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An Experimental Study of Some Repository Dosage Forms of Penicillin.

A. KATHRINE MILLER, W. F. VERWEY, AND DOROTHY L. WILMER.
(Introduced by L. E. Arnow.)

From the Department of Bacteriology, Medical Research Division, Sharp and Dohme, Inc., Glenolden, Pa.

The injection of repository dosage forms of penicillin results in a prolongation of plasma penicillin concentrations. Crystalline sodium or calcium penicillin suspended in peanut oil and beeswax,[1,2] procaine penicillin suspended in oil[3,4] or in water[5] are used for this purpose.

Experimental. During the course of a research program designed to develop an effective suspending vehicle for such repository dosage forms, dogs were given an intramuscular injection of 300,000 units of penicillin in the form of some of these products or of an experimental preparation consisting of procaine penicillin in peanut oil and aluminum monostearate gel, as well as of the non-repository dosage form of sodium penicillin in aqueous solution. Penicillin concentrations of heparinized plasma samples were determined by a modification of the Rammelkamp tube dilution assay method.[6,7] For purposes of arithmetic averaging, amounts of penicillin not detectable by the assay were considered as zero values. Table I lists the average penicillin values found following the injection of each of these products into 5 dogs. It will be seen that, of the preparations tested, the use of the oil-monostearate suspension resulted in the greatest prolongation of detectable levels of plasma penicillin.

It was believed that a test of ability to prolong a period of protection against an experimentally induced infection might offer a more definitive means of evaluation of these preparations than the study of duration of detectable plasma penicillin concentrations. Accordingly, the mouse protection test described below was developed.

Mice were divided into groups corresponding to the number of preparations to be tested. At zero time each mouse received a single intramuscular injection in the left hind leg of 15,000 units (0.05 cc) of the appropriate penicillin suspension. At stated time intervals after this treatment, 10 or 20 mice from each of the original groups were challenged with 1,000 minimum lethal doses of a 6-hour culture of Pneumococcus Type I. Following challenge, the mice were observed for seven days, at the end of which time the final calculations of percentage survival were made.

The effect of penicillin particle size on protective ability was investigated because of the report that oil-beeswax preparations were most effective in prolonging plasma penicillin concentrations when large particle size crystals were used in the suspension.[8] Two preparations of sodium penicillin and an oil-beeswax base were formulated: in one the majority of the crystals measured 50 microns and in the other the majority of the particles measured less than 5 microns. Similarly, large and small particle size samples of procaine penicillin were suspended in oil and aluminum monostearate. Several tests were run on each preparation, and the reported figures represent

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2 Romansky, M. J., and Rittman, G. F., *N.E.J. Med.*, 1945, **233**, 577.

3 Herrell, W. E., Nichols, D. R., and Heilman, Fordyce R., *Proc. Staff Meet. Mayo Clinic*, 1947, **22**, 567.

4 Boger, W. P., Oritt, J. E., Israel, H. L., and Flippin, H. F., *Am. J. Med. Sciences*, 1948, **215**, 250.

5 Whittlesey, P., and Hewitt, W. L., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 658.

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7 Miller, A. Kathrine, and Boger, W. P., *Am. J. Clin. Path.*, 1948, **18**, 421.

8 Dowling, H. F., Romansky, M. J., Welch, H., Robinson, J. A., Chandler, V. L., Zeller, W. W., and Hirsch, H. L., *J.A.M.A.*, 1948, **135**, 567.

TABLE I.
Effect of Dosage Form on Average Plasma Penicillin Concentration in Dogs Following a Single Intramuscular Injection (1.0 cc) of 300,000 Units of Penicillin.

Dosage form		Avg plasma penicillin conc. (u/cc) at different hrs following penicillin injection				
Penicillin salt, 300,000 u	Suspending vehicle	1	8	24	72	96
Sodium	Water*	22	0.06	.01		
Procaine	Oil	3.8	1.7	.55	.02	
"	Water	2.7	2.5	.19	.03	
"	Oil-monostearate	2.8	1.0	.51	.12	.09

* Solution.

TABLE II.
Effect of Dosage Form on the % Survival of Mice Receiving a Single Intramuscular Injection (0.05 cc) of Penicillin and Challenged with Pneumococci.

Dosage form			Time (hrs after treatment) of challenge with 1,000 M.L.D. Type I pneumococcus — % survival									
Penicillin salt 15,000 u	Suspending vehicle	Particle size	1	4	8	24	48	72	96	120	144	168
Sodium	Water*		100	100	45	0						
Procaine	Oil		100	100	100	13	0					
"	Water		100	100	75	7	0					
Sodium	Oil-beeswax	large				84	47	33	2			
		small				40	17	17	3			
Procaine	Oil-monostearate	large				66	30	3	0			
		small				100	97	87	66	34	13	0

* Solution.

calculations involving at last 20, and in most cases, 40 to 60 mice. Results of these experiments are included in Table II. It will be seen that none of the mice injected with aqueous solutions of penicillin were protected against an infection introduced 24 hours after the prophylactic treatment, but that 13% of the mice receiving procaine penicillin in oil and 7% of those receiving aqueous suspensions of the procaine salt retained sufficient penicillin for a 24-hour period to protect them against the pneumococcus infection given at that time. Large crystal sodium penicillin preparations protected a greater percentage of the test animals than did the small particle suspension, but, in contrast to these results, the small particle procaine penicillin in oil and aluminum monostearate was more effective than was the large crystal preparation, both in increasing the survival percentage and in prolonging the period of protection against infection. These data clearly demonstrate a remarkable superiority of small particle procaine penicillin suspended in oil and aluminum monostearate over all the other preparations tested in this manner.

Discussion. It is believed that the mouse protection test described here is a valid means of comparing the duration of therapeutic action of repository type penicillin preparations, and that it may be used to estimate the probable effectiveness of such preparations in producing prolonged plasma penicillin concentrations in human patients. Such a test has been found to be a useful tool in surveying experimental repository dosage formulas, and may be used as a guide in choosing preparations for clinical investigation.

Summary. A mouse protection test in which penicillin products are used prophylactically against subsequent challenge with Pneumococcus Type I is described. This test was used to evaluate repository type dosage forms of the antibiotic agent for their ability to prolong a period of protection against infection. Of the preparations tested the non-

repository aqueous solution of sodium penicillin was least effective, the suspensions of procaine penicillin in water or in oil were approximately equal in their activity, large particle size sodium penicillin in beeswax and oil was more effective, and small particle procaine penicillin suspended in oil and aluminum monostearate was the most effective preparation tested.

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Differentiation of Three Groups of Poliomyelitis Virus.*

JOHN F. KESSEL AND CHARLES F. PAIT.
(With the technical assistance of Robert George and Marilyn Thomas.)
From the Department of Bacteriology and Parasitology, School of Medicine, University of Southern California, and the Laboratory, Los Angeles County Hospital.

In a previous report in which 7 strains of poliomyelitis virus were compared by the method of challenging convalescent monkeys, Kessel and Pait[1] indicated that 4, BK, McK, Ca and Fr constituted one group, which was designated as Group A, or Group I.†

Two of the other 3 viruses studied, the La and Le, were distinct from the above 4 while the seventh, MV, appeared to exhibit an intermediate relationship. In 1946,[2] it was shown that La and Le were different by reciprocal neutralization tests.

Methods. In order to compare these relationships further, 4 of the viruses, McK, MV, La and Le were selected for reciprocal studies by 2 additional methods:

(a) challenge of vaccinated animals.

(b) Neutralization by pooled sera from the vaccinated animals. The vaccination was by the intramuscular route as recommended by Morgan,[3] the following schedule being used, 1.25 cc of 20% virus at 0, 1, 3 and 5 weeks, making a total of 1 g of infected monkey cord received by each animal. All animals were challenged with 100 $P.D._{50}$ of homologous virus by the intracerebral route at the seventh week. All immune animals were then challenged with 100 $P.D._{50}$ of a heterologous virus during the 13th week. Just prior to the heterologous challenge the monkeys were bled and their serums saved for neutralization

TABLE I.
Reciprocal Challenge of Vaccinated Animals.
Numerator equals number of monkeys showing symptoms, denominator equals number of monkeys inoculated.

Vaccinated with	Challenged with			
	McK	MV	La	Le
McK	0/12	1/3	4/5	*
MV	4/4	0/12	1/4	5/5
La	5/5	*	0/11	6/6
Le	*	*	2/4	0/4

100 PD_{50} used in challenge.
* Incomplete.

TABLE II.
Reciprocal Neutralization Tests.

Pooled serum from animals vaccinated with	Virus used in neutralization test			
	McK	MV	La	Le
McK	0/4	3/5	5/5	5/5
MV	4/4	0/4	0/4	4/4
La	5/5	2/4	0/5	4/5
Le	4/4	5/5	4/5	0/5
Controls	6/6	5/6	5/6	6/6

100 PD_{50} of virus was used.

* Aided by a grant from the National Foundation for Infantile Paralysis.

1 Kessel, J. F., and Pait, Charles E., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 606.

† More recently 5 additional strains, Mi, from Dr. Thomas Francis, Jr.; Gu, isolated in Los Angeles; Ko, and Cp, from Dr. William M. D. Hammon; and Br, from Dr. David Bodian, have been tested and found to belong to Group I.

2 Kessel, J. F., Moore, F. J., and Pait, Charles E., *Am. J. Hyg.*, 1946, **43**, 82.

3 Morgan, I. M., Howe, H. B., and Bodian, D., *Am. J. Hyg.*, 1947, **45**, 379.