Brit. J. Pharmacol. (1961), **17**, 70–81.

PHARMACOLOGY OF METHICILLIN

BY

P. ACRED, D. M. BROWN, D. H. TURNER AND D. WRIGHT

From the Beecham Research Laboratories, Brockham Park, Betchworth, Surrey

(*Received March* 7, 1961)

The pharmacology of a new antibiotic methicillin, 6(2:6-dimethoxybenzamido)-penicillanic acid, which is effective against staphylococci resistant to penicillin G, has been investigated. It is free from acute and chronic toxic effects, except that some pain may be caused following intramuscular injection. It is poorly absorbed orally, but after intramuscular injection the concentrations in the serum and in tissues are very similar to those found with penicillin G. It is excreted by the kidneys both by renal tubular secretion and glomerular filtration. It is also excreted in the bile in very high concentrations, the ratio of concentration in the bile to the blood being approximately 2.5 times that of penicillin G. From a study of the metabolism of the drug it is calculated that 75% is eliminated unchanged in the urine and that the remainder is probably destroyed after the excretion into the intestine via the bile.

With the isolation of 6-aminopenicillanic acid (Batchelor, Doyle, Nayler & Rolinson, 1959), it is now possible to synthesize a wide range of new penicillins having modified antibacterial properties. One of these new derivatives, 6-(2:6-dimethoxybenzamido)penicillanic acid, has been shown to possess remarkable stability to staphylococcal penicillinase, and recent clinical reports have confirmed its effectiveness in the treatment of infections caused by staphylococci resistant to penicillin G (Douthwaite & Trafford, 1960; Knox, 1960; Stewart, Harrison & Holt, 1960; and Stewart, Nixon, Coles, Kesson, Lawson, Thomas, Mishra, Mitchell, Semmens & Wade, 1960). Preliminary pharmacological and chemotherapeutic findings have been reported by Brown & Acred (1960). Full details of the pharmacological evaluation are now presented.

METHODS

In all experiments penicillin G and methicillin were 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 dilutions of the antibiotics were plotted against the log of the concentration, and from the regression line obtained the concentrations of the antibiotics in the specimens were estimated by interpolation. The appropriate dilutions of the controls and samples were made in phosphate buffer *p*H 7.0 (M/20) except in the experiments where serum concentrations were determined, in which case the controls were prepared in serum.

Acute toxicities

The acute toxicity was determined in male albino mice (18 to 22 g) after intravenous, subcutaneous and oral administration, and subcutaneously in rats (150 to 200 g). The effect on respiration and the electrocardiogram was studied in guinea-pigs and cats during the slow intravenous infusion of the antibiotic.

Prolonged administration

The effects of prolonged administration were investigated in rats and dogs.

(i) *Rats.* Two groups of 12 male rats, each animal weighing 80 to 100 g, were injected subcutaneously 5 days per week with methicillin at doses of 100 and 500 mg/kg in a volume of 0.1 ml./100 g body weight for a period of 12 weeks. A group receiving 0.1 ml./100 g body weight normal saline subcutaneously acted as controls. Daily food intake and the weight of each rat was recorded. Weekly records of the red and white blood cell counts and qualitative tests for sugar and protein in the urine were performed. Haemoglobin determinations and spectroscopic examinations of the blood were made on the first, sixth and twelfth week of the test.

At the end of 6 weeks, 6 rats from each group were killed and the remaining rats killed at 12 weeks. The weights of the livers, spleens, kidneys, testes and adrenals were recorded, and specimens of liver, spleen, kidney, lung, thyroid, heart, duodenum, stomach, pancreas, adrenal, testis and bone marrow were removed for histological examination.

(ii) *Dogs.* Methicillin (250 mg/kg) was administered subcutaneously twice daily for a period of 4 weeks to two dogs. The following biochemical and haematological estimations were made at weekly intervals: haemoglobin (g%), packed cell volume, total white cell count, blood urea, serum alkaline phosphatase, zinc sulphate turbidity and serum globulin and albumin. A differential blood cell count was carried out at the end of the first and final week of the test.

Local irritant action

Ten per cent. and 1% solutions were injected intramuscularly and intradermally into rats and guinea-pigs (3 per group). The solutions were administered in a volume of 0.1 ml. intramuscularly into the hind legs, and 0.05 ml. intradermally on a shaved area on the backs. After 24 hr the area of the injection was examined and the skin and subcutaneous tissues removed for histological examination.

The effect of methicillin on the eye was examined in a group of 3 rabbits. A 1% solution in normal saline was dropped into a pocket formed by pulling out the lower left eyelid. The solution was held over the eye for 1 min. Saline was similarly applied to the right eye. The eyes were examined at 1, 2, 4, 8 and 24 hr afterwards for signs of irritation.

Blood pressure and respiratory effects

The carotid blood pressure of 5 cats anaesthetized with a 4% urethane/1% chloralose mixture (5 ml./kg intravenously) was recorded manometrically on a smoked drum. Respiration was recorded by a lever connected by means of a thread which was sewn to the skin over the xiphisternum. Methicillin in physiological saline was administered intravenously through the femoral vein at intervals of 5 min.

Absorption

(a) *Oral*

(i) *Rabbits.* 100 and 500 mg/kg doses of methicillin were administered orally to groups of 5 rabbits. Blood samples for assay were removed from the lateral ear vein at 1, 2, 4 and 6 hr.

(ii) *Absorption from small intestine of rats.* The bile ducts of rats, 5 to a group, were cannulated as described by Harrison *et al.* (1960). The rats were restrained in close-fitting wire cages and the total bile and urine collected at 2, 4, 6 and 24 hr after the administration of 100 mg/kg methicillin directly into the duodenum. Blood specimens were taken from the tail vein at 1, 3, 5 and 24 hr after injection.

(b) *Intramuscular—serum and urine concentrations in dogs*

Methicillin and penicillin G were injected intramuscularly at a dose of 5 mg/kg, and blood and urine specimens were taken at intervals up to 6 hr after administration.

The blood specimens were removed by means of a sterile syringe from the radial vein, allowed to clot at room temperature and the serum transferred to sterile tubes and frozen. The urine was removed from the bladder, by means of a polythene cannula, at the same time as the blood samples were taken.

Distribution and elimination

(a) *Serum and cerebrospinal fluid concentrations in rabbits*

(i) *Serum concentrations.* Two groups of 5 rabbits were given intramuscular injections, in the hind leg, of 100 mg/kg methicillin and 100 mg/kg penicillin G ; 0.5 ml. samples of blood were removed from the lateral ear veins at 1, 2, 4 and 6 hr after administration. The samples were allowed to clot at room temperature and the serum was removed and kept at 4° C until assayed (at the end of 6 hr).

(ii) *Cerebrospinal fluid concentrations.* The cerebrospinal fluid concentrations were determined in anaesthetized rabbits after administration of 100 and 500 mg/kg methicillin intramuscularly. The rabbits were anaesthetized with urethane (1 g/kg intraperitoneally). The cerebrospinal fluid samples were withdrawn from the cisternum magnum by means of a sterile syringe and needle. Blood samples were taken from the lateral ear vein at the time of taking the cerebrospinal fluid samples. The concentrations of methicillin in the serum and the cerebrospinal fluid were assayed as before.

(b) *Blood levels and bile excretion in the anaesthetized rat*

Two groups of 5 rats (230 to 265 g) were anaesthetized with 1.0 ml./kg pentobarbitone intraperitoneally. After laparotomy the bile duct was cannulated with polythene tubing (0.4 mm internal diameter). 100 mg/kg methicillin and penicillin G was administered intramuscularly to each of the groups. The bile was collected over 30 min periods in sterile tubes which were cooled in iced water. 0.1 ml. samples of arterial blood were obtained at hourly intervals up to 5 hr from the right carotid artery and diluted with 0.4 ml. normal saline containing 100 units heparin.

(c) *Hen*

The technique described by Sperber (1949) was employed. The cloacal mucosae of 6-month-old laying Sussex hens were anaesthetized with a few drops of 2% cinchocaine hydrochloride in normal saline. A polythene funnel was held in position over each ureteral opening by stitching the mucosa to the free edge of a rubber washer which surrounded the head of the funnel. Short polythene tubes were inserted into the stems of the funnels and the urine from each kidney collected in sterile tubes which were cooled in iced water.

The antibiotics were dissolved in 2.0 ml. of 0.9% saline and injected into the muscles of the left leg. Probenecid, which blocks renal tubular secretion, was administered intravenously at a dose of 100 mg. The probenecid solution was prepared from 0.5 g tablets (Merck Sharpe & Dohme). Five tablets were powdered and triturated with 10 ml. 0.5 N sodium hydroxide and the *p*H adjusted to 7.4 with 1.0 N hydrochloric acid (2 to 3 ml.). The volume was made up to 100 ml. with 0.9% saline, giving a solution containing 25 mg probenecid per ml. Four ml. of this solution was injected into a wing vein 10 min prior to the administration of the antibiotic.

In order to increase urine flow, 100 ml. of warm tap water was given by crop tube at the commencement of the experiment. Samples of urine were taken for assay at 10 min intervals for the first hour after dosing and thereafter at hourly intervals up to 6 hr.

In the experiments 4 hens were employed, each of which received the following treatments on four separate occasions: (i) 100 mg methicillin ; (ii) 100 mg penicillin G ; (iii) 100 mg methicillin + 100 mg probenecid ; (iv) 100 mg penicillin G + 100 mg probenecid.

(d) *Distribution in tissues*

100 mg/kg methicillin was administered intramuscularly to groups of 10 rats, 1 group being killed at 0, ½, 1, 2, 4, 12 and 24 hr after the injection. The rats were exsanguinated by cutting the throat and the blood collected. The urine and faeces from each group were

collected and the amounts recorded. The following organs and tissues were removed and weighed: liver, spleen, kidney, lung, small intestine, large intestine and muscle at site of injection. The carcass was then weighed. All specimens were homogenized in a Waring blender. Appropriate dilutions of the homogenate were made with phosphate buffer *p*H 7.0 and specimens of the homogenate assayed. The total amount of antibiotic in each specimen was calculated. The specimens which were known to be contaminated were not specially treated, as we found that in general the assay figures obtained were not unduly influenced.

Serum binding—dialysis

Five ml. of bovine, horse or human serum containing 5 mg of either methicillin or penicillin G was placed in cellophane bags (Visking tubing ¾ in.) and suspended in 20 ml. sterile saline at 10° C for 48 hr. At the end of the period of dialysis the amounts of the antibiotic outside and inside the tubing were assayed. Five tubes were prepared of each antibiotic in each experiment.

RESULTS

Toxicities

(i) *Acute*

Methicillin when given intravenously to mice at doses up to 2.5 g/kg produces no signs of toxicity. When larger doses are given, 3 to 5 g/kg, occasional mild clonic convulsions are produced within 5 min of administration. These give way to a phase which may persist up to 4 hr in which the mice exhibit a loss of muscle tone. If disturbed during this phase, a mild muscular spasm is induced causing extension of the limbs. In the range 4 to 5 g/kg some deaths may occur due to cardiac and respiratory failure. With some animals respiration may cease for periods up to 10 sec, but this is followed by complete recovery. No toxic symptoms were noted in mice and rats given 4 g/kg of the antibiotic orally or subcutaneously.

(ii) *Prolonged administration*

(a) *Rats.* No untoward toxic symptoms were noted in the rats treated with methicillin. Post-mortem examination did not reveal any abnormalities and the

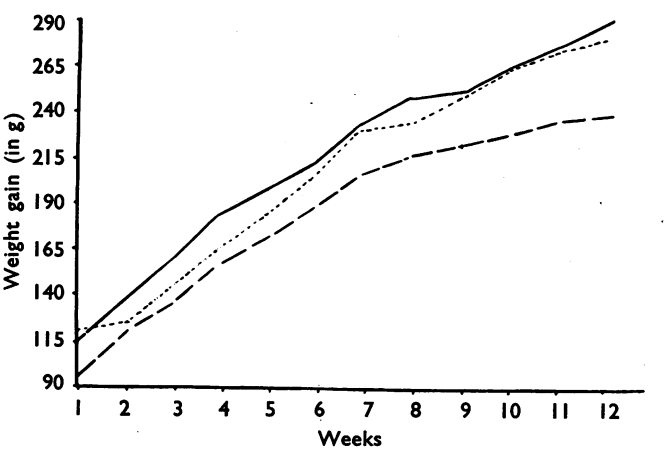

Fig. 1. Mean growth curves of rats—12 to a group—following daily intramuscular administration of (*a*) 500 mg/kg methicillin (——); (*b*) 100 mg/kg methicillin (- - - -); (*c*) 1.0 ml./kg normal saline (— — —).

histology of the organs examined was normal. The growth curves are shown in Fig. 1. The animals which received the antibiotic gained more weight than the controls.

(b) *Dogs.* No abnormal signs were observed in any of the tests carried out. The histology of the essential organs was normal.

Local irritant action

Macroscopically there was no evidence of tissue damage, except following the 10% intradermal injection, when there was a slight induration and erythema of the skin lasting 24 to 48 hr. Histologically there is a marked inflammatory reaction in the rat skin with necrosis of epidermis and hair follicles, following intradermal injection of 10% methicillin. A comparable inflammatory reaction is seen in the muscle. The changes in both skin and muscle of rats are much less marked with the 1% solution. The changes in the guinea-pig skin and muscle are similar; as severe in the skin at 10% but less marked in the muscle at that strength. The skin and muscle of guinea-pigs injected with the 1% solution showed no appreciable abnormality.

Cardiovascular and respiratory effects

When administered by slow intravenous injection to 5 guinea-pigs, 5 g/kg methicillin in saline given over a period of 25 min had no observable effect on the respiration or the electrocardiogram. When similarly administered to cats, 2.5 g/kg given over a 10 min period had no effect on the heart or respiration. In individual doses ranging from 10 to 500 mg/kg, methicillin was again without effect. The blood pressure response of cats to intravenous doses of adrenaline, noradrenaline, acetylcholine and histamine was unaffected by doses of 500 mg/kg methicillin. The pressor response to carotid occlusion was also unaffected by methicillin.

Absorption

(a) *Oral*

(i) *Rabbits.* No blood levels were detectable after 100 mg/kg, but significant blood levels were attained following administration of 500 mg/kg, viz., 3.0, 1.6, 1.6 and 1.45 μg/ml. at 1, 2, 4 and 6 hr respectively after administration.

(ii) *Absorption from small intestine of rats.* The concentration of methicillin found in the bile, urine and the blood after the intraduodenal administration of 100 mg/kg to rats is given in Table 1. Practically no blood levels were detectable, and only a total of 6.5% of the antibiotic was recovered—1.8% from the bile, 3.7% from the urine and 0.97% from the intestine.

(b) *Intramuscular—serum and urine concentrations in dogs*

The mean serum concentrations of penicillin G and methicillin obtained in three dogs after intramuscular injection of 5 mg/kg are shown in Fig. 2. Methicillin gives a slightly lower maximum blood concentration (2.9 μg/ml.) than penicillin G (4.05 μg/ml.). The peak also occurs slightly more in advance of penicillin G, but the fall-off in serum concentration of both antibiotics occurs at much the same rate. The excretion in the urine of both antibiotics is also very similar, 29% of penicillin G and 33% of methicillin being recovered in the urine in 6 hr.

TABLE 1

ABSORPTION OF METHICILLIN IN THE CONSCIOUS RAT AFTER INTRADUODENAL DOSING WITH 100 MG/KG

Rat	Dose in mg	Bile: % of dose excreted (hr) 0–2	2–4	4–6	6–24	Urine: % of dose excreted (hr) 0–2	2–4	4–6	6–24	Blood levels: concentration μg/ml. (hr) 1	3	5	24	% of dose remaining in intestine after 24 hr	Total % recovered
B	30	0·12	0·12	0·17	0·23	1·30	0·60	0·16	2·45	0·28	0·39	0	0	3·40	8·55
D	34	1·02	0·31	0·42	0·86	2·06	0·69	0·29	1·87	0	0	0	0	0·74	8·26
E	27	0·57	0·29	0·27	0·12	1·04	0·79	0·41	0·51	0	0	0	0	0	4·00
F	26	0·12	0·63	0·42	0·48	0·39	0·95	0·85	1·88	0	0	0	0	0·71	6·43
G	28	0·64	0·75	0·56	1·14	0·93	0·24	0·42	0·70	0	0	0	0	0	5·38
Mean % of dose excreted		0·49	0·42	0·36	0·56	1·14	0·66	0·42	1·48					0·97	6·52
Cumulative % excreted		0·49	0·91	1·27	1·83	1·14	1·80	2·22	3·70						

TABLE 2

DISTRIBUTION OF METHICILLIN IN THE RAT

Seven groups of 10 rats were injected intramuscularly with 100 mg/kg methicillin. One group was killed at the end of each time period. The mean concentration of methicillin is expressed in μg/g wet weight of tissue (column a) and the concentration ratio between the tissues μg/g wet weight to serum μg/ml. is shown in column b. The urinary excretion is expressed as a % of the dose administered

	0 hr		0·5 hr		1 hr		2 hr		4 hr		12 hr		24 hr	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Liver			32·8	1·01	23·8	0·79	12·9	2·36	4·1	22·8	0	—	0	—
Spleen			4·5	0·14	2·03	0·07	1·47	0·27	0	0	0·68	—	0	—
Kidneys			122·1	3·76	88·8	2·94	24·1	4·41	2·91	16·17	0·22	—	0	—
Lungs			15·6	0·42	8·8	0·29	2·34	0·43	1·36	7·56	0·19	—	0	—
Small intestine			74·9	2·3	133·9	4·43	149·6	27·4	54·7	304·0	1·05	—	0	—
Large intestine			5·31	0·16	6·84	0·23	7·76	1·42	31·41	14·45	14·5	—	0	—
Injection site	1,620·0		821·0	25·27	332·5	11·01	74·65	13·67	7·8	43·34	0·64	—	0	—
Carcass	2·0		11·8	0·29	9·39	0·31	2·52	0·46	3·7	20·6	0·71	—	0	—
Serum			32·5	1·0	30·2	1·0	5·46	1·0	0·18	1·0	0	—	0	—
Urine % excreted			13·39		32·6		42·8		48·9		74·6		68·4	

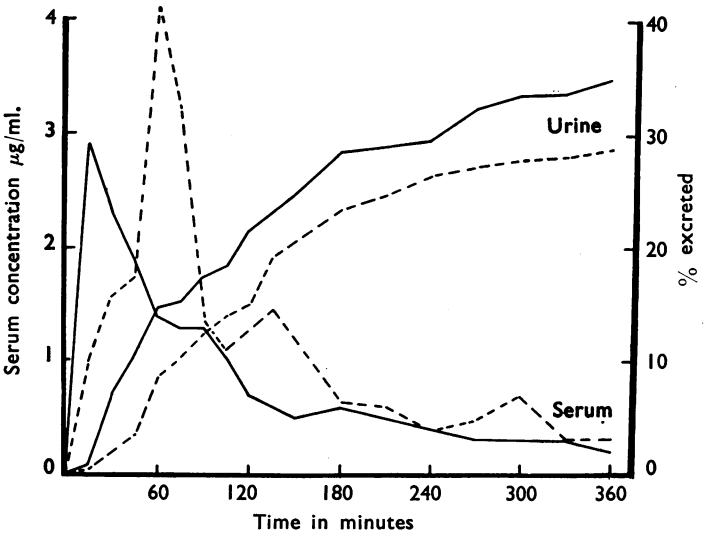

Fig. 2. Serum concentration and urinary excretion in dogs following intramuscular administration of 5 mg/kg methicillin (——) and penicillin G (– – –).

Distribution and elimination

(a) *Serum and cerebrospinal fluid concentrations in rabbits*

(i) *Serum concentrations.* The serum concentrations of penicillin G and methicillin after intramuscular injection to each of 5 rabbits are given in Table 3. The serum concentration picture of each antibiotic is similar.

TABLE 3

SERUM CONCENTRATIONS IN RABBITS RECEIVING 100 MG/KG METHICILLIN AND PENICILLIN G INTRAMUSCULARLY

	Serum concentration μg/ml. at hr after dosing			
Compound	1	2	4	6
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

(ii) *Cerebrospinal fluid concentration.* The concentration of methicillin obtained in the serum and cerebrospinal fluid after the intramuscular administration of 100 and 500 mg/kg is shown in Table 4. The concentration of antibiotic in the cerebrospinal fluid is approximately 100 times less that the concentration appearing in the

TABLE 4
MEAN CONCENTRATIONS IN SERUM AND CEREBROSPINAL FLUID OF RABBITS (3 PER DOSE LEVEL) AFTER INTRAMUSCULAR ADMINISTRATION OF METHICILLIN

Dose mg/kg	Tissue	Concentration μg/ml. at hr after dosing 0·5	1	2	3	6
100	C.s.f.	0·4	0·35	—	—	0
	Serum	34·0	24·0	—	—	0
500	C.s.f.	1·17	2·3	3·6	4·4	0
	Serum	130	136	288	130	1·0

serum. The time of maximum concentration of methicillin in the serum does not coincide, however, with the time of maximum concentration in the cerebrospinal fluid, the latter occurring somewhat later.

(b) *Blood levels and bile excretion in the anaesthetized rat*

The blood picture of the anaesthetized rat after the administration of penicillin G and methicillin is shown in Fig. 3. The blood levels of both antibiotics are similar and both are markedly concentrated in the bile, though at the end of 6 hours both the blood and bile levels of methicillin were a little higher than those of penicillin G.

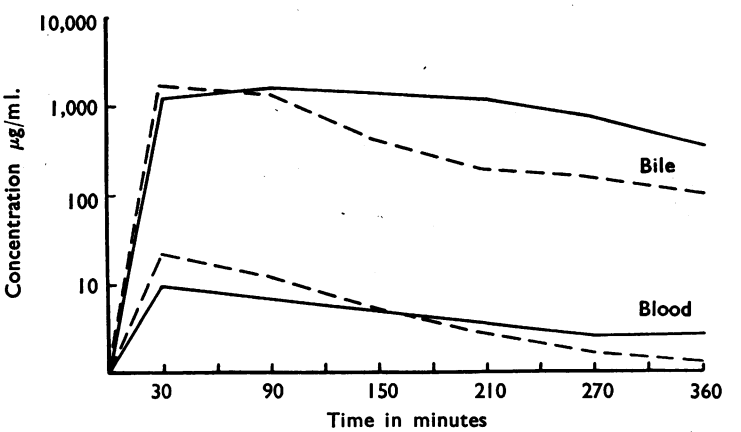

Fig. 3. Mean concentration in blood and bile of methicillin (——) and penicillin G (– – –) after intramuscular administration of 100 mg/kg of each antibiotic to groups of 5 anaesthetized rats.

(c) *Hen*

The excretion in urine of penicillin G and methicillin from the right and left kidneys following intramuscular administration into the left limb of 100 mg/kg of each antibiotic is illustrated in Fig. 4. The concentration of both antibiotics in the urine from the left kidney is considerably greater than the concentration in the urine from the right kidney. However, after the administration of 100 mg probenecid intravenously the concentration of both antibiotics in the urine from the right and left kidneys becomes identical.

(d) *Distribution in tissues*

The tissue concentrations of methicillin expressed as μg/g of wet weight of tissue are given in Table 2 (column a). In order to show the degree of concentration in the

various tissues and organs, the results have also been expressed as a ratio of the serum concentration (column b). Thirty min after the dose was given, the concentration occurring in the kidneys was approximately four times, that in the small intestine about double, and that in the liver about the same as that found in the serum. Lower concentrations occurred in the lungs, spleen and large intestine. The concentration of antibiotic in the serum falls off rapidly, but the concentration level in the other tissues apart from the spleen falls off more slowly, with the result that at the end of 4 hr the concentrations in the tissues are higher than those found in the serum. At the end of 12 hr there are only trace amounts left in most of the tissues,

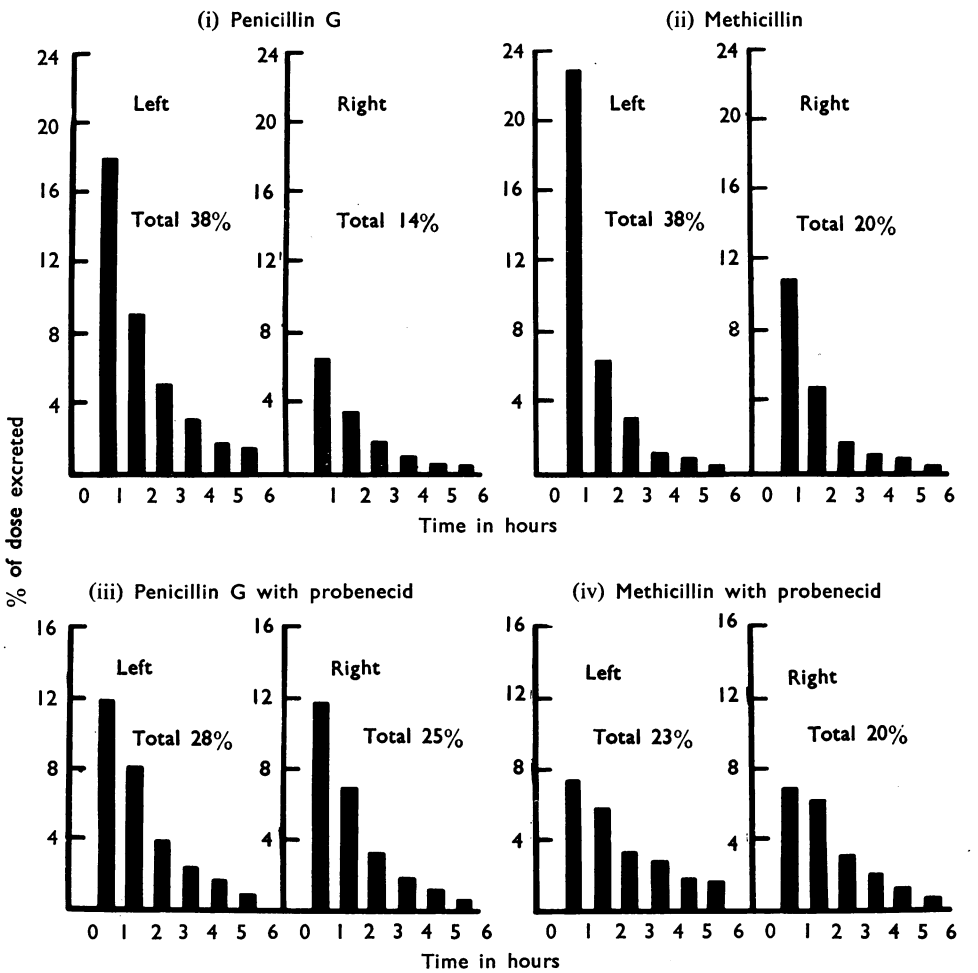

Fig. 4. Excretion of methicillin and penicillin G in the hen. The mean urinary excretion from the left and right kidneys of 4 hens each of which received, on four separate occasions: (i) 100 mg penicillin G; (ii) 100 mg methicillin; (iii) 100 mg penicillin G+100 mg probenecid; (iv) 100 mg methicillin+100 mg probenecid. The antibiotics were given intramuscularly and the probenecid intravenously.

none being detectable in the serum and liver. After 24 hr no antibiotic was detectable in any of the tissues. The urinary excretion is expressed as a percentage of the dose administered, a total of 74.6% being recovered in the first 12 hr. A total of 73.7%, 73.5%, 66.6%, 55.3%, 54.3%, 75.1% and 68.4% of methicillin was recovered from all tissues and body fluids at 0, 0.5, 1, 2, 4, 12 and 24 hr after administration.

Serum binding

Methicillin is considerably less bound to horse, bovine and human serum than penicillin G, the figures being respectively for each of the sera 10.2%, 4.5% and 17.7% for methicillin, and 21.0%, 27.3% and 43.4% for penicillin G.

DISCUSSION

An antibiotic should preferably be free from toxic effects. In this respect methicillin has remarkably few unwanted reactions. No toxic effects were noted in animals after large single intravenous doses. In one experiment a dose of 18 g was administered by slow intravenous infusion to a guinea-pig before serious toxic effects became apparent. When administered over a prolonged period both rats and dogs tolerated large doses, the only observable reaction being some discomfort at the time of injection. This is in agreement with clinical reports (Douthwaite & Trafford, 1960 ; Knudsen & Rolinson, 1960).

Methicillin is poorly absorbed from the gastro-intestinal tract, but it is well absorbed following parenteral administration, only small quantities remaining at the site of injection 12 hr afterwards. Good blood levels are obtained, and the antibiotic is distributed throughout the tissues of the body. There is a high concentration of antibiotic in the kidney, while the concentration in the liver is of the same order as that found in the serum for the first hour, but higher concentrations than those in the serum are subsequently found. The high concentrations appearing in the small intestine are probably accounted for by the presence of bile.

The total quantity of methicillin recovered from the intact animal falls steadily over the first 4 hr to 55%, but fully 75% is eventually recovered in the urine after 12 hr. It would seem that the antibiotic is taken up by some of the tissues in a non-assayable form and is then slowly released and then excreted in the urine.

That methicillin does combine with protein is shown by the dialysis experiments. There is considerable binding with serum protein, but it is less than that found with penicillin G. The degree of binding varies according to species, and it is possible that some of the antibiotic is more firmly bound to one type of protein than another and therefore it is more difficult to assay in this state. The antibiotic may be slowly released from this combination. Otherwise, it is difficult to postulate why there is a " loss " which is subsequently recovered in the urine.

The blood level picture and urinary excretion found for methicillin and penicillin G are practically identical, the pattern being similar in rabbits, dogs and conscious rats. In the anaesthetized rat the excretion is delayed, 50 to 55% of the antibiotics appearing in the urine of conscious rats during a 4 hr period, while only 25 to 35% is recovered in 6 hr from an anaesthetized animal, and as a consequence the blood

level is more prolonged. In view of this difference it would seem preferable to use non-anaesthetized animals for metabolism studies.

A marked difference is seen between the bile concentrations of methicillin and penicillin G. Comparison of the bile/blood ratio for the two antibiotics obtained in rats shows that methicillin is concentrated in the bile fully 2 to 2.5 times more than penicillin G. A total of 15 to 20% of the injected dose of methicillin is excreted in the bile—this we confirmed in both the anaesthetized and conscious rat—while 15% of penicillin G is similarly excreted. In view of the greater concentration ratio of methicillin from the blood to the bile, it might be expected that the difference between the amounts excreted would be more, but the initial blood levels of penicillin G are higher and thus proportionately a larger quantity appears in the bile.

In the normal animal one would expect that a considerable proportion of the antibiotic would be reabsorbed from the intestine after being excreted in the bile. However, the experiments where 100 mg/kg was injected into the duodenum of rats indicate that absorption even in the small intestine is poor—only 3.7% is recovered from the urine and 1.8% from the bile in 24 hr. The bulk of the dose appears to be destroyed, as only 0.97% can be accounted for in the intestine after 24 hr. From the observations on the distribution of the antibiotic in the intact animal one can therefore account for practically the whole of the dose administered, 75% being excreted in the urine and 15% lost in the intestine. We have found no evidence that 6-aminopenicillanic acid is formed in the body from methicillin; neither have we found that methicillin is excreted in a conjugated form in the bile as reported by Harrison *et al.* (1960).

The mode of elimination by the kidneys is well demonstrated in the hen experiment. Birds possess a renal portal system, and the venous return from the hind limbs is shunted through the parenchyma of the renal tubules. Therefore, when a substance is injected into a hind limb and both renal tubular secretion and glomerular filtration take place, the concentration of the substance excreted is greater in the urine from the ipsilateral kidney than in the urine from the contralateral kidney. If only glomerular filtration takes place, then the concentration in the urine excreted from both kidneys is identical. In our experiments the ipsilateral kidneys excreted more of the antibiotics than the contralateral kidneys, and blockage of renal tubular secretion resulted in equal concentrations of the antibiotics appearing in the urine from both kidneys. Therefore methicillin and penicillin G are excreted by the same mechanism, both glomerular filtration and renal tubular secretion taking place.

We wish to thank Dr A. C. Thackray, of the Bland Sutton Institute of Pathology, for expert histological comment; Dr A. A. G. Lewis, physician to the Connaught Hospital, Walthamstow, under whose skilled guidance the dog studies were conducted; and Mr F. P. Doyle and his colleagues for the preparation of the compound.

REFERENCES

BATCHELOR, F. R., DOYLE, F. P., NAYLER, J. H. & ROLINSON, G. N. (1959). The synthesis of penicillin: 6-aminopenicillanic acid in penicillin fermentations. *Nature (Lond.)*, **183**, 257–258.

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

DOUTHWAITE, A. H. & TRAFFORD, J. A. P. (1960). A new synthetic penicillin. *Brit. med. J.*, **ii**, 687–690.

HARRISON, PATRICIA M., WHITE, JEAN A. & STEWART, G. T. (1960). The excretion of sodium 6-(2,6-dimethoxybenzamido)penicillanate monohydrate in rats. *Brit. J. Pharmacol.*, **15**, 571–573.

KNOX, R. (1960). A new penicillin (BRL 1241). *Brit. med. J.*, **ii**, 690–693.

KNUDSEN, E. T. & ROLINSON, G. N. (1960). Absorption and excretion of a new antibiotic (BRL 1241). *Brit. med. J.*, **ii**, 700–703.

SPERBER, I. (1949). Investigations on the circulatory system of the avian kidney. *Zool. Bidrag. Uppsala*, **27**, 429–448.

STEWART, G. T., HARRISON, PATRICIA M. & HOLT, R. J. (1960). Microbiological studies on sodium 6-(2,6-dimethoxybenzamido) penicillanate monohydrate (BRL 1241) *in vitro* and in patients. *Brit. med. J.*, **ii**, 694–699.

STEWART, G. T., NIXON, H. H., COLES, H. M. T., KESSON, C. W., LAWSON, DAVID, THOMAS, R. G., MISHRA, J. N., MITCHELL, M. EILEEN, SEMENS, J. MARJORIE & WADE, T. H. H. (1960). Report on clinical use of BRL 1241 in children with staphylococcal and streptococcal infections. *Brit. med. J.*, **ii**, 703–706.