Brit. J. Pharmacol. (1953), **8**, 444.

THE ACTION OF 2 : 4-DIAMINO-6 : 7-DIBENZYLPTERIDINE AGAINST ANTIBIOTIC-RESISTANT STAPHYLOCOCCI

BY

H. O. J. COLLIER AND MARGARET PHILLIPS

From Research Division, Allen & Hanburys Ltd., Ware, Herts

(RECEIVED MAY 29, 1953)

2 : 4-Diamino-6 : 7-dibenzylpteridine was found (Collier and Waterhouse, 1952) to inhibit the growth of *Streptococcus faecalis* and *Staphylococcus aureus* when present in low concentration in the culture medium and to protect mice against infections of *Staph. aureus*; this action appeared to be potentiated by sulphathiazole. The therapeutic activity of the pteridine was not as high as that of penicillin against normal staphylococcal infections of mice; but we thought it desirable to explore its activity alone, and with sulphathiazole, against staphylococci resistant to penicillin and other antibacterial drugs.

MATERIALS AND METHODS

Strains.—Drug-resistant strains of *Staph. aureus* were obtained from three sources.

1. By serial transfer in the laboratory of strain CN491 in liquid media containing increasing concentrations of penicillin, sulphathiazole, dibenzylpteridine, or a mixture of equal weights of the two last-named.

2. Penicillin-resistant strains isolated by Dr. M. Barber at St. Thomas's Hospital, London, numbered D1, MID1, MID3, and W499.

3. Multiple-resistant strains Nos. 22 and 159 relatively insensitive to penicillin, streptomycin, aureomycin, chloramphenicol, terramycin, and sulphonamides, isolated at the Bristol Royal Infirmary (Clarke, Dalgleish, and Gillespie, 1952).

Methods in vitro.—The medium in all experiments had the following composition.

NaCl	5.0 g.
Na_2HPO_4	3.5 g.
KH_2PO_4	0.4 g.
"Vitamin free" casein hydrolysate (A. & H.) ...	5.0 g.
dl-Tryptophane	0.2 g.
Dextrose	2.0 g.
Aneurine	40 μg.
Nicotinic acid	400 μg.
Distilled water	to 1 litre

to *p*H 7.2–7.4

In the induction of drug-resistance, inocula were adjusted to give approximately 30,000 cells per ml. experimental medium. In sensitivity tests inocula of this size or of 1,000,000 cells per ml. of test medium were used. End-points were read visually and the minimal inhibitory concentrations expressed as the geometric means of several independent experiments. For other details, reference may be made to Collier and Waterhouse, 1952.

Methods in vivo.—Methods used in mice were as described in the above paper, except that the infective inocula were prepared from 5-hr. cultures of staphylococci. Rabbits were infected intravenously with 0.5 ml. of a 5-hr. culture of staphylococci washed and made up to initial volume. 1 and 24 hours after inoculation, we administered dibenzylpteridine or sulphathiazole, or their mixture in equal proportions, by mouth, or procaine penicillin in oil with aluminium stearate by intramuscular injection.

RESULTS

The Development of Resistance in vitro

In the course of 10–12 transfers, in liquid medium containing increasing concentrations of drug, *Staph. aureus* CN491 became more than a hundred times more resistant to penicillin, sulphathiazole, or dibenzylpteridine, but only about six times more resistant to a mixture of equal parts of sulphathiazole and pteridine (see Fig. 1). In these experiments an inoculum giving approximately 30,000 cells per ml. of test medium was used.

Cross-resistance in vitro

Artificially Produced Drug-resistant Strains of Staph. aureus *CN491.*—Using washed inocula of about 30,000 cells per ml. culture medium, we found that strains made resistant to any one of the three drugs remained sensitive to the other two. Each of these resistant strains was sensitive to the pteridine-sulphathiazole mixture.

Naturally Occurring Penicillin-resistant Strains.—Since penicillin-resistance may be more clearly seen when large inocula of staphylococci are used

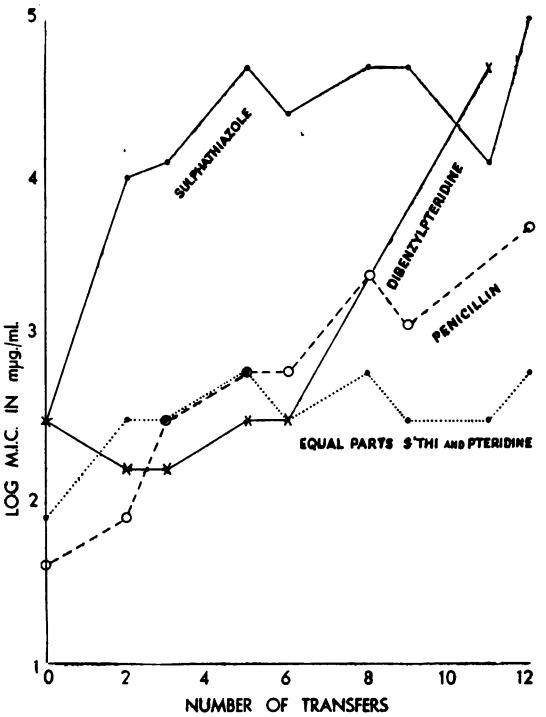

FIG. 1.—Development of resistance by *Staph. aureus* CN491 during exposure to sulphathiazole, dibenzylpteridine, and penicillin in serial subcultures. Ordinate: logarithm of the minimal inhibitory concentration (M.I.C.) in mμg./ml. Abscissa: number of transfers.

(see Barber, 1947) we employed inocula of about 1,000,000 as well as of about 30,000 washed cells per ml. medium when testing the cross-resistance of Barber's strains. The results of these experiments, illustrated in Table I, confirm the importance of inoculum size in the demonstration of penicillin resistance. They also show that, when a large inoculum was used, strain W499 exhibited a marked degree of resistance to dibenzylpteridine, but a mixture of sulphathiazole and dibenzylpteridine was effective against large inocula of this as well as of the other strains.

TABLE I

RESPONSES OF PENICILLIN-RESISTANT STAPHYLOCOCCI TO DIBENZYLPTERIDINE, SULPHATHIAZOLE, AND A MIXTURE OF THESE DRUGS, *IN VITRO*

The cultures were inoculated to give (*a*) about 1,000 000 cells/ml. and (*b*) about 30,000 cells/ml. respectively

Strain No.	Minimal Inhibitory Concentration of Drug in μg./ml. at 24 hr.						
	Penicillin		Dibenzyl-pteridine		Sulpha-thiazole		Pteri-dine+S'thi. 50:50
	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)
D1 ..	25·12	0·36	2·33	0·25	>100	1·26	0·45
MID1	8·91	0·5	0·79	0·05	>25	0·45	0·31
MID3	>10	0·63	2·5	0·16	>100	1·25	0·45
W499 ..	>100	0·78	>100	0·16	>100	0·35	1·1

Naturally Occurring Multiple-resistant Strains.—Even using an inoculum of the smaller size (*ca.* 30,000 cells per ml. medium) we found that strains 22 and 159 exhibited considerable resistance to penicillin, chloramphenicol, and sulphathiazole. Accordingly, using the same sized inoculum, we explored their sensitivity to dibenzylpteridine and to a pteridine-sulphathiazole mixture, with results as expressed in Table II. It will be seen that both strains are sensitive to dibenzylpteridine, but a significantly greater sensitiveness to the pteridine-sulphathiazole mixture is not shown.

TABLE II

RESPONSES OF MULTIPLE-RESISTANT STAPHYLOCOCCI TO VARIOUS DRUGS *IN VITRO*

Cultures inoculated to give about 30,000 cells/ml.

Strain No.	Drug	Minimal Inhibitory Concentration in μg./ml.	
		24 hr.	48 hr.
22	Chloramphenicol	50	50
	Sulphathiazole	50	71
	Penicillin	12·5	>100
	Dibenzylpteridine	0·552	0·78
	Pteridine+S'thi. 50:50 ..	0·78	0·78
159	Chloramphenicol	50	71
	Sulphathiazole	100	100–>100
	Penicillin	12·5	100
	Dibenzylpteridine	0·552	0·78
	Pteridine+S'thi. 50:50 ..	0·552	1·1

Therapeutic Experiments

Naturally Occurring Penicillin-resistant Strains.—The strains from Dr. Barber were not pathogenic to mice, but one of them, W499, was rapidly fatal to rabbits when given intravenously. In spite of the resistance of W499 to dibenzylpteridine, shown *in vitro*, we carried out some preliminary experiments on the protection of rabbits. In these experiments oral doses of 1.0 g. of sulphathiazole or of dibenzylpteridine failed to protect the animals. A mixture of equal parts of the two drugs, on the other hand, prolonged the lives of rabbits at doses of 0.2 and 1.0 g. per kg. However, in spite of the penicillin-resistance exhibited by this strain *in vitro*, an intramuscular dose of 100,000 units of procaine penicillin in oil per kg. protected the animals longer than did the pteridine-sulphonamide mixture.

Naturally Occurring Multiple-resistant Strains.—Strains 22 and 159 proved capable of killing

mice if inoculated intraperitoneally in large numbers with 5% mucin. In preliminary experiments we found that, given by mouth, sulphathiazole had little protective activity and that a sulphathiazole-pteridine mixture was no better than the pteridine alone.

The pteridine itself showed activity when given orally in large doses, but it was less active against either strain than chloramphenicol, in spite of the moderate degree of chloramphenicol resistance. This is illustrated in Fig. 2, which gives the results of an experiment with strain 159.

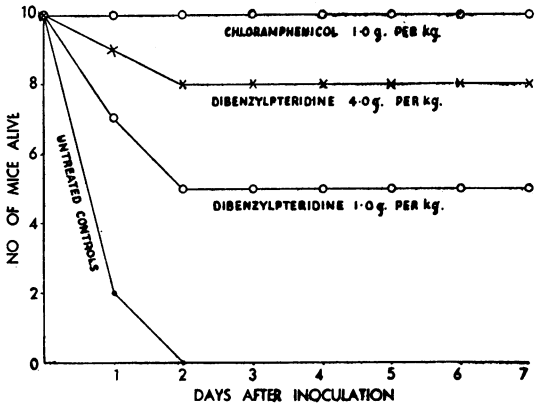

FIG. 2.—Protection of mice, infected with a multiple-resistant strain of *Staph. aureus* 159, by chloramphenicol and dibenzylpteridine administered orally 1 hr. after intraperitoneal inoculation. Ordinate: number of mice alive. Abscissa: days after inoculation.

The results of therapeutic tests in mice and in rabbits showed that dibenzylpteridine was less effective *in vivo* than might have been expected from its *in vitro* activity, while the converse was true of penicillin and chloramphenicol. One of the questions which therefore arose was how far body fluids antagonized the antibacterial activity of the pteridine.

TABLE III

ANTAGONISM OF DIBENZYLPTERIDINE, SULPHATHIAZOLE, AND THEIR MIXTURE BY VARIOUS SERA

Strain W499 inoculated to give about 30,000 cells/ml.

Drug	Serum Included at 5% in Synthetic Medium	Minimal Inhibitory Concentration in μg./ml. at 16 hr.
Dibenzylpteridine ..	None	0·046
	Rabbit	2·5–>10
	Mouse	0·88
	Man	1·25
Sulphathiazole ..	None	0·625
	Rabbit	>10
	Man	>10
Equal parts ..	None	0·039
Dibenzylpteridine +	Rabbit	0·625
Sulphathiazole ..	Man	0·625

Effect of Serum on Drug Activity

When 5% serum from man, mouse, or rabbit was added to the synthetic medium, strain W499 showed a marked decrease in sensitiveness to dibenzylpteridine, to sulphathiazole, and to a mixture of the two drugs. As will be seen from Table III, the antagonistic effect was least with a mixture of pteridine and sulphathiazole.

DISCUSSION

The experimental work *in vitro*, illustrated in Fig. 1, provides yet another example of resistance developing more slowly to a mixture of drugs than to one alone, as has been described for other drugs by Carpenter, Bahn, Ackerman, and Stokinger (1945) and more recently by, for example, Purcell, Wright, and Finland (1953).

Collier and Waterhouse (1952) showed that dibenzylpteridine exerted some therapeutic effect in mice against an antibiotic-sensitive strain of *Staph. aureus*. By the oral route, its activity was slight, but it was considerably more effective when given intraperitoneally. In the present therapeutic tests with antibiotic-resistant strains, the pteridine showed about the same activity by mouth as before. The intraperitoneal route of administration was not used, as it was thought that there might be some direct action of the drug on the staphylococci before their absorption into the blood stream. The potentiation of dibenzylpteridine by sulphathiazole, previously reported by Collier and Waterhouse for a normal strain of staphylococcus, was also found in those of the present strains of staphylococci not already resistant to sulphathiazole.

The relative ineffectiveness of the dibenzylpteridine *in vivo*, as compared with its performance *in vitro* in a synthetic medium, is at least partly explained by the antagonistic action of serum. This finding contrasts with the failure of oxalated horse blood to antagonize markedly the action of pteridines against *Str. faecalis* (Collier and Waterhouse, 1952).

SUMMARY

1. Strains of *Staph. aureus* made separately resistant in the laboratory to sulphathiazole, penicillin, and dibenzylpteridine respectively, showed no cross-resistance.

2. Exposure of serial subcultures to a mixture of dibenzylpteridine and sulphathiazole produced resistance less readily than did exposure to either drug separately, or to penicillin alone.

3. Resistance to dibenzylpteridine was found in one of four naturally occurring penicillin-resistant strains.

4. Two naturally occurring strains resistant to various antibiotics and to sulphonamides were sensitive to dibenzylpteridine.

5. A mixture of dibenzylpteridine and sulphathiazole given by mouth protected rabbits against infection with a moderately penicillin-resistant strain of *Staph. aureus*. Penicillin was also effective when given intramuscularly in high doses.

6. Dibenzylpteridine protected mice against two multiple-resistant strains of *Staph aureus*, but was less effective than chloramphenicol, when both drugs were administered by mouth.

7. Some antagonism of dibenzylpteridine by serum was demonstrated *in vitro*.

We wish to thank Miss M. Boothroyd and Mrs. E. Harris for technical assistance, and the directors of Messrs. Allen & Hanburys Ltd. for permission to publish this paper. The dibenzylpteridine was kindly prepared by Mr. J. H. Dunsmuir.

REFERENCES

Barber, M. (1947). *J. Path. Bact.*, **59**, 373.
Carpenter, C. M., Bahn, J. M., Ackerman, H., and Stokinger, H. E. (1945). *Proc. Soc. exp. Biol., N.Y.*, **60**, 168.
Clarke, S. K. R., Dalgleish, P. G., and Gillespie, W. A. (1952). *Lancet*, **1**, 1132.
Collier, H. O. J., and Waterhouse, P. D. (1952). *Brit. J. Pharmacol.*, **7**, 161.
Purcell, E. M., Wright, S. S., and Finland, M. (1953). *Proc. Soc. exp. Biol., N.Y.*, **82**, 124.