tail in the absence of challenge (Fig. 1).

Effect of systemic therapy. We have found that oral or subcutaneous administration of sulfadiazine in doses of 0.15 to 0.5 g per kg, and streptomycin subcutaneously 0.1 g per kg body weight, given daily for 5 to 10 days (beginning 6 hours after challenge), will pro-

duce survival of 90 to 100% of burned mice challenged locally with pseudomonas strain 180(1). It was observed that this treatment brought about a pronounced increase in viable tail length, with 30 to 47% viable after 16 days and 35 to 50% after 30 days. Chloramphenicol, penicillin, and tetracyclin, which were without effect on survival when administered systemically, gave results similar to the untreated controls (Fig. 2); this absence of effect of chloramphenicol is in contrast with its activity when applied locally, as shown above. The results with systemic sulfadiazine and streptomycin are comparable to those obtained with effective local treatment. These findings indicate that sulfadiazine and streptomycin administered systemically not only protect the animals from generalized infection with pseudomonas 180, but also suppress the local infection in the tail. They also stress the importance in wound healing of pathogenic bacterial contamination.

The technics described here may prove useful for the study of various types of injury such as irradiation and chemical trauma, as well as the study of therapeutic agents on wound healing in the presence or absence of infection.

The length of the rodent tail makes it quite vulnerable to injury applied over its entire length, so that viable tail may afford a more sensitive index of tissue damage than other areas of body surface. A feature of the present study is the striking extent to which an injury, normally leading to the death of tissue, may be reversed by therapeutic measures.

Summary. A simple technic is described for measurement of wound healing following a standardized burn of the mouse tail. A procedure was also developed for the application of local therapy to the burned area. A marked influence on viable tail length was obtained by local protective measures, and by administration of local and systemic antibacterial therapy.

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Drug Interactions Between Disulfiram and α-Methyldopa and Related Agents in Reserpine-Pretreated Rats.* (31485)

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Tyramine owes its sympathomimetic effects to the release of norepinephrine from storage sites in postganglionic adrenergic nerves(1-3). The pressor response to tyramine and adrenergic nerve stimulation is reduced or abolished in animals pretreated with reserpine (1,4). The sympathetic nerve blockade by reserpine can be partially reversed by injection of dopa, dopamine, or norepinephrine (5,6). The α-methylated analogues of the catecholamines also reverse the nerve blockade by reserpine(6,7) and the tyramine blockade by reserpine(6,8).

The enzyme, dopamine-β-hydroxylase, catalyzes the conversion of dopamine to norepinephrine and other analogue of phenylethylamine to their corresponding β-hydroxylated derivatives (See review, 9). Disulfiram and other copper chelating agents are inhibitors of this enzyme *in vivo* as well as *in vitro*(10-12).

In the following experiment the effect that disulfiram and its active metabolic product, diethyldithiocarbamic acid(10), has on dopa, dopamine, α-methyldopamine and α-methyl-

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