In the Laboratory

The Differentiation of Penicillins G and K by an Assay Method *in Vivo*

MARGARET BUCK, ANNETTE C. FARR, and R. J. SCHNITZER

Chemotherapy Laboratory, Hoffmann-La Roche, Inc. Nutley, New Jersey

The activity of penicillin toward experimental infections of mice with Borrelia formed the basis for a rapid *in vivo* assay method of penicillin used in this laboratory during the last two years.

It was found that a single subcutaneous injection of 15,000 or 25,000 units penicillin G/kg. (dependent on the strain of Borrelia used) administered to mice in the active multiplication phase of the spirochetes in the blood decreased the number of spirochetes gradually, until almost all parasites had disappeared within 3 hours. This reduction of parasites remained constant for at least 20 hours; the ratio of the 3-hour value to the 20-hour value was 1.0 or not higher than 1.1.

Recent observations by Eagle and Musselman (*2*) and Coghill, Osterberg, and Hazel (*1*) showed that penicillin K was considerably less active *in vivo* (rabbit syphilis, streptococcal and pneumococcal infections) than penicillin G and produced very low blood levels. In experimental Borrelia infections of mice penicillin K[1] was found to be much less active than penicillin G: subcutaneous treatment with doses of 15,000–50,000 units/kg. was without effect; a temporary reduction of the number of parasites in the blood was observed after administration of 100,000 units/kg.

These findings seemed to indicate that the *in vivo* assay with Borrelia might be useful for the determination of penicillin K in mixtures.[2]

Experiments were carried out with artificial mixtures of crystalline penicillin G sodium salt (supplied by M. W. Goldberg and W. E. Scott from the Roche Chemical Research Laboratories) and crystalline penicillin K (about 90 per cent pure K) prepared on a weight basis. The unitage of the mixtures was calculated on the basis of 1,666 units/mg. G and 2,000 units/mg. K and then checked by the cup test method. The figures for the potency *in vitro*, for which we are indebted to B. Tabenkin and G. Fels, were in close agreement with the calculated values, showing an occasional variation of not more than 5 per cent.

Tests were carried out with a strain of Borrelia isolated from *Ornithodorus turicata* in this laboratory about four years ago and with the laboratory strain of *Borrelia novyi* (kindly supplied by Q. M. Geiman, of Harvard Medical School). The latter strain was somewhat less sensitive toward penicillin than the younger strain isolated from ticks but produced consistently more severe infections. Only the results with this strain are presented.

PROCEDURE

Albino mice of 16–21 grams were infected intra-abdominally with 0.5 cc. of a suspension of mouse blood containing 8–10 parasites/microscope field (dark-field illumination; approx. 600 lin. magnification), corresponding to approximately 30,000 parasites/mm^3.

The infected animals showed a well-developed infection of the peripheral blood after 22–24 hours. The initial count was 700–900 parasites/100 fields. Infections with less than 400 or more than 1,500 parasites/100 fields seemed to be less suitable for this type of experiment.

The animals, divided into groups of five, received a single subcutaneous injection of 25,000 units/kg., using the solutions of the pure penicillins or their mixtures.

Counts were originally taken every hour up to 3 hours and after 20 hours. Since experience indicated that the decisive values were obtained 3 hours and 20 hours after the treatment, we confined ourselves to determining the number of parasites at these two intervals. The actual counting was done by dark-field microscopy. In cases of heavy infections it seemed sufficient to count 10–20 fields, but if the number of parasites was very small, 60–100 fields had to be examined.

RESULTS

The results as given in Table 1 are based, without exception, on at least six but generally more (up to 20) experiments. Due to the scarcity of penicillin K at our disposal fewer experiments were performed in which larger doses of K were required.

The figures representing per cent reduction from the initial count show that the activity of penicillin G was

[1] We are indebted to Charles Pfizer and Company for the penicillin K employed.

[2] In a recent publication by J. Williamson and E. M. Lourie (*Brit. med. J.*, 1946, 828–829) evidence is brought forth that penicillin III (= X) is also less active in Borrelia infections than penicillins F and G.

very consistent and produced an almost complete reduction of the number of parasites for 20 hours. Penicillin K, on the other hand, was of very low activity even if a four times higher dose was given. The presence of 30 per cent penicillin K in mixtures of G and K was evident not only by the lower initial reduction but especially by the increase of parasites after 20 hours. The ratio t 3 : t 20 was in all instances greater than 1.1. If 50 per cent K were present in the mixtures, a still greater drop in activity was observed. In the untreated controls the number of parasites increased steadily and was generally 50–100 per cent higher after 3 hours and three to five times higher than the initial count after 20 hours. Similar results were obtained with the other Borrelia strain.

TABLE 1

ASSAY OF PENICILLINS G AND K AND MIXTURES OF G AND K IN *B. novyi* INFECTIONS OF MICE
(Initial count/100 fields: 798 ± 160; single subcutaneous treatment)

Penicillin		Dose units/kg.	Reduction from initial parasite count (%)		Ratio t 3 : t 20
% G	% K		t 3*	t 20†	
100	0	25,000	98.7 ± 1.8	92.5 ± 3.6	1.06
70	30	25,000	93.4 ± 5.3	75.6 ± 9.2	1.22
50	50	25,000	84.3 ± 6.9	46.0 ± 2.3	1.83
0	90	100,000	76.0 ± 3.7	53.7 ± 11.8	1.43

* 3-hour interval.
† 20-hour interval.

From these experiments the conclusion was drawn that 25,000 units/kg. of a penicillin containing more than 70 per cent penicillin G would reduce the initial number of *B. novyi* by not less than 95 per cent (usually more) within 3 hours, the reduction lasting 20 hours.

Although there are indications that this *in vivo* assay technic might be developed to a method of higher sensitivity, it seems that approximately 30 per cent K could be determined in a mixture of active penicillins with the present procedure.

Routine assays with penicillin mixtures from production batches demonstrated that the Borrelia test carried out with crystalline G and an artificial mixture of 70 per cent G and 30 per cent K as standards was sufficiently sensitive for practical purposes, e.g. for the study of the influence of precursors.

Whether the *in vivo* assay will be preferable to the differential assay with *Bacillus subtilis* R and *Staphylococcus aureus* (*3*) for the purpose of production control cannot yet be decided. In case of artificial mixtures there was a fairly good agreement of the *in vitro* and *in vivo* determinations.

The question arose whether other *in vivo* assay methods, e.g. with bacterial infections, could be used for the determination of penicillin K. The low activity of penicillin K in pneumococcal and streptococcal infections as demonstrated by Eagle and Musselman (*2*) seemed to suggest such a possibility. It may be seen from Table 2 that the presence of 50 per cent K in an artificial mixture of pure penicillins could easily be detected; the presence of 30 per cent K did, however, not interfere significantly with the antibacterial activity.

TABLE 2

ACTIVITY OF PENICILLINS G AND K AND THEIR MIXTURES IN EXPERIMENTAL INFECTIONS OF MICE WITH 1,000 MLD OF TYPE 1 PNEUMOCOCCI (STRAIN 6301) AND β-HEMOLYTIC STREPTOCOCCI (STRAIN #4)

Penicillin		Total dose units/kg.	Organism	Number of mice survivors		Survivors (%)
% G	% K					
100	0	3,000	type 1 pneumococci	20	14	70
75–70	25–30	3,000		10	9	90
50	50	3,000		10	2	20
25	75	3,000		10	3	30
0	90	6,000		10	1	10
0	90	12,000		10	4	40
..	..	Controls		20	0	0
100	0	1,000	β-hemolytic streptococci	10	9	90
70	30	1,000		10	7	70
50	50	1,000		10	4	40
..	..	Controls		10	0	0

Even if it would be possible to increase the sensitivity of an *in vivo* assay method in bacterial infections sufficiently for the determination of smaller quantities of penicillin K, these methods would always require at least a five-day observation period before a definite result could be obtained.

The advantage of the Borrelia assay technic is that it requires no more time than the *in vitro* methods.

References

1. COGHILL, R. D., OSTERBERG, A. E., and HAZEL, G. R. *Science*, 1946, **103**, 709–710.
2. EAGLE, H., and MUSSELMAN, A. *Science*, 1946, **103**, 618–620.
3. SCHMIDT, W. H., WARD, G. E., and COGHILL, R. D. *J. Bact.*, 1945, **49**, 411–412.

Apparatus for the Prolonged Sterile Culture *in Vitro* of Whole Plants or Excised Plant Tissues

R. S. DE ROPP[1]

Department of Animal and Plant Pathology
The Rockefeller Institute for Medical Research
Princeton, New Jersey

An apparatus for prolonged sterile culture *in vitro* of whole plants or excised plant tissues should meet

[1] Research officer, Agricultural Research Council, Great Britain, and visiting investigator, The Rockefeller Institute for Medical Research.