Inhibition of Supraeschar and Subeschar *Pseudomonas* Infection by Silver Sulfadiazine Dry Foam

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Abstract □ The results of an *in vivo* evaluation of silver sulfadiazine dry foam are described. Using burned guinea pigs infected with *Pseudomonas aeruginosa*, silver sulfadiazine was applied every 12 hr as the dry foam or ointment. After 72 hr of therapy with the medicated dry foam, only one of the 15 burns remained infected while seven of the 15 burns remained positive for *Pseudomonas* after treatment with the corresponding medicated ointment ($p < 0.05 > 0.01$). It is suggested that the medicated dry foam provided significantly greater activity in treating a supraeschar burn wound infection of recent onset. In addition, a modified crossover study demonstrated that both the medicated dry foam and ointment are less effective in treating subeschar burn wound infections.

Keyphrases □ Silver sulfadiazine dry foam—inhibition of supraeschar and subeschar *Pseudomonas* infection, guinea pigs □ Burn therapy—inhibition of supraeschar and subeschar *Pseudomonas* infection by silver sulfadiazine dry foam □ *Pseudomonas* infections (burns)—inhibition by silver sulfadiazine dry foam □ Foams, dry—silver sulfadiazine, inhibition of *Pseudomonas* burn infections

Earlier, the formulation and in *vitro* evaluation of antibacterial dry foams were described (1). These data showed that the efficacy of the topical dry foams was not less than equivalent to that of corresponding medicated ointments. Furthermore, those dry foams containing silver salts appeared to possess significantly greater activity ($p < 0.001$) than that of ointments containing silver salts when therapeutic activity was monitored by *in vitro* methods. To determine further the efficacy of dry foams, the results of an *in vivo* evaluation of silver sulfadiazine dry foam are described.

EXPERIMENTAL

***In Vivo* Evaluation**—Each of 15 male guinea pigs was inflicted with four thermal injuries as previously described (2). All burns were challenged with an overnight broth culture of *Pseudomonas aeruginosa*[1], and each burn was assigned a specific therapeutic regimen. Therapy was initiated 30 min after inoculation and continued every 12 hr for 72 hr. One burn per animal was treated with 0.1 g of 2% silver sulfadiazine ointment, applied by means of a sterile blade. The second burn per animal was medicated with 2% silver sulfadiazine dry foam, applied in the form of 2.5-cm squares. An oil-in-water ointment[2] and dry foam control were applied to the third and fourth burns per animal, respectively. Swab cultures were obtained immediately prior to the application of the dosage forms. The results of this study are listed in Table I.

After 72 hr of treatment, a crossover study was initiated. The burn areas previously treated with the unmedicated dry foam were assigned therapy with the silver sulfadiazine dry foam, applied every 12 hr for 72 hr. In a similar manner, those areas previously treated with the oil-in-water ointment were assigned a treatment regimen of silver sulfadiazine ointment. Swab cultures were obtained every 12 hr for 72 hr immediately prior to the application of the dosage forms. The results are listed in Table II.

[1] ATCC 9721.

[2] Neobase Ointment, Burroughs Wellcome & Co., Research Triangle Park, N.C.

Table I—Results of Silver Sulfadiazine Therapy on Infected Burns

Hours[a]	Number of Animals[b] with Positive *Pseudomonas* Cultures[c] after Treatment[d] with: Silver Sulfadiazine Dry Foam	Silver Sulfadiazine Ointment	Dry Foam Control	Ointment Control
12	14	13	15	15
24	8	10	15	15
36	1	9	14	15
48	1	6	15	15
60	1	7	15	15
72	1[e]	7[e]	15	15

[a] Times listed are the hours after inoculation with an overnight broth culture of *Ps. aeruginosa*. [b] Each of 15 animals received four full thickness burns. [c] A positive culture was considered to be one in which five or more *Ps. aeruginosa* colonies were isolated and identified from a burn. [d] Treatment consisted of 2% silver sulfadiazine ointment, 2% silver sulfadiazine dry foam, an oil-in-water ointment control, or dry foam control applied to the involved site every 12 hr for 72 hr. [e] $p < 0.05 > 0.01$.

Identification of *Pseudomonas*—The isolation, identification, and quantification of the test bacteria were accomplished using visual, UV, and biochemical methods, as previously described (2).

RESULTS AND DISCUSSION

Earlier *in vitro* (1) and *in vivo* (2) studies demonstrated that 8.5% mafenide dry foam and 8.5% mafenide ointment were equally effective in treating *Pseudomonas* burn wound infections. However, since *in vitro* comparisons of the dry foam and ointment containing silver nitrate or silver sulfadiazine showed significant differences in therapeutic activity, *in vivo* studies were performed to document these preliminary conclusions.

Table II—Results of Crossover Silver Sulfadiazine Therapy on Infected Burns

Hours[a]	Number of Animals[b] with Positive *Pseudomonas* Cultures[c] after Treatment[d] with: Dry Foam/Silver Sulfadiazine Dry Foam	Ointment/Silver Sulfadiazine Ointment
12	15	15
24	15	15
36	14	15
48	15	15
60	15	15
72	15	15
84	13	15
96	10	12
108	7	11
120	8	8
132	6	7
144	4	5
156	4[e]	5[e]

[a] Times listed are the hours after inoculation with an overnight broth culture of *Ps. aeruginosa*. [b] Each of 15 animals received four full thickness burns. [c] A positive culture was considered to be one in which five or more *Ps. aeruginosa* colonies were isolated and identified from a burn. [d] Treatment consisted of dry foam control or an oil-in-water ointment control applied to the involved sites every 12 hr for 72 hr. This was followed by the application of 2% silver sulfadiazine dry foam or 2% silver sulfadiazine ointment to the involved sites every 12 hr for 72 hr. [e] $p = 0.1$.

After the initial 72 hr of treatment, all unmedicated control areas continued to demonstrate the presence of *Pseudomonas*. Of the 15 burns treated with silver sulfadiazine dry foam, one remained infected with the test bacteria. In contrast, seven of the 15 burns remained positive for *Pseudomonas* after treatment with the corresponding medicated ointment. Chi-square computations demonstrated the significance of these data ($p < 0.05 > 0.01$). However, at the conclusion of the *in vivo* crossover study, four burns treated with the medicated dry foam remained infected while five of the areas treated with the medicated ointment remained infected ($p = 0.1$).

In assessing the results of the initial *in vivo* study with those of the subsequent crossover study, several factors must be considered. The 72-hr delay in instituting therapy in the crossover study may have led to an infected environment not readily affected by rates of release from the dosage form. The proliferation of the test bacteria during this initial 72 hr probably resulted in widespread subeschar colonization. This movement of the bacteria into tissues below the burn produced a medium for growth not easily treated with topical agents.

In clinical use, both local and systemic therapy would be indicated to combat the advancing bacteria successfully. Furthermore, surgical excision of the devitalized eschar would be performed as an aid in the elimination of the infecting organism. The presence of eschar provides an excellent medium for bacterial growth and also provides a barrier between the applied drug product and the subeschar colonies, possibly preventing therapeutic contact and effect. Moreover, the protein binding characteristics of silver ion and the sulfonamide may prevent deep percutaneous absorption (3).

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Growth of Calcium Oxalate in Gel Systems

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Abstract □ Methods are described for growing calcium oxalate in silica and gelatin gels under different conditions. The results obtained indicate that, in silica gel, calcium oxalate grows into single individual crystals, twins, and rosettes. Bipyramidal calcium oxalate dihydrate crystals similar to those present in the urine of stone formers were prepared in the silica gel system. The gelatin gel offered a suitably structured substrate on which calcium oxalate monohydrate crystals grow into aggregates. The orientation pattern of calcium oxalate crystals suggests that the growth process is controlled by the stereospecificity of the gelatin medium supporting growth.

Keyphrases □ Calcium oxalate—growth of crystals in silica and gelatin gels, relevance to stone formation □ Crystal growth—calcium oxalate in silica and gelatin gel systems, types of growth □ Silica—substrate for growing calcium oxalate crystals □ Gelatin—substrate for growing calcium oxalate crystals

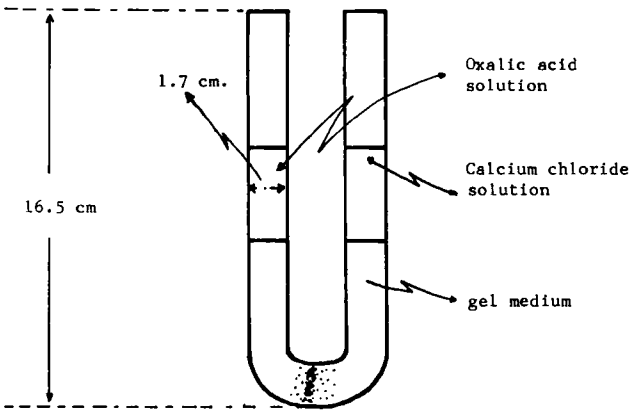

Figure 1—*Dimensions of the U-shaped tubes selected for the gel growth studies.*

Recent reports on the growth of calcium oxalate *in vitro*, either by precipitation from solution or from urine, generated valuable information on growth kinetics and the factors controlling crystal growth (1, 2). Attempts to grow crystal aggregates, similar to natural concretion, have so far met with little success (3, 4). This report describes a method for growing single crystals and artificial concretions of calcium oxalate. It is believed that this *in vitro* experimental model could be of fundamental significance in understanding the complex mechanisms of stone formation.

Studies on the crystallization of poorly soluble salts are often slowed down by the lack of methods for growing single crystals that permit close observations and understanding of the growth process and the factors controlling such phenomena. Interest in the gel medium for crystal growth has been greatly stimulated by the work of Henisch (5). Single crystals of calcium tartrate, tungstate, carbonate, and sulfate were grown using silica gel as the growth medium (6–10). This technique offered new opportunities for the production of useful single crystals and presented new observations in mechanism studies.

The growing pattern of crystals is dependent on the structure of the gel and the nature of the additives present in the gel medium. For very poorly soluble salts, the growth rate is essentially a diffusion process and the chemical reaction is not the rate-de-