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PHARMACOLOGY AND CHEMOTHERAPY OF AMPICILLIN—A NEW BROAD-SPECTRUM PENICILLIN

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The pharmacology and chemotherapy of a new penicillin, 6[D(−)-α-aminophenylacetamido] penicillanic acid, are described. It is non-toxic, is absorbed orally and is distributed throughout the body in a manner similar to other penicillins. It is eliminated unchanged from the body in high concentrations in the bile and urine. Almost all of the antibiotic can be accounted for in the urine and intestinal contents 2 hr after intramuscular administration but not after oral administration. It is concluded that the antibiotic is not metabolized within the body. Studies with infected animals show that it is as effective as the existing oral penicillins against *Staphylococcus pyogenes* Smith (penicillin sensitive), *Streptococcus pyogenes* Group A and *Diplococcus pneumoniae*. It is ineffective against penicillin-resistant *Staphylococci*. When tested in mice infected with the gram-negative organisms, *Salmonella typhimurium* and *Klebsiella pneumoniae*, it was considerably more active than tetracycline and chloramphenicol.

There is a need for a penicillin which is effective orally and having activity against both gram-negative and gram-positive organisms. Benzylpenicillin, although lacking in toxicity, is unfortunately not absorbed after oral administration and its activity against gram-negative organisms is limited. Doyle, Nayler & Smith (1961) have synthesized a new penicillin, ampicillin (6[D(−)-α-aminophenylacetamido] penicillanic acid), which largely overcomes these disadvantages. The preliminary pharmacology and chemotherapy has been reported by Brown & Acred (1961). Full details of the pharmacological and chemotherapeutic evaluation are now presented.

METHODS

In all experiments benzylpenicillin was administered as its sodium salt. Ampicillin, which was 84% pure, was also administered as its sodium salt, all doses being given in terms of the pure compound. 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.

Chromatographic studies were carried out using 1 cm wide strips of Whatman no. 1 filter papers, 53 cm long with the origin at 11 cm. The solvent used was ethyl acetate-isopropanol-water in the proportions 4:2:1. 100 ml. of solvent was used in the trough of each tank.

All tanks were lined with Whatman no. 3 mm filter paper. The chromatograms were run from 16 to 20 hr at 20° C after equilibrating for at least 1 hr.

Detection of the penicillin 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 penicillin was seen as clear zones of growth inhibition on the plates. Control strips were set up using an aqueous ampicillin solution.

Acute toxicities

The acute toxicity was determined in male albino mice (Edwards strain—18 to 22 g) after intravenous, subcutaneous and oral administration, and in Sprague-Dawley rats (150 to 200 g) after subcutaneous and oral administration.

Prolonged administration

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

(i) *Rats.* Doses of 500 and 100 mg/kg were administered orally by stomach tube to groups of 12 male rats, 5 days per week for a period of 12 weeks. The dose was administered as a freshly prepared aqueous solution in a volume equivalent to 0.5 ml./100 g body weight. The control group received an equivalent volume of tap water. 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 examination of the blood were made on the first, sixth and twelfth week of the test. At the end of 12 weeks all the rats were killed and the weights of the livers, spleens, kidneys, testes and adrenals were recorded. Specimens of liver, spleen, kidney, lung, thyroid, heart, duodenum, stomach, pancreas, adrenal, testis and bone marrow were removed from 6 animals in each group for histological examination.

(ii) *Dogs.* Ampicillin (250 mg/kg) was administered orally twice daily for a period of 4 weeks to 2 dogs. The following biochemical and haematological estimations were made at weekly intervals: haemoglobin (%), 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

5% and 1% solutions were injected intramuscularly and intradermally into 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 in a shaved area on the back. After 24 hr the area of the injection was examined and the skin and subcutaneous tissues were removed for histological examination.

The effect of ampicillin on the eye was examined in a group of 3 rabbits. A 25% 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 pressures of 5 cats anaesthetized with a 4% urethane/1% chloralose mixture (5 ml./kg intravenously) were recorded manometrically on a smoked drum. Respiration was recorded by a lever connected to a thread which was sewn to the skin over the xiphisternum. Ampicillin in physiological saline was administered intravenously through the femoral vein at intervals of 5 min.

Absorption, distribution and elimination

(a) Absorption—oral and intramuscular

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

(ii) *Dogs.* Ampicillin was administered orally and intramuscularly to groups of 5 dogs. A dose of 5 mg/kg was given intramuscularly and a dose of 20 mg/kg orally. Blood specimens were taken up to 5 hr after administration. Following the intramuscular administration, a comparison was made with benzylpenicillin, and following the oral administration a comparison was made with phenoxymethyl penicillin. The blood specimens were removed by means of a sterile syringe from the radial vein and were allowed to clot at room temperature and the serum was transferred to sterile tubes and frozen.

(iii) *Rats—intestinal administration.* Male rats (290 to 430 g) were starved overnight. After anaesthetizing with ether, laparotomy was performed and the bile duct was cannulated with polythene tubing (0.40 mm bore). The latter was passed through a small aperture in the abdominal wall and the skin and muscle incisions were sutured. The animals were then dosed and placed in close-fitting restraining cages of wire mesh. Blood, bile and urine specimens were obtained at intervals throughout the 24 hr following injection of ampicillin direct into the duodenum (100 mg/kg, 1.0 ml./100 g body weight). At the termination of the experiment, homogenized samples of the following were assayed: (*a*) small intestine from pylorus to ileo-caecal valve, (*b*) caecum, colon, rectum and faeces.

(b) Distribution and elimination

The methods and doses used in investigating the concentrations in the cerebrospinal fluid of rabbits, the mode of urinary excretion in the hen and the tissue distribution in rats were the same as those used by Acred, Brown, Turner & Wright (1961). In all experiments the antibiotic was administered intramuscularly, but in the distribution experiments it was also given orally. The bile, blood and urine concentrations in conscious rats were determined using the method described above for intestinal administration, the antibiotic being given orally by stomach tube.

Protein binding

5 ml. of bovine, horse or human serum containing 5 mg of either ampicillin or phenoxymethyl penicillin was placed in cellophane bags (Visking Tubing $\frac{1}{4}$ in. diameter) 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 of each antibiotic were prepared in each experiment.

Chemotherapy

The protective effect of ampicillin was determined in mice infected with *Klebsiella pneumoniae, Salmonella typhimurium, Diplococcus pneumoniae, Streptococcus pyogenes* Group A, *Staphylococcus aureus* Smith (benzylpenicillin sensitive), and *Staphylococcus aureus* 52–75 (benzylpenicillin resistant, minimal inhibitory concentration 125 μg/ml.). The experimental infections were produced in groups of 10 mice by injections of the bacteria using from 100 to 500 median lethal doses of the bacterial culture, prepared in 5% hog gastric mucin to enhance the virulence (Table 1). The median lethal dose (LD50) was determined by

TABLE 1

THE MEDIAN LETHAL DOSE (MLD) OF BACTERIA AND THE MULTIPLE OF THIS DOSE ADMINISTERED TO INFECT THE ANIMALS IN THE PROTECTION TESTS (SEE TEXT)

Organism	Median lethal dose (LD50) organism/ml.	Infecting dose
Klebsiella pneumoniae	1×10^1	100 × MLD
Salmonella typhimurium	1×10^1	100 × MLD
Diplococcus pneumoniae	1×10^4	100 × MLD
Streptococcus pyogenes A	1×10^6	100 × MLD
Staphylococcus aureus Smith	1×10^2	500 × MLD
Staphylococcus aureus 52–75	1 in 2 dilution of an overnight broth culture with 5% hog gastric mucin	

preparing seven 10-fold dilutions of 18 to 25 hr cultures of the pathogens in 5% hog gastric mucin. 0.5 ml. of the dilutions was injected intraperitoneally into male albino mice (18 to 22 g), one dilution being administered to a group of 10. The animals were observed for 4 days and deaths were recorded each day. The median lethal dose was determined graphically by plotting the dilution factor of the culture against the percentage deaths, using log probit paper.

The median curative dose (CD50). Ampicillin, tetracycline, chloramphenicol, phenoxymethyl penicillin, benzylpenicillin and phenethicillin were administered subcutaneously and orally to the groups of mice. A 4:1 dose ratio was used, the doses being administered in 0.2 ml. normal saline immediately following the infection. The mice were observed for 4 days and the deaths were recorded daily. The percentage deaths were plotted against log dose on log probit paper, and the dose of compound (mg/kg) giving protection to 50% of the mice (CD50) was read off from the graph.

RESULTS

Toxicity

(i) *Acute.* Ampicillin is non-toxic to mice and rats when administered either orally or subcutaneously in doses of 5 g/kg.

As regards intravenous dosage for mice, 2 g/kg has been administered without lethal effects, although muscle tremors, slowed respiration and mild clonic convulsions have sometimes occurred. The amount which can be administered intravenously has been limited by solubility, and the maximum dose we have been able to give has been 2.5 g/kg. At this dose level 3 out of a group of 10 mice have died.

(ii) *Prolonged administration.* No toxic symptoms were noted in rats treated with ampicillin. Similarly there were no observable toxic symptoms in dogs apart from a slight loosening of the stools during the early days of the test. Post-mortem examination did not reveal any abnormalities in either species, and the histology of the organs examined was normal.

Local irritant and pharmacological action

No macroscopical signs of damage were observed apart from a slight area of erythema at the site of injection. Microscopical changes in the skin and muscle were minimal and difficult to find. They consisted only of very sparse inflammatory cell infiltration and slight accompanying interstitial fluid accumulation.

Doses up to 80 mg/kg, administered intravenously to cats, had no effect on blood pressure or respiration. Neither had they any effect on the blood pressure response to an injection of adrenaline, acetylcholine or histamine.

Absorption

(i) *Rabbits.* The blood levels obtained after oral and intramuscular administration of phenoxymethyl penicillin and ampicillin are given in Table 2. After intramuscular dosing the peak level of phenoxymethyl penicillin at 1 hr is higher than ampicillin, but at subsequent periods the concentrations of both antibiotics are similar. On the other hand, after oral administration the concentrations of ampicillin are considerably greater than phenoxymethyl penicillin.

TABLE 2

MEAN BLOOD CONCENTRATIONS FOLLOWING ORAL AND INTRAMUSCULAR ADMINISTRATION OF 100 MG/KG AMPICILLIN AND PHENOXYMETHYL PENICILLIN TO GROUPS OF 5 RABBITS

Antibiotic	Concentration μg/ml. of blood at hr 1	2	3	4	Route
Ampicillin	3·0	2·91	0·73	0·13	Intramuscular
Phenoxymethyl penicillin	4·7	2·42	0·65	0·22	
Ampicillin	2·16	0·92	0·27	0·06	Oral
Phenoxymethyl penicillin	0·67	0·5	0·03	0	

(ii) *Dogs.* The mean serum concentrations after 20 mg/kg of ampicillin and phenoxymethyl penicillin given orally and of 5 mg/kg ampicillin and benzylpenicillin given intramuscularly are shown in Table 3. Following the oral administration of phenoxymethyl penicillin, the highest blood concentration recorded occurred 30 min after administration, whereas with ampicillin the highest concentration occurred 1 hr afterwards. Throughout the 5 hr period of the test, when

TABLE 3

MEAN SERUM CONCENTRATIONS IN GROUPS OF 5 DOGS AFTER ORAL ADMINISTRATION OF 20 MG/KG AMPICILLIN AND PHENOXYMETHYL PENICILLIN AND INTRAMUSCULAR ADMINISTRATION OF 5 MG/KG AMPICILLIN AND BENZYLPENICILLIN

Antibiotic	Route	Concentration μg/ml. at hr after dosing 0·5	1·0	1·5	2·0	2·5	3·0	3·5	4·0	4·5	5·0	5·5	24
Ampicillin	Oral	4·87	7·23	3·67	3·51	2·15	0·96	0·4	0·32	0·22	0·19	0·11	0
Phenoxymethyl penicillin	Oral	2·58	1·48	0·59	—	0·15	0·1	0·08	0·07	0·07	0·04	0·04	0
Ampicillin	Intramuscular	4·6	3·3	1·55	0·95	—	0·32	0·13	0·13	0·09	0·09		
Benzylpenicillin	Intramuscular	2·01	0·76	0·48	0·26	—	—	0·06	0·04	0·03	0·01		

blood samples were taken regularly, the concentration of ampicillin was always considerably higher than phenoxymethyl penicillin. After intramuscular administration the peak serum concentrations of ampicillin and benzylpenicillin occur at 30 min. Throughout the period of the test the concentrations of ampicillin were greater than benzylpenicillin.

(iii) *Rat—intestinal absorption.* The percentage of dose excreted in the bile and urine and that remaining in the gut after the 25 hr period of experimentation are shown in Table 4. Maximum biliary excretion occurred in the 2 to 4 hr period after dosing and there was no excretion in the bile after 23 hr. No urine samples were obtained during the first 2-hr period, and maximum urinary excretion occurred within 4 hr of dosing. Over the 25 hr period approximately twice as much ampicillin was excreted in the urine as compared with that excreted in the bile. In only one rat (no. 3) was there any appreciable quantity of ampicillin remaining in the small intestine after 25 hr. Similarly, the colon, caecum, rectum and faeces of only one rat contained a significant quantity of ampicillin after 25 hr. Approximately 7% of the dose could be accounted for in the bile, urine and faeces.

TABLE 4

ABSORPTION AND EXCRETION OF AMPICILLIN IN THE CONSCIOUS RAT AFTER INTRADUODENAL DOSING AT 100 MG/KG

[0* no sample obtained]

Rat no.	Dose mg	Bile. % of dose excreted (hr)					Urine. % of dose excreted (hr)					Blood levels. Concentration μg/ml. (hr)				% of dose remaining in gut after 25 hr	
		1–2	2–4	4–6	6–23	23–25	1–2	2–4	4–6	6–23	23–25	1	3	5	24	Small intestine	Colon, caecum, rectum, faeces
1	32	0·21	0·83	0·19	0·25	0	0*	0·53	0·04	0·63	0*	0·52	0·36	0·21	0	0·01	0·09
2	43	0·66	0·78	0·28	0·70	0	0*	0*	2·39	2·06	0·04	0·94	0·52	0·46	0	0	2·64
3	29	0·72	0·38	0·10	0·21	0	0*	1·32	0·35	0·84	0	1·35	0·56	0·28	0·11	2·71	0
4	30	0·16	0·71	0·18	0·06	0	0*	0·97	2·49	0·26	0	3·00	0·42	0·17	0	0	0
5	41	0·98	1·80	1·37	0·16	0	0*	3·76	0*	2·95	0·01	0·60	0·88	0·76	0	0·06	0
Mean		0·55	0·90	0·42	0·28	0	0*	1·65	1·32	1·35	0·01	1·28	0·55	0·38	0·02	0·56	0·55
Cumulative % excretion		0·55	1·45	1·87	2·15			1·65	2·97	4·28	4·29						

TABLE 5

MEAN CONCENTRATIONS IN BLOOD, URINE AND BILE, % OF DOSE EXCRETED IN URINE AND BILE, AND RATIO OF URINE AND BILE TO BLOOD CONCENTRATIONS AFTER ORAL ADMINISTRATION OF 100 MG/KG OF AMPICILLIN AND PHENOXYMETHYL PENICILLIN TO GROUPS OF 10 CONSCIOUS RATS

Time (hr)	Blood concentration μg/ml.		Urine concentration μg/ml.		Bile concentration μg/ml.		% of dose excreted in urine		% of dose excreted in bile		Ratio of urine to blood concentration		Ratio of bile to blood concentration	
	Ampicillin	Phenoxymethyl penicillin	Ampicillin	Phenoxymethyl penicillin	Ampicillin	Phenoxymethyl penicillin	Ampicillin	Phenoxymethyl penicillin	Ampicillin	Phenoxymethyl penicillin	Ampicillin	Phenoxymethyl penicillin	Ampicillin	Phenoxymethyl penicillin
0–2	0·25	1·42	171·4	546·5	65·9	441·0	0·69	3·55	0·27	3·05	690	385	265	311
2–4	0·41	1·27	234·8	384·6	95·3	503·6	1·46	3·90	0·65	4·19	569	303	231	397
4–6	0·19	0·41	235·2	225·8	83·7	96·6	1·08	0·66	0·41	0·55	1,238	551	441	236

Distribution

Cerebrospinal fluid. Ampicillin penetrates into the cerebrospinal fluid with difficulty. The concentrations in the cerebrospinal fluid after doses of 500 mg/kg intramuscularly at 1, 2, 4 and 6 hr following administration are respectively 1.4, 0.12, 0.05 and 0.13 μg/ml., whereas the corresponding levels in the serum are 54.0, 14.25, 4.2 and 5.8 μg/ml.

Elimination in the conscious rat. Table 5 shows the mean blood, urine and bile concentrations of phenoxymethyl penicillin and ampicillin at 2-hr intervals following the administration of 100 mg/kg orally of each antibiotic. The concentration ratio from the blood to the urine, and from the blood to the bile, is also given. The mean ratios show that there is little difference between the two antibiotics regarding the blood/bile concentration, the bile concentration being approximately 300 times that found in the blood. However, the concentration of ampicillin in the urine is twice that of phenoxymethyl penicillin, and the mean blood to urine concentration ratios of each antibiotic are 800 for ampicillin and 400 for phenoxymethyl penicillin.

Urinary excretion—the hen. In hens treated with ampicillin only, almost all of the antibiotic administered is excreted in the urine during the 6-hr period following intramuscular administration into the left leg. Four to five times more is excreted by the left kidney than by the right kidney. After probenecid which blocks renal tubular filtration the mean total amount excreted by the left kidney is reduced to about 1.6 times that excreted by the right kidney; moreover, following probenecid approximately 66% of the injected dose only can be accounted for during the period of test (see Table 6).

TABLE 6

EXCRETION OF AMPICILLIN IN THE HEN

The mean urinary excretion from the left and right kidneys of 4 hens each of which received 100 mg ampicillin and 100 mg ampicillin + 100 mg probenecid. Ampicillin was administered into the left leg muscles and probenecid into a wing vein

Treatment	Time (hr)	% of dose excreted	
		Left	Right
Ampicillin	0–1	36·78	6·05
	1–2	19·10	4·49
	2–3	9·07	1·19
	3–4	7·34	1·40
	4–5	3·85	1·56
	5–6	1·64	1·99
	Total 0–6	77·78	16·68
Ampicillin with probenecid	0–1	18·56	12·75
	1–2	10·07	6·85
	2–3	5·51	1·99
	3–4	3·52	1·41
	4–5	1·93	1·87
	5–6	1·16	0·85
	Total 0–6	40·75	25·72

Tissue distribution. The amounts of ampicillin recovered from the various tissues after oral and intramuscular administration are shown in Tables 7 and 8. The amounts are expressed in μg/g wet weight (column a) and as the ratio of concentration in the tissues to the concentration in the serum (column b). The values

TABLE 7

DISTRIBUTION OF AMPICILLIN IN RATS AFTER ORAL ADMINISTRATION

Seven groups of 10 rats were given orally 100 mg/kg ampicillin. One group was killed at the end of each time period. The mean concentration of ampicillin 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

a = concentration μg/ml. b = $\frac{\text{concentration in tissue}}{\text{concentration in serum}}$ *=% recovered from whole carcass

		0·5 hr		1 hr		2 hr		4 hr		12 hr		24 hr	
Organ	0 hr	a	b	a	b	a	b	a	b	a	b	a	b
Liver		15·82	3·58	18·0	9·18	8·16	9·27	2·17	27·13	0·08	6·4	0	—
Spleen		2·22	0·5	0·39	0·2	0·33	0·38	0·12	1·5	0·03	2·64	0·05	—
Kidneys		13·22	2·99	9·86	5·03	6·31	7·17	1·04	13·0	0·06	5·0	0	—
Lungs		6·17	1·4	1·72	0·88	1·29	1·47	1·08	13·5	0·03	1·8	0·08	—
Stomach		706·0	159·7	372·4	190·0	198·0	225·0	5·65	70·63	8·75	700·0	0·09	—
Small intestine		1,230·0	278·3	1,260·0	642·9	666·0	756·8	37·66	470·7	1·15	92·0	0·13	—
Caecum, colon		2·41	0·55	0·35	0·18	531·9	604·5	1,482·0	18,525·0	73·5	5,880·0	2·77	—
Faeces		0	0	0	0	0·104	0·12	660·8	8,260·0	26·18	2,094·0	77·5	—
Carcass		3·57	0·81	0·71	0·36	1·95	2·22	0·68	8·5	0·125	10·0	0	—
Serum		4·42	1·0	1·96	1·0	0·88	1·0	0·88	1·0	0·012	1·0	0	—
% urine excreted		0·2		0·87		3·11		4·34		6·63		3·7	
% dose recovered	*60·38	64·8		57·9		45·03		42·65		9·61		4·31	

TABLE 8

DISTRIBUTION OF AMPICILLIN GIVEN INTRAMUSCULARLY, IN RATS

Five groups of 10 rats were injected intramuscularly with 100 mg/kg ampicillin. One group was killed at the end of each time period. The mean concentration of ampicillin 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.

$$a = \text{concentration } \mu\text{g/ml.} \qquad b = \frac{\text{concentration in tissue}}{\text{concentration in serum}}$$

Organ	0 hr		0·5 hr		1 hr		2 hr		4 hr	
	a	b	a	b	a	b	a	b	a	b
Liver			175·0	2·7	70·48	4·6	5·76	6·1	0·98	5·4
Spleen			8·3	0·13	3·4	0·22	0·76	0·8	0·24	1·3
Kidneys			288·0	4·4	146·2	9·6	6·56	6·9	0·75	4·2
Lungs			28·6	0·44	6·7	0·44	1·18	1·3	0·52	2·9
Small intestine			77·4	1·2	111·7	7·3	70·8	75·3	15·56	86·4
Large intestine			6·74	0·1	5·24	0·34	20·1	21·4	105·8	587·7
Site of injection	1,700·0	204·6	132·0	2·01	31·4	2·1	3·86	41·1	1·86	10·3
Carcass	2·64	0·32	17·9	0·28	4·54	0·3	0·78	0·8	0·85	4·7
Urine			2,548·0	3·9	4,105·0	268·7	2,626·0	2,793·6	1,452·0	8,066·6
Serum	8·31	1·0	64·4	1·0	15·28	1·0	0·94	1·0	0·18	1·0
Faeces							13·0	13·8	23·32	129·5
% recovered in urine	—		52·0		84·4		112·0		95·5	
% dose recovered	85·6		73·2		99·4		116·9		99·9	

represent the mean of 10 rats. The amount of the antibiotic recovered expressed as a percentage of the dose administered is shown in the tables.

After oral administration the concentration of ampicillin fell off more rapidly in the serum than any other tissue, with the exception of the spleen. The high concentrations in the livers and kidneys were probably accounted for by the presence of bile and urine in these organs. The passage of the antibiotic along the alimentary canal is well illustrated. The antibiotic passed very rapidly from the stomach to the small intestine. There were only trace amounts found in the colon for the first 2 hr of the test, but at 4 hr large quantities were recovered in the colon and faeces. Throughout the period of the test the total quantity recovered decreased until at 12 and 24 hr only 9.6% and 4.3% of the doses administered were recoverable ; the majority of these quantities were in the urine, 6.6% and 3.7% respectively. After oral administration 43 to 65% can be recovered in the first 4 hr.

After intramuscular administration there is a rapid increase of serum level during the first half-hour following administration ; however, this is followed by a very rapid decrease in serum levels. Within 4 hr serum levels have been reduced from 64.4 μg/ml. to 0.18 μg/ml. Practically the whole of the amount injected is recovered in the urine within 1 to 2 hr after administration. The concentrations occurring in the liver and the kidneys are also initially very high, and they decline very rapidly but not quite at the same speed as the serum levels. The concentrations in the spleen and lung during the first hour never reach the concentration found in the serum, but the decline in the concentrations in these two organs is much less than in the serum, so that at the end of 4 hr the concentrations are higher than the serum. The amount found in the small intestine and the large intestine resulting probably from biliary excretion does not exceed 6.5% of the dose administered. After intramuscular injection 73 to 117% can be recovered in the first 4 hr.

Protein binding

Ampicillin is considerably less bound to serum than phenoxymethylpenicillin (Table 9).

TABLE 9

PROTEIN BINDING

Mean % of ampicillin and phenoxymethyl penicillin bound to bovine, horse and human serum

	Mean % serum bound		
Antibiotic	Bovine	Horse	Human
Ampicillin	17·15	7·9	17·0
Phenoxymethyl penicillin	51·37	39·37	68·7

Chemotherapy

Ampicillin is effective against a number of experimental infections produced by gram-negative and gram-positive organisms in mice (Table 10). Against the penicillin-sensitive staphylococci (*Staphylococcus aureus* Smith) ampicillin, benzylpenicillin and phenethicillin, given subcutaneously, are the most active antibiotics followed by phenoxymethyl penicillin and tetracycline ; chloramphenicol is inactive.

TABLE 10

ACTIVITY OF AMPICILLIN, PHENOXYMETHYL PENICILLIN, PHENETHICILLIN, BENZYLPENICILLIN, TETRACYCLINE AND CHLORAMPHENICOL AGAINST GRAM-POSITIVE AND GRAM-NEGATIVE INFECTIONS

Expressed in terms of the dose of antibiotic calculated to protect 50% of a group of infected mice (CD50 mg/kg)

n=number of observations

Organism	Antibiotic	*In vitro* M.I.C. μg/ml.	Route–Oral Mean CD50 mg/kg	n	Route–Subcutaneous Mean CD50 mg/kg	n
Staphylococcus aureus Smith	Ampicillin	0·1	0·3	4	0·3	4
	Phenoxymethyl penicillin	0·02	0·3	3	0·5	1
	Benzylpenicillin	0·02	5·8	1	0·3	3
	Phenethicillin	0·05	0·3	2	0·1	1
	Tetracycline	0·1	5·2	1	6·0	1
	Chloramphenicol	50·0	Inactive 100	2	Inactive 100	2
Staphylococcus aureus 52–75	Ampicillin	>200·0	Inactive		Inactive	
	Phenoxymethyl penicillin	>200·0	Inactive		Inactive	
	Benzylpenicillin	>200·0	Inactive		Inactive	
	Phenethicillin	>200·0	Inactive		Inactive	
	Tetracycline	>200·0	Inactive		Inactive	
	Chloramphenicol	>200·0	Inactive		Inactive	
Streptococcus pyogenes Group A	Ampicillin	0·1	0·1	1	0·025	
	Phenoxymethyl penicillin	0·05	0·1	1	0·1	1
	Benzylpenicillin	0·05	—		—	
	Phenethicillin	0·2	0·5	1	0·1	1
	Tetracycline	0·2	0·5	1	0·5	1
	Chloramphenicol	2·0	3·2	1	3·2	1
Diplococcus pneumoniae	Ampicillin	0·05	0·25	2	0·5	2
	Phenoxymethyl penicillin	0·05	0·6	2	0·9	2
	Benzylpenicillin	0·02	—	2	0·6	2
	Phenethicillin	0·05	0·4	1	0·2	1
	Tetracycline	0·2	13·0	1	5·0	2
	Chloramphenicol	2·0	>100·0	1	Inactive 100	1
Klebsiella pneumoniae	Ampicillin	0·5	11·6	9	35·4	8
	Phenoxymethyl penicillin	50·0	Inactive 400	4	Inactive 400	4
	Benzylpenicillin	10·0	—		Inactive 400	3
	Phenethicillin	500·0	Inactive 400	2	Inactive 400	2
	Tetracycline	0·5	Inactive 400		61·0	14
	Chloramphenicol	1·0	165·0		280·0	2
Salmonella typhimurium	Ampicillin	1·0	18·0	10	12·8	10
	Phenoxymethyl penicillin	200·0	Inactive 400	4	Inactive 400	4
	Benzylpenicillin	10·0	Inactive 400	3	82·0	3
	Phenethicillin	>100·0	Inactive 400	2	Inactive 400	2
	Tetracycline	2·0	62·4	5	59·2	16
	Chloramphenicol	5·0	310·0	1	250·0	3

When given by the oral route ampicillin, phenoxymethyl penicillin and phenethicillin are equally active, but the activity of benzylpenicillin is reduced to that of tetracycline ; chloramphenicol again is inactive. None of the antibiotics is active against staphylococci resistant to benzylpenicillin.

Against streptococci, ampicillin, phenoxymethyl penicillin, phenethicillin and tetracycline are all very active when given orally or subcutaneously, although

ampicillin is the most active when given subcutaneously. Again, chloramphenicol is the least active.

Against pneumococci, ampicillin is the most active, followed by phenoxymethyl penicillin, phenethicillin and benzylpenicillin. Tetracycline is less active and chloramphenicol is only slightly active.

In the animals infected with gram-negative organisms, benzylpenicillin, phenethicillin and phenoxymethyl penicillin are inactive, while ampicillin is considerably more active than tetracycline, which in turn is more active than chloramphenicol, both orally and subcutaneously.

DISCUSSION

Ampicillin is the first semi-synthetic penicillin which is effective orally against gram-negative and gram-positive organisms. Unlike other broad-spectrum antibiotics, which are usually limited in their usefulness by untoward side-effects, ampicillin is non-toxic. As with benzylpenicillin, high doses can be administered safely by injection and by mouth to laboratory animals. In the prolonged toxicity studies the only observable symptoms occurred in dogs, where the initial dosing resulted in the passage of semi-liquid stools. In contrast to tetracycline and chloramphenicol, which produce severe tissue damage at the sites of injection (Hanson, 1961), ampicillin after intramuscular and intradermal administration has little irritant effects.

In the rat after oral and intraduodenal administration, ampicillin and phenoxymethyl penicillin are poorly absorbed. However, in dogs after oral administration, ampicillin gives higher blood levels than phenoxymethyl penicillin. The levels are also higher than those obtained after administration of much larger doses to rats. In humans, ampicillin (Knudsen, Rolinson & Stevens, 1961) also gives good blood levels after oral administration ; and 30% of the antibiotic can be recovered in the urine within 6 hr. The results indicate that the rat is an unsuitable animal for the study of oral absorption of penicillin derivatives.

After oral administration a large proportion of the antibiotic is destroyed in the intestine. Since the antibiotic is not stable to bacterial " penicillinase " it can be assumed that it is destroyed by the intestinal flora. There would appear to be no destruction of the antibiotic within the body, as bile and urine chromatography revealed only unchanged ampicillin.

After intramuscular administration almost all of the antibiotic is recovered in the urine in 2 hr. This is in marked contrast to the oral results, where the total recovery in the urine does not exceed 10%. The concentration of ampicillin in the bile and urine during the first 4 hr after administration to rats is 600 and 250 times respectively the corresponding serum concentrations, these concentration ratios being similar to those found with methicillin. However, in the experiments in which the bile ducts were cannulated, it would appear that the stress of the operation reduces the urinary excretion considerably, since only 23.4% of the antibiotic is recovered in 4 hr, whereas 95% of the antibiotic is recovered in the urine in the unoperated animals within the same time period.

Ampicillin is rapidly cleared from the blood by the kidneys. It is removed both by renal tubular secretion and glomerular filtration. This was demonstrated in the hen, which possesses a renal portal system (Sperber, 1949). 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, prior to the block in tubular secretion by probenecid, 95% of the dose of ampicillin administered is excreted within 6 hr following administration, and of this 78% is excreted by the kidney nearest to the injection site, while only 16.6% is excreted by the contralateral kidney. On the other hand, after the administration of probenecid, although the amount excreted by the contralateral kidney is increased and that excreted by the other kidney is reduced, the kidneys still do not excrete equal amounts, suggesting incomplete block of renal tubular secretion. Knudsen (personal communication) has found that in humans a single dose of probenecid similarly does not block completely renal tubular secretion of ampicillin. A second dose is required to produce complete block.

Ampicillin is distributed throughout the body tissues, but is concentrated only in the kidney and liver, the high values in these organs probably being attributed to the presence of urine and bile respectively. As with other penicillins, only small quantities penetrate the cerebrospinal fluid.

Benzylpenicillin, phenoxymethyl penicillin, phenethicillin and ampicillin all have the same order of activity *in vitro* against the gram-positive organisms, and this relationship in activity is born out *in vivo*. Tetracycline likewise gives a reasonable parallelism between *in vivo* and *in vitro* activity. On the other hand, chloramphenicol has a CD50 of 3.2 mg/kg against the streptococcal infection, but is inactive in doses of 100 mg/kg against *Staphylococcus aureus* Smith and *Diplococcus pneumoniae*, although *in vitro* the activity of chloramphenicol against the three organisms differs by only one tube dilution.

Against the gram-negative organisms which were investigated the activity of ampicillin *in vivo* corresponds to its *in vitro* activity, but both tetracycline and chloramphenicol are considerably less active *in vivo* than ampicillin, even though their *in vitro* activities are only slightly less than ampicillin.

There is no valid explanation for these differences at present. They cannot be attributed simply to ampicillin being better absorbed, being less protein-bound or being distributed throughout the body more effectively than either tetracycline or chloramphenicol, since if this were so it would be expected that the difference in the *in vivo* and *in vitro* activities of these antibiotics would be the same against the gram-positive as well as the gram-negative organisms. In the assessment of the activity of chemotherapeutic agents, therefore, these results emphasize the need to correlate all the *in vitro* and *in vivo* experimental results.

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