Brit. J. Pharmacol. (1951), **6**, 430.

SOME BIOLOGICAL PROPERTIES OF CEPHALOSPORIN P_1

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(Received April 23, 1951)

Cephalosporin P_1 is an antibiotic from a species of *Cephalosporium*. Its production is described by Crawford, Heatley, Boyd, Hale, Kelly, Miller, and Smith (1951) and its isolation and chemical properties by Burton and Abraham (1951). This substance was subjected to a fairly extensive biological investigation, because as its properties were progressively revealed it seemed possible that the substance might be of interest for therapeutic purposes. The cephalosporin used was lyophil-dried material that had been prepared by chromatography and countercurrent distribution between solvents. Its purity was appraised by analysis of the curves obtained from the distribution experiments. As will be shown later, there was little or no difference in antistaphylococcal activity between this material and crystalline cephalosporin.

Bacteriological Investigations

Antibacterial activity of lyophil-dried cephalosporin P_1

The sensitivity of a number of species of bacteria was tested by the serial dilution method. The staphylococci, bacilli, micrococcus, bacterium, and saprophytic mycobacteria were grown in heart extract broth; the streptococci, neisseria, corynebacteria, and brucellae in 10 per cent serum broth; and the erysipelothrix in Difco tryptose broth. In each case one drop of a 24-hour culture, diluted or undiluted, was added to each of a series of tubes containing twofold serial dilutions of cephalosporin in 2 ml. of the same medium. With the clostridia one drop of a four-day growth in heart extract broth was used, and with *Myco. tuberculosis* 0.2 ml. of a six-day growth in Dubos and Davis's broth was added to 1.8 ml. of medium. The presence or absence of growth was determined by naked eye observation.

It will be seen in Table I that cephalosporin P_1 inhibits the growth of staphylococci, corynebacteria, and *Cl. tetani* at a considerable dilution. Some organisms were inhibited at moderate dilutions, but streptococci and the Gram-negative bacteria tested were but little affected by the concentrations used.

Antibacterial activity of crystalline cephalosporin P_1

The sensitivity of several strains of staphylococcus (N.C.T.C. 6571, C.N. 491, Hallet, Goodwin, Thurston, N.C.T.C. 8178, C.N. 1202) to crystalline cephalosporin was determined in the same way. No substantial difference was found between the activity of crystalline cephalosporin and that of the lyophil-dried material.

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TABLE I

ANTIBACTERIAL ACTIVITY OF LYOPHIL-DRIED CEPHALOSPORIN P_1 *in vitro*

Organism	Dilution of culture used for inoculum	Highest dilution giving complete inhibition of growth after incubation for:		Lowest dilution giving no inhibition of growth after incubation for:	
		24 hr.	48 hr.	24 hr.	48 hr.
Staph. aureus (N.C.T.C.6571) ..	1:1,000	2,560,000	1,280,000	10,240,000	2,560,000
,, ,, (C.N. 491) ..	1:1,000	2,560,000	1,280,000	10,240,000	2,560,000
,, ,, (Hallet)	1:1,000	2,560,000	1,280,000	10,240,000	2,560,000
,, ,, (Lally)	1:1,000	1,280,000	640,000	5,120,000	1,280,000
,, ,, (Welch)	1:1,000	1,280,000	640,000	5,120,000	1,280,000
,, ,, (Newall) ..	1:1,000	1,280,000	640,000	2,560,000	1,280,000
,, ,, (Broad)	1:1,000	1,280,000	640,000	2,560,000	1,280,000
,, ,, (Goodwin) ..	1:1,000	1,280,000	640,000	2,560,000	1,280,000
,, ,, (Willowchowski)	1:1,000	640,000	320,000	1,280,000	640,000
Str. pneumoniae (A 2128) ..	1:1	10,000	<10,000	40,000	<10,000
,, ,, (A 2163) ..	1:1	10,000	<10,000	40,000	<10,000
,, *viridans* (140)	1:1	<10,000	<10,000	20,000	<10,000
,, ,, (135)	1:1	<10,000	<10,000	20,000	<10,000
,, *haemolyticus* group A ..	1:1	10,000	<10,000	40,000	<10,000
,, ,, ,, B ..	1:1	10,000	<10,000	40,000	<10,000
,, ,, ,, C ..	1:1	10,000	<10,000	40,000	<10,000
,, ,, ,, D ..	1:1	10,000	<10,000	20,000	<10,000
,, ,, ,, G ..	1:1	10,000	<10,000	40,000	<10,000
,, ,, ,, K ..	1:1	10,000	<10,000	40,000	<10,000
C. diphtheriae var. *gravis* ..	1:1	2,560,000	1,280,000	5,120,000	2,560,000
,, ,, ,, *intermedius*	1:1	5,120,000	2,560,000	10,240,000	5,120,000
,, ,, ,, *mitis* ..	1:1	>10,240,000	>10,240,000	>10,240,000	>10,240,000
,, *xerosis*	1:1	1,280,000	40,000	2,560,000	80,000
,, *hofmanni*	1:1	5,120,000	1,280,000	10,240,000	2,560,000
,, *pyogenes*	1:1	2,560,000	1,280,000	5,120,000	2,560,000
Cl. welchii	1:1		80,000		160,000
,, *tetani*	1:1		640,000		1,280,000
,, *sporogenes*	1:1		20,000		40,000
,, *septique*	1:1		80,000		160,000
M. lysodeikticus	1:100	160,000	80,000	320,000	160,000
B. subtilis	1:1,000	80,000	40,000	160,000	80,000
,, *anthracis*	1:1,000	160,000	80,000	320,000	160,000
N. meningitidis	1:1	10,000	<10,000	40,000	<10,000
,, *gonorrhoeae*	1:1	40,000	40,000	80,000	80,000
,, *pharyngis sicca*	1:10	20,000	<10,000	40,000	<10,000
Br. abortus	1:1	<10,000	<10,000	<10,000	<10,000
,, *melitensis*	1:1	<10,000	<10,000	<10,000	<10,000
Bact. coli	1:1,000	<10,000	<10,000	<10,000	<10,000
Ery. monocytogenes	1:1	20,000	20,000	80,000	40,000
Myco. phlei	1:100		<10,000		<10,000
,, *smegmatis*	1:100	80,000	80,000	160,000	160,000
,, *tuberculosis* (human H37)*	1:1		10,000		20,000
,, ,, (,, P.N.)	1:1		<10,000		<10,000
,, ,, (bovine Branch)	1:1		<10,000		<10,000
,, ,, (,, S 182)*	1:1		20,000		40,000

Where " Highest dilution . . ." and " Lowest dilution . . ." do not come next to each other in the twofold series, the intermediate tubes showed partial inhibition of growth. A blank indicates that the test was not read.

* After six days' incubation cephalosporin 1 : 10,000 did not inhibit growth.

Crystalline cephalosporin was also used to estimate the drug's action against several Gram-negative organisms not previously tested, *Bact. friedländeri*, *Bact. aerogenes* (63169), *Bact. aerogenes* (62691), *Salm. typhi* (B5K), *Salm. enteritidis* gaertner, *Sh. flexneri*, *Sh. sonnei*, *Sh. shigae*, *Ps. pyocyanea*, and *V. cholerae*. The organisms were grown in heart broth and serial dilution tests set up as before, one drop of a 24-hour culture being used as inoculum. In no case was growth affected, even by the highest concentration of cephalosporin used, 1 : 10,000. It is thus confirmed that cephalosporin is without activity against Gram-negative organisms.

Activity of cephalosporin against penicillin-resistant staphylococci

Table I shows that cephalosporin P_1 was highly active against penicillin-resistant staphylococci. The Willowchowski strain was resistant to concentrations of penicillin up to 500 units per ml., Goodwin to concentrations up to 250 units, and Lally, Welch, Newell, and Broad to concentrations up to 100 units per ml. The N.C.T.C. 6571, C.N. 491, and Hallet strains were highly sensitive to penicillin.

Effect of inoculum size

The effect of varying the size of the inoculum was investigated in similar serial dilution tests, a 24-hour culture being diluted with water as required. Table II shows that the size of the inoculum made a considerable difference to the end-point

TABLE II

EFFECT OF INOCULUM SIZE ON ANTIBACTERIAL ACTIVITY OF CEPHALOSPORIN P_1

Organism	Dilution of culture used for inoculum	Highest dilution giving complete inhibition of growth after incubation for:		Lowest dilution giving no inhibition of growth after incubation for:	
		24 hr.	48 hr.	24 hr.	48 hr.
Staph. aureus (N.C.T.C. 6571)	1 : 1	320,000	160,000	640,000	320,000
	1 : 1,000	2,560,000	1,280,000	10,240,000	2,560,000
	1 : 1,000,000	5,120,000	2,560,000	10,240,000	5,120,000
C. diphtheriae var. *gravis*	1 : 1	2,560,000	1,280,000	5,120,000	2,560,000
	1 : 100	2,560,000	1,280,000	10,240,000	2,560,000
	1 : 10,000	>40,960,000	2,560,000	>40,960,000	5,120,000

when *Staph. aureus* (N.C.T.C. 6571) was used, but that it was unimportant with *C. diphtheriae* var. *gravis* when the results were read after 48 hours' incubation.

Effect of serum

Concentrations of cephalosporin of 1 : 10,000, 1 : 50,000, and 1 : 250,000 in water, in 50 per cent serum, and in 90 per cent serum were incubated at 37° C. for five hours, and the antibacterial activity of the solutions against *Staph. aureus* (N.C.T.C. 6571) tested by the cylinder-plate method. From the zone size it appeared that in both 50 per cent and 90 per cent serum the activity was reduced by about 60 per cent.

Development of resistance by staphylococci in vitro

Staph. aureus (N.C.T.C. 6571) was cultivated in tubes of broth containing serial dilutions of cephalosporin. After 24 hours cocci from the tube with the highest

concentration that allowed visible growth were subcultured into another series of tubes containing serial dilutions. After three such subcultures the staphylococci were not inhibited by cephalosporin at a concentration of 1 : 25,000. It was thus clear that they had rapidly acquired considerable resistance to the substance *in vitro*.

In view of the work to be described later it is interesting to note that when treated in a similar way *Staph. aureus* (C.N. 491) acquired no resistance to aureomycin hydrochloride when grown in its presence, even after six subcultures. This finding agrees with that of Price, Randall, and Welch (1948).

Mode of action in vitro

Tubes containing serial dilutions of cephalosporin in 2.0 ml. of broth were inoculated with one drop of an undiluted 24-hour culture of *Staph. aureus* (N.C.T.C. 6571). The tubes were incubated at 37° C., and from time to time the number of viable organisms in each culture was estimated. Fig. 1 shows the results. A concentration of cephalosporin of 1 : 400,000 or less inhibited growth only temporarily, but with a concentration of 1 : 200,000 or more no viable organisms were found after 24 hours.

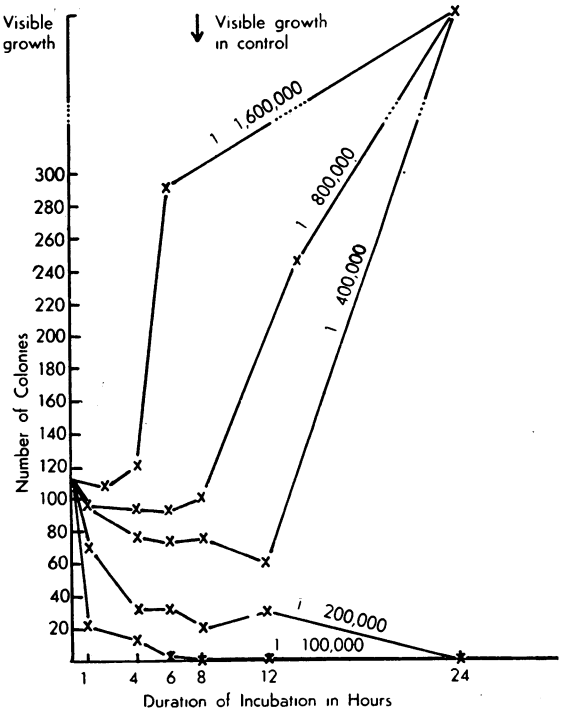

FIG. 1.—Mode of action of cephalosporin P_1 *in vitro*. The number of staphylococci surviving after cultivation in media containing cephalosporin. Each point is the average of three measurements.

This means that under the conditions of the test with the small inoculum used cephalosporin is bactericidal at a concentration of 1 : 200,000 or more, while in lower concentrations it is bacteriostatic.

Comparison with helvolic acid, aureomycin, and terramycin

There are certain similarities between the biological behaviour of helvolic acid (Florey, Chain, Heatley, Jennings, Sanders, Abraham, and Florey, 1949, page 332) and cephalosporin, and, as will be seen later, it became desirable to compare the action of cephalosporin *in vivo* with that of aureomycin and terramycin. Some comparative *in vitro* tests were therefore made.

The sensitivity of two strains of staphylococcus to helvolic acid, aureomycin hydrochloride, and terramycin hydrochloride was tested by the dilution method in the same way as was their sensitivity to cephalosporin. The highest dilution of helvolic acid completely inhibiting growth after 48 hours' incubation was 1 : 160,000 for *Staph. aureus* (C.N. 491) and 1 : 320,000 for *Staph. aureus* (N.C.T.C. 6571). Both these strains were inhibited by dilutions of terramycin up to 1 : 2,560,000. Aureomycin is destroyed when in solution in the medium used for these tests, and so in the tests with this substance readings were made earlier. The growth of both strains was inhibited for 12 hours by concentrations up to 1 : 1,280,000 and for 24 hours by concentrations up to 1 : 640,000 for *Staph. aureus* (C.N. 491) and up to 1 : 1,280,000 for *Staph. aureus* (N.C.T.C. 6571). If these findings are compared with those shown in Table I it is seen that the antistaphylococcal action of cephalosporin *in vitro* is stronger than that of helvolic acid, but of the same order as that of aureomycin and terramycin.

C. diphtheriae var. *gravis* was found to be completely inhibited by dilutions of helvolic acid up to 1 : 5,120,000.

A comparison with Table I in the paper by Chain, Florey, Jennings, and Williams (1943) shows that cephalosporin has a similar antibacterial range to that of helvolic acid.

Pharmacological Investigations

Acute toxicity

To mice.—As supplies of material were limited, only the effects of intravenous injection were determined. Cephalosporin P_1 is not freely soluble, the maximum concentration obtainable in isotonic saline at *p*H 7 being 10 mg./ml. This necessitated the injection of relatively large volumes of fluid. The injection of 1.5 ml. of a saturated solution, i.e., 15 mg. of substance, intravenously into mice weighing 20 g. caused the death of the four mice used, two after 16 hours, one after 19 hours, and one after 22 hours. All had epileptiform convulsions immediately after the injection, from which they passed into a semi-comatose condition. The injection of 1.5 ml. of isotonic saline did not affect two control animals.

Thirteen mice of 20 g. weight were given 1 ml., i.e., 10 mg. Immediately after injection they became ill and were semi-comatose, but eleven gradually improved during the six hours after the injection and eventually recovered fully. They were well at the end of three weeks when killed for histological examination. No lesions attributable to the cephalosporin were found in liver, kidney, or spleen. Of the two that died, one lived for 16 and one for 28 hours. Thus the LD50 after intravenous injection is between 10 and 15 mg. per mouse, i.e., between 500 and 750 mg./kg.

To leucocytes.—The effect of cephalosporin on human leucocytes was determined by the method of Abraham, Chain, Fletcher, Florey, Gardner, Heatley, and Jennings

(1941). Concentrations of 1 : 220 to 1 : 880 killed about half the cells in four hours, but with concentrations of 1 : 1,000 or less the survival time did not differ from that of the cells in control preparations, most cells surviving more than six hours.

To tissues.—No gross abnormality was seen at the site of injection in mice killed from one to eleven hours after a single subcutaneous dose of 1 or 2 mg. of cephalosporin, nor any lesion of the gut in mice given a single dose of 1, 2.5, or 5 mg. by mouth in 0.6 ml. of water from one to fifteen hours before death. Mice given 5 mg. aureomycin hydrochloride by mouth in the same volume of fluid showed injection of the small intestine when killed after five and seven hours, and mice given 5 mg. terramycin hydrochloride slight " blushing " of the gut when killed after nine hours.

Chronic toxicity

To mice.—Five mice were given 4 mg. cephalosporin a day for four days by subcutaneous injection, 1 mg. at 0900 hours, 1 mg. at 1600 hours, and 2 mg. at 2300 hours. Three weeks after the first injection, when killed for histological examination, all were still well. No animal showed any gross lesion *post mortem*, nor any histological abnormality in liver, kidney, or spleen that could be attributed to the cephalosporin.

Cephalosporin, 5 mg. 12-hourly for 5½ days, was given by mouth to five mice. No abnormalities were noted, and all were well when killed three weeks after the first dose. No gross lesion was found *post mortem*, nor any histological abnormality in liver, kidney, or spleen that could be attributed to the cephalosporin.

To tissues.—In the animals used for the chronic toxicity tests no lesion was found at the site of injection in those that had been given cephalosporin subcutaneously, and no abnormality was detected in the gut of those given the drug by mouth.

Effect on blood pressure

Cephalosporin P_1 injected intravenously in doses of up to 200 mg. in 20 ml. affected neither the blood pressure nor the respiration of a decerebrate cat weighing 2.8 kg.

Effect on smooth muscle

The effect of adding cephalosporin P_1 to a bath in which an isolated guinea-pig uterus was contracting rhythmically was the same for all concentrations tested from 1 : 100,000 to 1 : 10,000. At first the rate and excursion of the contractions were increased, and then, after a few minutes, the contractions became slower and of great amplitude. The original type of contraction was restored after removal of the cephalosporin.

Absorption

After subcutaneous injection.—The presence of cephalosporin in the blood was detected either by the slide method of Garrod and Heatley (1944) or by the hole-plate method (Florey *et al.*, 1949, page 130). The latter method gave adequate information, and as it is easier to perform was generally used. Mice weighing 18 to 20 g. were killed at various intervals after the injection of cephalosporin, and

undiluted serum from the blood was collected and placed in holes punched in a plate sown with *Staph. aureus* (C.N. 491). The zones of inhibition were read after 18 hours' incubation at 37° C. The results are shown in Fig. 2. Cephalosporin was absorbed from the subcutaneous tissues to produce easily detectable concentrations in the blood. As a very rough approximation it can be taken that a zone of

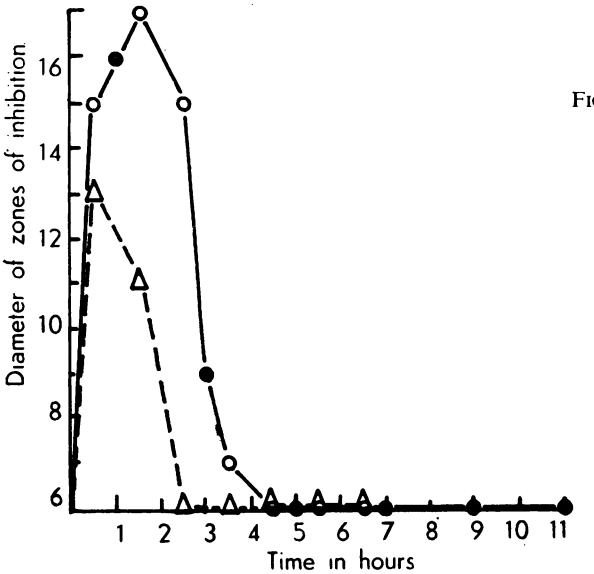

FIG. 2.—Serum levels in mice after the injection of cephalosporin P_1 subcutaneously. Lower curve (△---△) after 1 mg.; upper curve (O———O) after 2 mg. Blood serum taken at different times after injection was tested for activity in hole-plates seeded with *Staph. aureus*. (N.B. A reading of 6 mm. represents the diameter of the hole and indicates no inhibition.) Each point in the 1 mg. curve is the average of measurements on the sera of two mice; the hollow circles on the 2 mg. curve are measurements from one mouse, the solid circles measurements on the pooled sera of three mice.

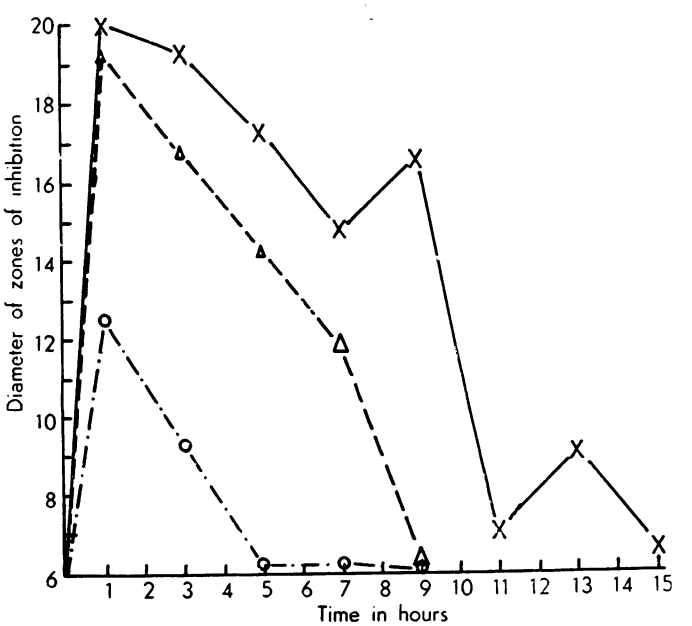

FIG. 3.—Serum levels in mice after the administration of cephalosporin P_1 by mouth. Lowest curve (o-.-.-o) after 1 mg.; middle curve (△- - -△) after 2.5 mg.; highest curve (X———X) after 5 mg. Activity was measured by the hole-plate method using 6 mm. holes and plates seeded with *Staph. aureus*. Each point on the curves is the average of measurements on four mice, except the last three points on the 5 mg. curve which are the average for three mice.

inhibition of diameter 18–19 mm. represents a concentration of cephalosporin of about 1 : 100,000 in the serum.

After oral administration.—Single doses of 1, 2.5, and 5 mg. dissolved in 0.6 ml. of water were administered by stomach tube to mice weighing 18 to 20 g. The mice were killed at intervals and their sera assayed as before. Fig. 3 shows the results.

About 15 hours after the administration of 5 mg. cephalosporin by mouth none was present in the stomach and little in the jejunum, but a considerable amount was present in the colon. There was little in any of these situations after the administration of 1 or 2.5 mg.

It is thus clear that cephalosporin is readily absorbed from the gastro-intestinal tract, leading to the appearance of well-sustained concentrations in the blood.

Comparison of absorption of cephalosporin with aureomycin and terramycin

As will be seen later, it became desirable to compare the effects of aureomycin, terramycin, and cephalosporin on experimental staphylococcal infection in mice.

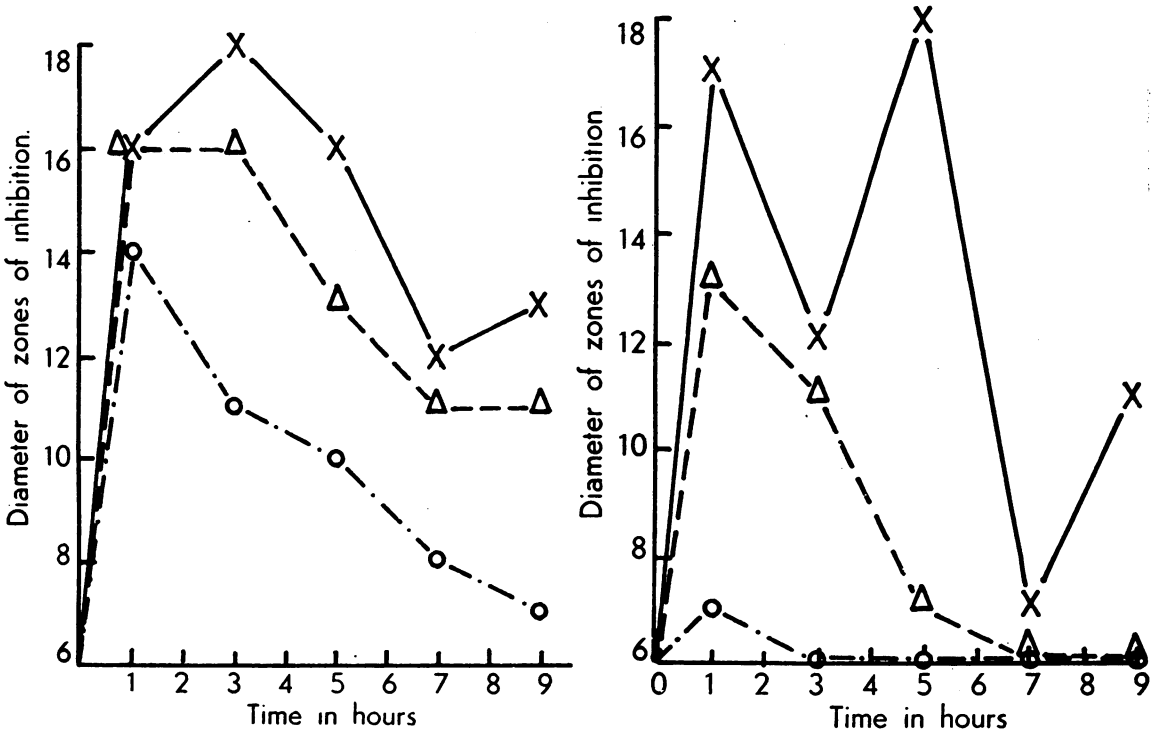

FIG. 4.—Serum levels in mice after the administration of aureomycin by mouth. Lowest curve (o–.–.–o) after 1 mg.; middle curve (△– – –△) after 2.5 mg.; highest curve (X———X) after 5 mg. Activity was measured by the hole-plate method using 6 mm. holes and plates seeded with *Staph. aureus*. Each point represents the measurement obtained from the serum of one mouse.

FIG. 5.—Serum levels in mice after the administration of terramycin by mouth. Lowest curve (o–.–.–o) after 1 mg.; middle curve (△– – –△) after 2.5 mg.; highest curve (X———X) after 5 mg. Activity was measured by the hole-plate method using 6 mm. holes and plates seeded with *Staph. aureus*. Each point represents the measurement obtained from the serum of one mouse.

Comparisons were made of absorption of the three substances when given by mouth under the same conditions. Figs. 4 and 5 show the results.

It would appear that under the conditions of the experiment cephalosporin and aureomycin are retained in the blood for about the same time, slightly longer than is terramycin.

Excretion

In the urine.—Twenty-one mice of 20 g. weight were given a single subcutaneous injection of 2 mg. cephalosporin, and the urine excreted in the next five hours collected. The amount of cephalosporin excreted in the urine was measured by estimating the urinary concentration of the drug by the hole-plate method. The average amount excreted in five hours was less than 0.01 mg., a very small proportion of the injected dose.

The urine was collected from two decerebrate cats used for investigating the effect of cephalosporin on the blood pressure. Although it is not possible to give accurate quantitative results from these experiments, they agree with those obtained in mice in that only a small amount appeared in the urine. The first cat, which received 200 mg. intravenously in divided doses during 3 hours 53 minutes, secreted less than 1 mg. of cephalosporin in the urine collected during the 4½ hours after the first injection, and the second cat, which received 350 mg. intravenously in 3 doses during 43 minutes, secreted about 2 mg. in the 3 hours after the first injection.

In the bile and by the gastro-intestinal tract.—Bile was also collected from the two cats just mentioned. The cat that was given 200 mg. cephalosporin during 3 hours 53 minutes secreted about 1.2 mg. of cephalosporin in the 4½ hours after the first injection, and the cat that was given 350 mg. during 43 minutes about 2.8 mg. in the 3 hours after the first injection.

Ten mice that were given a single subcutaneous injection of 2 mg. cephalosporin were killed 5 hours later, and the stomach, a length of jejunum, and a length of colon were each washed out with 0.25 ml. saline. Activity was detected by the hole-plate method in the colonic washing from every mouse, in the jejunal washing from all but one, and in the stomach washing from four. It is possible therefore that in mice cephalosporin is excreted by the alimentary canal, but it is more likely that its presence there is due to excretion in the bile.

Thus the relatively rapid elimination of cephalosporin from the blood does not seem to be adequately accounted for by urinary or biliary secretion. Further attempts to ascertain its fate in the body were not made.

Protection Experiments

The staphylococcus is among the organisms most sensitive to the action of cephalosporin P_1, and though the activity of the drug is reduced in the presence of serum it is not abolished. In this cephalosporin resembles aureomycin, the efficacy of which is also reduced in the presence of serum (Chandler and Bliss, 1948). Terramycin is less affected (Bliss, Warth, and Chandler, 1950). The foregoing experiments made it clear that cephalosporin was sufficiently non-toxic to mice to administer for a long time in sufficient dosage to maintain a level in the blood that inhibits the growth of the staphylococcus *in vitro*. There were, therefore,

reasonable grounds for supposing that it might suppress the growth of the staphylococcus in the body. Experiments were done to test this supposition, using the strain of *Staph. aureus* (C.N. 491) that had been used to estimate the level of cephalosporin in the blood of normal animals.

A 24-hour culture was diluted as required and one vol. of the diluted culture added to nine vols. of a 7 per cent suspension of mucin. The mice were infected by injecting 0.5 ml. of this suspension intraperitoneally. As the virulence of the organism varied from time to time different dilutions of the culture were used in different experiments. An approximate LD50 determined on small numbers of animals is given in each Table of results.

In the first experiment the cephalosporin was made up at a strength of 5 mg. per ml. Each mouse was given three subcutaneous injections daily, 1 mg. of cephalosporin at 0900 hours, 1 mg. at 1600 hours, and 2 mg. at 2300 hours, beginning one hour after infection. Table III shows that the cephalosporin increased the length of life but not the survival rate.

TABLE III

ANTISTAPHYLOCOCCAL ACTION OF CEPHALOSPORIN P_1 *in vivo*

4 mg. given daily in three doses subcutaneously. LD50 was between 1 : 10,000 and 1 : 100,000 dilutions. Dilution of culture: 1 : 1,000

	Control	Cephalosporin
Mice alive at start	10	10
,, ,, after 24 hours	1	10
,, ,, ,, 48 ,,	1	9
,, ,, ,, 3 days	1	9
,, ,, ,, 4 ,,	1	7
,, ,, ,, 5 ,,	1	3
,, ,, ,, 6 ,,	1	0
,, ,, ,, 7 ,,	1	
,, ,, ,, 14 ,,	1	
,, ,, ,, 21 ,,	1*	

* Gross staphylococcal lesions in survivor.

Table IV shows the findings in the second experiment. Cephalosporin was given subcutaneously in the same way as before and in the same dose (4 mg. a day), but the size of the inoculum of staphylococcus was varied. As in the first experiment, the drug increased the length of life but not the number of animals surviving. Decreasing the inoculum size a thousandfold did not affect the result.

It seemed likely that the dose of cephalosporin given in these experiments was insufficient, for it had been shown (Fig. 2) that a single dose of 1 mg. subcutaneously produced a detectable concentration in the serum for only two hours or so, and a single dose of 2 mg. a level detectable for only about three and a half hours. In these protection experiments there was an interval of seven hours after each dose of 1 mg. and an interval of ten hours after the dose of 2 mg. A further experiment was therefore done in which 5 mg. of cephalosporin was given by mouth every 12 hours. A single oral dose of this size gave a detectable serum concentration for about 15 hours (Fig. 3), and so repeated doses should maintain a detectable level throughout. The results of the experiment are shown in Table V. Under

TABLE IV

EFFECT OF INOCULUM SIZE ON ANTISTAPHYLOCOCCAL ACTION OF CEPHALOSPORIN P_1 *in vivo*

4 mg. given daily in three doses subcutaneously. LD50 was at a dilution of about 1 : 1,000,000

Dilution of culture:	1 : 1,000		1 : 5,000		1 : 10,000		1 : 50,000		1 : 100,000	
	Control	Cephalosporin	Control	Cephalosporin	Control	Cephalosporin	Control	Cephalosporin	Control	Cephalosporin
Mice alive at start ..	5	5	5	5	5	5	5	5	5	5
,, ,, after 24 hours	2	5	2	5	4	5	3	5	2	5
,, ,, ,, 48 ,,	0	4	1	5	0	5	2	1	0	4
,, ,, ,, 3 days		2	1	5		5	2	1		4
,, ,, ,, 4 ,,		1	0	5		4	1	1		4
,, ,, ,, 5 ,,		0		4		1	0	1		1
,, ,, ,, 6 ,,				3		1		1		1
,, ,, ,, 7 ,,				3		0		1		1
,, ,, ,, 14 ,,				2				1		1
,, ,, ,, 21 ,,				2*				1*		1*

* No staphylococcal lesions in survivors.

TABLE V

ANTISTAPHYLOCOCCAL ACTION OF CEPHALOSPORIN P_1 *in vivo*

5 mg. given by mouth every 12 hours for 5½ days. LD50 was at a dilution between 1 : 100,000,000 and 1 : 1,000,000,000

Dilution of culture:		1 : 10,000		1 : 100,000		1 : 1,000,000		Combined result	
		Control	Cephalosporin	Control	Cephalosporin	Control	Cephalosporin	Control	Cephalosporin
Mice alive at start ..		10	10	10	10	10	10	30	30
,, ,, after 24 hours		3	8	2	8	2	10	7	26
,, ,, ,, 48 ,, ..		2	7	2	7	2	10	6	24
,, ,, ,, 3 days..		2	7	2	6	2	10	6	23
,, ,, ,, 4 ,, ..		2	6	2	6	2	9	6	21
,, ,, ,, 5 ,, ..		2	6	2	6	2	9	6	21
,, ,, ,, 6 ,, ..		2	6	2	6	2	9	6	21
,, ,, ,, 7 ,, ..		2	4	2	6	2	9	6	19
,, ,, ,, 14 ,, ..		1	4	2	6	2	9	5	19
,, ,, ,, 21 ,, ..		1	3	1	5	2	9	4	17
Staphylococcal lesions in survivors	None	1	1		2		6	1	9
	Slight		1	1				1	1
	Gross		1		3	2	3	2	7

these conditions the cephalosporin not only increased the length of life but enabled some of the animals to survive the infection. The size of the inoculum of staphylococcus seemed to make some difference to the numbers surviving.

Nevertheless, the results were disappointing, for it is probable that an inhibitory level of cephalosporin was maintained in the blood for some five and a half days, and the defences of the animals might have been expected to deal with the cocci in that time, even if the drug was only present in bacteriostatic concentrations. Only with the small inoculum of staphylococci did the defences seem adequate.

TABLE VI

COMPARISON OF THE ANTISTAPHYLOCOCCAL ACTION OF CEPHALOSPORIN P_1, AUREOMYCIN, TERRAMYCIN, AND PENICILLIN *in vivo*

Cephalosporin, aureomycin, and terramycin were given orally and penicillin subcutaneously. The dose shown was repeated 12-hourly for 4½ days. LD50 was at a dilution of about 1 : 10,000,000

Drug and dose:	Control	Cephalosporin (mg.)		Aureomycin (mg.)			Terramycin (mg.)			Penicillin 17,000 units ≡ 10 mg.
		5	10	1	2.5	5	1	2.5	5	
A. Dilution of culture 1 : 100,000										
Mice alive at start ..	10	5	5	5	5	5	5	5	5	5
,, ,, after 24 hours	2	1	4	5	5	5	5	5	5	5
,, ,, ,, 48 ,,	0	1	2	5	5	5	5	5	5	5
,, ,, ,, 3 days		1	2	5	5	5	5	5	5	5
,, ,, ,, 4 ,,		1	2	5	5	5	5	5	5	5
,, ,, ,, 5 ,,		1	2	5	5	5	5	5	5	5
,, ,, ,, 6 ,,		1	2	5	5	5	5	5	5	5
,, ,, ,, 7 ,,		1	2	5	5	5	5	5	5	5
,, ,, ,, 14 ,,		1	2	5	5	5	5	4	5	5
,, ,, ,, 21 ,,		1	2	5	5	5	5	4	5	5
Staphylococcal lesions in survivors: None			1	3	5	5	5	3	4	5
Staphylococcal lesions in survivors: Slight									1	
Staphylococcal lesions in survivors: Gross		1	1	2				1		
B. Dilution of culture 1 : 1,000										
Mice alive at start ..	10	5	5	5	5	5	5	5	5	5
,, ,, after 24 hours	2	4	2	5	5	5	5	5	5	5
,, ,, ,, 48 ,,	0	3	0	5	5	5	5	4	5	5
,, ,, ,, 3 days		3		5	5	5	5	4	5	5
,, ,, ,, 4 ,,		3		5	5	5	5	4	5	5
,, ,, ,, 5 ,,		3		5	5	5	5	4	5	5
,, ,, ,, 6 ,,		3		5	5	5	5	4	5	5
,, ,, ,, 7 ,,		3		5	5	5	5	4	5	5
,, ,, ,, 14 ,,		3		5	5	5	4	4	5	5
,, ,, ,, 21 ,,		1		5	5	5	1	4	5	5
Staphylococcal lesions in survivors: None				1	4	2			1	4
Staphylococcal lesions in survivors: Slight		1		3	1	1			1	1
Staphylococcal lesions in survivors: Gross				1		2	1	4	3	

As aureomycin and terramycin, both drugs which are stated to be bacteriostatic *in vitro* at concentrations likely to be met with in the body, are being successfully used clinically for the treatment of staphylococcal infections resistant to penicillin, it was desirable to see how these drugs performed under the experimental conditions in which cephalosporin had been examined. An experiment was therefore done in which the effects of various doses of the three drugs given by stomach tube in 0.6 ml. water were compared. Two sizes of inoculum of the staphylococcus were used, and as before the injections were started one hour after infection. In addition, penicillin was administered subcutaneously to two groups of mice, large doses

being given, as there were 12-hour intervals between injections. Table VI records the results.

It is clear that both aureomycin and terramycin performed much better under these conditions than did cephalosporin.

Development of resistance in vivo

It will be recalled that resistance of the staphylococcus to cephalosporin was readily induced by suitable culture *in vitro*. It was possible therefore that the failure of cephalosporin in the protection tests was connected with a similar induction of resistance.

The sensitivity of a staphylococcus recovered from the blood of a mouse which died two and a half weeks after infection, and which had been treated by 5 mg. of cephalosporin by mouth every 12 hours for 4½ days, was compared with that of the culture used to infect the mouse. The two strains were equally sensitive.

The possibility remains that the organism became resistant during treatment but in the time that elapsed between the cessation of treatment and death again became sensitive. This possibility was not tested.

Discussion

The administration of penicillin has always been guided by the idea that the dose necessary, that is the level to be attained in the blood, bears a close relationship to the sensitivity *in vitro* of the organism causing the disease being treated. That this view holds good for penicillin has recently been reaffirmed by the extensive experimental results of Eagle, Fleischman, and Musselman (1950).

Evidence is accumulating, however, that it is not always possible to forecast the effect of an antibiotic *in vivo* from its performance *in vitro*. Thus helvolic acid, which inhibited the growth of the staphylococcus *in vitro* at considerable dilutions, was relatively ineffective *in vivo* against experimental disease, even though doses could be administered which were sufficient to confer on the blood strong inhibitory powers against the staphylococcus. The reason for this lack of therapeutic power was not elucidated, but at the time it was thought that it might be due to the fact that, unlike penicillin, the drug was bacteriostatic and not bactericidal. However, since those experiments were done aureomycin and terramycin, two drugs that are said to be bacteriostatic at concentrations likely to be met with *in vivo*, have been shown to be effective against both experimental infection in animals and natural infection in man.

Like helvolic acid, to which it has a number of chemical and biological points of similarity, cephalosporin P_1 has been found relatively ineffective *in vivo*, though strongly bacteriostatic *in vitro*, and even bactericidal at concentrations greater than 1 : 200,000. Cephalosporin P_1 is less toxic than aureomycin or terramycin when given intravenously (Harned, Cunningham, Clark, Cosgrove, Hine, McCauley, Stokey, Vessey, Yuda, and SubbaRow, 1948 ; P'an, Scaduto, and Cullen, 1950 ; Schoenbach, Bryer, and Long, 1950) ; sufficient can be given to maintain a concentration in the blood which strongly inhibits the infecting organism ; and it has much the same degree of antibacterial activity *in vitro* as aureomycin and terramycin. Nevertheless, *in vivo* it is greatly inferior to either.

The poor results given by cephalosporin P_1 *in vivo* do not appear to be determined by the development of resistance of the infecting organism, though this readily occurs *in vitro*, and for the present no satisfactory reason for its failure can be offered.

Just as there is a lack of correlation between the performance of cephalosporin *in vitro* and *in vivo*, so there is a similar though converse lack with aureomycin. This drug has been shown by Klein, Schorr, Tashman, and Hunt (1950) to be more active *in vivo* than its activity *in vitro* would give reason to suspect, especially when one considers the very considerable depression of its activity by serum *in vitro*. The work on aureomycin recorded in this paper accords with that of Klein *et al.* It is clear that there is something yet to be learnt about the exact mode of behaviour of cephalosporin P_1 and aureomycin *in vivo*. Perhaps the observations of Bigger and Ware (1950) on a factor in Lemco which makes sulphathiazole bactericidal may eventually throw some light on this matter. It may be, also, that there is some confusion in the use of the terms " bacteriostatic " and " bactericidal." It appears that aureomycin, like a number of other antibiotics, kills a very considerable proportion of the population of a sensitive species *in vitro* (Spicer, 1950), though not all. If it acts in the same way *in vivo* it would as a consequence cause a great diminution in the number of bacteria present in a lesion though not killing them all ; that is, its action *in vivo* would be essentially " bactericidal." However, further work is required on the mode of action of these substances *in vivo*.

At the present time an antibiotic has to have very particular powers to compete with those already introduced into medicine, and it might be reasoned that cephalosporin P_1, even if fully effective against staphylococcal infection in mice, would not have had much use owing to the limited range of bacteria against which it is effective. Such a substance, however, with limited range would be very useful if it did not interfere with the growth of intestinal organisms or cause the side-effects such as nausea, vomiting, and diarrhoea which may result from the administration of aureomycin and terramycin, and especially if its range included penicillin-resistant staphylococci. It is perhaps a measure of progress in the treatment of bacterial disease that little more than 10 years ago cephalosporin P_1 would have been considered a substance of outstanding qualities.

Summary

In vitro cephalosporin P_1 inhibits the growth of staphylococci, corynebacteria and *Cl. tetani* at considerable dilution. It has little effect on streptococci, Gram-negative organisms, or tubercle bacilli. Penicillin-resistant staphylococci are sensitive to the drug ; serum reduces but does not abolish its action. By suitable repeated culture staphylococci are easily made resistant to its action *in vitro*. In concentrations of more than 1 : 200,000 cephalosporin P_1 is bactericidal, in weaker concentrations it is bacteriostatic. It is more active against the staphylococcus than helvolic acid, and has the same order of activity as aureomycin and terramycin.

Given intravenously, cephalosporin P_1 is less toxic than aureomycin or terramycin, the LD50 for 20 g. mice being between 500 and 750 mg./kg. No gross or microscopic lesions were found *post mortem* in mice given 5 mg. 12-hourly by mouth for 5½ days. The drug did not affect the blood pressure of a decerebrate cat, but did modify the contractions of isolated guinea-pig uterine muscle. It is well absorbed

both when given subcutaneously and when given by mouth. After oral administration serum levels are attained and maintained which are greater than those necessary to inhibit the staphylococcus *in vitro*. The drug disappears from the blood rather quickly, although little is excreted in either urine or bile.

Mouse protection experiments show that the drug has some protective action against staphylococcal infections, but that this action is much weaker than that of either aureomycin or terramycin.

No evidence is available to explain this lack of antistaphylococcal activity *in vivo* compared with the high activity *in vitro*.

We are indebted to the Medical Research Council for grants towards technical assistance and materials used in this work. The cephalosporin P_1 was brewed and partially purified at the Antibiotics Research Station of the Medical Research Council at Clevedon. The final preparation was done by Dr. H. S. Burton. We have received invaluable technical help from Mr. J. Kent and Miss M. Lancaster. We are indebted to Dr. M. A. Jennings, Dr. E. P. Abraham, and Dr. N. G. Heatley for help in the preparation of this paper.

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Note added in proof: Just before proofs of this paper were received we became aware of the fact that the aureomycin used in these experiments was not pure. It was taken from capsules made for oral administration to man, and it appears that in these it is mixed with some other material. The same is probably true of the terramycin used. A capsule that contains 250 mg. of aureomycin contains about 130 mg. of other solids. This fact makes no difference to the arguments in this paper, but it does mean that pure aureomycin and terramycin hydrochlorides are more active against the staphylococcus than appears from the figures given, and that the doses administered in the protection experiments were smaller than was supposed.