THE THERAPEUTIC ACTIVITY OF PENICILLINS F, G, K, AND X IN EXPERIMENTAL INFECTIONS WITH PNEUMOCOCCUS TYPE I AND STREPTOCOCCUS PYOGENES

By HARRY EAGLE, M.D.

With the Technical Assistance of Arlyne D. Musselman

(From the Laboratory of Experimental Therapeutics of the United States Public Health Service and The Johns Hopkins School of Hygiene, Baltimore)

(Received for publication, September 25, 1946)

It is known that penicillin as produced in culture by strains of *Penicillium notatum* or *chrysogenum* consists of at least four distinct molecular species (1), and there is reason to suspect that additional penicillins may be present, not as yet chemically identified. There is the further possibility that by adding appropriate precursor substances to the nutrient medium, the mold may be induced to synthesize penicillins not formed on the media ordinarily used; and a large number of penicillin derivatives, biologically active, have been formed by appropriate chemical treatment of the natural product.

The question has of course arisen as to whether these penicillins differ significantly in their bactericidal activity *in vitro* and therapeutic action *in vivo*. There is already affirmative evidence on both these points. The four "natural" penicillins have been shown to vary widely in their *in vitro* activity against staphylococci (1), streptococci (2), and spirochetes (2). Further, penicillin X has been found to be several times more active than commercial penicillin against streptococci, gonococci, meningococci, and pneumococci *in vitro* (3, 4), and more effective than G also in the treatment of gonorrhea in man (3).

The present paper will describe the relative activities of penicillins F, G, K, and X in the treatment of experimental infections with *Streptococcus pyogenes* and Pneumococcus Type I. The significant differences observed suggest the desirability of using specific types of penicillins against specific infections. Apparent discrepancies between the relative bactericidal action of the several penicillins *in vitro* and their therapeutic efficacy against the same organism *in vivo* have been shown to be related to the widely varying blood levels obtained when these penicillins are administered in equal dosage.

Methods and Materials

Penicillins.—The crystalline penicillins used in these studies were generously provided by the following companies:

F—The Upjohn Company—Lot 175EANW6; G—E. R. Squibb and Sons—Lot CRA-214-20; K—Abbott Laboratories—Lot RP309P2 and 509A030; Charles Pfizer and Co., Inc.—Lot (5-2-46); X—Lederle Laboratories, Inc.—Lot CA3242-IC.

Because penicillin K was not at the time available in adequate amounts, in some of the ex-

periments a preparation was used (Abbott 506A030) containing approximately 65 per cent of inert material, but in which penicillin K was said to constitute more than 90 per cent of the contained penicillin as determined by chromatographic adsorption (13).

Bacterial Infections.—Mice weighing 18 to 22 gm., averaging 20 gm., were inoculated intraperitoneally with 0.2 cc. of diluted 18 to 24 hour blood-broth cultures of *Streptococcus pyogenes* (strain C-203) or Pneumococcus Type I. The exact dilutions used as inoculum and the minimum lethal dose represented by those dilutions are given in the tables.

Treatment with Penicillin.—Treatment with penicillin was begun 2 hours after inoculation, and was repeated every 3 hours to a total of ten injections. The dosages were varied serially twofold, using similarly varying concentrations in a fixed volume of 0.2 cc. per injection. Although the penicillin solutions were injected into the muscles of the hind leg, there was considerable leakage into the soft tissues, and the treatment should be considered a combination of intramuscular and subcutaneous administration.

Observation.—Mice were observed for 7 to 14 days before being adjudged cured. Control animals died regularly in 24 to 48 hours; and the majority of the treatment failures had died within 3 to 5 days after the last injection of penicillin.

In Vitro Assays of Bactericidal Activity of Penicillins.—The method used for the assay of streptococcidal activity has been described in detail elsewhere (2, 5).

The method used for the assay of pneumococcidal activity was a combination of turbidimetric and colorimetric, using 0.1 per cent dextrose-broth with an internal indicator (brom cresol purple in 0.003 per cent concentration). The medium was further enriched by the addition of one-tenth volume of heated (56°C. for 2 hours) rabbit serum. Varying amounts of a known dilution of penicillin in broth (0.8, 0.72, 0.06, 0.48, 0.4, etc. in a total volume of 0.8 cc.) were inoculated with 0.4 of a 1:1,000 dilution of a 24 hour culture. Results were read after 18 hours in the incubator; and the end-point was usually sharply demarcated, a clear tube with no visible growth and no change in indicator color being followed by a turbid tube in which there had been an obvious production of acid.

EXPERIMENTAL RESULTS

A. Therapeutic Activity of Penicillins F, G, K, and X in Pneumococcal and Streptococcal Infections in White Mice

Pneumococcal Infections.—In Table I are summarized the results of three experiments done at different times. Although the inoculum in those three experiments varied from a low of $\begin{matrix}>10^3\\<10^4\end{matrix}$ M.L.D. to a high of $\begin{matrix}>10^4\\<10^5\end{matrix}$ M.L.D., the results in the three experiments did not differ significantly, and they have been combined in the table. A single lot each of penicillin F, G, and X was used throughout. Two lots of penicillin K (Abbott) were used, one a purified preparation, and one in which K was said to constitute more than 90 per cent of the active material, but which contained approximately 65 per cent inert ingredients (*cf.* above). The therapeutic activity of these two preparations differed by less than 25 per cent, and they have been pooled in Table I.

The total curative doses (CD_{50}) of penicillins F, G, K, and X were 4.6, 3.8, 20, and 2.4 mg. per kg., respectively. These figures correspond to relative activities per milligram of 83, 100, 19, and 160, referred to G as 100. If the comparison is made on the basis of "units" instead of milligrams, the relative

therapeutic activities of F, G, K, and X become 92, 100, 14, and 295, respectively. However expressed, penicillin X was clearly the most active of the four penicillins, K was by far the least active, and F and G were essentially equal in activity.

TABLE I

The Therapeutic Activity of Penicillins F, G, K, and X in Pneumococcal Infections of White Mice

Mice weighing an average of 20 gm. (18 to 22 gm.) were inoculated intraperitoneally with 0.2 cc. of diluted 24 hour blood-broth culture of Type I pneumococci. Intramuscular treatment with penicillin (0.2 cc. per injection) was begun 2 hours later and repeated every 3 hours for ten doses. In three individual experiments, the dilutions used for inoculation were 1:500, 1:400, and 1:200, and these were found to contain 10^4, $\begin{matrix}>10^3\\<10^4\end{matrix}$, and $\begin{matrix}>10^4\\<10^5\end{matrix}$ M.L.D., respectively. Despite the variations in the size of the inoculum, the therapeutic results did not vary significantly, and the three experiments have been pooled in the table.

Total penicillin	Proportion of survivors with penicillins			
	F	G	K	X
mg. per kg.				
0.5	1/20	0/20		1/20
1	2/20	2/20		1/20
2	6/20	3/20	0/6	9/20
4	6/20	10/20	0/20	16/20
8	15/20	17/20	5/20	19/20
16			6/20	
32			18/20	
CD_{50}, *mg.*, *kg.**	4.6	3.8	20	2.4
Therapeutic activity, relative to G as 100,				
per mg	83	100	19	160
per "unit"	92	100	14	295

* Calculated after method of Reed and Muench (12).

Streptococcal Infections.—Two experiments in which the inocula were $\begin{matrix}>10^4\\<10^5\end{matrix}$ and $\begin{matrix}>10^5\\<10^6\end{matrix}$ M.L.D. gave similar results, and have been combined in Table II.[1]

[1] In a third small scale experiment carried out at a later date, in which the inoculum was 10^4 M.L.D., and in which the organisms were significantly less virulent, judged by the time required for death in the control animals, the curative doses of G, K, and X were consistently lower than in the first two experiments; but their relative activities referred to penicillin G as 100 (100, 12.5, and 320) agreed satisfactorily with those shown in Table II. The penicillin K used in this experiment was made by Pfizer, and was as inactive as the Abbott preparation used in the experiments of Table II.

The total curative doses (CD_{50}) of penicillins F, G, K, and X were 2.6, 1.3, 14.0, and 0.5 mg. per kg. These values correspond to relative activities of 50, 100, 9, and 260, respectively, referred to G as 100. If the penicillins are compared on the basis of units rather than milligrams, the relative therapeutic activities of F, G, K, and X become 54, 100, 7, and 480. Penicillin X was

TABLE II

The Therapeutic Activity of Penicillins F, G, K, and X in Streptococcal Infections of White Mice

Mice weighing an average of 20 gm. (18 to 22 gm.) were inoculated intraperitoneally with 0.2 cc. of a 1:20 dilution of an 18 hour blood-broth culture of *Streptococcus pyogenes* (C-203). Intramuscular treatment with penicillin was begun 2 hours later and repeated every 3 hours for 10 doses. Two experiments with quantitatively similar results have been pooled in the table. In one, the inoculum was found to represent $\begin{matrix}>10^4\\<10^5\end{matrix}$ M.L.D., and in the other $\begin{matrix}>10^5\\<10^6\end{matrix}$ M.L.D.

Total penicillin	Proportion of survivors with penicillins			
	F	G	K*	X
mg. per kg.				
0.25				1/12
0.5	0/12	0/12		6/12
1	2/12	6/12		11/12
2	4/12	10/12	0/6	5/6
4	9/12	10/12	0/12	6/6
8			0/12	
16			8/12	
32			9/12	
CD_{50}, *mg. per kg.*‡	2.6	1.3	14.0	0.5
Activity relative to G as 100				
per mg.	50	100	9	260
per "unit"	54	100	7	480

* Crystalline K (Abbott).

‡ Calculated after method of Reed and Muench (12).

again the most active of the four penicillins, K the least active (1/29th as active as X gravimetrically, and 1/68th as active as X per unit), with F and G again intermediate in activity.

B. Correlations between the Relative Bactericidal Activity of Penicillins F, G, K, and X in Vitro, and Their Therapeutic Efficacy in Vivo

If penicillins F, G, K, and X were identical in their pharmacological behavior, their therapeutic efficacy would be determined solely by their relative bactericidal activity *in vitro*. However, if one compares the activities of these

penicillins against streptococci (or pneumococci) *in vitro* with their curative doses against the same organism *in vivo*, it becomes apparent that there are consistent and significant differences between the several penicillins with respect to that ratio.

Experiments to determine the relative bactericidal activity of penicillins F, G, K, and X are summarized in Table III. The comparison of their *in vitro* activity with their therapeutic efficacy is given in Table IV.

1. As is there shown, the greatest discrepancy between bactericidal activity and therapeutic efficacy is observed with penicillin K. *In vitro*, penicillin K was 1.15 times more active than G against streptococci, and 2 times more active than G against pneumococci. Against the latter organism, it was the most

TABLE III

The Bactericidal Activities of Penicillins F, G, K, and X against Pneumococcus Type I and Streptococcus pyogenes in Vitro

(All values have been expressed relative to that of penicillin G as 100.)

Penicillin species	Relative bactericidal activity *in vitro versus*		
	Pneumococcus Type I		*Streptococcus pyogenes* (C-203)
	Individual experiments	Mean	Mean*
F	50, 67, 56, 73	60	75
G		100	100
K	160, 160, 200, 160, 200, 200	180	115
X	133, 121, 120, 160, 150	135	145

* Averages of experiments reported in a previous communication (2) and others carried out subsequently.

active of the four penicillins here studied. *In vivo*, however, it was one-fifth as active as G against pneumococci, one-eleventh as active against streptococci, and in both cases, approximately one-tenth as effective as would be implied by its direct bactericidal activity *in vitro*.

The therapeutic inactivity of penicillin K, first noted by Chesney (9) and Mahoney and Arnold (10) in the treatment of experimental syphilis, is therefore not peculiar to that disease, but is observed in any infection, and reflects the anomalous pharmacologic behavior of penicillin K. This has been considered in detail in the foregoing two papers of this series (5, 6). Like penicillins F, G, and X, penicillin K is inactivated by serum and plasma *in vitro*. Unlike those penicillins, the rate of K inactivation accelerates as its concentration is reduced; and at concentrations of less than 1 microgram per cc., an effectively bactericidal level for most organisms and the usual therapeutic range, it is inactivated so rapidly as to be of little value. Because of a similar inactivation by blood and perhaps by the tissues *in vivo*, it gives lower and more

evanescent blood curves than either F, G, or X. Thus, after the intramuscular injection in rabbits of 0.6 mg. per kg., plasma levels of 0.1 microgram per cc. or more were sustained for 0.5 hours, as compared with 1.4 for G and 2.1 for X. In man, the corresponding values after intravenous injection were 0.5, 1, and 1.3 hours. For a concentration of 0.1 microgram per cc., the disparity would be even greater. Corresponding to its rapid inactivation by serum and plasma

TABLE IV

A Comparison between the Bactericidal Activity of Penicillins F, G, K, and X in Vitro, and Their Therapeutic Efficacy in Vivo

Penicillin species	Infecting organism						Remarks
	Streptococcus pyogenes			Pneumococcus Type I			
	Bactericidal action *in vitro**	Therapeutic efficacy *in vivo*‡	Ratio§ of activity $\frac{In\ vivo}{In\ vitro}$	Bactericidal action *In vitro**	Therapeutic efficacy *In vivo*‖	Ratio§ of activity $\frac{In\ vivo}{In\ vitro^*}$	
F	75	50	0.67	60	83	1.4	
G	100	100	1.0	100	100	1.0	
K	115	9	0.08	180	19	0.11	The activity of penicillin K *in vivo* was approximately one-tenth as great as that *in vitro*
X	145	260	1.71	135	160	1.2	The therapeutic activity of penicillin X was greater than would be implied by its bactericidal activity *in vitro*

* *Cf.* Table III.

‡ *Cf.* Table II.

§ All activities have been referred to that of penicillin G as 100. Of necessity, the ratio of *in vitro: in vivo* activity for penicillin G was 1.

‖ *Cf.* Table I.

in vitro, and its rapid disappearance from the blood *in vivo*, the urinary recovery of active penicillin in rabbits averaged only 33 per cent for K, as compared with 61 for F, 87 for G, and 74 for X. The corresponding recoveries in man were 28 per cent for K, as compared with 86 and 93 per cent, for G and X, respectively.

In summary, penicillin K is rapidly inactivated by blood or plasma, and presumably in consequence of a similar inactivation *in vivo* disappears from the circulating blood far more rapidly than F, G, or X. Corresponding to its rapid inactivation, the therapeutic efficacy of K in the two infections here

studied averaged only one-tenth of its bactericidal activity against the same organisms *in vitro*.

2. In contrast to penicillin K, X was somewhat more effective therapeutically than one would have anticipated from its bactericidal action *in vitro*. Against streptococci, X was 1.45 times more active than G *in vitro*, but 2.6 times more active *in vivo* (Table II). Against pneumococci, X was 1.35 times more active than G *in vitro*, but 1.6 times more active *in vivo*. In both infections, X was more active therapeutically than would be implied by its bactericidal activity *in vitro*.

An adequate explanation is provided by the fact that, as shown in the preceding paper ((6); *cf.* also references 3, 4, 7), penicillin X regularly gives higher and more sustained blood levels than does G in equal (gravimetric) dosage. In rabbits, intramuscular injections of 0.6 mg. per kg. provided levels of 0.1 microgram per cc. for 1.4 hours with G, and 2.1 hours with X. In man, the corresponding values were 1.6 and 2.3 hours, respectively. The more sustained plasma levels probably reflect the fact (5) that penicillin X is inactivated more slowly by serum and plasma, and perhaps by the body tissues, than is; *e. g.*, penicillin G.

Although the foregoing differences seem slight, they are of considerable practical importance. The proportion of surviving organisms after exposure to a given concentration of penicillin for time t usually varies with the time as an exponential factor (8). Even if one omits from consideration the somewhat higher average levels provided by X as compared with G, a 1.5-fold difference in the time for which a given concentration is maintained may represent more than a two-fold difference in the number of organisms surviving.

In summary, in the two infections here studied, penicillin X was more effective therapeutically than its bactericidal activity *in vitro* would imply, probably because it provides somewhat higher and more sustained blood levels than penicillin G administered in equal gravimetric dosage.

C. A Comparison of the Relative Susceptibility of Streptococci and Pneumococci to Penicillin in Vitro and in Vivo

One may assume as a working hypothesis that the therapeutic efficacy of penicillin rests primarily on its direct bactericidal action, and that the defense mechanisms of the host contribute but little to that therapeutic action. Under such circumstances, the widely varying curative dose of penicillin in different infections could be determined largely by the similarly varying susceptibility of the particular organisms. This is obviously an oversimplification, and fails to take cognizance of such important factors as the varying number of organisms to be killed, their possibly varying accessibility to penicillin, and the rapidity and extent of the patient's immune response. It is therefore of interest to note that in the experiments described in the present paper, the curative dose against

a given penicillin was in fact directly related to the varying susceptibility of those organisms *in vitro*.

TABLE V

The Correlation between the Susceptibility of Streptococci and Pneumococci to Penicillin in Vitro, and the Curative Dose in Vivo

The factor under consideration is the relative susceptibility of different organisms to the *same* penicillin *in vitro* and *in vivo*. In the table, as well as in Fig. 1, comparisons are to be made reading horizontally, and not vertically.

Penicillin species	Streptococci			Pneumococci		
	Concentration of penicillin which inhibited growth *in vitro**		Curative dose *in vivo*	Concentration of penicillin which inhibited growth *in vitro*		Curative dose *in vivo*
	Individual experiments	Mean		Individual experiments	Mean	
	γ *per cc.*	γ *per cc.*	*mg. per kg.*	γ *per cc.*	γ *per cc.*	*mg. per kg.*
F	0.01 0.012 0.012 0.013 0.01 0.014 0.012 0.012	0.012	2.6	0.03 0.03 0.03 0.028	0.03	4.6
G	0.008 0.01 0.012 0.011 0.007 0.009 0.009 0.015	0.01	1.3	0.015 0.02 0.02 0.02 0.017 0.025	0.019	3.8
K	0.007 0.009 0.009 0.009 0.007 0.008 0.007 0.008	0.008	14	0.011 0.008 0.01 0.013 0.013 0.008	0.01	20
X	0.005 0.005 0.007 0.004 0.007 0.005 0.005	0.005–0.006	0.5	0.02 0.017 0.015 0.012 0.013 0.017	0.016	2.4

* Under the conditions of the *in vitro* assay technic here used.

Conclusion: The greater resistance of pneumococci to penicillin *in vitro* was regularly reflected in a higher curative dose *in vivo*.

This is shown in Table V and Fig. 1. The average minimum concentration of (*e.g.*) penicillin G which inhibited the growth of streptococci and pneumococci *in vitro* under the conditions of the assay method here used was 0.01 and 0.019 microgram per cc., respectively. Corresponding to this twofold difference in the susceptibility of the organisms *in vitro*, the curative doses of G in the two infections were 1.3 and 3.8 mg. per kg. With penicillin X, a threefold difference in the inhibiting concentration for streptococci and pneumococci

in vitro (0.005 and 0.016 microgram per cc.) was reflected in a fivefold difference in the curative dose (0.5 and 2.4 mg. per kg.); and a similar parallelism was noted with penicillin F (inhibitory concentrations of 0.012 and 0.03 microgram per cc., and curative doses of 2.6 and 4.6 mg. per kg.). Penicillin K is not considered in Fig. 1 because of the complication introduced by its rapid inactivation *in vivo*.

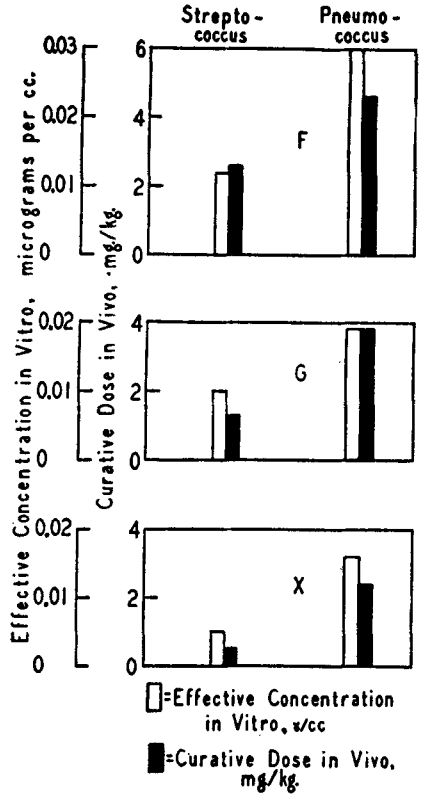

FIG. 1. The correlation between the susceptibility of streptococci and pneumococci to penicillin *in vitro* and the curative dose *in vivo* (after data of Table V).

The implication is clear that the curative dose of penicillin in a given infection is in large part determined by the susceptibility of the particular organism to penicillin *in vitro*. It must nevertheless be emphasized that in the two experimental infections here studied, the host species, the method of inoculation, and the schedule of administration were identical throughout; the type of infection was similar (a massive fulminating septicemia), and comparable numbers of organisms had to be killed in order to effect cure (inocula of the same order of magnitude, 10^4 to 10^6 M.L.D., were used in all the experiments). Although

the activity of penicillin in these controlled experimental infections paralleled its activity *in vitro*, wide differences in the inoculum or in the age of the infection would probably be reflected in a correspondingly varying curative dose; and if the organisms were at areas not readily accessible to penicillin, or if the immune response were of a different order of magnitude in time or extent, the susceptibility of the organism to penicillin might no longer be the factor which solely or even primarily determined the curative dose.

DISCUSSION

The data of the present and preceding papers indicate that in suitably controlled experiments the therapeutic activity of a given penicillin species in a given infection is materially affected by two factors: the intrinsic bactericidal action of the penicillin against the particular organism, as measured by its activity *in vitro*, and the pharmacological behavior of the penicillin *in vivo*, measured by the height and amplitude of the blood penicillin curve.

Of the four natural penicillins so far identified, X is the penicillin of choice in the treatment of streptococcal and pneumococcal infections. Not only is it intrinsically more active against these organisms than F or G ((2); page 179); but in addition, milligram for milligram, penicillin X yields higher and more sustained blood levels than those penicillins (5, 3, 4, 7)). In consequence, the curative dose of X in milligrams has been here found to be four-tenths that of G in streptococcal infections, and two-thirds that of G in pneumococcal infections. Compared on the basis of units rather than milligrams, X was therapeutically three times as active as G against pneumococci, and five times as active against streptococci. Against gonococci also, X has been found to be the most active of the four natural penicillins *in vitro* (11), and more effective than G in the treatment of the human disease (3).

In vitro, penicillin K has perhaps the highest over-all activity of all four penicillins, being the most active against staphylococci (1) and pneumococci (page 179), and the second most active against streptococci (2) and spirochetes (2). *In vivo*, however, it had only a minor therapeutic activity in two of these infections, due to the fact that it is rapidly inactivated *in vivo*, and provides significant blood levels for relatively short periods. The therapeutic inactivity of K thus has a pharmacological basis, is observed in every infection so far studied, and removes that penicillin from serious consideration as a therapeutic agent for systemic administration. It may perhaps be of value in topical application, if it is present in sufficient concentration (the rate of inactivation varies inversely with its concentration), and particularly if exudates are found to be less active than is serum in its inactivation.

The commercial production of penicillin has already gone through several stages. At the present writing, the trend is to the production of a highly refined and perhaps crystalline product, consisting almost entirely of penicillin

G. This has certain definite advantages. It permits the administration of penicillin on a gravimetric basis, as a drug, instead of on an arbitrary scale of staphylococcidal units which has no necessary meaning in other infections. Equally important, penicillin G is the major single penicillin component produced by the presently available strains of mold. The proportion of G relative to the other penicillins can be further increased by the addition of suitable precursor substances to the nutrient medium, and it is formed in the deep tank cultures which facilitate large scale production.

It must nevertheless be emphasized that in many infections, penicillin G is not the penicillin of choice. Of the three natural penicillins which are to be considered, F, G, and X, penicillin G is known to be distinctly inferior to X in the treatment of at least three infections (streptococcal, pneumococcal, and gonococcal). If the difference between G or X were solely quantitative, this would be of only minor importance. Thus, if it required 120,000 units of G as compared to 25,000 units of X to cure a case of gonorrhea, or if it required 800,000 units of G instead of 200,000 units of X to cure a case of pneumonia, the problem would simply be one of adequate dosage. There is, however, a real possibility that the difference may be qualitative as well as quantitative. It may well be that of 100 cases of subacute bacterial endocarditis, perhaps only *e.g.* 70 can be cured with G, no matter in what dosage it is employed, while perhaps 90 could be cured with X. This qualitative difference cannot be studied in animals, for in the experimental infections susceptible to penicillin, all the animals (>95 per cent) are cured if adequate doses are administered. It can, however, be studied in man, given an adequate supply of the pure penicillins.

The fact that such wide differences in bactericidal activity and pharmacological behavior have been demonstrated among the first four natural penicillins to be identified suggests that additional penicillins may yet be discovered which are more active against specific organisms, or have more favorable pharmacological properties, than any of those now available.

SUMMARY

1. The relative bactericidal activities of penicillins F, G, K, and X against Type I pneumococcus *in vitro* were 60, 100, 180, and 135. The corresponding activities against *Streptococcus pyogenes*, strain C-203, were 75, 100, 115, and 145, respectively.

2. The total curative doses (CD_{50}) of penicillins F, G, K, and X in pneumococcal infections of white mice (ten injections at 3 hour intervals) were 4.6, 3.8, 20, and 2.4 mg. per kg., respectively, or relative activities of 83, 100, 19, and 160, referred to G as 100.

3. The corresponding curative doses in streptococcal infections of white mice were 2.6, 1.3, 14.0, and 0.5 mg. per kg., or relative activities of 50, 100, 9, and 260.

4. Penicillin K was therefore one-tenth as active *in vivo* as would be implied by its bactericidal activity *in vitro*. This probably reflects its rapid inactivation *in vivo*, evidenced by the low and evanescent blood levels observed in both rabbits and man, and the low urinary recovery of this species of penicillin.

5. Penicillin X was significantly more active therapeutically than its bactericidal activity *in vitro* would imply. This probably reflects its slower inactivation *in vivo*, evidenced by the somewhat higher and more prolonged blood levels afforded by this penicillin in comparison with penicillin G. Judged by the mouse infections with the strains here used, penicillin X is the penicillin of choice in the treatment of infections with pneumococcus Type I and hemolytic streptococci.

6. The curative dose of penicillin in streptococcal and pneumococcal infections paralleled the varying susceptibility of these organisms to penicillin *in vitro*.

BIBLIOGRAPHY

1. Eagle, H., and Musselman, A. D., *Science*, 1946, **103**, 618.
2. Eagle, H., *J. Bact.*, 1946, **52**, 81.
3. Welch, H., Putnam, L. E., Randall, W. A., and Herwick, R. P., *J. Am. Med. Assn.*, 1944, **126**, 1024.
4. Ory, E. M., Meads, M., and Finland, M., *J. Am. Med. Assn.*, 1945, **129**, 257.
5. Eagle, H., *J. Exp. Med.*, 1947, **85**, 141.
6. Eagle, H., *J. Exp. Med.*, 1947, **85**, 163.
7. Coghill, R. D., Osterberg, A. E., and Hazel, G. R., *Science*, 1946, **103**, 709.
8. Eagle, H., unpublished data.
9. Chesney, A. M., personal communication.
10. Mahoney, J. F., and Arnold, R. C., personal communication.
11. Hill, J., personal communication.
12. Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.
13. Coghill, R. D., personal communication.