

## Fluid and Penicillin G Dynamics in Polyethylene Chambers Implanted Subcutaneously in Rabbits

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Chemical and cellular characteristics of fluid within subcutaneously implanted polyethylene chambers in rabbits were studied over a 3-month period. The fluid attained a relatively stable protein and cellular composition which was consistent with a mononuclear exudate. After a single dose of intramuscular penicillin G, the antibacterial activity of chamber fluid was found to be dynamic and similar to the serum antibacterial activity. This animal model may be useful for in vivo studies of the interaction of microorganisms with antimicrobial agents.

A procedure for implanting polyethylene chambers subcutaneously in rabbits has recently been described by Arko for the study of experimental *Neisseria gonorrhoeae* infections (1). Similar chambers have been implanted into a variety of laboratory animals (2) and fluid and pharmacological dynamics (5, 6), as well as infections with *Treponema pallidum* (7), have been studied. The present study was undertaken to determine the protein content and cellular characteristics of the fluid which filled the chambers after implantation into rabbits, and to determine the dynamics of penicillin G in the chamber fluid after single intramuscular (i.m.) injections of three-dose regimens.

### MATERIALS AND METHODS

Male and female New Zealand white rabbits weighting 2 to 5 kg were used. Two polyethylene chambers (practice golf balls; Sportsotron, Inc., Smithtown, N.Y.) with numerous holes in their walls were sterilized by autoclaving and surgically implanted subcutaneously in the dorsolumbar region of each animal, one on each side, according to the method described by Arko (1). After implantation, the chambers gradually filled with pale amber fluid. Specimens of chamber fluid from 10 rabbits were aspirated at 14, 28, 60, and 90 days after implantation with a syringe equipped with a 25-gauge needle and the following were determined: total protein content, erythrocyte count, leukocyte count, and differential.

The dynamics of penicillin G in the rabbit serum and chamber fluid after a single i.m. injection were determined after the chambers had been implanted for a minimum of 3 months. Groups of three rabbits with implanted chambers each received aqueous potassium penicillin G in doses of 1,000, 10,000, and 100,000 U per kg of body weight. Serum and chamber fluid specimens were obtained before penicillin administration, and at 30 min, 1, 2, 3, 4, 5, 6, 8, and 24 h

after injection, and were subsequently assayed for antibacterial activity (ABA) using a microdilution technique (4). Specimens (0.05 ml) were serially diluted in 0.05-ml amounts of Trypticase soy broth, pH 7.2, using standard microdilution U plates (Linbro Chemical Co., New Haven, Conn.). Five one-hundredths milliliters of a 6-h broth culture of *Staphylococcus aureus* ATCC 25923 (penicillin G minimal inhibitory concentration 0.03 µg per ml) diluted 1:1000 in Trypticase soy broth was added to each well. The microdilution plates were sealed and then incubated at 37 C for 18 h. The ABA was read as the highest serum or chamber fluid dilution which completely inhibited growth of the assay organism as determined with a reading mirror (Cooke Laboratory Products, Alexandria, Va.).

### RESULTS

Mean values of total protein determinations, erythrocyte and leukocyte counts, and leukocyte differential counts of 20 chamber fluid samples are shown in Table 1. Chamber fluid total protein content decreased slightly (0.2 g per dl) during the 90-day period after implantation. A more rapid decrease in erythrocyte and leukocyte counts to 10,000 per mm<sup>3</sup> and 900 per mm<sup>3</sup>, respectively, occurred during the same period. Initially (day 14), the leukocytes were predominantly polymorphonuclear, with a mean of 67%, but within 1 month, mononuclear cells predominated (90%).

Results of serum and chamber fluid ABA determinations are shown in Fig. 1. The peak ABA in chamber fluid occurred 1.5 to 3.0 h after the peak serum ABA, and the ABA persisted longer in chamber fluid than in serum for the three doses tested.

Peak penicillin G levels are shown in Table 2 and were estimated by multiplying the ABA of the specimen by the minimal inhibitory concentration of the assay organism. Chamber fluid

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TABLE 1. Results of protein and cellular analysis of fluid obtained from subcutaneously implanted chambers in rabbits<sup>a</sup>

Analysis	Days after implantation			
	14	28	60	90
Total protein (g per dl)	3.2	3.2	3.1	3.0
Erythrocytes per mm <sup>3</sup>	55,300	30,000	17,500	10,000
Leukocytes per mm <sup>3</sup>	7,000	2,300	1,500	900
Leukocyte differential				
% Polymorphonuclear leukocytes	67	10	8	9
% Mononuclear leukocytes	33	90	92	91

<sup>a</sup> Mean of 20 determinations (10 rabbits, two chambers per rabbit).

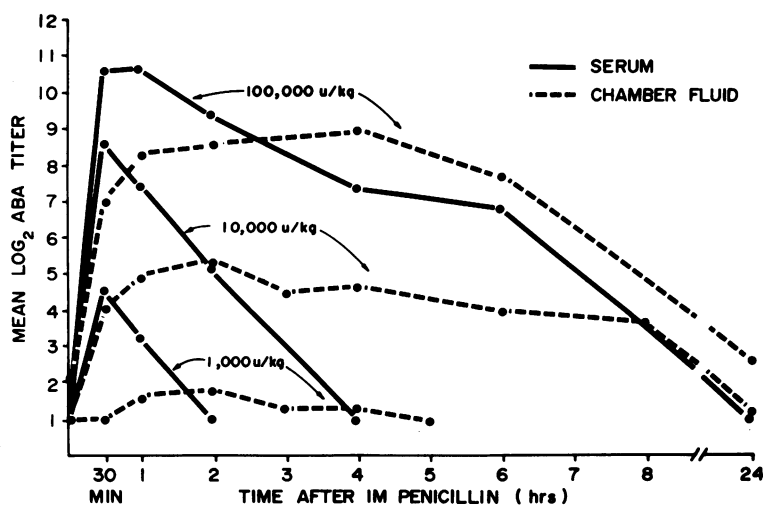


FIG. 1. Serum and chamber fluid ABA after *i.m.* injections of 1,000, 10,000, or 100,000 U per kg of aqueous penicillin G in rabbits.

TABLE 2. Peak serum and chamber fluid levels after single intramuscular injection of 1,000, 10,000, and 100,000 U per kg of aqueous penicillin G in rabbits

Substance	Penicillin G (U/kg)		
	1,000	10,000	100,000
Serum	0.7 <sup>a</sup> (30 min) <sup>b</sup>	12.5 (30 min)	50.0 (1 h)
Chamber fluid	0.1 (2 h)	1.3 (2 h)	15.4 (4 h)

<sup>a</sup> Estimated (minimal inhibitory concentration of assay organism  $\times$  ABA of specimen) in micrograms per milliliter.

<sup>b</sup> Time in parentheses, Time after injection.

peak levels were 0.1, 1.3, and 15.4  $\mu$ g per ml for doses of 1,000, 10,000, and 100,000 U per kg, respectively, and represented 14.3, 10.4, and 30.8% of the corresponding peak serum levels.

## DISCUSSION

Within 14 days after the implantation of the chambers, sufficient fluid had accumulated within the chambers to enable serial sampling with a syringe and needle via one of the holes in the chamber walls. Initially, the fluid was slightly pink, presumably due to hemoglobin, but 2 to 3 months after implantation became pale yellow. The protein content remained relatively stable, although the cellular composition decreased throughout the 3-month period. After the first month, the leukocyte differential remained relatively stable.

The dynamics of penicillin G in chamber fluid were similar to those observed in serum, and although peak chamber fluid levels were considerably lower than the corresponding peak serum levels, the antibiotic persisted longer in chamber fluid than in serum. Excluding the initial 2- to 3-h period after injection, chamber fluid ABA exceeded the corresponding

serum ABA (Fig. 1). After i.m. injection of 100,000 U per kg of penicillin G, therapeutic levels up to 15.4  $\mu\text{g}$  per ml were achieved in chamber fluid. Chisholm et al. (3) have reported similar results with ampicillin, gentamicin, tobramycin, sulfamethoxazole, and trimethoprim, utilizing silastic capsules implanted subcutaneously in dogs. These investigators have proposed that the chamber fluid approximates interstitial fluid. They suggested that chamber fluid antibiotic levels may provide a better estimate of the "tissue level" of an antibiotic than of serum levels, a possibility that could be of considerable theoretical and clinical importance in the rational approach to antibiotic therapy.

Previous studies (1, 7) have shown that chambers such as those used in this study could be infected with a variety of bacteria in a controlled manner and would support growth of the organisms without seriously affecting the animal. The present study has shown that therapeutic levels of penicillin G were achievable in chamber fluid after an i.m. injection. Therefore, it is possible that this model could be useful for further studies of the interaction of microorganisms with antimicrobial agents, in-

cluding studies of the pathogenesis, immunology, and treatment of a variety of experimental infections.

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