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Flucloxacillin, a New Isoxazolyl Penicillin, Compared with Oxacillin, Cloxacillin, and Dicloxacillin

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Summary: Flucloxacillin, a new isoxazole penicillin, is active against penicillinase-producing strains of *Staphylococcus aureus* and is well absorbed in man after oral and intramuscular administration. Compared with isoxazole penicillins in current clinical use—namely, oxacillin, cloxacillin, and dicloxacillin—flucloxacillin has proved as active against Gram-positive cocci, including penicillin-resistant staphylococci. The extent of binding of flucloxacillin to the protein of human serum was similar to that of oxacillin and cloxacillin and less than that of dicloxacillin. In man flucloxacillin given orally produced total and free serum levels higher than those obtained with oxacillin and cloxacillin; total serum levels similar to those of dicloxacillin, and free levels greater than those of dicloxacillin. Similarly, after intramuscular injection the free serum levels of flucloxacillin were higher than those of oxacillin, cloxacillin, and dicloxacillin.

Introduction

The isoxazolyl series of semisynthetic penicillins, first synthesized and evaluated in these laboratories (Doyle *et al.,* 1961;

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Hanson *et al.,* 1965), combines resistance to penicillinase with acid stability and activity against Gram-positive bacteria. The isoxazole penicillins in current clinical use—namely, oxacillin, cloxacillin, and dicloxacillin—are absorbed when given by mouth or by injection and show significant activity against penicillin-resistant staphylococci and other Gram-positive cocci. The therapeutic efficacy of the isoxazole penicillins in the treatment of staphylococcal and streptococcal infections is now well established.

Oxacillin, cloxacillin, and dicloxacillin have closely related chemical structures (Fig. 1), and the antibacterial activities of these compounds are also similar, though oxacillin is somewhat less active than cloxacillin and dicloxacillin against penicillin-resistant staphylococci, and cloxacillin is rather less active than oxacillin and dicloxacillin against pneumococci (Knudsen *et al.,* 1962; Bennett *et al.,* 1965; Knott *et al.,* 1965). The main differences among the isoxazole penicillins lie in the serum concentrations obtained after administration of the compounds and in the extent to which they are bound to serum protein.

Oxacillin, the first member of the series, gives rise to serum concentrations which are substantially lower than those obtained with cloxacillin both by mouth and by injection (Knudsen *et al.,* 1962; Bunn and Milicich, 1964; Gravenkemper *et al.,* 1964), while dicloxacillin results in serum levels higher than those of cloxacillin when given by mouth (Bennett *et al.,* 1965; Knott *et al.,* 1965). Dicloxacillin,

however, is more highly bound to serum protein than is cloxacillin (Gloxhuber *et al.*, 1965; Kunin, 1966), and this factor may be held to detract from the superior levels of total antibiotic measured in the plasma (Gloxhuber *et al.*, 1965; Rolinson, 1967; *Medical Letter*, 1968).

In this present paper the properties of a further member of the isoxazole series is reported. This compound (3-(2-chloro-6-fluorophenyl)-5-methyl-4-isoxazolyl-penicillin has the generic name flucloxacillin (Floxapen) (Fig. 1) and details are given

Flucloxacillin, 3-(2-chloro-6-fluorophenyl)-
5-methyl-4-isoxazolylpenicillin, sodium salt
monohydrate

Molecular weight 493·9
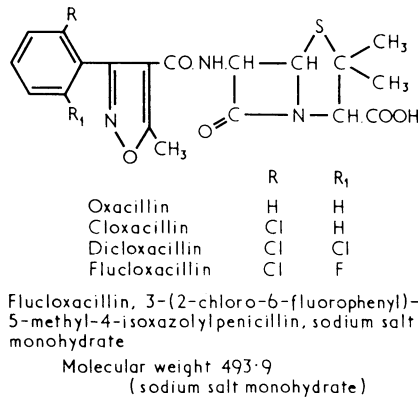(sodium salt monohydrate)

FIG. 1.—Isoxazolyl penicillins in clinical use.

regarding antibacterial activity, protein binding, and serum levels of this new penicillin in comparison with oxacillin, cloxacillin, and dicloxacillin.

Materials

Flucloxacillin is available as the sodium salt monohydrate, in which form it is a white or off-white crystalline powder. It is freely soluble in water, and a solution of 10% w/v flucloxacillin in phosphate buffer (pH 7·0) retains 98% of its initial potency after storage for 14 days at 5° C. A 10% solution of flucloxacillin in water has a pH of 5-7.

Antibacterial Activity of Flucloxacillin

Minimum inhibitory concentrations (M.I.C.) required to prevent growth of the test organisms for 24 hours at 37°C. were determined by serial dilution in nutrient broth (Oxoid No. 2) or nutrient agar (blood agar base, Oxoid). Horse blood (5%) was incorporated in the media for tests with streptococci, pneumococci, and *Haemophilus* species. For tests in liquid medium the inoculum was normally one drop (0·03 ml.) of an overnight broth culture of the test organism (about 10⁷ cells) in 5 ml. of medium. Serial dilutions in agar were poured into Petri dishes and inoculated with a multiple-inoculating device delivering a drop (0·003 ml.) of an overnight broth culture (about 10⁶ cells).

Antibacterial Spectrum.—The antibacterial spectrum of flucloxacillin compared with cloxacillin and benzylpenicillin is shown in Table I, where it can be seen that flucloxacillin was active against the Gram-positive bacilli tested and against Gram-positive cocci, with the exception of *Streptococcus faecalis.* In general, the Gram-negative bacteria tested were relatively insensitive to flucloxacillin, the enterobacteriaceae requiring concentrations of 500 μg./ml. or greater for inhibition. Strains of *Haemophilus influenzae, Bordetella pertussis, Pasteurella septica,* and *Brucella abortus* were more susceptible, but were only inhibited by 5·0-12·5 μg/ml. In contrast, the gonococci and meningococci were sensitive to flucloxacillin, and were inhibited at a concentration of 0·1 μg./ml. Flucloxacillin was as active as cloxacillin against the test organisms and was generally five to ten times less active than benzylpenicillin

TABLE I.—*Antibacterial Spectra of Flucloxacillin, Cloxacillin, and Benzylpenicillin*

Organism	M.I.C. (μg./ml.)		
	Flucloxacillin	Cloxacillin	Benzylpenicillin
*Staphylococcus aureus**	0·1–0·25	0·1–0·25	0·02
Staphylococcus aureus†	0·25–0·5	0·25–0·5	>50
β-Haemolytic streptococcus	0·05–0·1	0·1	0·01
α-Haemolytic streptococcus	0·5	0·5	0·05
Streptococcus pneumoniae	0·25	0·25–0·5	0·01
Streptococcus faecalis	25	25	5·0
Bacillus subtilis	0·5	0·5	0·1
Bacillus anthracis	0·5	0·5	0·05
Sarcina lutea	0·25	0·25	0·005
Listeria monocytogenes	1·25	1·25	0·05
Corynebacterium diphtheriae	0·25	0·1	0·02
Erysipelothrix rhusiopathiae	0·25	0·1	0·05
Clostridium tetani	0·25	0·5	0·02
Clostridium welchii	0·25	0·5	0·02
*Neisseria gonorrhoeae**	0·1	0·1	0·01
Neisseria gonorrhoeae†	2·5	2·5	0·1
Neisseria meningitidis	0·1	0·1	0·01
Haemophilus influenzae	5·0–12·5	12·5–25	0·5
Bordetella pertussis	12·5	25	0·5
Pasteurella septica	12·5	12·5	0·1
Brucella abortus	5·0	5·0	0·1
Escherichia coli	>500	>500	50
Proteus mirabilis	>500	500	5·0
Salmonella typhi	>500	500	5·0
Shigella sonnei	>500	500	12·5
Klebsiella aerogenes	>500	>500	>500
Pseudomonas aeruginosa	>500	>500	>500
Vibrio cholerae	500	250	1·25
Candida albicans	>500	>500	>500

*Penicillin-sensitive. †Penicillin-resistant.

against Gram-positive bacteria, with the exception of penicillinase-producing strains of staphylococci which were resistant to benzylpenicillin but sensitive to flucloxacillin.

Activity against Streptococci.—Flucloxacillin was inhibitory to β-haemolytic streptococci at concentrations of 0·05-0·1 μg./ml., and inhibited most of the pneumococci tested at concentrations of 0·1-0·25 μg./ml. (Table II). Against these organisms flucloxacillin was as active as dicloxacillin and oxacillin, and was slightly more active than cloxacillin.

Activity against Staphylococci.—All four isoxazole penicillins showed a similar level of activity against penicillin-sensitive strains of staphylococci compared with 0·02 μg./ml. for benzylpenicillin (Table III). In the case of the penicillin-resistant staphylococci most strains were inhibited by flucloxacillin at a concentration of 0·25-0·5 μg./ml.; both flucloxacillin and dicloxacillin were marginally more active than cloxacillin, while oxacillin was the least active of the four isoxazole penicillins (Table III). The stability of flucloxacillin to staphylococcal penicillinase compared with benzylpenicillin was further illustrated by determining the effect of altering the inoculum size on the relative activities of the penicillins against a penicillinase-producing strain of *Staphylococcus aureus.* In this test, benzylpenicillin was active against an inoculum of a 1/1,000 dilution of an overnight culture at a concentration of 0·25 μg./ml., but against an undiluted culture the M.I.C. of benzylpenicillin was raised to 125 μg./ml. as a result of the penicillinase activity of the large inoculum. In contrast, the M.I.C. of flucloxacillin increased only twofold, from 0·25 to 0·5 μg./ml., under the same conditions.

Methicillin-resistant Staphylococci. — Naturally occurring strains of methicillin-resistant staphylococci were also resistant to flucloxacillin as well as to cloxacillin and dicloxacillin (Table IV). The resistance of these strains of staphylococci was not due to inactivation of the penicillin in question but to an intrinsic resistance. Such strains comprised mixed populations of cells of which only a minority was resistant while the majority was of normal sensitivity (Sutherland and Rolinson, 1964). As a result of this heterogeneity a small inoculum appeared sensitive to the isoxazole penicillins, whereas a large inoculum appeared resistant.

pH and Antibacterial Activity of Flucloxacillin.—The M.I.C.s of flucloxacillin against *Staph. aureus* were determined in nutrient broth incorporating M/20 phosphate buffer at pH 5·8, pH 7·2, and pH 8·0. The activity of flucloxacillin

TABLE II.—*M.I.C. Values of Flucloxacillin, Cloxacillin, Dicloxacillin, and Oxacillin Against Streptococci and Pneumococci*

Organism	No. of Strains	Penicillin	M.I.C. (μg.ml.) and No. of Strains									
			>1·25	1·25	0·5	0·25	0·1	0·05	0·02	0·01	0·005	0·002
β–haemolytic streptococcus	10	Flucloxacillin					5	5				
		Cloxacillin					10					
		Dicloxacillin					6	4				
		Oxacillin					4	5	1			
		Benzylpenicillin									7	3
Str. pneumoniae	18	Flucloxacillin			1	12	3	1		1		
	18	Cloxacillin			5	9	3	1		1		
	18	Dicloxacillin			1	11	4	1		1		
	8	Oxacillin					6	2				
	8	Benzylpenicillin								7	1	

TABLE III.—*Relative Activities of Flucloxacillin, Cloxacillin, Dicloxacillin, and Oxacillin Against Staphylococcus aureus*

Penicillin	No. of Strains	Mean	M.I.C. (μg./ml.) and No. of Strains						
			1·25	0·5	0·25	0·1	0·05	0·02	0·01
Penicillin-sensitive Staphylococci									
Flucloxacillin	65	0·16		6	14	43	2		
Cloxacillin ..	65	0·21		10	29	26			
Dicloxacillin	65	0·17		2	27	33	3		
Oxacillin ..	43	0·15		2	13	26	2		
Benzyl-penicillin	38	0·03					12	25	1
Penicillin-resistant Staphylococci									
Flucloxacillin	72	0·31	1	22	43	4	2		
Cloxacillin ..	72	0·34	1	29	37	5			
Dicloxacillin	72	0·28	1	17	44	9	1		
Oxacillin ..	72	0·41	6	39	23	4			

TABLE IV.—*Relative Activities of Flucloxacillin, Cloxacillin, and Dicloxacillin Against a Naturally Occurring Methicillin-resistant Strain of Staphylococcus aureus 1757*

Inoculum (No. of Cells)	M.I.C.* (μg./ml.)					
	Flucloxacillin		Cloxacillin		Dicloxacillin	
	24 hr.	48 hr.	24 hr.	48 hr.	24 hr.	48 hr.
20 × 10⁶	2·5	50	2·5	25	1·25	50
20	0·1	1·25	0·25	0·5	0·25	0·5

*Serial dilution in nutrient broth.

was not greatly influenced by the pH of the medium, the penicillin being generally two to four times more active at pH 5·8 than at pH 7·2 or above.

Bactericidal Activity.—In viable-count experiments both flucloxacillin and cloxacillin showed a similar degree of bactericidal activity against a penicillin-resistant strain of *Staph. aureus,* and concentrations only slightly greater than the M.I.C. resulted in a substantial reduction in the viable count during the first few hours (Fig. 2).
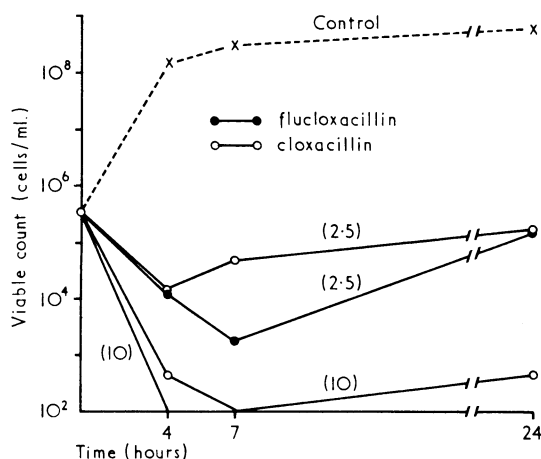

FIG. 2.—Bactericidal activities of flucloxacillin and cloxacillin against a penicillinase-producing strain of *Staphylococcus aureus* 1089. Figures in parentheses are concentrations (μg./ml.).

Effect of Serum on Antibacterial Activity.—Flucloxacillin was 10 to 20-fold less active in 95% human serum than in nutrient broth, and a similar reduction in activity was also seen with cloxacillin and dicloxacillin. Thus the minimum bactericidal concentrations of these penicillins against two penicillinase-producing strains of *Staph. aureus* increased from 0·15 μg./ml. in nutrient broth to 1·5–3·0 μg./ml. in human serum.

Binding of Flucloxacillin to Serum Proteins

The extent of binding of flucloxacillin and the other isoxazole penicillins to the proteins of human serum was measured by an ultrafiltration technique (Rolinson and Sutherland, 1965) in which pooled human serum, pH 7·4, containing 50 μg. of the test penicillin per ml. was filtered through Visking viscose-cellulose dialysis tubing. The penicillin content of the protein-free ultrafiltrate was measured by microbiological assay, and this quantity represented the free unbound fraction of penicillin in serum. The amount of protein-bound penicillin was derived by subtracting the level of free penicillin from the known total concentration in serum. The results of the ultrafiltration experiments are shown in Table V, where each value repre-

TABLE V.—*Extent of Binding of Isoxazolyl Penicillins in Human Serum*

Penicillin	% Bound	% Free
Oxacillin	93·1	6·9
Cloxacillin	94·0	6·0
Dicloxacillin	96·9	3·1
Flucloxacillin	94·7	5·3

sents the mean of at least three determinations. It will be seen that the unbound levels of flucloxacillin, cloxacillin, and oxacillin were very similar. Dicloxacillin, on the other hand, was bound to serum protein to a significantly greater extent, the unbound fraction of dicloxacillin being almost exactly half that of cloxacillin.

Sensitivity Disc Tests

Flucloxacillin sensitivity discs (5 μg.) produced inhibition zones in tests with penicillin-sensitive and penicillinase-producing strains of *Staph. aureus* which were of the same order as those obtained with 5 μg. cloxacillin sensitivity discs. Naturally occurring strains of *Staph. aureus,* resistant to methicillin and to the isoxazole penicillins, often appeared to be sensitive to flucloxacillin discs, as with cloxacillin discs, though resistant colonies were sometimes observed within the inhibition zones when a large inoculum of cells was examined after overnight incubation at 37° C., and were common after incubation for 48 hours at 37° C. Dilute inocula almost invariably appeared sensitive when tested at 37°C., as is the case for methicillin and cloxacillin (Sutherland and Rolinson, 1964). Resistance to flucloxacillin was generally readily demonstrated when sensitivity tests were incubated at 30°C. (Annear, 1968) or involved the use of 5% NaCl agar (Barber, 1964; Sutherland, 1964), as is the case with other penicillinase-stable penicillins.

Absorption and Excretion in Human Volunteers

Assay in Serum and Urine

The concentrations of flucloxacillin and other isoxazole penicillins in serum and urine were measured by standard cup-plate microbiological assay with *Sarcina lutea* N.C.T.C. 8340 as assay organism (Knudsen *et al.*, 1962). For the assay of serum specimens, standard solutions of flucloxacillin, cloxacillin, or dicloxacillin were prepared in pooled human serum to give a range of 0·5-50 μg./ml.; for urine specimens the standard solutions were prepared in M/20 phosphate buffer, pH 7·0, over a range of 0·25-20 μg./ml. Oxacillin was more active than the other penicillins against *Sarcina lutea* N.C.T.C. 8340, and was diluted in serum to give a range of 0·1-10 μg./ml. or in buffer from 0·05 to 5 μg./ml. The plates were incubated overnight at 30° C., the inhibition zone diameters were measured, and the concentrations of the test specimens were derived from the standard line constructed from the standard solution.

Oral Administration

The penicillins were given in gelatin capsules as a single dose of 250 or 500 mg. to healthy fasting volunteer subjects. Blood specimens were taken at ½, 1, 2, 3, 4, or 6 hours after administration of the dose, and urine was collected over the 0 to 6-hour period. Levels of total antibiotic were measured by microbiological assay, and the serum concentrations of free, unbound antibiotic were calculated from these total levels and from the values for the extent of binding of the isoxazole penicillins in human serum.

Data in Table VI represent the total and free serum levels obtained in numerous studies with fasting subjects given single oral doses of flucloxacillin, dicloxacillin, cloxacillin, or oxacillin. It will be seen that the total serum levels of oxacillin were substantially lower than those of cloxacillin, and likewise the free levels of oxacillin were less than those of cloxacillin. With dicloxacillin, a 250-mg. dose produced total serum levels comparable to those attained with the 500-mg. dose of cloxacillin. Dicloxacillin, however, is bound to serum protein to a significantly greater extent than cloxacillin, and consequently when this factor is taken into account the peak free levels of dicloxacillin were only about half those of cloxacillin. In the case of flucloxacillin, the total levels obtained with a 250-mg. dose were similar to those obtained with 250 mg. of dicloxacillin; but because flucloxacillin is bound to serum protein to a lesser degree than dicloxacillin the free serum levels of flucloxacillin were considerably higher than those of dicloxacillin. The total levels of flucloxacillin after administration of a 250-mg. dose were almost equal to those obtained with 500 mg. of cloxacillin, and as the extent of binding of both penicillins is very similar, the unbound levels of flucloxacillin were only slightly lower than those of cloxacillin.

The mean values for flucloxacillin and cloxacillin obtained from three separate cross-over studies involving 43 healthy volunteers are shown in Fig. 3. A single dose of 500 mg. was given in capsule form to the fasting subjects. It will be seen that at all times the serum levels of total antibiotic were considerably higher in the case of flucloxacillin than with clox-
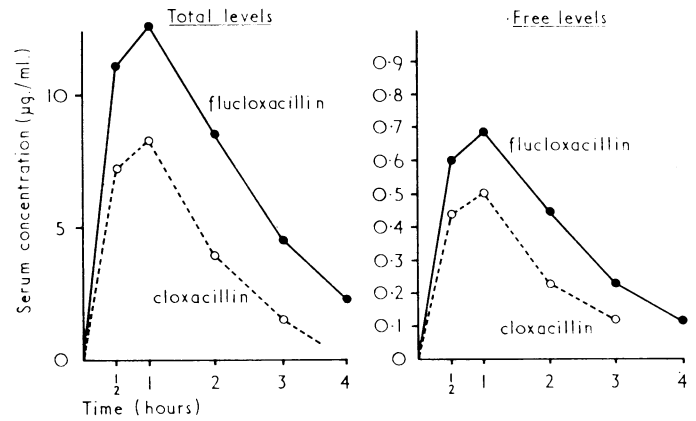

FIG. 3.—Serum concentrations of flucloxacillin and cloxacillin after a single 500-mg. oral dose to fasting human subjects. Cross-over studies in 43 subjects.
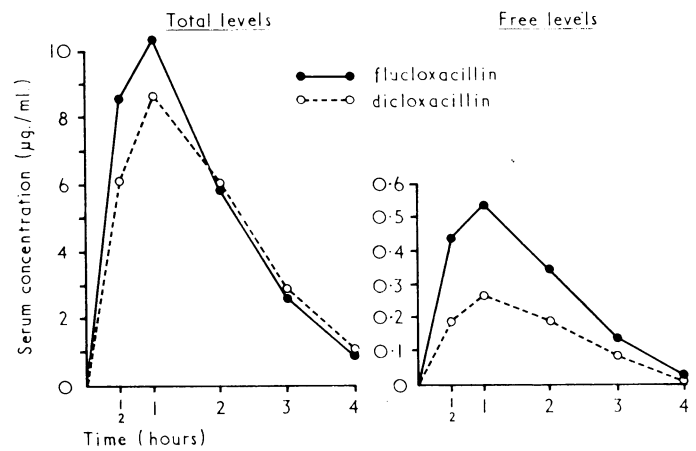

FIG. 4.—Serum concentrations of flucloxacillin and dicloxacillin after a single 250-mg. oral dose to fasting human subjects. Cross-over studies in 32 subjects.

acillin. Levels of free flucloxacillin were also significantly higher than those of cloxacillin, since the extent of binding of these two compounds is similar.

Similarly, data in Fig. 4 show the mean values for flucloxacillin and dicloxacillin obtained in two separate cross-over studies involving 32 subjects in the fasting state. In these studies a single dose of 250 mg. was given by mouth in capsule form. It will be seen that the serum levels of total antibiotic were similar for the two penicillins. As a result of the difference in serum binding, however, free levels of flucloxacillin were substantially higher than those of dicloxacillin. At the peak levels this difference was about twofold.

In these studies the absorption of the isoxazole penicillins showed considerable variation between one volunteer and another, and there was also considerable variation in the time at which peak blood levels were reached, though such variation is not apparent from the mean values as shown in Figs. 3 and 4. For example, in the cross-over studies with flucloxacillin and cloxacillin, at a dose of 500 mg. the peak levels of total antibiotic for the 43 subjects involved ranged from 3·4

TABLE VI.—*Serum Concentrations in Volunteers (A Single Dose in the Form of a gelatin capsule was Taken by Volunteers in the Fasting State)*

Penicillin						Dose (mg.)	No. of Subjects	Mean Total Level (μg./ml.)					Mean Free Level* (μg./ml.)				
								½ hr.	1 hr.	2 hr.	3 hr.	4 hr.	½ hr.	1 hr.	2 hr.	3 hr.	4 hr.
Oxacillin	..	..	..	..	..	500	25	5·6	4·9	1·5	0·6	0·2	0·39	0·34	0·1	0·04	0·01
Cloxacillin	..	..	..	..	..	500	122	8·4	9·2	4·5	<2·0	<2·0	0·5	0·55	0·27	—	—
Flucloxacillin	..	..	..	..	..	500	53	13·9	14·5	6·7	4·4	2·2	0·73	0·76	0·35	0·23	0·12
Flucloxacillin	..	..	..	..	..	250	48	6·9	8·8	5·0	2·2	<1·0	0·36	0·46	0·26	0·12	—
Flucloxacillin	..	..	..	..	..	125	11	1·9	5·8	2·9	1·1	<1·0	0·1	0·31	0·15	0·06	—
Dicloxacillin	..	..	..	..	..	250	84	6·4	9·3	6·7	3·1	1·4	0·2	0·29	0·2	0·1	0·04

*Free levels in serum samples calculated from the total levels and extent of binding. Free concentrations in human serum were found to be oxacillin 6·9%, cloxacillin 6·0%, dicloxacillin 3·1%, and flucloxacillin 5·3%.

to 26·5 µg./ml. in the case of flucloxacillin and from 1·5 to 30·0 µg./ml. with cloxacillin. Mean peak levels, irrespective of the time the peak was reached, were 14·7 µg./ml. for flucloxacillin and 9·6 µg./ml. for cloxacillin, and as a result of a similar degree of serum binding the peak levels of unbound flucloxacillin were also significantly higher than those of cloxacillin. In the cross-over studies with flucloxacillin and dicloxacillin, at a dose of 250 mg., a wide range of peak values (from 3·0 to 26·0 µg./ml. with both penicillins) was again seen among the subjects involved. Mean peak total levels of flucloxacillin and dicloxacillin were similar (11·0 µg./ml.), but as a result of the difference in protein binding the mean peak levels of free flucloxacillin were considerably higher than those of dicloxacillin.

Urinary Excretion

In the cross-over study with flucloxacillin and cloxacillin at 500 mg. (Fig. 2), 50·5% and 39·5% of the doses respectively were recovered in the urine collected over 0-6 hours. In the study with flucloxacillin and dicloxacillin at 250 mg. (Fig. 4) the recovery was 55% and 59% respectively over the same period.

Effect of Food on Absorption

Flucloxacillin was given at a 250-mg. or 500-mg. dose to volunteer subjects one hour after they had eaten a standard breakfast. Absorption of flucloxacillin was delayed and the peak serum concentrations were about half those obtained in fasting subjects (Table VII). Similar effects have been

TABLE VII.—*Effect of Food on Oral Absorption of Flucloxacillin*

Dose (mg.)	State	No. of Subjects	Average Serum Concentrations (µg./ml.)						Excretion 0–6 hr. (%)
			½ hr.	1 hr.	2 hr.	3 hr.	4 hr.	6 hr.	
250	Fasting	48	6·9	8·8	5·0	2·2	<1·0	—	59
	Non-fasting	8	<1·0	2·6	5·2	4·6	2·1	—	56
500	Fasting	43	11·1	12·6	8·4	4·6	2·2	—	50
	Non-fasting	12	<1·0	3·2	7·9	—	3·6	<1·0	35

reported for cloxacillin and oxacillin (Knudsen *et al.*, 1962; Gravenkemper *et al.*, 1964).

Intramuscular Administration

Total and free serum levels are shown in Fig. 5 for cloxacillin, dicloxacillin, and flucloxacillin administered as single intramuscular injections of 250 mg. to healthy volunteers. The total levels of flucloxacillin were slightly higher than those of dicloxacillin, which in turn were higher than those of cloxacillin. Taking the extent of binding into consideration it will be seen that the free levels of flucloxacillin were appreciably higher than those of cloxacillin or dicloxacillin.

Data for oxacillin, cloxacillin, and flucloxacillin after intramuscular injection of a single 500-mg. dose to volunteers are shown in Fig. 6. Total and free levels of the three compounds were similar with regard to the peak values, but the levels of flucloxacillin were considerably more prolonged than those of oxacillin and slightly more prolonged than with cloxacillin.

Discussion

Our results show that flucloxacillin was generally similar to oxacillin, cloxacillin, and dicloxacillin, in terms of spectrum and level of activity against Gram-positive bacteria. Thus
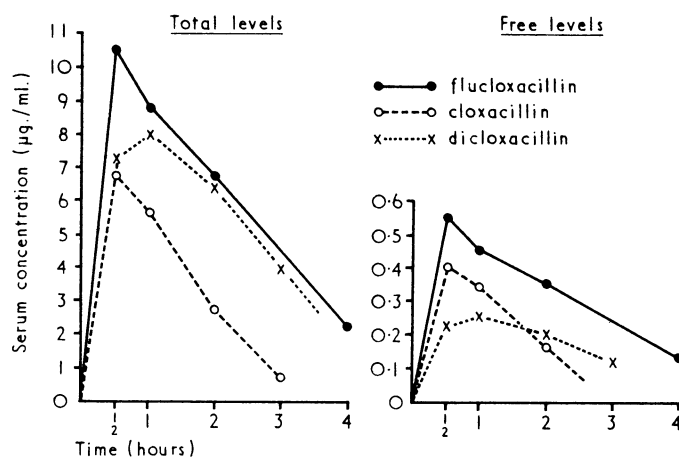

FIG. 5.—Serum concentrations after a single intramuscular injection of 250 mg. in human subjects.
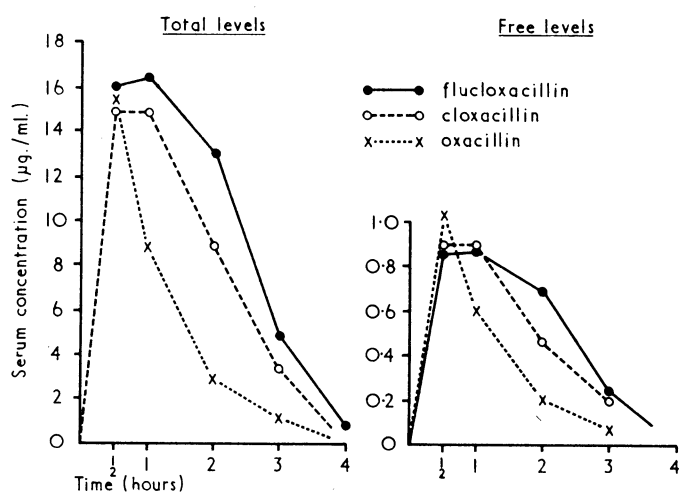

FIG. 6.—Serum concentrations after a single intramuscular injection of 500 mg. in human subjects.

flucloxacillin was as active as cloxacillin and dicloxacillin against penicillinase-producing strains of *Staph. aureus* and was more active than oxacillin in this respect. The four penicillins were equally active against penicillin-sensitive staphylococci, while cloxacillin was the least active against β-haemolytic streptococci and pneumococci.

In common with oxacillin, cloxacillin, and dicloxacillin, flucloxacillin showed a high degree of binding to serum proteins. In the tests reported here flucloxacillin was bound to serum protein to a similar extent as oxacillin and cloxacillin, but to a markedly lesser extent than dicloxacillin. Thus, for the same level of total antibiotic in serum, the unbound levels of oxacillin, cloxacillin, and flucloxacillin would be about twice as high as the unbound levels of dicloxacillin.

Flucloxacillin was well absorbed in people after administration by both oral and intramuscular routes. When given by mouth flucloxacillin gave rise to total serum levels equivalent to those obtained with dicloxacillin, and superior to those of cloxacillin and oxacillin. Moreover, when the extent of serum binding of these penicillins was considered the free levels of flucloxacillin were superior to those of dicloxacillin as well as those of cloxacillin and oxacillin. The relationship of the total serum levels for a given oral dose of oxacillin, cloxacillin, dicloxacillin, and flucloxacillin might be expressed approximately as 1:2:4:4, respectively, but the levels of unbound penicillins would be 1:2:2:4. Thus the superior absorption of dicloxacillin compared with cloxacillin is counterbalanced by the higher serum binding of the former (Kunin, 1966; *Medical Letter*, 1968) whereas the higher total levels of

flucloxacillin also represent higher free levels of active penicillin. Hence the administration of oral flucloxacillin results in serum levels of unbound drug higher than those obtained with oxacillin, cloxacillin, or dicloxacillin, thus providing higher concentrations of active antibiotic for tissue distribution (Scholtan and Schmid, 1962; Kunin, 1965, 1966; Verwey *et al.*, 1966). Similarly, after administration by intramuscular injection the serum levels of total flucloxacillin were superior to those of the other isoxazole penicillins, and consequently the levels of unbound antibiotic are also greater for flucloxacillin than for oxacillin, cloxacillin, or dicloxacillin.

In addition to our data, experiments carried out in these laboratories by Mr. P. Acred and Mrs. P. Hunter have shown that flucloxacillin is highly active against experimental infections in mice with penicillin-resistant staphylococci. Consideration of the particular properties of flucloxacillin—namely, antibacterial activity, protein binding, and absorption in human subjects—shows that the compound is superior to oxacillin, cloxacillin, and dicloxacillin, and suggests that the clinical efficacy of flucloxacillin may be expected to compare favourably with those isoxazole penicillins at present in clinical use.

We wish to thank Dr. J. H. C. Nayler and his colleagues for preparing the penicillins used in these studies, and Mrs. Eleanor Fairclough for skilled technical assistance.

Addendum

D. M. BROWN, Beecham Research Laboratories, writes: Acute toxicity determinations were carried out in the mouse and in the dog. In the mouse the LD_{50} was established at 3·8 g./kg. orally and 2·2 g./kg. subcutaneously. In the dog, doses up to 10 g./kg. were administered orally, but all dogs vomited when they received 4·5 g./kg. and a lethal effect in the species could not be established. Long-term toxicity studies were carried out in the rat and in the dog. Doses of 200, 500, and 2,000 mg./kg. orally and 250 mg./kg. subcutaneously were administered to both species, but in the dog study the

high dose had to be reduced to 1,000 mg./kg. as most of the animals vomited after receiving the drug.

Laboratory studies, which included urine and blood biochemistry and haematology, were carried out at 0, 4, 12, and 25 weeks in the rat and at 0, 6, 12, 18, and 24 weeks in the dog. At termination (25th week) necropsy was carried out on all animals and the essential organs were removed for histopathology. No adverse reactions were noted except very slight irritation at the site of the subcutaneous injections.

Teratological studies were performed in the rat and the mouse, doses of 200 and 500 mg./kg. being administered orally during days 6-15 of pregnancy in the rat and days 8-17 of pregnancy in the mouse. No adverse reactions were noted in the mothers or the fetuses except in the rat at the high dose, which was greatly in excess of the therapeutic dose, where there was an increased incidence of resorptions.

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