Brit. J. Pharmacol. (1963), **21**, 339–354.

FURTHER PHARMACOLOGY AND CHEMOTHERAPY OF CLOXACILLIN

BY

P. ACRED AND D. M. BROWN

From the Department of Pharmacology and Chemotherapy, Beecham Research Laboratories, Brockham Park, Betchworth, Surrey

(*Received June* 21, 1963)

In concentrations greatly in excess of therapeutic blood levels cloxacillin has a slight hypotensive action. As with other antibiotics, it sometimes causes diarrhoea in rabbits but, in doses up to 200 mg/kg, it does not have a teratogenic effect on the rabbit foetus. Cloxacillin is distributed throughout the body. High concentrations are found only in the liver and kidney, these reflecting the high concentrations in the bile and urine respectively. It differs from other penicillins investigated in that there appears to be little or no renal tubular secretion as demonstrated in experiments on the hen. Cloxacillin is excreted as the unchanged drug and as an active metabolite in the urine and bile. After oral administration it is metabolized in the caecum giving a penicillin which differs from the urinary metabolite and which has a greater antibiotic action against *Sarcina lutea* than the parent penicillin. The activity of the caecal metabolite against resistant and sensitive strains of *Staphylococcus*, however, remains similar to that of cloxacillin. Against infections due to resistant and sensitive *Staphylococcus* in animals cloxacillin is active by both the oral and subcutaneous routes and it is more effective orally than an equal subcutaneous dose of methicillin.

Nayler, Long, Brown, Acred, Rolinson, Batchelor, Stevens & Sutherland (1962) have recently reported on the chemistry, toxicology, microbiology and pharmacology of a new semi-synthetic penicillin, cloxacillin. This penicillin is stable to staphylococcal penicillinase and is active orally. Further studies on the chemotherapy and pharmacology are described in this paper.

METHODS

In all the experimental work cloxacillin was used as its pure sodium salt monohydrate, which is freely soluble in cold water giving a solution stable at 5° C for 1 week. Methicillin (Celbenin) and penicillin G (Solupen) were also administered as their pure sodium salts.

The antibiotics were assayed by the cup-plate technique using *Sarcina lutea* as the test organism. The zone diameters obtained for the control dilution of the antibiotics were plotted against the log of the concentration, and from the regression lines obtained the concentrations of the antibiotics in the specimens were estimated by interpolation. The appropriate dilutions of the controls and samples were made in 0.05 M-phosphate buffer at *p*H 7.0, except that in the experiments where serum concentrations were determined the controls were prepared in serum. Before assay all specimens were stored at 4° C.

Chromatographic studies were carried out using 1 cm wide strips of Whatman No. 1 filter paper 53 cm long with the origin at 11 cm. The solvent was a saturated solution of ether in water buffered to *p*H 6.2 (Karnovsky & Johnson, 1949). 100 ml. of solvent were used

in the troughs of each tank. All tanks were lined with Whatman No. 3 filter paper. The chromatograms were run from 16 to 20 hr at 20° C after equilibration for at least 1 hr.

Detection of the penicillins on the chromatograms was carried out by placing the strips on agar plates seeded with *Sarcina lutea* and incubating overnight at 30° C. The location of the penicillins was seen as clear zones of inhibition of growth on the plates. Control strips were set up using an aqueous solution of cloxacillin.

Cardiovascular and respiratory effects

The carotid arterial blood pressure of cats anaesthetized with a 1% solution of chloralose was recorded with a mercury manometer on a smoked drum. Respiration was recorded by a lever connected by means of a thread sewn to the skin over the xiphisternum. Compounds in 0.9% saline were administered intravenously through a cannula in a femoral vein. The actions of cloxacillin on the blood pressure responses to acetylcholine (4 μg), adrenaline (10 μg) and histamine (2 and 0.5 μg) were observed. The effect on the blood pressure response to stimulation of the uncut cervical vagus nerve (a rectangular-wave stimulus of 2 V and 5 msec duration, at a rate of 20 shocks/sec for 5 sec every 5 min) was also observed.

The electrocardiogram was recorded by an Ediswan pen recorder linked to hypodermic needle electrodes, placed subcutaneously 2 cm on either side of the midline of the anterior thorax. After the administration of cloxacillin, tracings for 30 sec periods were obtained at 2 min intervals. Simultaneous records of carotid arterial blood pressure were obtained by means of a capacitance manometer, and respiratory movements by a thermistor device (Bainbridge, Warren & Wiggins, 1962).

The action of cloxacillin on the superior cervical ganglion of cats was studied by applying rectangular-wave stimuli of 5 msec duration at a rate of 20 shocks/sec for 30 sec every 10 min to the preganglionic trunk. The voltage was adjusted to give just maximal contractions of the nictitating membrane. A similar stimulus was applied to the postganglionic trunk 2 min after the preganglionic stimulation. Responses were recorded by a frontal writing lever on a smoked drum. The effect of intravenous injection of adrenaline (10 μg) on the nictitating membrane was also observed.

Blood concentrations of cloxacillin were determined in each of six cats which had had successive doses of cloxacillin, samples being taken from the left femoral vein every 10 min after the administration of the penicillin.

Pregnancy tests

Six female rabbits of mixed breed and six New Zealand white females of proved fertility were mated. From the 8th day of pregnancy nine of the rabbits (three mixed breed and six New Zealand) were injected intramuscularly with 250 mg/kg of cloxacillin in aqueous solution, and three (mixed breed) were given 180 mg/kg of thalidomide orally. Treatment continued until the 16th day of pregnancy. The animals were observed daily for abortions during the whole course of pregnancy. The animals which came to term had their litters normally.

Absorption of penicillins

Rabbits. 100 mg/kg of cloxacillin, methicillin and penicillin G were administered intramuscularly to groups of five rabbits. Blood samples were removed from the lateral ear vein at 0.5, 1, 2, 4 and 6 hr after administration. The samples were allowed to clot at room temperature and the serum removed for assay.

Dogs. Cloxacillin was administered orally at a dose of 100 mg/kg. Blood samples (0.3 ml.) for assay were taken at 0.5, 1, 2, 3, 4 and 6 hr after administration, by means of a sterile syringe from the radial vein and placed in heparinized tubes containing 5.0 U of heparin in 0.05 ml. of saline.

Distribution and elimination of cloxacillin

Distribution in tissues. The distribution of cloxacillin throughout the body was determined in groups of five male rats, weighing 100 to 200 g, after oral and intramuscular administration of 100 mg/kg. Groups of rats were killed by exsanguination at 0, 0.5, 2, 4, 6 and 24 hr after administration of the penicillin. The urine and faeces from each group were collected and the amounts recorded. The following organs and tissues from the rats receiving the intramuscular dose were removed and weighed: liver, spleen, kidneys, lungs, small intestine, large intestine, caecum, muscles from the site of injection and carcass.

After oral administration of cloxacillin the stomach also was removed. All specimens were homogenized in a "Waring blendor" and appropriate dilutions were made with phosphate buffer at *p*H 7.0 for assay.

Urinary excretion. The mode of urinary excretion in the hen was examined as described by Acred, Brown, Turner & Wright (1961), by stitching a polyethylene funnel over each ureteral opening to collect urine from each kidney. Cloxacillin was dissolved in 2.0 ml. of 0.9% saline and injected into the muscles of the left leg at a dose of 100 mg/kg. Probenecid, which blocks renal tubular secretion, was administered intravenously at a dose of 100 mg.

Biliary excretion. Groups of male rats (weighing 250 to 350 g) were starved overnight. After they had been anaesthetized with ether laparotomy was performed and the bile duct was cannulated with polyethylene tubing (0.4 mm bore). The tubing was passed through a small aperture in the skin at the back of the neck, and the skin and muscle incisions were sutured. The animals were then given an intramuscular injection of 100 mg/kg of cloxacillin and placed in metabolism cages. The polyethylene tubing was taken through the top of the cage and led into a 10 ml. cylinder for collection of the bile. Throughout the 24 hr period samples of the bile and urine were taken.

Cerebrospinal fluid concentrations. The cerebrospinal fluid concentrations of cloxacillin were determined in rabbits after intramuscular administration of 500 mg/kg. 0.5, 1, 2 and 4 hr after administration of cloxacillin two rabbits were anaesthetized with urethane (1 g/kg, intravenously) and cerebrospinal fluid samples were withdrawn from the cisterna magna by means of a sterile syringe and needle. Blood samples were taken from the lateral ear veins at the time of taking the cerebrospinal fluid samples. The blood samples were allowed to clot at room temperature. The serum and cerebrospinal fluid samples were kept at 4° C until assayed.

Investigation of metabolite

In vivo. 100 mg/kg of cloxacillin was administered orally to groups of five male albino rats (weighing 100 to 200 g). At 2, 4 and 6 hr after administration one group was killed and the alimentary tract was removed. The small intestine, caecum and large intestine were separated and the contents removed by washing out with saline. The different parts of the intestine were weighed and homogenized in the same way as in the distribution studies and the homogenates assayed for penicillin. The contents obtained from each section of the intestine were also assayed by the cup-plate technique using *Sarcina lutea*, *Staphylococcus pyogenes* Oxford and *Staphylococcus pyogenes* Russell (the Russell *Staphylococcus* being resistant to penicillin G). The results were expressed as a concentration in μg/g of tissue or μg/ml. of content and also expressed as a percentage of the dose administered. The extracts and the washings were chromatographed.

In vitro. Homogenates weighing 1 g from various sections of the alimentary canal and the contents were suspended in 3.5 ml. of Krebs solution. To the suspension 0.5 ml. of a 1 mg/ml. solution of cloxacillin was added to give a final concentration of the penicillin in the suspension of 100 μg/ml. The homogenates were placed in a water-bath heated to 37° C and shaken throughout the period of the test. Samples were removed from each homogenate at 0, 2 and 4 hr after adding the penicillin and assayed for penicillin concentration by the usual cup-plate technique using *Sarcina lutea* as the test organism.

Chemotherapy

Thigh lesion tests

Single infection. The protective effect of cloxacillin was determined using the method of Selbie & O'Grady (1954) as modified by Brown & Acred (1960). Groups of ten mice were infected by injecting into the muscle sheath of the left thigh 0.2 ml. of a 1:3 dilution of an overnight growth of *Staphylococcus pyogenes* Russell, type 80. Cloxacillin and methicillin were administered in doses of 50 and 200 mg/kg orally and subcutaneously daily for 3 days, the first dose being given immediately after the infection. The infected control group received no treatment. A non-infected, no treatment, control group was set up with each experiment. The maximum thigh diameters of each mouse were measured with callipers on the 1st, 2nd and 6th days after infection.

Double infection. A double lesion test was also carried out in which the animals were infected with *Staphylococcus pyogenes* Russell in the left hind-limb, and with *Staphylococcus pyogenes* 2187 (penicillin G-sensitive) in the muscles of the right hind-limb. 0.2 ml. of a 1:3 dilution of an overnight culture of each organism was injected into each mouse. Cloxacillin, methicillin and penicillin G were administered in doses of 100 mg/kg orally and subcutaneously, the dose schedule being the same as for the single lesion test.

Daily thigh enlargement was assessed as the difference in thigh diameters between an infected group of animals and the thigh diameters of the non-infected, no treatment, control group. The results were expressed as the percentage protection calculated in the following manner:

$$\frac{\text{Mean daily thigh enlargement of infected control} - \text{Mean daily thigh enlargement of penicillin group}}{\text{Mean daily thigh enlargement of infected control}} \times 100$$

CD50 tests

The effects of cloxacillin, methicillin and penicillin G were also determined in mice infected with *Staphylococcus pyogenes* Smith (penicillin G-sensitive). The method for determination of the LD50's of the culture and the amount of organism given in the infectivity tests were identical to those described by Acred, Brown, Turner & Wilson (1962). For the antibiotics a 4:1 dose ratio was used, the dose being administered in 0.2 ml. of 0.9% saline immediately after the infection. The mice were observed for 4 days and deaths were recorded daily. The percentage of deaths was plotted against dose on log probit paper and the dose of the compound in mg/kg giving protection to 50% of the mice (CD50) was read off from the graph.

RESULTS

Cardiovascular and respiratory effects

Intravenous doses of 25 to 400 mg/kg of cloxacillin administered to anaesthetized cats lowered the blood pressure. With doses below 200 mg/kg the effects were transient and recovery occurred within 5 to 10 min; after 200 and 400 mg/kg the hypotension was more prolonged, persisting for more than 10 min. Responses to intravenous administration of adrenaline, acetylcholine and histamine, and to electrical stimulation of the uncut cervical vagus nerve, were not significantly altered by cloxacillin. Doses up to 50 mg/kg of cloxacillin had no significant action on the electrocardiogram, while larger doses extended the PQRS complex by 30 to 50% of the control value. Recovery to normal occurred within 8 min of administration of the drug. No significant effects on respiratory movements were demonstrated.

Increasing doses of cloxacillin in the same animal caused an inhibition of up to 67% of the responses of the nictitating membrane to pre- and postganglionic nerve stimulation. A single dose of 200 mg/kg in a previously untreated animal

caused a 32% reduction of the response, but within 5 min it had returned to normal. The responses of the nictitating membrane to adrenaline were, however, unaffected.

Pregnancy tests

Nine out of twelve rabbits in the test became pregnant, six of which received cloxacillin and three received thalidomide. All the rabbits given cloxacillin delivered normal litters; the litter size for the individual animals being (i) 5, (ii) 14, (iii) 8, (iv) 5, (v) 7, and (vi) 1+. The last rabbit (vi) had a litter but, before inspection could be made, it had partially eaten all the young except one, which appeared normal. Examination of the remaining limbs of the young which had been killed revealed no abnormalities. In rabbits (iv) and (v) there was evidence that two embryos in one, and three embryos in the other, had not been maintained after implantation. Of the rabbits which received thalidomide, one showed signs of abortion on the 20th day; this rabbit was killed and there were five underdeveloped foetuses of which four showed spinal deformities or limb abnormalities; two other foetuses had died and at these sites there was bleeding. This condition had given the external appearance of impending abortion. In the second rabbit given thalidomide there was a complete abortion on the 22nd and 23rd days of pregnancy, and in the third rabbit ten undersized foetuses were delivered normally. Four of these showed deformities of the hind-limbs. The mother was killed and the uterus examined. One dead foetus was found which consisted of a mass of disorganized tissue and blood, enclosed by the amniotic membrane.

Absorption

Blood levels after intramuscular administration of the penicillins into rabbits. The blood levels after the intramuscular administration of 100 mg/kg of penicillin G, methicillin and cloxacillin are given in Table 1.

TABLE 1

SERUM CONCENTRATIONS IN GROUPS OF FIVE RABBITS RECEIVING 100 MG/KG OF CLOXACILLIN, METHICILLIN AND PENICILLIN G INTRAMUSCULARLY

		Serum concentration (μg/ml.) at time after dosing			
Penicillin		1 hr	2 hr	4 hr	6 hr
Cloxacillin		10·0	6·6	5·0	4·5
		23·5	17·0	3·6	3·0
		7·2	3·9	4·1	3·1
		8·8	5·0	2·5	4·1
		11·5	7·4	2·1	2·6
	Mean	12·2	8·0	3·5	3·5
Methicillin		12·5	3·65	0·51	0
		18·0	3·65	0·3	0
		14·0	4·2	0·74	0
		18·0	5·0	0·34	0
		14·0	6·2	1·3	0
	Mean	15·3	4·48	0·64	0
Penicillin G		11·0	5·1	2·65	1·13
		9·0	5·0	1·17	0·5
		16·0	10·5	1·0	0·1
		10·5	4·7	0·48	0
		9·8	6·1	1·15	0·23
	Mean	11·26	6·28	1·29	0·39

The patterns of blood level after methicillin and penicillin G are similar, maximum concentrations of 15.3 and 11.3 μg/ml. respectively being obtained 30 min after administration. After 30 min from administration of cloxacillin a concentration of 12.2 μg/ml. was found, but the levels of cloxacillin were more prolonged than those of methicillin or penicillin G, and significant concentrations (3.5 μg/ml.) were detected 6 hr after administration.

Blood levels after oral administration of cloxacillin into dogs. The mean blood levels after oral administration of 100 mg/kg of cloxacillin into dogs are shown in Table 2. A maximum concentration of 4.4 μg/ml. was found 30 min after administration.

TABLE 2

WHOLE BLOOD CONCENTRATIONS OF CLOXACILLIN (μG/ML.) IN DOGS FOLLOWING ORAL ADMINISTRATION OF 100 MG/KG

The dogs weighed from 10 to 12 kg. *No sample

	Whole blood concentration (μg/ml.) at time (hr) after dosing							
Dog	0·5	1·0	1·5	2·0	2·5	4·0	5·0	6·0
1	5·2	3·1	1·8	2·5	0	0	0	0
2	5·4	6·5	3·0	1·0	0	0	0	0
3	3·0	1·8	2·4	0·8	0	0	0	0
4	6·1	*	1·5	1·4	1·0	0	0	0
5	1·8	2·6	1·7	1·0	0·8	0	0	0
6	4·4	2·6	1·3	1·6	1·0	0·8	0	0
7	4·7	2·8	1·7	1·2	1·0	1·1	0·9	0
Mean	4·4	3·2	1·9	1·3	0·54	0·27	0·1	0

Distribution and elimination

Distribution in tissues. The distribution of cloxacillin after oral and intramuscular administration of 100 mg/kg to groups of five male rats is given in Tables 3 and 4. Following intramuscular and oral administration there was no preferential concentration of the penicillin in any particular organ, except in the liver and kidney, the organs specifically concerned with the excretion of the penicillin from the body. The high concentrations found in the sections of the alimentary canal following oral dosing are a consequence of the route of administration. After 6 hr from intramuscular administration, 42.5% of the antibiotic had been recovered in the urine and during the following 18 hr a further 10.6% was obtained.

Following oral administration the total percentage recovered from all sites increased to a maximum of 124% at 6 hr, falling to 63% at 24 hr. While most of the antibiotic was recovered in the urine following intramuscular administration, only approximately 30 to 50% of the total recovered was found in the urine after oral administration. As the antibiotic moved along the alimentary canal from the stomach to the large intestine the concentration of the antibiotic, instead of decreasing as would be expected due to absorption, dilution and loss, actually increased, until at 6 hr the concentration in the large intestine (3,750 μg/ml.) was greater than that found in the stomach at the beginning of the experiment (2,800 μg/ml.).

Urinary excretion in the hen. The mean percentage of the dose excreted by the left kidney during the 1st hr after administration was only 5.8% greater than that excreted from the right kidney (Table 5). Thereafter there was no difference in the

TABLE 3

DISTRIBUTION OF CLOXACILLIN IN TISSUES OF MALE ALBINO RATS AFTER ORAL ADMINISTRATION OF 100 MG/KG

Seven groups of five rats (weighing from 100 to 200 g) were given orally 100 mg/kg of cloxacillin. One group was killed at the end of each time period. The mean concentrations of cloxacillin are expressed in μg/g wet weight of tissue or in μg/ml. of fluids (columns *a*) and the concentration ratios between the tissues (μg/g wet weight) or fluids (μg/ml.) to serum (μg/ml.) are shown in columns *b*. The urinary excretion is expressed as a percentage of the dose administered

Tissue and fluid	Time after administration: 0 hr		0·5 hr		1 hr		2 hr		4 hr		6 hr	24 hr
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*a*
Liver			30·1	3·7	16·5	3·5	9·6	1·3	8·3	2·7	4·8	0
Spleen			2·7	0·3	0	0	2·6	0·4	0	0	1·8	16·7
Kidneys			2·15	0·27	28·0	5·9	12·6	1·8	7·5	2·5	22·1	7·4
Lungs			8·7	1·1	10·2	2·2	9·4	1·3	5·7	1·9	9·9	0
Stomach	2,800·0	143·5	620·0	76·5	485·0	103·2	245·0	34·5	23·5	7·8	11·3	0
Small intestine			440·0	54·3	810·0	172·3	345·0	48·6	142·0	47·3	39·5	15·3
Caecum, large intestine			5·0	0·62	0	0	850·0	119·7	2,000·0	666·6	3,750·0	710·0
Faeces			2·2	0·27	3·3	0·7	4·3	0·6	756·0	252·0	400·0	1,990·0
Carcass	18·2	0·93	4·8	0·6	3·8	0·80	4·5	0·6	3·6	1·2	3·2	2·0
Serum	19·5	(1·0)	8·1	(1·0)	4·7	(1·0)	7·1	(1·0)	3·0	(1·0)	0	0
Urine			176·0	21·7	50·5	10·7	490·0	69·0	360·0	120·0	445·0	267·5
Total recovered (%)	89		48		59		69		83		124	63
% recovered in urine			0·79		1·54		20·2		12·5		29·1	39·8

TABLE 4

DISTRIBUTION OF CLOXACILLIN IN TISSUES OF MALE ALBINO RATS AFTER INTRAMUSCULAR INJECTION OF 100 MG/KG

Seven groups of five rats (weighing from 100 to 200 g) were injected intramuscularly with 100 mg/kg of cloxacillin. One group was killed at the end of each time period. The mean concentrations of cloxacillin are expressed in μg/g wet weight of tissue or in μg/ml. of fluids (columns *a*) and the concentration ratios between the tissues (μg/g wet weight) or fluids (μg/ml.) to serum (μg/ml.) are shown in columns *b*. The urinary excretion is expressed as a percentage of the dose administered

Tissue and fluid	Time after administration: 0 hr		0·5 hr		1 hr		2 hr		4 hr		6 hr	24 hr
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
Liver			29·0	2·1	15·2	2·3	2·4	1·3	0	0	0	0
Spleen			48·5	3·6	4·2	0·6	0	0	0	0	0	1·8
Kidneys			242·5	17·6	76·0	11·7	15·2	8·0	3·9	2·1	1·8	1·8
Lungs			15·0	1·1	10·4	1·6	0	0	0	0	0	0
Small intestine			117·0	8·6	166·0	25·5	59·6	31·4	11·1	6·2	6·5	0
Large intestine, caecum			6·0	0·4	0	0	7·6	4·0	127·0	70·5	75·0	1·8
Faeces							0	0	70·0	38·9	359·0	26·4
Right leg (site of injection)	165·0	97·1	260·0	19·3	127·5	19·6	14·0	7·4	1·9	1·0	4·3	0
Carcass	4·7	0·3	23·3	1·7	18·5	2·8	14·2	7·5	0	0	2·3	0
Urine			1,710·0	126·6	2,400·0	369·2	5,400·0	2,842·0	4,780·0	2,665·5	695·0	425·0
Serum	17·0	(1·0)	13·5	(1·0)	6·5	(1·0)	1·9	(1·0)	1·8	(1·0)	0	0
Total recovered (%)	83·4		39·1		40·5		29·8		17·8		51·3	53·7
% recovered in urine			4·2		20·5		15·8		13·7		42·5	53·1

TABLE 5

URINARY EXCRETION OF CLOXACILLIN IN THE HEN

Values are urinary excretions from the left and right kidneys of five hens each of which received 100 mg of cloxacillin. Cloxacillin was administered into the left leg muscles

	Cumulated % of dose excreted at time (hr) after administration											
	Left kidney						Right kidney					
Hen	1	2	3	4	5	6	1	2	3	4	5	6
A	12·4	20·8	24·6	26·2	29·1	29·4	9·1	15·2	19·2	23·6	24·2	24·2
B	9·2	12·3	15·4	17·7	19·7	21·3	17·0	22·7	25·3	26·8	28·8	29·3
C	25·6	30·9	35·3	37·5	40·9	41·7	25·2	32·0	36·8	39·6	40·9	41·9
D	29·6	34·1	38·0	41·5	43·3	44·4	17·2	27·3	30·2	33·4	35·0	36·1
E	35·8	44·6	48·4	51·5	53·6	55·4	15·1	15·3	18·4	20·0	22·1	23·7
Mean	22·5	28·5	32·3	34·8	37·3	38·4	16·7	22·5	26·0	28·7	30·2	31·0

accumulated percentage excreted from the left and right kidneys. After the intravenous administration of 100 mg of probenecid, which blocks renal tubular secretion, the percentages of the antibiotic excreted by the right and left kidneys were almost identical.

Biliary excretion. The concentration of cloxacillin and the percentage of the antibiotic recovered in the bile at hourly intervals over 6 hr, in groups of eight rats administered 100 mg/kg intramuscularly, are given in Table 6. 23% of the antibiotic was recovered in the bile in 6 hr. From an experiment put up in parallel using twelve rats in pairs with collection of urine, 16% of the antibiotic was recovered.

TABLE 6

THE CONCENTRATION OF CLOXACILLIN AND PERCENTAGE RECOVERED IN BILE FOLLOWING INTRAMUSCULAR ADMINISTRATION OF 100 MG/KG

Values are means for groups of eight rats weighing between 250 and 350 g

	Cloxacillin recovered at time (hr) after dosing					
Cloxacillin	0–1	1–2	2–3	3–4	4–5	5–6
Concentration (μg/ml.)	3,862	2,173	978	437	99	51
Cumulated recovery (%)	10·4	18·1	21·1	22·6	22·8	22·9

Cerebrospinal fluid concentrations. After intramuscular administration of 500 mg/kg of cloxacillin the concentration in the cerebrospinal fluid was considerably less than that found in the serum. The results are shown in Table 7.

TABLE 7

CONCENTRATION OF CLOXACILLIN IN SERUM AND CEREBROSPINAL FLUID OF RABBITS AFTER INTRAMUSCULAR ADMINISTRATION OF 500 MG/KG

Values are means of two rabbits per time period

	Concentration (μg/ml.) at time after dosing		
Fluid	1 hr	2 hr	4 hr
Cerebrospinal fluid	2·3	2·3	3·3
Serum	440	142·5	112·5

Metabolism

In vivo. The concentrations of the antibiotic in the intestinal contents and the intestinal walls are given in Tables 8 and 9. As the antibiotic entered the caecum there was a very considerable increase in amount assayable against *Sarcina lutea*,

TABLE 8

CONCENTRATION OF CLOXACILLIN IN THE TISSUES OF RATS AFTER ORAL ADMINISTRATION OF 100 MG/KG

The concentrations were determined by assaying in parallel against *Sarcina lutea*, *Staphylococcus pyogenes* Russell (penicillin-resistant) and *Staphylococcus pyogenes* Oxford (penicillin-sensitive)

Tissue and fluid	Concentration (μg/ml.) at time after dosing								
	2 hr			4 hr			6 hr		
	Sarcina	*Staph.* Oxford	*Staph.* Russell	*Sarcina*	*Staph.* Oxford	*Staph.* Russell	*Sarcina*	*Staph.* Oxford	*Staph.* Russell
Small intestine, walls	88·0	76·0	135·0	18·0	0	0	24·0	0	0
Small intestine, contents	1,600·0	1,400·0	1,950·0	245·0	165·0	225·0	110·0	30·0	74·0
Caecum, walls	65·0	3·6	0	460·0	49·0	78·0	210·0	13·0	26·0
Caecum, contents	1,005·0	160·0	56·0	7,300·0	650·0	545·0	8,400·0	350·0	365·0
Large intestine, walls	0	0	0	26·0	0	0	79·0	6·5	16·0
Large intestine, contents	0	0	0	5,150·0	53·0	44·0	4,150·0	505·0	418·0
Urine	935·0	735·0	1,025·0	1,170·0	380·0	650·0	270·0	160·0	230·0

TABLE 9

TOTAL PERCENTAGE RECOVERY OF CLOXACILLIN FROM ALIMENTARY CANAL AND URINE OF RATS GIVEN 100 MG/KG ORALLY

Assays were as in the experiments summarized in Table 8

Time after dosing	Total recovery (% of dose)		
	Staph. Russell	*Staph.* Oxford	*Sarcina*
2 hr	35·03	37·5	43·4
4 hr	17·18	19·5	64·4
6 hr	16·5	12·7	135·43

but when assayed against the two strains of *Staphylococcus* there was a considerable reduction in the relative amount recovered. In relation to the amount of metabolite in the intestinal contents the amount in the walls of the intestine was negligible. When the urine from the same animals was also assayed, the concentrations obtained when assayed against the three organisms were of the same order or size. Chromatograms of the samples showed clearly two zones of inhibition for the samples from the caecum and large intestine, one zone corresponding with the parent compound. The second large zone was near the origin (Fig. 1). The R_F value for this latter zone differed from that of the metabolite isolated from the urine.

In vitro. After incubation of the homogenates of various tissues and intestinal contents with cloxacillin there was a considerable increase in the antibiotic activity in the caecal and large intestine contents (Table 10). The initial concentration was 100 μg/ml. but at 4 hr the concentration in the caecal contents was 360 μg/ml. and in the sample from the large intestine was 300 μg/ml. There was a similar

increase in concentration on incubating cloxacillin with faeces. No increase in concentration was recorded with the caecal and large intestine walls, or with any other section of the alimentary canal.

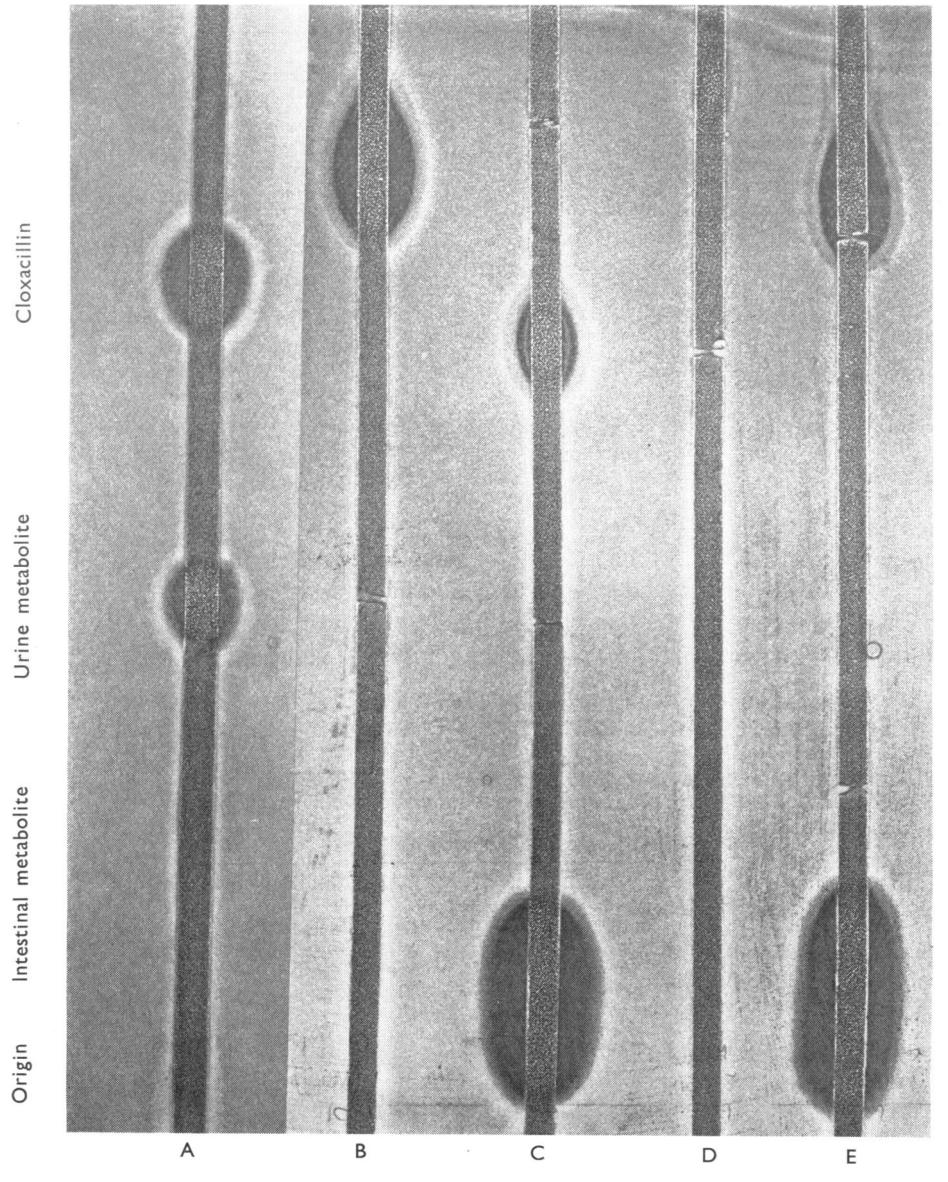

Fig. 1. Chromatography of urine and intestinal homogenates obtained from rats 4 hr after administration of 100 mg/kg of cloxacillin, showing the intestinal metabolite (C and E) and urine metabolite (A). The chromatography paper strips were placed on agar plates seeded with *Sarcina lutea* and incubated at 30° C. A, urine; B, aqueous control; C, caecal contents; D, caecal wall; and E, large intestine contents.

TABLE 10

STABILITY OF CLOXACILLIN IN HOMOGENATES OF RAT TISSUES

1 g of the tissue was suspended in 3.5 ml. of Krebs solution. Cloxacillin was added to give a concentration of 100 μg/ml. and the suspension was incubated at 37° C. At 0, 2 and 4 hr, samples were removed for assay

Tissue and fluid	Concentration (μg/ml.) at time		
	0 hr	2 hr	4 hr
Pancreas	91·0	69·0	66·0
Liver	92·0	62·0	50·0
Small intestine, contents	91·0	71·0	68·0
Small intestine, walls	90·0	73·0	63·0
Duodenum, contents	82·0	43·0	52·0
Duodenum, walls	84·0	86·0	74·0
Control	90·0	91·0	90·0
Caecum, walls	120·0	100·0	70·0
Caecum, contents	110·0	210·0	360·0
Large intestine, walls	115·0	90·0	70·0
Large intestine, contents	105·0	190·0	300·0
Faeces	—	230·0	250·0
Control	96·0	91·0	72·0

Chemotherapy

Thigh lesion test

Single infection. The results of oral and subcutaneous administration of 50 and 200 mg/kg of cloxacillin and of subcutaneous injection of 50 and 200 mg/kg of methicillin on the size of the thigh lesion in mice are given in Table 11. Cloxacillin (200 mg/kg by either route) gave virtually complete healing. Following oral and

TABLE 11

PERCENTAGE PROTECTION AGAINST INFECTIONS IN MICE DUE TO PENICILLINASE-PRODUCING *STAPHYLOCOCCUS PYOGENES* RUSSELL

Doses of 200 and 50 mg/kg of cloxacillin or methicillin were administered to groups of ten mice. Cloxacillin was given orally and subcutaneously, methicillin subcutaneously only

Penicillin	Protection (%)			
	Oral dose		Subcutaneous dose	
	200 mg/kg	50 mg/kg	200 mg/kg	50 mg/kg
Cloxacillin	100·0	60·6	97·7	76·8
	100·0	72·5	100·0	98·1
	100·0	61·7	100·0	93·8
	97·0	61·4	97·5	91·7
	99·0	49·3	99·0	75·9
	100·0	52·0	96·8	84·0
	100·0	54·9	100·0	97·8
Mean	99·4	58·9	98·7	88·3
Methicillin			73·2	24·4
			84·1	31·8
			91·7	61·2
			56·7	43·3
			69·2	38·5
			86·0	36·0
			85·7	57·1
Mean			78·1	41·8

subcutaneous administration of 50 mg/kg, the healing was reduced to 59% and 88% respectively. Administered subcutaneously, 50 mg/kg of cloxacillin gave better protection than 200 mg/kg of methicillin administered by the same route, while 50 mg/kg orally of cloxacillin was superior to 50 mg/kg of methicillin given subcutaneously. Methicillin was inactive orally.

Double infection. The effects of cloxacillin, methicillin and penicillin G, administered subcutaneously and orally to groups of mice, against infections of a penicillin-sensitive *Staphylococcus* in one hind-limb and a resistant *Staphylococcus* in the other hind-limb, are shown in Table 12 and Fig. 2. Cloxacillin was highly effective against both organisms, by both routes. It was not as effective as penicillin G against the sensitive staphylococcal infection, but was considerably more active than penicillin G against the resistant *Staphylococcus.*

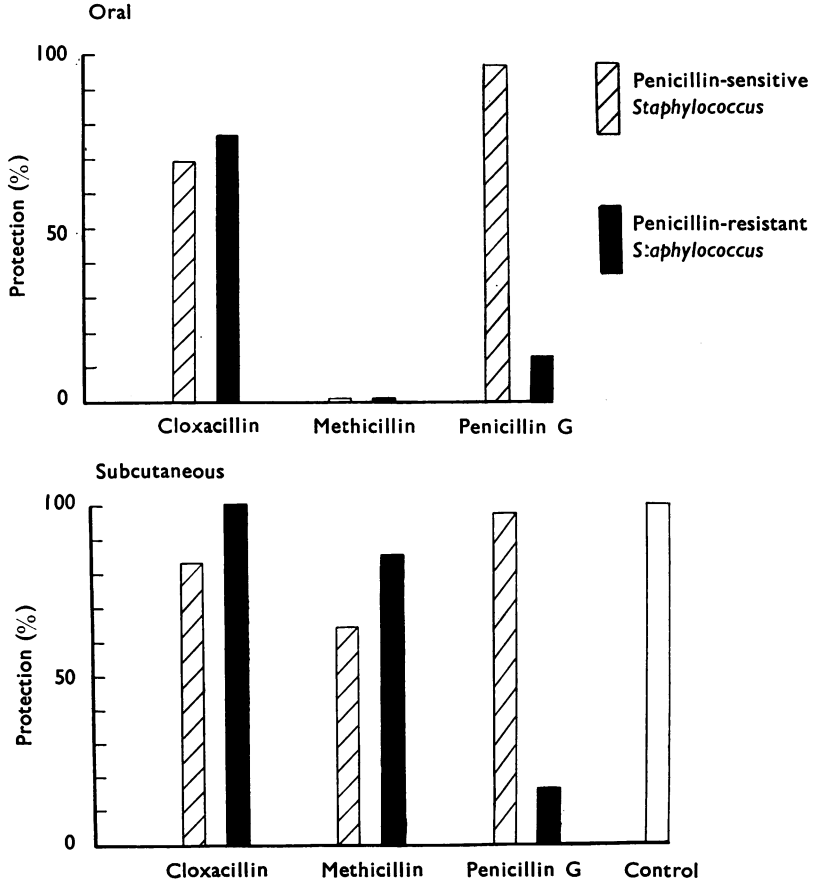

Fig. 2. Histogram showing percentage protection of mice (ten per group) infected intramuscularly with *Staphylococcus pyogenes* Russell (penicillin-resistant) in the left hind-limb and *Staphylococcus pyogenes* 2187 (penicillin-sensitive) in the right hind-limb. 100 mg/kg of cloxacillin, methicillin and penicillin G were administered orally (upper histogram) and subcutaneously (lower histogram) daily for 3 days. The controls were non-infected mice.

TABLE 12

PERCENTAGE PROTECTION AGAINST INFECTIONS IN MICE DUE TO PENICILLINASE-PRODUCING *STAPHYLOCOCCUS PYOGENES* RUSSELL INJECTED INTO THE LEFT THIGH AND PENICILLIN G-SENSITIVE *STAPHYLOCOCCUS PYOGENES* 2187 INJECTED INTO THE RIGHT THIGH

Doses of 100 mg/kg of cloxacillin, methicillin and penicillin G were administered orally and subcutaneously into groups of ten mice. The percentage protection was calculated as described in Methods

	Protection (%) against			
	Staph. pyogenes Russell		*Staph. pyogenes* 2187	
Penicillin	Oral penicillin	Subcutaneous penicillin	Oral penicillin	Subcutaneous penicillin
Cloxacillin	75·6	100·0	69·5	83·1
Methicillin	0	85·2	0	64·4
Penicillin G	13·8	17·3	95·6	98·4

TABLE 13

ACTIVITY OF CLOXACILLIN, METHICILLIN AND PENICILLIN G AGAINST *STAPHYLOCOCCUS PYOGENES* SMITH (PENICILLIN-SENSITIVE)

Activity is expressed in terms of the dose of antibiotic calculated to protect 50% of a group of infected animals (CD50, mg/kg)

	CD50 (mg/kg)	
Penicillin	Oral	Subcutaneous
Cloxacillin	39·0	11·5
Methicillin	Inactive	1·3
Penicillin G	5·8	0·2

CD50 tests

The CD50 values for cloxacillin, methicillin and penicillin G against *Staphylococcus pyogenes* Smith (penicillin G-sensitive) are given in Table 13. Penicillin G was the most effective when given both orally and subcutaneously. Methicillin was only effective by injection, but against this strain of *Staphylococcus* was more effective than cloxacillin.

DISCUSSION

Cloxacillin is one of a series of isoxazol penicillins which is well absorbed orally and is highly effective against staphylococci resistant to penicillin G. After intravenous injection it slightly lowered blood pressure due to a direct action on the heart, and probably to an inhibition of sympathetic nerve conduction. Concentrations in the blood at which these effects were found were in the region of 850 μg/ml. and were therefore very much in excess of those required to produce a therapeutic effect (over 2.5 μg/ml.) and highly unlikely to be encountered clinically.

Cloxacillin, when administered to rabbits, did not induce abnormalities in the foetus, although the mothers may have been affected due to an alteration in the intestinal flora, all the rabbits under test showing varying degrees of diarrhoea. The number of resorption sites which occurred in two of the rabbits was well within the normal expected limits (Adams, 1960), and is of no significance. On the other hand thalidomide killed a number of the foetuses with resulting abortion and in most of the survivors there were signs of limb and other abnormalities.

The effectiveness of cloxacillin against both penicillin-resistant and penicillin-sensitive strains of *Staphylococcus in vivo* has been clearly demonstrated. Cloxacillin when given orally was more active than the same dose of methicillin administered by injection. Against the sensitive *Staphylococcus* cloxacillin was less active than penicillin G. The effectiveness of penicillin G, when administered orally, against the sensitive *Staphylococcus* was probably due to the massive dose of penicillin administered, which would ensure that some of the antibiotic passed through the stomach and was subsequently absorbed giving rise to concentrations in the blood adequate to overcome the infection.

The distribution in the body and the excretion in the bile followed the same pattern as seen with other penicillins (Acred *et al.*, 1961, 1962), and the high concentrations found in the kidney and liver merely reflect the concentrations in the urine and bile. The total amount excreted in the bile was similar to that found with methicillin, ampicillin and penicillin G. The excretion of cloxacillin by the hen's kidney, however, differed in that there was very little renal tubular secretion.

After oral administration of cloxacillin there was an unexpected increase in the amount of antibiotic recovered in the caecum and large intestine, the antibiotic activity in terms of cloxacillin being greater than the amount administered. From these results it was evident that a metabolite was being formed which was more active than cloxacillin against *Sarcina lutea,* but the increased activity was not seen when resistant and sensitive strains of *Staphylococcus* were used in the cup-plate assay. The relative activities of the metabolite of cloxacillin against the two strains of *Staphylococcus* were identical and hence it can be inferred that the metabolite was also stable to staphylococcal penicillinase.

The intestinal metabolite was not absorbed since the conversion occurred in the large intestine and caecum where active absorption is minimal. However, the body can also metabolize cloxacillin producing another active metabolite, which is probably formed in the liver (Mansford, personal communication). This metabolite, which appears in the urine and the bile of rats, is distinct from the intestinal metabolite. Other workers (Vanderhaeghe, Van Dijck, Claesen & De Somer, 1961 ; Rolinson & Batchelor, 1962 ; Rollo, Somers & Burley, 1962) have reported the presence of penicillin metabolites in the urine of humans and it is possible therefore that a number of other penicillins can be metabolized in the body. In estimating penicillins in body tissues, therefore, it is necessary to verify chromatographically as well as by bioassay that the activity measured is due to the administered penicillin and not due to a mixture of the parent penicillin and metabolites. If a mixture is present, spuriously high or low values can be registered since a metabolite can show a different relative activity against the test organism compared to the parent penicillin, as we have found with the intestinal metabolite of cloxacillin when this is assayed against *Sarcina lutea.* Under these circumstances, to obtain values of absolute concentrations it is necessary to isolate the metabolite and determine its *in vitro* activity against the assay organism. In relation to therapeutic activity it is also essential to determine the antibiotic spectrum of the metabolite since it could be more effective against some organisms and less effective against others than the parent penicillin.

The authors would like to express their thanks to Dr J. H. C. Nayler and his colleagues for the preparation of cloxacillin, to Mr K. R. L. Mansford and Mr R. Crowne for the chromatography, and to Mrs L. Mizen, Mr B. F. Clark and Mr M. J. Wilson for their assistance in these studies.

REFERENCES

ACRED, P., BROWN, D. M., TURNER, D. H. & WILSON, M. J. (1962). Pharmacology and chemotherapy of ampicillin—a new broad spectrum penicillin. *Brit. J. Pharmacol.*, **18**, 356–369.

ACRED, P., BROWN, D. M., TURNER, D. H. & WRIGHT, D. (1961). Pharmacology of methicillin. *Brit. J. Pharmacol.*, **17**, 70–81.

ADAMS, C. E. (1960). Studies on prenatal mortality in the rabbit, *Oryctolagus cuniculus*: the amount and distribution of loss before and after implantation. *J. Endocr.*, **19**, 325–344.

BAINBRIDGE, J. G., WARREN, E. & WIGGINS, K. E. (1962). An inexpensive blood pressure transducer. *J. sci. Instrum.*, **39**, 29–30.

BROWN, D. M. & ACRED, P. (1960). Chemotherapeutic studies on a new antibiotic BRL 1241. *Lancet*, **ii**, 568–569.

KARNOVSKY, M. L. & JOHNSON, M. J. (1949). Filter paper chromatography of penicillin broths. *Analyt. Chemist*, **21**, 1125–1133.

NAYLER, J. H. C., LONG, A. A. W., BROWN, D. M., ACRED, P., ROLINSON, G. N., BATCHELOR, F. R., STEVENS, S. & SUTHERLAND, R. (1962). Chemistry, toxicology, pharmacology and microbiology of a new acid-stable penicillin, resistant to penicillinase (BRL 1621). *Nature (Lond.)*, **195**, 1264–1267.

ROLINSON, G. N. & BATCHELOR, F. R. (1962). Penicillin metabolites. In *Antimicrobial Agents and Chemotherapy*, pp. 654–660. Ann Arbor: American Society for Microbiology.

ROLLO, I. M., SOMERS, G. F. & BURLEY, D. M. (1962). Bacteriological and pharmacological properties of phenoxybenzylpenicillin. *Brit. med. J.*, **i**, 76–88.

SELBIE, F. R. & O'GRADY, F. (1954). A measurable tuberculous lesion in the thigh of the mouse. *Brit. J. exp. Path.*, **35**, 556–565.

VANDERHAEGHE, H., VAN DIJCK, P., CLAESEN, M. & DE SOMER, P. (1961). Preparation and properties of 3,4-dichloro-α-methoxybenzyl penicillin. In *Antimicrobial Agents and Chemotherapy*, p. 581. Detroit: American Society for Microbiology.