MICROBIOLOGICAL STUDIES ON SODIUM 6-(2,6 DIMETHOXYBENZAMIDO) PENICILLANATE MONOHYDRATE (BRL 1241) IN VITRO AND IN PATIENTS

BY

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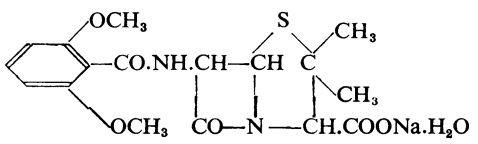
A report in 1959 by Batchelor *et al.* on the isolation of 6-aminopenicillanic acid drew attention to the possibility of synthesizing new forms of penicillin by the introduction of side-chains. Derivatives prepared in this way may or may not possess antibacterial activity, but we were particularly impressed by the range and mode of action of one derivative, supplied to us in 1959 as BRL 1241 ("celbenin"). The compound—sodium 6-(2,6 dimethoxybenzamido)penicillanate monohydrate—may be represented by the following structural formula:



In the report which follows, this compound is compared with natural penicillins and with phenoxypropionamidopenicillin ("broxil"), another synthetic derivative of 6-aminopenicillanic acid.

Our results led us at an early stage to believe that BRL 1241 was a compound of therapeutic importance. Part of the present report is therefore concerned with results obtained in patients treated with it in this hospital as described in the accompanying publication (Stewart *et al.*, 1960).

Methods

Sensitivity tests in liquid and in solid media were performed by standard techniques with the assay nutrient broth and agar supplied by the Southern Group Laboratories.

(*a*) In liquid media, the various penicillins were made up in broth on a w/v basis from refrigerated stock solutions. The bactericidal or bacteriostatic effects were compared by colony-counts of aliquots from 3-ml. control and test cultures at various intervals after inoculation. To assess sterility of a clear culture, the entire volume was added to 200 ml. of broth containing an excess of penicillinase (see below). In some tests the broth was reinforced by the addition of a thin layer of human or horse blood.

(*b*) Solid media were used for screening tests by impregnating 10-mm. disks of Ministry of Health blotting-paper No. 8A 08371 with 1 μg., 10 μg., or more of the drug under test. The organisms were grown on pour-plates, reinforced by blood products when necessary (haemophilus, pneumococcus, etc.) and the inhibitory zones measured with callipers.

(*c*) A few tests of lysis were performed by adding a suspension of washed bacterial cells to concentrations of the drugs in M/15 phosphate buffer at *p*H 7 and reading the changes in turbidity of the suspensions at 575 mμ.

Assays of BRL 1241 in body fluids were performed on large assay-plates, seeded with a test organism, usually *Sarcina lutea*. The unknown fluid was compared in each assay with a range of controls in water, serum, plasma, or albumin, and the result read by interpolation from the linear semi-log graph of concentration against zone diameter. In the case of urine the sample was heated to 75° for 10 minutes to minimize contamination and diluted with water to 1:100 or more.

Chromatography of benzylpenicillin and BRL 1241 in urine was performed by the ascending technique on 25-cm. squares of Whatman No. 1 paper, using as solvent the top layer of butanol-ethanol-phosphate buffer (40:20:50) mixture. After an overnight run, the chromatograms were dried out and "developed" on assay-plates seeded with *Sarcina lutea* in agar.

Penicillins.—BRL 1241 and phenoxypropionamidopenicillin were supplied as pure amorphous powders. Phenoxymethylpenicillin (penicillin V) and benzylpenicillin (penicillin G) were obtained in the crystalline form. All comparative tests were made on a w/v basis. Dosage of BRL 1241 in patients was normally 100 mg./kg./day in four six-hourly doses.

BRL 1241 was supplied to us as a pale cream slightly hygroscopic powder, absorbing 0.5% moisture at room temperature in 24 hours. It was remarkably soluble in cold water, from which it did not precipitate on boiling. The resulting solution was neutral and the solubility decreased with fall of *p*H, sharply at *p*H 4 or lower. The acid precipitate redissolved readily in alkali. In solution at *p*H 7, BRL 1241 lost 30% of its potency after 10 minutes at 80° C., and 75% at 100° C., being in this respect rather less heat-labile than benzylpenicillin. When dried on paper disks, BRL 1241 was much less heat-labile, retaining approximately 75% of its potency after one hour at 160° C.

Penicillinase was obtained in the form of a broth filtrate of *Bacillus licheniformis* and crystalline "neutrapen," in which the penicillinase is derived from *Bacillus cereus*. A number of experiments were also performed with penicillinase-forming strains of *Staphylococcus aureus*.

Inoculum.—As a routine, the inoculum used in our experiments was a standard drop of a 24-hour culture of the organism in appropriate medium, diluted sufficiently to cause no visible turbidity in the test media. In the case of routine tests with *Staph. aureus*, the final inoculum produced by this means was 200,000-300,000 viable organisms per ml. of test medium. In particular experiments the inoculum was varied from 600 organisms per ml. up to 500 million organisms per ml. to test the influence of inoculum-size.

Results

A preliminary survey of the comparative sensitivities of various pathogens, isolated consecutively and without selection from patients admitted to this hospital, showed that there were some important differences between the four penicillins (Table I). Thus, 43% of strains of *Staph. aureus* were completely resistant by paper-disk assay to 10 μg. of benzylpenicillin, whereas all of these strains were inhibited by this quantity or less of BRL 1241 and an intermediate figure (74%) by phenoxypropionamidopenicillin. On the other hand, the other pyogenic Gram-positive cocci were, in general, more

TABLE I.—*Effect of Four Forms of Penicillin Upon a Number of Pathogenic Bacteria, Consecutively Isolated from Patients, Excluding Re-isolations. The Figures Indicate No. Sensitive /No. Tested.*

Bacteria	μg. Drug in Assay Disks	Benzyl-penicillin	Phenoxy-methyl-benzyl-penicillin	Phenoxy-propion-amido-penicillin	BRL 1241	% Sensitive to BRL 1241
Staph. aureus	10	171/301	100/137	223/301	301/301	100
,, *albus*	10	35/42	27/37	36/42	42/42	100
Haemolytic streptococcus group A ..	1	108/108	52/52	108/108	108/108	100
Haemolytic streptococcus group D ..	10	8/12	4/6	7/12	0/12	0
Haemolytic streptococcus other groups	1	6/6	6/6	6/6	6/6	100
Non-haemolytic streptococcus	10	7/12	4/8	6/12	7/12	58
Pneumococcus ..	1	60/60	30/30	60/60	60/60	100
Proteus		7/8	0/2	0/8	0/8	0
Coliforms	10	2/14	0/5	0/14	0/14	0
Ps. pyocyanea ..		0/6	0/2	0/6	0/6	0
Shigella and Salmonella		0/7	0/2	0/7	0/7	0
Haemophilus	10	14/17	7/10	6/17	14/17	82
Clostridium welchii ..	1	1/1	1/1	1/1	1/1	

sensitive to the natural penicillins than to the two synthetic penicillins. Streptococci of group A and pneumococci (various types) were uniformly sensitive to the synthetic penicillins, as to benzylpenicillin, but other streptococci were as often as not resistant to BRL 1241. Gram-negative bacteria were uniformly—and highly—resistant to BRL 1241 except for some strains of haemophilus.

Tests were then performed in nutrient and non-nutrient liquid media to distinguish between the bactericidal and bacteriostatic powers of the different penicillins. Since the exact molecular weight of compound BRL 1241 was at that time unknown to us, we compared the compounds in w/v solution on the assumption that their molecular weights were fairly close to each other. These tests showed that there were important differences between the actions of the penicillins upon different organisms. In general, the two natural penicillins were similar to each other but different in some important respects from the synthetic penicillins.

Staphylococcus aureus

The most important finding was the remarkable uniformity of all our strains in their sensitivity to BRL 1241. Irrespective of their level of resistance to benzylpenicillin, these strains were all inhibited in liquid media by 1–2.5 μg./ml. Many of the strains tested (Fig. 1) were resistant to relatively high concentrations (20 μg./ml. or more) of benzylpenicillin and many were resistant in addition to the tetracyclines and other antibiotics. All were isolated from patients with active infections, and many of the strains were penicillinase-formers from hospital-acquired infections, including some due to type 80.

BRL 1241 was invariably bactericidal in its action, often more so than the other penicillins (Fig. 2), though never to the extent of killing all the cells in an inoculum. The rate of action was relatively constant. At its optimal concentration of 2.5–5 μg./ml. BRL 1241 killed approximately half the cells of an inoculum in nutrient media in one hour, 90–95% in two hours, and 95–99% in four hours (Fig. 3). This bactericidal action was slower and of a lower order than that of benzylpenicillin upon sensitive strains, but it was uniform with all strains tested.

In its action against *Staph. aureus*, phenoxypropionamidopenicillin occupied an intermediate position between benzylpenicillin and BRL 1241. At 2.5–10 μg./ml. it inhibited many, though not all, strains resistant to these concentrations of benzylpenicillin. At low concentrations (less than 1 μg./ml.) it was less rapidly bactericidal toward sensitive organisms than benzylpenicillin. Phenoxymethylpenicillin showed, in general, only minor differences from benzylpenicillin.

Experiments were performed with suspensions of washed cells of *Staph. aureus* in non-nutrient M/15 phosphate buffer at *p*H 7. Addition of any of the penicillins produced some acceleration in the natural death rate of the bacterial cells, under these conditions, but no significant reduction in the turbidity of suspensions in a four-hour period, indicating that the bactericidal effect upon resting cells was not accompanied by immediate lysis of the cell-substance.

Streptococcus Group A

All four penicillins had a bactericidal action upon all strains tested. The extreme sensitivity shown by this organism toward low concentrations (0.01–0.1 μg./ml.) of benzylpenicillin was not demonstrable in the case of the two 6-aminopenicillanic acid derivatives, but there was a marked uniformity of the inhibitory level, particularly of BRL 1241, at 0.1–1 μg./ml. The bactericidal action of BRL 1241 was less rapid than that of benzylpenicillin (Fig. 4), but was sometimes more complete; with two strains, partial sterility was easily and rapidly produced in growing 3-ml. cultures by concentrations of 0.01–5 μg. benzylpenicillin/ml., but a few residual

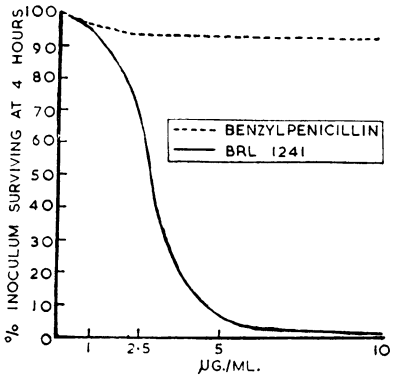


FIG. 1

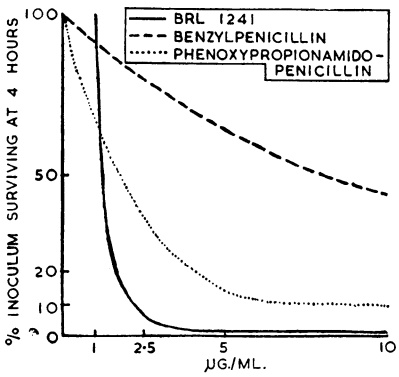


FIG. 2

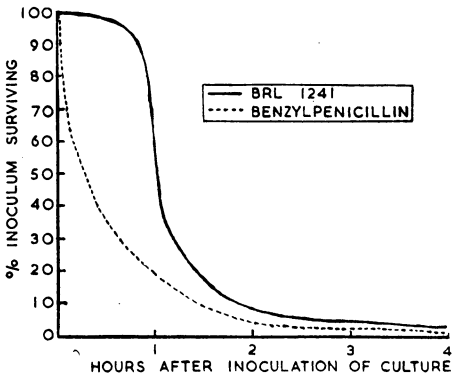


FIG. 3

FIG. 1.—Comparison of bactericidal action of BRL 1241 and benzylpenicillin upon a penicillin-resistant strain of *Staph. aureus*. FIG. 2.—Comparison of bactericidal action of three penicillins upon a partially sensitive strain of *Staph. aureus*. FIG. 3.—Rate of bactericidal action of BRL 1241 and benzylpenicillin upon a penicillin-sensitive strain of *Staph. aureus*.

staphylococci were found to be surviving after 24 hours, when the entire volume was transplanted to 200 ml. of broth and subcultured; whereas, with BRL 1241 (5 μg./ml.), 24 hours' incubation produced under similar conditions complete sterility of these strains.

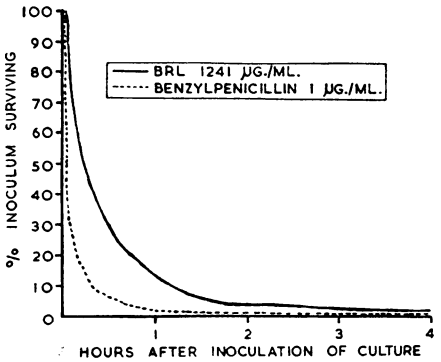


FIG. 4

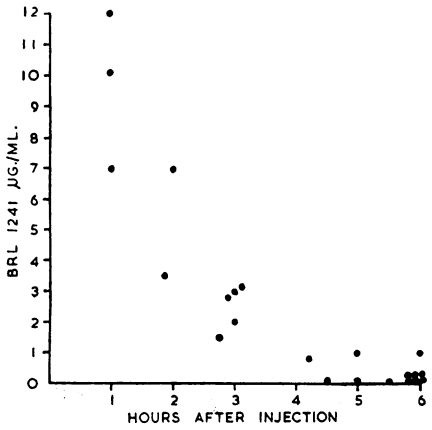


FIG. 5

FIG. 4.—Comparison of bactericidal action of BRL 1241 and benzylpenicillin upon a streptococcus of group A. FIG. 5.—Concentration of BRL 1241 in the sera of children receiving 100 mg./kg./day in four six-hourly intramuscular injections.

Streptococci of other groups did not show the uniform sensitivity of Group A strains, and were less sensitive to BRL 1241 than to either phenoxypropionamidopenicillin or benzylpenicillin. Group D streptococci appeared to be particularly resistant.

Pneumococcus

All strains tested were sensitive to all the penicillins, in a fashion similar to streptococci of group A. In terms of effective concentrations, the order of activity was benzylpenicillin > phenoxypropionamidopenicillin > BRL 1241.

All the penicillins had a strong bactericidal effect, demonstrable in two hours or less with benzylpenicillin and two to four hours with the others. Cultures of types 9 and 19 were completely sterilized by benzylpenicillin and BRL 1241; cultures of types 3 and 18 were sterilized by benzylpenicillin but not by BRL 1241.

To check this difference *in vivo*, groups of mice were infected with pneumococci of types 3 and 9 and treated with benzylpenicillin and BRL 1241. The results (Table II) showed that BRL 1241 was again less active than benzylpenicillin.

TABLE II.—*Effect of Three Daily Subcutaneous Injections of BRL 1241 (100 mg./kg.) Compared with Benzylpenicillin (100 mg./kg.) in Mice Infected Intraperitoneally with Pneumococci of Types 3 and 9*

Drug	No. of Mice Infected	No. Surviving	
		2 Days	4 Days
Benzylpenicillin ..	10	9	8
BRL 1241 ..	11	9	3
Nil	11	4	2

Comparison Between Tests in Solid and Liquid Media

The precise concentration of drug required to produce bactericidal action, and the extent of this action, can only be assessed in liquid media. For screening purposes, however, solid media are much more convenient. Under the conditions described above, we found that 10 μg. of BRL 1241 dried on a 10-mm. blotting-paper disk produced a 16–30 mm. clear inhibitory zone on blood-agar pour-plates seeded with any strain of *Staph. aureus*. This corresponded to a bactericidal concentration of about 2.5 μg./ml. in nutrient broth. With group A streptococci and pneumococci, zones of 20–35 mm. were constantly produced in pour-plates by 1 μg. of BRL 1241 on a disk. "Halo" effects at the perimeters of the zones were common with BRL 1241 as with benzylpenicillin, but not with phenoxypropionamidopenicillin.

In our experience, paper disks have always been unreliable for assessment of sensitivity of staphylococci to benzylpenicillin in that some organisms resistant to disks at 10 μg. may be readily inhibited by lower concentrations in liquid media; alternatively, a large inoculum of a penicillin-destroying organism may grow after a few hours in liquid media more vigorously than around a 10-μg. disk. These discrepancies, which are doubtless due to the cumulative variables of inoculum-size, penicillinase-formation, and the emergence of resistant persisters, were much less liable to occur with BRL 1241 than with benzylpenicillin or phenoxypropionamidopenicillin. For practical purposes, therefore, disks of 1 μg. and 10 μg. of BRL 1241 were reasonably reliable for screening purposes, and were comparable to inhibitory concentrations of 1–2.5 μg./ml. which were readily attainable in the plasma.

Effect of Penicillinase

Penicillinase was added, in varying concentration, to cultures of *Staph. aureus* and other organisms containing graded concentrations of the different penicillins. It was found that, in general, penicillinase was much less active against BRL 1241 than against benzylpenicillin, while phenoxypropionamidopenicillin occupied an intermediate position. Within this broad limit, there was considerable variation in the interaction of the enzyme and its substrates, according to the origin and amount of enzyme added, the stage of growth and size of inoculum in a culture and the strain of organism used, among other factors.

The relative immunity of BRL 1241 to staphylococcal penicillinase is best shown by the low concentration (2.5 μg./ml.) at which it inhibits many, if not all, strains of penicillinase-forming staphylococci, even when a very large inoculum is added; it does, however, act as a penicillinase-inducer and as a substrate, and after 24–48 hours' growth it may suffer some inactivation (Table III) while a potent penicillinase may be demonstrable in the filtrate.

With therapeutic concentrations of BRL 1241 (2.5–5 μg./ml.) this enzymic inactivation was insufficient to

TABLE III.—*Comparison of Effect of Benzylpenicillin and BRL 1241 Upon a Penicillinase-producing Strain of Staph. aureus*

Type of Penicillin	Minimum Inhibitory Conc. (μg./ml.) (100,000 Organisms/ml.)	Inoculum (Organisms/ml.) Giving, in 10 μg./ml.		% Penicillin Inactivated by Growth at Sub-inhibitory Concentration
		Full Growth	No Growth	
Benzylpenicillin ..	25	100,000	1,650	100
BRL 1241 ..	2·5	?	> 500 million	10

inhibit the antibacterial effect during the first 24 hours' incubation, though not thereafter. In this respect, inoculum size was of little importance (Table III), for, whereas benzylpenicillin would inhibit a penicillin-forming organism only in a critically small inoculum, BRL 1241 maintained its bactericidal effect, in the therapeutic range, more or less irrespective of the inoculum-size in nutrient media, serum, plasma, and whole blood.

Bioassays after incubation with two other forms of penicillinase, derived from *B. licheniformis* and *B. cereus*, showed that, despite its resistance to staphylococcal penicillinase, BRL 1241 could be completely inactivated (Table IV). Penicillinase from *B. cereus* was more active in this respect against BRL 1241, though still much less active than against benzylpenicillin.

TABLE IV.—*Effect of Different Forms of Penicillinase Upon Benzylpenicillin and BRL* 1241

Type of Penicillinase	Minimum Concentration Inactivating 1 μg. of	
	Benzylpenicillin	BRL 1241
B. licheniformis	1 unit	100 units
B. cereus	0·1 ,,	10 ,,

To test the effect of additional sources of penicillinase, as in mixed infections, a comparison of benzylpenicillin and BRL 1241 was made upon growing cultures of penicillinase-forming staphylococci and penicillin-sensitive staphylococci, with and without added penicillinase. This experiment showed (Table V) that BRL 1241 retained its inhibitory power in the presence of two independent types of penicillinase.

TABLE V.—*Effect of Penicillinase from B. cereus and Penicillinase from Staph aureus Upon Benzylpenicillin and BRL* 1241

Strain	Penicillinase (Units) Required to Neutralize 1 μg. of:	
	Benzylpenicillin	BRL 1241
Penicillinase-forming (H.99) ..	1·0	10
,, ,, (902) ..	0·01	10
Penicillin-sensitive (Oxford) ..	1·0	10
,, ,, (57)	1·0	50

When solutions of benzylpenicillin and BRL 1241 were incubated at 37° C. with appropriate concentrations of (cereus) penicillinase, assays of the mixtures at intervals showed that benzylpenicillin was partly inactivated in 15 minutes and completely inactivated in one hour, whereas BRL 1241 did not begin to lose potency until two to four hours.

Tests for Drug Resistance

Attempts were made to induce two strains of *Staph. aureus* to become resistant to BRL 1241 *in vitro* by repeated passage through liquid and solid media containing rising concentrations of the compound. This process was started by subculturing cells which had been exposed for 48 hours to a sub-inhibitory concentration of 1 μg./ml. in broth into progressively higher concentrations (2.5, 5, 10, and 25 μg./ml.) for 48-hour periods at 37° C., after which the (fractional) surviving inoculum was reassayed against BRL 1241 along with a control line of the same strain which had not been exposed to the drug. By this technique no resistance developed *in vitro*; the test line firmly retained its original sensitivity to 2.5 μg./ml. of the drug.

A second set of experiments was then performed on solid media, by subculturing colonies growing nearest to the edge of a ditch containing BRL 1241 (10 μg./ml.) in agar on to agar containing a gradient of concentration up to 25 μg./ml. By this means the organism was "trained" to grow to the edge of the original (10 μg./ml.) ditch but no further. No growth occurred, in any media, at higher concentrations. The "trained" colonies retained their normal morphology and biochemical reactions on subculture on drug-free media.

Thirdly, all strains of *Staph. aureus* re-isolated from patients receiving BRL 1241 were reassayed for sensitivity. These re-isolated organisms mainly came from nasal swabs, since the main lesions were usually sterilized fairly rapidly, but in a few instances organisms were obtained also from the lesions during the first two to four days of treatment. Again no evidence of acquired resistance was found, even in organisms re-isolated (from the nose) after two weeks of treatment. In four cases streptococci of group A were also reassayed similarly: in two cases after re-isolation from the lesions during the first three days of treatment and in two other cases on relapse immediately after five-day courses. Again there was no change in the minimum inhibitory level of the drug *in vitro*.

Effect of Combinations of Drugs

Benzylpenicillin and BRL 1241 were tested in "chessboard" fashion in combination, each with the other and with streptomycin, tetracycline, erythromycin, and chloramphenicol. Two strains of *Staph. aureus* were used as test organisms, and the drugs were tested at concentrations of 1 μg./ml. and 2 μg./ml. individually and 1 μg./ml. of each of a pair in combination. The results showed that there was no additive or synergistic effect between the two penicillins or between these drugs and streptomycin or tetracycline. The immediate bactericidal action of the two penicillins was, if anything, weaker in the presence of erythromycin and chloramphenicol. None of these drugs showed any interference with the bacteriostatic actions of the two penicillins over a 24-hour period of incubation together.

In the case of a penicillinase-forming strain of *Staph. aureus* highly resistant to benzylpenicillin, there was again no evidence of an additive or synergistic effect between this antibiotic and BRL 1241. Under these conditions BRL 1241 produced the same degree of inhibition at 2 μg./ml. alone as it did in the presence of benzylpenicillin.

Assay of BRL 1241 in Body Fluids

As we were dealing with children, we did not attempt to assay repeated samples of blood from any one patient. Individual samples were, however, taken from a number of children at various intervals after injection, and, since dosage was standardized at 100 mg./kg./day, these samples give some indication of the levels of the drug obtained in the serum. In some instances, sera and plasmas from the same samples were assayed together, but there were no obvious differences in the concentrations of the drug. For this purpose, standards were made in normal serum and plasma as well as in distilled water.

The results (Fig. 5) showed that inhibitory levels of BRL 1241 (1 μg./ml. or more) were regularly detectable in the serum three to four hours after injection but not usually thereafter. In one patient, a temporary rise of dose to 150 mg./kg./day produced a level of 1.5 μg./ml. after six hours.

Assays of urine showed that large quantities of the drug were rapidly excreted. In 12-hour samples of urine, 60–75% of the two doses of drugs injected during that period could regularly be recovered, much of it during the three hours after injection. The urinary excretion was assayed serially in four children receiving 10- and 20-day courses of injections; in each case the concentration of drug dropped rapidly in the 12-hour sample collected after the last injection, and in subsequent 12-hour samples collected during the next three days no drug was detectable. Chromatographic studies

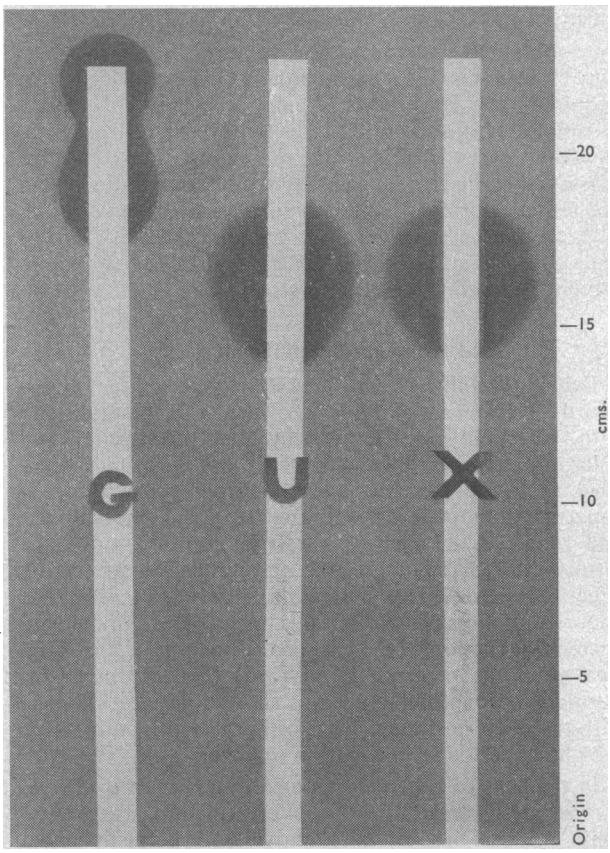


FIG. 6.—Chromatographic bio-assay of urine from a patient receiving BRL 1241 (100 mg./kg./day). The clear areas indicate the zones of migration of BRL 1241 as excreted in the patient's urine (U) compared with BRL 1241 (X) and benzylpenicillin (G) added to normal urine. The Rf is calculated from the scale (cm.) on the right. For details of method, see text.

(Fig. 6) on the urine indicated that BRL 1241 was excreted mainly or entirely in unchanged form, and that no active metabolite was found.

Five samples of C.S.F. were assayed in four patients receiving 100 mg./kg./day at intervals of half to five and a half hours after injection. In no instance was an effective inhibitory concentration detected. In another patient receiving the same dose, a haematoma, remote from the site of injection, was aspirated. On assay, this gave a concentration of 10 μg./ml.

Samples of bile, obtained by duodenal intubation, were taken from a patient who had received a dose of 1 g. of BRL 1241 an hour previously. Aliquots of three samples (which were being obtained for another diagnostic purpose), taken at 75, 80, and 90 minutes after injection, were assayed and showed a content of 3–4.5 μg./ml. During this time 15 ml. of bile was easily withdrawn, indicating that the excretion of the drug in the bile in a period of 15 minutes was at least 53 μg.

Hypersensitivity

A test injection of 10 μg. of BRL 1241 was given intradermally to one adult volunteer who was known to be hypersensitive to benzylpenicillin and in whom this quantity of benzylpenicillin had provoked a violent urticarial reaction a few months previously. The response to BRL 1241 was identical—immediate local redness, then oedema, followed by an extensive urticarial rash of the arm and shoulder in the course of the next two hours. Ephedrine and " piriton " were given during this time, and the allergic reaction gradually subsided.

Discussion

These results show that BRL 1241 has a pronounced bactericidal action against all strains of *Staph. aureus* freshly isolated from lesions in 301 children in hospital, including strains which are resistant to benzylpenicillin and form penicillinase. This bactericidal level is remarkably constant, at 2.5–5 μg./ml., in liquid media including serum, plasma, and whole blood, and persists in the presence of large inocula of penicillinase-forming organisms, and, to a considerable extent, with two independent forms of penicillinase acting together.

The action of the drug on staphylococci has therefore two components: (*a*) an intrinsic bactericidal power, and (*b*) an ability to withstand breakdown by various forms of penicillinase. The bactericidal action is slower and less complete than that of benzylpenicillin against strains sensitive to both drugs, but the fraction of inoculated cells (5–10%) which persist for a few hours thereafter show no lessening of their intrinsic sensitivity, and apparently only a very restricted capacity to develop resistance by serial passage through the drug. This inability to develop resistance *in vitro* is paralleled, in these studies, by a comparable inability on the part of pathogenic staphylococci re-isolated from patients treated with BRL 1241 to develop resistance *in vivo*.

The other synthetic penicillin tested in these experiments—phenoxypropionamidopenicillin—shows properties intermediate to those of the natural benzylpenicillins (G and V) and BRL 1241; it is active *in vitro* against a higher proportion of strains of *Staph. aureus* than benzylpenicillin and is less readily destroyed by penicillinase, though the superiority under either heading is marginal and far short of BRL 1241. The similarity of trend in the two synthetic penicillins does, however, suggest that the presence of an appropriate side-chain in the 6-amino position may be an important key to the mode of action of the penicillins and of penicillinase, for which both compounds are good inducers, despite their immunity to it.

The bactericidal activity of BRL 1241 extends to other Gram-positive cocci, including many if not all strains of group A streptococci and pneumococci. As with staphylococci, BRL 1241 is less rapid in action than benzylpenicillin, but it may sometimes be equally complete in that it is capable of sterilizing a relatively large inoculum of some strains of streptococci and pneumococci. There is, however, little or no useful activity against Gram-negative bacteria against which benzylpenicillin often shows a measurable effect, *in vivo* and *in vitro*.

Inhibitory levels of BRL 1241 (2.5 μg./ml. or more) are readily attained in the plasma and are retained for three to four hours when six-hourly injections at the rate of 100 mg./kg./day are given to children. The peak, in the first hour after injection, is much higher, but the

drug does not appear to pass into the C.S.F. during this time. A high proportion of an injected dose is excreted within a few hours in the urine in a form which appears to be chemically and microbiologically unchanged. At the end of a course of treatment there is apparently no retention of an excretable product beyond 12 hours in children. Further studies will be required to elucidate the fate of the remaining 30–40% of the injected dose not detected in the urine. From the amount detected in the bile, it would appear that very little is metabolized. In rats, Harrison and Stewart (1960) found that the concentration in the bile during the first two hours after an injection was one-third to one-sixth that in the urine. The result reported here indicates that a considerable proportion of the drug is excreted via the bile in man also, so that obviously very little, if any, is retained or metabolized in the body.

The results obtained *in vitro* with staphylococci and streptococci of group A, together with the fact that inhibitory levels can readily be attained in the plasma, indicate that BRL 1241 should be active therapeutically in infections due to these organisms, and this is borne out by the clinical results obtained in the accompanying paper (p. 703).

The compound also possesses a promising degree of activity—as high as that of the tetracyclines and erythromycin—against the pneumococcus, but the results obtained in mice infected experimentally with this organism show that it is much less active than benzylpenicillin. Its intrinsic bactericidal activity, even against sensitive organisms, is therefore of a lower order than that of benzylpenicillin and its range is narrower. Furthermore, injections have to be given at least six-hourly to maintain effective plasma concentrations, and there is no evidence that the compound is active by mouth.

There is close agreement in the results presented by the other contributors (Rolinson *et al.*, 1960; Knox, 1960; Thompson *et al.*, 1960; Elek and Fleming, 1960) that all strains of *staph. aureus*, from a variety of sources, are uniformly sensitive to BRL 1241. In tests on solid media, as in our experiments and in those of Elek and Fleming (1960), drug resistance develops with difficulty, if at all, when *Staph. aureus* is exposed to a gradient of increasing concentration. In liquid media, on the other hand, as in the experiments of Knox (1960) and Rolinson *et al.* (1960), a proportion of the cells in a given inoculum acquire resistance fairly readily. This discrepancy *in vitro* probably arises from differences in technique, and, from the practical viewpoint, the stringent tests applied by Elek and Fleming in their spraying experiments proves that resistance does not develop easily *in vivo*. This result is in keeping with our finding that specific strains of *Staph. aureus* re-isolated from patients treated with BRL 1241 show no decrease in sensitivity to the drug.

It is obviously impossible at the present stage to define the full therapeutic role of BRL 1241, but the evidence at present available shows good correlation between results obtained *in vitro* and *in vivo*. On this basis it is reasonable to conclude that the compound has a high and unusually consistent degree of activity against staphylococci and possibly some other pyogenic cocci in concentrations which can readily be attained in the serum.

Summary

BRL 1241, a synthetic derivative of 6-aminopenicillanic acid, has a strong bactericidal action on *Staphylococcus aureus*, streptococci of group A, and other pyogenic cocci in concentrations of 1–5 μg./ml.

Concentrations of this order are readily attained in the serum when children are given four intramuscular injections at a dose of 100 mg./kg./day. Two-thirds of this dosage is excreted in the urine unchanged within a few hours of injection, while a considerable proportion of the remainder is excreted in the bile.

BRL 1241 is much more resistant to the action of staphylococcal penicillinase, and other forms of penicillinase, than benzylpenicillin. It is as active against penicillinase-forming staphylococci as against penicillin-sensitive staphylococci.

In short-term experiments and clinical trials, staphylococci and streptococci did not acquire significant resistance *in vitro* or *in vivo* to the drug.

Allergic hypersensitivity to benzylpenicillin was accompanied in one patient by comparable hypersensitivity to BRL 1241.

In common with another derivative of 6-aminopenicillanic acid, BRL 1241 shows a narrower range of activity and less intrinsic activity than natural penicillins against various bacteria. The main place of these synthetic compounds at present is therefore in the treatment of staphylococcal infections.

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From time to time mental health officers and psychiatric social workers have asked the Council of Social Services to find homes, under the boarding-out scheme, for elderly people who are, or have been, patients in mental hospitals. During the past six years homes have been found for 10 such elderly people, one of whom had been in hospital for 20 years. The procedure for boarding out people from mental hospitals is the same as for any elderly person. A social worker from the Council of Social Service visits them and gets to know them well, suggests a home and takes them to see it. The only difference is that these elderly people perhaps need more support during and immediately after the move, or in any time of minor crisis such as the holiday of the people with whom they live. In four cases there has been a recurrence of mental illness necessitating further hospital treatment, but in two cases the patients were able to leave again after a short period. Two elderly people from mental hospitals have been boarded out happily for six years and the others for periods ranging from one to three years. The success of this experiment has led in the past two years to the Council of Social Service being asked to help to find homes for younger people who have had a mental illness. Nine people under 50 years of age have been found homes, and support has been given in much the same way as with elderly people. The understanding and patient help of the people who have offered homes has been very largely responsible for the happy arrangements that have been made. (Exeter Council of Social Service: "Mental Health—New Needs and Opportunities.")