AMPICILLIN IN TREATMENT OF CERTAIN GRAM-NEGATIVE BACTERIAL INFECTIONS

BY

W. P. U. KENNEDY, M.B.
Senior House Officer

A. T. WALLACE, M.D.
Bacteriologist

J. McC. MURDOCH, F.R.C.P.Ed., M.R.C.P.Glasg.
Consultant Physician

Department of Infectious Diseases, City Hospital, Edinburgh

Ampicillin, the first semisynthetic penicillin active against Gram-negative pathogens, is bactericidal *in vitro* to *Escherichia coli*, certain *Proteus* spp., some *Klebsiella* strains, salmonellae, and shigellae. The susceptible range of Gram-positive organisms is the same for ampicillin as for benzylpenicillin, although ampicillin is much less potent, weight for weight. Ampicillin is also destroyed by penicillinase, and is therefore inactivated by many strains of *Staphylococcus aureus* and by certain penicillinase-producing strains of *Proteus* and *Klebsiella* (Rolinson and Stevens, 1961 ; Acred *et al.*, 1962).

Ampicillin is acid-stable and is well absorbed from the gastro-intestinal tract. Maximum serum concentrations are reached after one and a half to three hours, falling to lower but still significant levels in four to six hours (Knudsen *et al.*, 1961 ; Stewart *et al.*, 1961). Renal excretion of ampicillin produces very high concentrations in the urine (Brown and Acred, 1961 ; Acred *et al.*, 1962). The levels attained are very much higher in bile than in serum, and as the drug is not detoxicated in the liver its bactericidal activity is unaffected (Stewart and Harrison, 1961 ; Acred *et al.*, 1962). Biliary concentrations of ampicillin are much greater than those of chloramphenicol, streptomycin, or neomycin (Harrison and Stewart, 1961).

In experimental animals it is evenly distributed throughout the body, except for the kidneys and liver, where tissue concentrations are high (Brown and Acred, 1961). Diffusion into the cerebrospinal fluid is poor (Stewart *et al.*, 1961 ; Acred *et al.*, 1962). The present investigation is an attempt to assess the therapeutic effect of ampicillin in certain infections of the urinary tract and various types of salmonellosis.

Patients and Methods

Forty-eight patients, aged 6 weeks to 79 years, were selected for treatment, and they fell into three main groups.

Group A: Infections of Urinary Tract

Thirty-one adults and six children in this group had infections of the urinary tract confirmed by the finding of 100,000 or more viable organisms per ml. of urine in at least two fresh midstream specimens (Kass, 1956 ; MacDonald *et al.*, 1957). Twenty-eight women and three men were aged 22 to 79, and all but three of them had suffered recurrent episodes of infection over periods varying from 5 months to 35 years. Six patients dated their symptoms from childhood. Antibacterial drugs, often in repeated courses, had been given on previous occasions to 30 of the patients. The sulphonamides had proved unsuccessful in at least 15, chloramphenicol in 11, nitrofurantoin in eight, streptomycin in six, tetracycline in four, cycloserine in three, oral penicillin in two, and cephalosporin C in one.

The six children in this group were two girls aged 5 and 6 years, and four boys aged 6 weeks to 10 years. Both girls had recurrent urinary symptoms, the main complaint being diurnal and nocturnal urinary incontinence. One of them had previously been treated with tetracycline and nitrofurantoin. Three of the boys were admitted with acute febrile illnesses, with diarrhoea and vomiting for two to three days ; the fourth was found to have an infection of the urinary tract during his convalescence from infective hepatitis. Two of the boys had marked phimosis, and were later circumcised.

Proteus mirabilis was isolated in 21 patients, *E. coli* in 10, enterococci in seven, coliforms in two, and *Haemophilus vaginalis* in one. Four of these were mixed growths. All organisms were inhibited *in vitro* by a concentration of 16 μg./ml. or less of ampicillin.

Blood urea nitrogen levels and creatinine clearances were measured twice prior to treatment. Urine specific gravity was estimated daily when possible. Four patients had advanced chronic pyelonephritis with a high blood urea nitrogen and very low clearances ; one of these was a woman of 26 who had undergone a nephrectomy two years previously.

Radiography was possible in 31 patients. Structural abnormality was demonstrated in 17 of them by intravenous pyelography. Six of these had undergone surgery before referral to this hospital, while a further five were operated upon before the ampicillin was given. Radiography was not carried out in three women because of pregnancy, in two children on account of extreme youth, and in a diabetic woman of 73 with severe renal damage.

All patients were given ampicillin in an intensive short-term course. The dose was 750 mg. six-hourly in 12 adults, 500 mg. six-hourly in 19 adults, and from 62.5 to 500 mg. six-hourly in the six children, according to body weight (about 50 mg./kg./24 hours). Treatment was given for 14 days except in the case of a man of 79 whose treatment was continued for 21 days after transvesical prostatectomy. The daily fluid intake was restricted to 1,400 ml. during treatment. The usual clinical criteria of response were noted, and accurate bacteriology of serial urine cultures was observed.

Fourteen patients were treated for 14 days only, but the remaining 23 were also given ampicillin in doses of 250 mg. on alternate days for a prolonged period. All were asked to attend for out-patient review at intervals of not more than three months.

Group B: Salmonella Infections

Of the 10 patients included in this group three were suffering from acute typhoid fever with *Salmonella typhi* present in both stools and blood before treatment began. One patient had less severe enteric fever with diarrhoea, caused by *Salm. montevideo*. One man had suffered a diarrhoeal illness two weeks previously, and had no symptoms when admitted, although *Salm. paratyphi B* was present in his stools. A girl of 9 years had acute *Salm. paratyphi B* enteritis and septicaemia, and her 11-year-old brother had a clinically similar illness with a strongly positive Widal reaction, although the organism was not isolated prior to treatment. *Salm. paratyphi B* was constantly present in the faeces of the remaining three patients, who

had become asymptomatic "carriers." These three patients had already been treated with chloramphenicol and were being followed up by daily culture of the stools. One of them, a 40-year-old woman, produced 29 positive stools over a period of one month, and was found to have gallstones, for which a cholecystectomy was performed before ampicillin was given. The pathogens were cultured from the bile in this case. The other two "carriers" produced positive stools less frequently: nine in one month and 11 in three months respectively.

The details of treatment in this group are summarized in Table I. All patients had follow-up tests by stool culture. Six specimens were cultured during the three weeks following the course of treatment, and after leaving hospital the patients were screened at regular intervals by the public health department.

Group C: Severe Systemic Gram-negative Infections

This patient was a 41-year-old man with *Kl. pneumoniae* septicaemia originating from acute colonic diverticulitis. The organism was inhibited *in vitro* by 4 μg./ml. of kanamycin sulphate, and treatment was started with this antibiotic in six-hourly doses of 0.25 g. intramuscularly. His condition greatly improved, and in view of the risk of ototoxicity kanamycin was discontinued after a total dose of 43 g. Two days later he relapsed both clinically and bacteriologically, again with *Kl. pneumoniae* septicaemia. The minimum inhibitory concentration (M.I.C.) of ampicillin for the infecting organism was 2 μg./ml. and ampicillin was given in doses of 750 mg. six-hourly for a total of 96 days, a period long enough to allow resection of the affected colonic segment to be carried out. Thereafter the patient was immobilized in a plaster shell because of the operative finding of osteomyelitis of the fifth lumbar vertebral body, presumably the result of direct mesenteric extension of infection. Since then he has been maintained on long-term ampicillin in doses of 250 mg. daily.

Bacteriological Studies

Group A.—Midstream specimens of urine were collected in wide-mouthed jars and were either delivered immediately to the laboratory or stored for not more than four hours at 4° C. Three specimens were cultured prior to treatment, and similar specimens were examined twice during treatment—on the two days immediately after completion of treatment, and at every out-patient review. Culture, viable counts of organisms, and determination of antibiotic sensitivities were carried out by standard methods (Gould and Bowie, 1952; Murdoch *et al.*, 1959, 1962). The M.I.C. of ampicillin for each strain was estimated by serial tube-dilutions, with concentrations of ampicillin increasing from 2 to 64 μg./ml. At least one early-morning specimen of urine from each patient was examined by direct staining and by culture for tubercle bacilli. Serum and urine concentrations of ampicillin were determined two or three times weekly during treatment. Venous blood was withdrawn for assay three hours after an oral dose of ampicillin; urine specimens were 10-ml. aliquots of 24-hour collections. Antibiotic concentrations were estimated by a doubling dilution method (Murdoch *et al.*, 1962), using the standard Oxford *Staph. aureus* (Figs. 1 and 2).

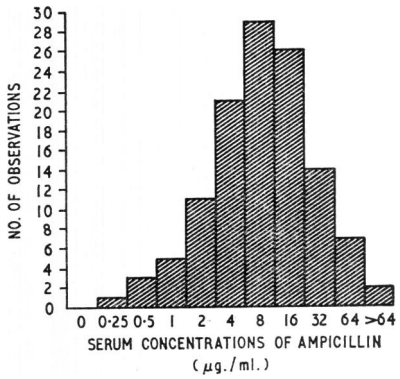

FIG. 1.—Distribution of 119 estimations of serum concentrations of ampicillin. (Note variation in oral dose and possible variation in absorption in young children.)

Groups B and C. — Specimens of stool, urine, and blood were cultured as previously described (Murdoch *et al.*, 1962). Serum and urine concentrations of ampicillin were estimated as for group A.

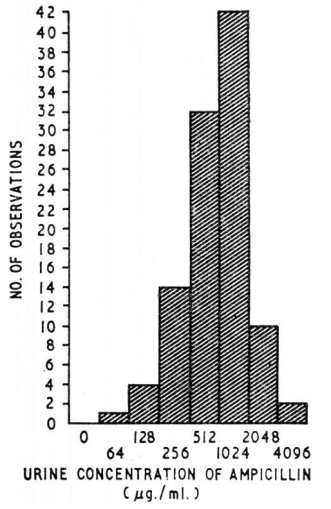

FIG. 2.—Distribution of 105 estimations of urine concentrations of ampicillin.

Results

Group A

The ampicillin sensitivity of 364 strains of urinary pathogens isolated during the period of study is shown in Table II. Ampicillin was given only to patients whose organisms had been inhibited *in vitro* by 16 μg./ml. or less. Serum concentrations of ampicillin, measured three hours after an oral dose, were widely distributed, but 84% of the 119 results fell between 2 and 32 μg./ml. (Fig. 1). In 105 estimations of average urine concentration over 24 hours, 93% of the results were from 256 to 2,048 μg./ml. (Fig. 2).

The results of treatment in this group are set out in Table III. Two patients were withdrawn from the trial

TABLE I.—*Ampicillin Therapy in Salmonelloses*

Order in Text	Sex	Age (yr.)	Previous Therapy	Organism	Ampicillin M.I.C. (μg./ml.)	Daily Dose of Ampicillin (g.)	Duration of Treatment (days)	Follow-up	
								Results	Time
1	M	55	Chloramphenicol	*Salm. typhi*	0·25	3	36	Negative	14 months
2	F	35		,,	4	3	21	Relapse	6 days
						4*	14	Negative	2 months
3	F	49		,,	<2	3	14	Relapse	12 days
						4*	21	Negative	2 months
4	F	70	Sulphonamide and neomycin	*Salm. montevideo*	4	3	14	Relapse	14 days
5	M	41		*Salm. paratyphi B*	<2	4	21	Negative	3 months
6	F	9	Chloramphenicol	,, ,,	4	2	18	,,	7 ,,
						1†	28		
7	M	11	,,	Positive Widal	—	2	18	,,	7 ,,
						1†	28		
8	F	40	,,	*Salm. paratyphi B*	0·5	3	21	Relapse	44 days
9	M	18	,,	,, ,,	2	2	28	,,	20 ,,
10	F	25	,,	,, ,,	4	2	28	Negative	14 months

* Second courses of treatment following relapse. † Treatment continued in reduced doses as out-patients.

after urticarial rashes had developed within a short time of starting treatment.

Ampicillin failed to eradicate proteus organisms from the urine of one patient with radiological "clubbing" of the calices even though the *in vitro* M.I.C. for the organism was 2 μg./ml. Ampicillin successfully eradicated organisms from the urine of the remaining 34 patients. Two patients who were given short-term treatment became reinfected with organisms sensitive to ampicillin. Of the 12 failures in the long-term treated group, 11 relapsed or were reinfected while taking ampicillin, and one was reinfected within one month of stopping treatment. The organisms

TABLE II.—*Ampicillin Sensitivity of 364 Strains of Urinary Pathogens (Tube-dilution Method)*

Organism	No. of Isolates	M.I.C. of Ampicillin (μg. ml.)							
		<2	2	4	8	16	32	64	>64
E. coli	194	6	10	59	73	28	1	5	12
Coliforms ..	56	2	1	8	31	—	—	3	11
P. vulgaris ..	79	19	12	15	22	8	2	1	—
Kl. pneumoniae ..	16	1	—	2	1	—	—	2	10
Ps. pyocyanea ..	10	—	—	—	—	—	—	—	10
Staph. aureus ..	5	1	1	3	—	—	—	—	—
Enterococci ..	4	3	—	1	—	—	—	—	—

TABLE III.—*Results of Treatment of Infections of Urinary Tract*

Ampicillin Treatment	No. of Patients	No. with Sterile Urine		Total Failures	With-drawals
		Immediate Response	Long-term Response		
14-day course ..	14	11	8	3	3*
Long-term course ..	23	23	11	12	0

*Two patients were withdrawn because of skin sensitization reactions. One patient died in cardiac failure within three weeks.

responsible for failure were resistant to ampicillin at levels greater than 64 μg./ml. *in vitro* in eight patients, and sensitive to the drug at levels of 16 μg./ml. or less in four patients. These 15 patients represent a long-term failure rate of 40%. The 76-year-old diabetic woman died in congestive cardiac failure three weeks after completing 14 days' treatment.

Eight patients given short-term and 11 given long-term therapy had a sterile urine at follow-up periods ranging from 1 to 13 months. Thirty-one patients (84%) have been followed up for three months or more.

Group B

The M.I.C. of ampicillin was determined *in vitro* for 12 salmonellae isolates and was 4 μg./ml. or less for each patient, with the exception of the boy treated on the basis of a positive Widal reaction.

The results of treatment are shown in Table I. Ampicillin was primarily successful in eradicating *Salm. typhi* from one of the patients with acute typhoid fever, but the other two relapsed and required a second course with larger doses of the antibiotic. The patient with enteric fever due to *Salm. montevideo* relapsed within four days of finishing treatment. Two of the three carriers of *Salm. paratyphi B* relapsed shortly after treatment was completed. The boy aged 11 years produced *Salm. paratyphi B* in his stools on the thirty-first day of ampicillin therapy, but thereafter all cultures were negative and he remained well. A total of five relapses therefore occurred in the 10 patients, and two of these were dealt with by a second course of the antibiotic. The extent of follow-up has so far been between 2 and 14 months; seven of the patients remain free from infection.

Group C

The patient in this group made a satisfactory recovery after 96 days of treatment with ampicillin. He has continued on long-term ampicillin, and his urine has been sterile at each follow-up examination. He remained in good health 18 months after treatment was started.

Side-effects

No severe toxicity directly attributable to ampicillin was observed in this series, and none of the children suffered any ill effects. Three patients complained of looseness of the stools during the first few days of treatment, but normal bowel function returned without any reduction in the dose of the antibiotic being necessary. One of the patients with enteric fever developed a "black tongue" on the thirteenth day of ampicillin treatment, which resolved after withdrawal of the drug. Urticarial rashes, typical of hypersensitivity to penicillin, occurred in five patients. There was a definite history of allergic reaction to penicillin in one of these, while the others had all had previous courses of penicillin without allergic manifestations. The rashes faded after withdrawal of ampicillin and treatment with antihistamines.

Discussion

Comment has previously been made on the unsatisfactory results of current antibacterial treatment of infections of the urinary tract (Kass, 1955; Murdoch *et al.*, 1959; Syme *et al.*, 1961; Zangwill *et al.*, 1962). Chronic and recurrent infections are particularly difficult to eradicate (*Lancet*, 1963). To assess a new antibacterial drug in the treatment of infections of the urinary tract drainage defects should first be detected and corrected where possible. It may be that long-term suppressive antibiotic or chemotherapy will protect the renal tract from further inflammatory damage. Ampicillin is a bactericidal antibiotic with no apparent toxicity which can be given for long periods of time with relative safety, and has potential value in this form of treatment. The present series of 37 patients gives an indication of the success of this drug in initial treatment only. All of the patients had urinary pathogens shown to be sensitive *in vitro* to ampicillin, and the immediate attainment of urine sterility in 34 (92%) is comparable to the results of Brumfitt *et al.* (1962) and of Trafford *et al.* (1962). Nevertheless even in this small series of patients there has been a long-term failure rate of 15 (40%).

Long-term treatment will protect some patients from future infection, but in others it will fail. This may be explained in several ways. First, the treatment will eradicate susceptible organisms to reveal an underlying infection with resistant strains, or, second, the organisms may develop resistance while under treatment. The third possibility is reinfection by different species naturally resistant to the antibiotic being given. It may be that long-term ampicillin treatment in low doses provides optimum conditions for one or other of these possibilities. The conclusion can be drawn that ampicillin, in common with other antibacterial agents, should not be used for indiscriminate long-term treatment of infections of the urinary tract without regular bacteriological examination of the urine.

The place of ampicillin in infections of the urinary tract should be limited to the initial and long-term treatment of sensitive proteus infections, because many strains of the other common urinary pathogens are ampicillin-resistant. Ampicillin is not as effective as cycloserine in treating *E. coli* infections and is much more expensive—a matter of particular importance in long-term therapy.

Reports have been published of the successful treatment with ampicillin of a carrier of *Salm. typhi*, but of failure in six carriers of *Salm. typhimurium* (Stewart *et al.*, 1961 ; Trafford *et al.*, 1962). Ampicillin has failed in three carriers of salmonellae, but a fourth patient remained negative one month after cholecystectomy and ampicillin therapy (Tynes and Utz, 1962). The five relapses in the present series of 10 patients may have been due to the use of an inadequate dose, as they all occurred after treatment with less than 1 g. six-hourly. The administration of a larger dose in two of them resulted in a clinical and bacteriological cure. It has been suggested that ampicillin may not reach the lower ileum in a concentration inhibitory to pathogenic bacteria, owing to rapid absorption from the upper part of the small intestine, and possibly to local destruction by penicillinase (Stewart and Harrison, 1961). This may contribute to failure of the drug in acute salmonelloses.

Chronic salmonella infections are notoriously difficult to treat (Main, 1961). In acute salmonelloses chloramphenicol is unsatisfactory in preventing the development of the carrier state (Douglas, 1950 ; Good and Mackenzie, 1950 ; Woodward *et al.*, 1950). This is due to the bacteriostatic action of chloramphenicol and the low concentrations achieved in bile. Ampicillin, being bactericidal and highly concentrated in bile, may be a useful drug in carrier states when given in an adequate dose of at least 1 g. six-hourly for 21 days. It probably represents no advance in the treatment of acute salmonelloses, although more extensive trials are necessary (*Brit. med. J.*, 1961). At the time of writing there was a large epidemic of paratyphoid infection in the Edinburgh area and ampicillin treatment was being compared with chloramphenicol for both the acute infection and the carrier state in over 130 patients. It is hoped to publish the results of this trial after adequate long-term follow-up studies have been completed.

Side-effects similar to those seen with other penicillins occurred in seven (19%) of the patients but were readily reversible. It seems likely that cross-sensitization exists among all penicillins, and it is important that patients should be questioned about previous penicillin allergy before ampicillin or any other penicillin is given.

Summary

Forty-eight patients were treated with ampicillin, 37 suffering from infections of the urinary tract, 10 from salmonelloses, and one from *Kl. pneumoniae* septicaemia. The ampicillin sensitivity of the causative organisms has been studied, and concentrations of ampicillin in blood and urine have been estimated. In the treatment of infections of the urinary tract ampicillin should be reserved for infections due to *Proteus* spp. It is not the drug of first choice for *E. coli* infections, being less effective and more costly than cycloserine. The majority of strains of *Klebsiella* are resistant. A follow-up study showed a 38% long-term failure rate, the possible causes of which are discussed.

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INDOMETHACIN: A NEW NON-STEROID ANTI-INFLAMMATORY AGENT

BY

F. DUDLEY HART, M.D., F.R.C.P. **P. L. BOARDMAN, M.B., M.R.C.P.**

The Rheumatism Unit, Westminster Hospital, London

The measurement of joint-swelling in the human subject is not easy, but in the assessment of drugs purporting to have an anti-inflammatory effect in conditions characterized by the presence of chronic inflammatory swelling, such as rheumatoid arthritis, some clinical measure is essential. We have found that the only practical and reliable measurement which can be done repeatedly and reasonably quickly in the wards is finger-swelling measured by jewellers' rings (Hart and Clark, 1951). All patients with active rheumatoid arthritis entering our wards have finger-swelling measured in this way twice weekly by the same clinician at approximately the same time of day as a routine measure. Also, the patient's own assessment of pain, stiffness, the number of analgesic tablets taken daily, the time taken to limber-up in the morning. and the clinician's assessment of grip strength, joint tenderness, and sedimentation rate are done routinely on all patients as measures of progress irrespective of the treatment given throughout their stay in hospital.

Measurable reduction of joint-swelling occurs regularly and demonstrably with steroid therapy, but not with salicylates, phenacetin, paracetamol, or the pyrazoles (phenylbutazone or oxyphenbutazone) as measured by this method ; and since the early use of the corticosteroids and corticotrophin no other therapeutic substances of the many we have tried have produced a measurable reduction in swelling of the interphalangeal joints. It was therefore a pleasant surprise when we found that in indomethacin (MK 615) we had the first non-corticosteroid agent which produced a predictable and measurable reduction in joint-swelling in most cases of active rheumatoid arthritis.

Chemistry

Indomethacin is a non-steroid anti-inflammatory and antipyretic agent. Its activity does not depend upon pituitary-adrenal stimulation and it is fully active in adrenalectomized animals. Chemically it is 1-(*p*-chloro-