(2,500 mg./kg. bodyweight). These results are shown in Fig. 3. It is clear that the increased dosage has greatly increased the effectiveness of the treatment.

## Discussion

The results obtained show that this compound is effective in the treatment of *Staph. pyogenes* infections in mice. When its activity was compared with other antibiotics it was less active than erythromycin and of comparable activity to chloramphenicol and streptomycin. However, in the case of erythromycin it is worth noting that 50 mg./kg. is a large dose, and a comparatively small increase would be apt to give rise to toxic symptoms in the animals, but the dosage of BRL 1241 could be greatly increased without risk of intoxication (Brown and Acred, 1960). The surprisingly satisfactory response to streptomycin in view of the *in vitro* resistance of the organism to this drug may be explained by the fact that the intracellular streptomycin level is only about one-tenth that of the extracellular level (Mackaness, 1952), and consequently the extracellular level (in effect the therapeutic level) would be effective in combating the infection.

The exceedingly low toxicity of BRL 1241 (mice have been given single inoculations of 0.5 g. without ill effect) prompted us to try the therapeutic effect of doses in excess of 50 mg./kg. bodyweight. The most striking feature of this experiment was that single doses of 50 mg. per 20-g. mouse (2,500 mg./kg.) was curative, while the same total dosage divided into five daily doses of 10 mg. per mouse was only slightly less effective (Fig. 3). It would appear that it is better to treat infections with a single overwhelming dose of this drug. No experiments have been done in animals using penicillin-sensitive strains of *Staph. pyogenes,* but since Thompson *et al.* (1960) found no strains resistant to BRL 1241 among a wide range examined it seems unlikely that treatment of these would be any less effective.

## Summary

BRL 1241 is shown to be effective in the treatment of penicillin-resistant staphylococcal infections in mice.

The results of treatment with the drug are compared with results obtained in the treatment of similar infections with antibiotics in common use.

We are grateful for supplies of BRL 1241 to Beecham Research Laboratories Limited, whose registered trade name for this drug is "celbenin."

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Measures to protect workers against the inhalation of dusts, fumes, or other impurities likely to be injurious are discussed in a recently published Ministry of Labour booklet, *Toxic Substances in Factory Atmospheres* (H.M.S.O., price 1s. net).

# SENSITIVITY OF STAPHYLOCOCCUS PYOGENES TO BENZYLPENICILLIN AND BRL 1241

BY

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The isolation of pure 6-aminopenicillanic acid (Batchelor *et al.,* 1959) has made it possible to synthesize a large number of penicillin compounds by the substitution of different radicals on the side-chain of the penicillanic acid nucleus. One such compound, BRL 1241 ("celbenin"), is of special interest in that it is virtually non-toxic, has an antibiotic spectrum similar to penicillin itself, is not destroyed by staphylococcal penicillinase, and is active against staphylococcal infections *in vivo* (Thompson *et al.,* 1960).

We have compared the activity of compound BRL 1241 with crystalline penicillin G against all isolations of *Staphylococcus pyogenes* in this hospital in the nine-months period between June, 1959, and April, 1960.

## Materials and Methods

### Staphylococcus pyogenes

All coagulase-positive staphylococci were regarded as *Staph. pyogenes* and the organisms were subcultured on to broth-agar slopes for storage purposes at the time of isolation. The strains examined were all isolated from the patients and staff of this hospital between June, 1959, and April, 1960, in the course of routine bacteriological investigations. The origin and distribution of these strains may be seen in the Table. It must be stressed that in some cases the same phage type was isolated from the same lesion on more than one occasion. Such repeated isolations occurred on 206 occasions. However, the antibiotic sensitivity of all isolations of *Staph. pyogenes* was examined.

*Distribution of Phage Types of the Staph. pyogenes Cultures Isolated from In-patient and Out-patient Sources. Repeated Isolations of the Same Phage Type from Any One Lesion are Not Included.*

| | Group | | | | | |
|---|---|---|---|---|---|---|
| | I* | Type 80 Only | II | III | IV | NT† |
| **In-Patients** | | | | | | |
| Wound infection, post-op., etc. | 65 | 84 | 11 | 64 | 1 | 51 |
| Primary infection (carbuncles, etc.) | 16 | 29 | 2 | 5 | — | 9 |
| Nose, throat, etc. (except staff) | 28 | 15 | 10 | 22 | 2 | 35 |
| Sputum | 15 | 11 | 2 | 16 | 1 | 8 |
| Maternity (mothers and babies) | 17 | 2 | 14 | 9 | — | 9 |
| Staff—nose, throat, hands (not infected) | 41 | 11 | 12 | 14 | — | 38 |
| **Out-Patients** | | | | | | |
| Wound infection, post-op., etc. | 37 | 9 | 15 | 17 | 1 | 5 |
| Primary infection (carbuncles, etc.) | 39 | 17 | 27 | 24 | — | 25 |
| Nose, throat, etc. (except staff) | 3 | 5 | 7 | 4 | — | 8 |
| Total | 261 | 183 | 100 | 175 | 5 | 188 |

* Strains, excluding type 80, reacting with phages of group I.
† Not typable with phages available.

*Phage-typing.*—This was carried out by methods of Anderson and Williams (1956) using phages supplied by Dr. R. E. O. Williams, of the Staphylococcal Reference Laboratory, Colindale. The distribution of the different groups is shown in the Table. In view of its special epidemiological significance, phage type 80 has been recorded separately.

*Crystalline Penicillin G.*—A solution of crystalline penicillin G was prepared in distilled water to contain 6 μg. of the antibiotic per ml. (10 units/ml.).

*Compound BRL 1241.—Staph. pyogenes* (Oxford) is known to be sensitive to crystalline penicillin G at a concentration of 0.012 μg./ml. Preliminary titrations of compound BRL 1241 against *Staph. pyogenes* (Oxford) showed that the growth of the organisms was inhibited by a concentration of between 2 and 3 μg. of the compound per ml. A solution of comparable activity to one containing 6 μg. of crystalline penicillin G per ml. was therefore prepared containing 1.25 mg. of compound BRL 1241 per ml. in distilled water.

### Methods of Sensitivity

Qualitative tests were carried out on broth-agar of uniform composition and poured in 12-ml. amounts into flat-bottomed disposable polystyrene Petri dishes 85 mm. in diameter. These plates were dried before use. Two strips of filter paper 65 by 5 mm. were impregnated, one with crystalline penicillin G and the other with compound BRL 1241, and placed upon plates pre-inoculated with streaks of six strains of staphylococci. The filter paper was placed at right angles to the line of inoculation and the two strips were well separated. The result was recorded after overnight incubation at 37° C. by measuring the diameter of the zones of inhibition of the cultures by superimposing the culture plates on 1-mm. graph paper.

Subsequently the sensitivity to compound BRL 1241 of groups containing the most sensitive and least sensitive strains as assessed by the inhibition zone diameters, which ranged from 19 mm. to 5 mm., was determined quantitatively by the method of Selbie *et al.* (1945) to establish the effective range of the compound against staphylococci.

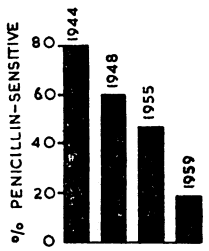
## Results

A total number of 1,118 isolations of pathogenic staphylococci were examined. All of these proved sensitive to compound BRL 1241, whereas only 202 (18%) were sensitive to crystalline penicillin G. All the organisms were sensitive to compound BRL 1241 in concentrations between 1.6 and 3.2 μg./ml.

## Discussion

The potential role of compound BRL 1241 in the treatment of staphylococcal infections in hospitals is one of the greatest importance in view of these results. The fact that none of the strains of staphylococci examined showed any signs of resistance to this compound was in sharp contrast to the results of the sensitivity tests of these organisms with the parent compound, crystalline penicillin G.

The number of strains of penicillin-sensitive *Staph. pyogenes* has steadily fallen since the introduction of penicillin into this hospital in 1944. In the original survey Selbie *et al.* (1945) found 80% of staphylococci sensitive to penicillin. Subsequent surveys carried out in 1948 and 1955 (unpublished) show a steady decrease, only 47% being sensitive in 1955. Now only 18% of organisms isolated are sensitive (see Chart). These results show that penicillin is of very doubtful therapeutic value in the treatment of staphylococcal infection in this hospital, and if the present trend persists the drug will soon become valueless for this purpose. It is possible that this trend could be checked and even reversed by banning the use of penicillin for a limited period as suggested by Barber *et al.* (1960).



Histogram showing the decline in strains of *Staph. pyogenes* sensitive to penicillin between 1944 and the end of 1959.

Of special interest in the distribution of phage types of staphylococci is the large number (183) of isolations of phage type 80. This organism, which was described by Rountree and Freeman (1955), is unusually virulent, and is becoming more and more prevalent in hospital infections. Characteristically this type is penicillin-insensitive, and compound BRL 1241 may prove of great value in the control and possibly in the elimination of this type, since all staphylococci, regardless of their phage type, appear to be equally sensitive to the drug.

It is worthy of note that whereas 20% of staphylococci examined at the time of introduction of penicillin (Selbie *et al.*, 1945) were resistant to the antibiotic, we have found no strains of *Staph. pyogenes* resistant to compound BRL 1241 on its introduction. However, previous experience with antibiotics in general use has shown that, sooner or later, resistant strains of staphylococci appear and, having appeared, increase steadily in the hospital environment. So far, we have not encountered a strain of staphylococcus which is resistant to compound BRL 1241; but while it is possible that such a strain will appear, with proper control of the therapeutic use of compound BRL 1241 the number of resistant strains will be kept at a low level.

## Summary

The total isolations of *Staph. pyogenes* in the Middlesex Hospital in the latter half of 1959 and first quarter of 1960 were tested for sensitivity against crystalline penicillin G and the new penicillin compound BRL 1241. Only 18% were sensitive to crystalline penicillin G, whereas all were sensitive to compound BRL 1241.

We are grateful to Beecham Research Laboratories Limited for supplies of BRL 1241.

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The National Deaf Children's Society's *Annual Report and Accounts, 1959–60,* says that the financial position of the Society has improved during the year as a result of legacies, an appeal, and an increase in subscriptions and donations. New premises have been purchased in Gloucester Place, and this should enable the Society to carry out a greatly increased amount of necessary work for deaf children.