sidered likely in one case and possible in another. Nevertheless, it seems strange that such a specific disease pattern should suddenly be produced by the many different drugs recorded. As more reports appear, moreover, the incidence of cases due to drugs seems to diminish. In the present series of cases—the largest yet reported—it seems clear that the disease was not a septicaemia. On the contrary, my findings suggest that it is caused by phage-type-71 *Staph. aureus*, which produces a local skin condition, together with a secondary toxæmia, comparable to that produced by the streptococcus in scarlet fever. Possibly the staphylococcus produces an altered, potentiated, or new toxin, which in a non-immune person gives rise to toxic epidermal necrolysis. Though a search for rashes in other members of the household has not been made routinely, four cases were associated with impetigo, and, recently, routine nasal swabbing revealed phage type 71 in one parent in three different cases. Furthermore, the simultaneous involvement of two brothers and a sister, and the high incidence in children, would suggest an infection in those without resistance. Again, the geographical incidence supports infection, and a small region like Northern Ireland, with a population of under 1,000,000, has a relatively large number of cases, since 41† have now been published and the number is undoubtedly higher.

Summary

Thirty-one cases of Lyell's toxic epidermal necrolysis are described. It is suggested that the condition starts as a local skin infection, followed by an extensive erythema and superficial exfoliation resembling scalding. This critical period of illness

† Ten reported by Beare (1962).

is succeeded by the sudden onset of recovery, reminiscent of the crisis course formerly seen in cases of pneumonia.

Death occurred in a 3-day-old infant, but all other patients recovered completely without recurrence.

The primary skin lesion was found to be caused by phage-type-71 *Staphylococcus aureus*, and it is postulated that the "scalded skin syndrome" is due to a unique toxic effect of this organism. Thus, while a drug or other cause may be considered, a search for this organism is indicated in all cases.

Treatment consists of skilled nursing together with the administration of antibiotics, systemic and topical; corticosteroid drugs are not indicated.

I am grateful to Dr. C. Field (consultant paediatrician) for inviting me to see several cases under his care, and for referring some cases to my unit. I am also indebted to Dr. F. F. Kane (consultant physician, Northern Ireland Fever Hospital) for allowing me to have details of Cases 4, 5, and 6. My thanks are also due to Mrs. Landau (senior scientific officer of the hospital laboratory) for her enthusiastic co-operation regarding bacteriology and phage-typing in these cases. Since results are largely dependent on skilled and devoted nursing, I am particularly grateful to the nursing staff, and especially to Sister Adams and Staff Nurse Burton. Finally, I am greatly indebted to my senior secretary, Mrs. McKnight.

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Laboratory and Pharmacological Studies in Man with Hetacillin and Ampicillin

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Hetacillin is the generic name for 6-(2,2-dimethyl-5-oxo-4-phenyl-1-imidazolidinyl)penicillanic acid, which is prepared by the reaction of ampicillin with acetone (Hardcastle *et al.*, 1966) (Fig. 1). The antibacterial spectrum of hetacillin has not been reported in detail, but Bunn *et al.* (1966) have stated that hetacillin has essentially the same spectrum of activity in vitro as ampicillin. Absorption and excretion studies with hetacillin in man have been reported by Bunn *et al.* (1966) and by Tuano *et al.* (1966). The results of clinical trials with hetacillin were reported at the Sixth Interscience Conference on Antimicrobial

Agents and Chemotherapy (Philadelphia, U.S.A., 1966—proceedings in press).

The experiments reported here were designed to investigate the hydrolysis of hetacillin to ampicillin in vitro and in man, and to compare the antibacterial activities, and the absorption and excretion in man, of hetacillin and ampicillin.

Hydrolysis of Hetacillin to Ampicillin

In aqueous solution hetacillin hydrolyses to form ampicillin and acetone. The formation of ampicillin was demonstrated by thin-layer chromatography as described by Hardcastle *et al.* (1966). The formation of acetone was demonstrated by gas chromatography. Fig. 2 shows the rate of hydrolysis of hetacillin to ampicillin at 37° C. in aqueous solution at pH 7. Under these conditions hydrolysis of hetacillin was rapid, so that within 15 minutes at 37° C. more than 50% of an aqueous solution of 1 mg. of hetacillin per ml. in M/40 phosphate buffer, pH 7, had hydrolysed to ampicillin, and at the end of 60 minutes there was virtually no unchanged hetacillin in solution. The rate of hydrolysis under acid conditions (pH 2) was less rapid, but after 60 minutes there was about 50% conversion of hetacillin to ampicillin.

FIG. 1.—Structure of hetacillin.

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FIG. 2.—Hydrolysis of hetacillin to ampicillin in aqueous solution. The formation of ampicillin from a solution of 1 mg. hetacillin ml. in M/40 phosphate buffer at pH 7.0 and 37° C. was measured by hydroxylamine assay.

Antibacterial Activities of Hetacillin and Ampicillin

The minimum inhibitory concentrations of hetacillin and ampicillin required to prevent growth of bacteria for 24 hours at 37° C. were determined by serial dilution of the compounds in nutrient broth and in nutrient agar. The effect of serum on the antibacterial activities of the compounds was measured by serial dilution of the compounds in nutrient broth and in 95% human serum. After overnight incubation at 37° C., subcultures were made on to antibiotic-free agar, and the end-

TABLE I.—Relative Antibacterial Activities of Ampicillin and Hetacillin

Organism	M.I.C. (μg./ml.)	
	Ampicillin	Hetacillin
<i>Escherichia coli</i> O111	2.5	2.5
<i>Klebsiella aerogenes</i> A*	500	500
<i>Salmonella typhi</i>	1.25	1.25
<i>Salmonella typhimurium</i>	1.25	1.25
<i>Shigella flexneri</i>	2.5	2.5
<i>Proteus mirabilis</i>	1.25	1.25
<i>Proteus mirabilis</i> 889*	> 500	> 500
<i>Proteus morganii</i> H	50	50
<i>Pseudomonas pyocyanea</i>	> 500	> 500
<i>Haemophilus influenzae</i>	0.5	0.5
<i>Staphylococcus aureus</i>	0.05	0.05
<i>Staphylococcus aureus</i> *	250	250
<i>Streptococcus pyogenes</i>	0.02	0.02
<i>Streptococcus pneumoniae</i>	0.05	0.05
<i>Streptococcus faecalis</i>	1.25	1.25

* Penicillinase-producing strain.

TABLE II.—Effect of Serum on the Antibacterial Activities of Ampicillin and Hetacillin Against *Staphylococcus aureus* Smith

Compound	% Bound in Human Serum	M.I.C. (μg./ml.)	
		In Nutrient Broth	In Human Serum
Ampicillin	16	0.03	0.03
Hetacillin	16	0.03	0.03

TABLE III.—Absorption and Excretion Studies with Hetacillin and Ampicillin in Man

Study	No. of Subjects	Dose	Route	Compound	Mean Serum Concentration (μg./ml.)							Urinary Excretion 0-6 hr. % of dose
					½ hr.	1 hr.	2 hr.	3 hr.	4 hr.	6 hr.	8 hr.	
A	12	250 mg.	Oral	Hetacillin*	0.35	1.09	2.45	—	0.96	0.28	—	36
				Ampicillin†	1.34	3.18	3.80	—	0.97	0.31	—	63
B	12	250 mg.	Oral	Hetacillin†	< 0.05	0.31	0.93	0.91	0.57	0.23	—	21
				Ampicillin†	0.48	1.49	2.09	1.33	0.69	0.24	—	40
C	15	500 mg.	Oral	Hetacillin*	0.51	1.94	2.96	—	0.99	0.51	—	39
				Ampicillin†	1.71	3.46	4.62	—	1.40	0.53	—	55
D	11	500 mg.	Oral	Hetacillin†	0.35	1.19	2.68	1.80	1.30	0.59	0.30	25
				Ampicillin†	0.86	2.69	4.82	2.32	1.32	0.44	0.21	50
E	12	500 mg.	Oral	Hetacillin†	0.24	1.23	2.46	2.49	2.16	0.72	0.35	48
				Ampicillin†	1.97	4.27	4.92	4.44	2.63	0.77	0.25	71
F	12	500 mg.	Oral (non-fasting)	Hetacillin†	0.12	0.70	1.06	1.07	1.20	0.68	0.46	27
				Ampicillin†	1.17	2.56	2.98	2.36	1.26	0.38	0.17	41
G	8	250 mg.	Intramuscular	Hetacillin*	1.23	2.3	3.2	—	1.7	0.66	—	84
				Ampicillin†	5.7	5.8	2.9	—	0.8	0.29	—	88
H	8	500 mg.	Intramuscular	Hetacillin*	1.9	3.7	5.0	—	3.6	1.9	—	75
				Ampicillin†	7.4	9.2	6.7	—	2.4	1.2	—	86

* Material prepared in these laboratories by Dr. J. H. C. Nayler.

† Versapen, Bristol Laboratories Limited.

‡ Penbritin, Beecham Research Laboratories.

points were read after overnight incubation. The extent of binding of the compounds to the protein of human serum was measured by ultrafiltration of serum containing hetacillin or ampicillin (Robinson and Sutherland, 1965).

The minimum inhibitory concentrations of hetacillin and ampicillin against a range of bacteria are given in Table I, where it can be seen that the antibacterial activities and spectra of both compounds were identical. Similarly, both compounds were bound to the protein of human serum to the same extent and showed the same activities in the presence of human serum (Table II).

Absorption and Excretion of Hetacillin and Ampicillin in Human Subjects

The subjects who took part in the studies described below were healthy men or women aged 18 to 49 years. Cross-over studies were carried out in which the same groups were given either hetacillin or ampicillin, followed by the alternative drug one week later. Blood specimens were normally taken half, one, two, four, and six hours after administration of the compound. In certain studies three-hour and eight-hour specimens were also collected. After separation of the sera the specimens were assayed on the same day, except when eight-hour specimens were collected and these were kept overnight at 4° C. before assay. During the six-hour period after administration of the compounds urine was collected for measurement of antibiotic content.

Antibiotic serum concentrations were measured by a standard agar-diffusion assay technique with *Sarcina lutea* as assay organism (Knudsen *et al.*, 1961). Serum specimens were suitably diluted in normal human serum, or in some studies in 4% bovine plasma albumin, fraction V, and standard solutions of hetacillin and ampicillin were prepared in the same diluents. The plates were incubated overnight at 30° C., when the diameters of the inhibition zones were measured.

Serum and urine samples from subjects who received ampicillin were assayed against standard solutions prepared from ampicillin. Similarly, samples from subjects who received hetacillin were assayed against hetacillin standards, though, in fact, standard solutions prepared from hetacillin and ampicillin gave the same inhibition-zone diameters in this assay. This is not surprising in view of the speed at which hetacillin hydrolyses to ampicillin.

Conversion of Hetacillin to Ampicillin in Man

The amounts of unchanged hetacillin appearing in the blood stream of human subjects after the oral and intramuscular administration of hetacillin were measured. Hetacillin was separated from ampicillin by high-voltage agar-gel electrophoresis—essentially the technique described by Lightbown and

de Rossi (1965) being used—and the compounds were demonstrated by bioautography.

After oral administration of hetacillin, ampicillin alone was detected in the blood stream for the first 30 minutes after dosing. Thereafter, unchanged hetacillin was detected in small and variable amounts in some subjects up to 90 minutes after administration, after which ampicillin alone was present in the blood stream. The amount of unchanged hetacillin present at any time represented a small fraction of the total antibiotic, and at the time of peak antibiotic serum concentrations ampicillin only was present. On intramuscular administration of hetacillin, small amounts of unchanged hetacillin were first detected after 30 minutes, declining to trace quantities 120 minutes later. Whenever hetacillin was detected this was generally only a small fraction of the total antibiotic in the blood stream.

Oral Absorption Studies

The compounds were administered as ampicillin free acid and hetacillin free acid in 250-mg. gelatin capsules. The results of these studies are summarized in Table III.

Studies A, B, C, and D (Figs. 3 and 4).—In these studies hetacillin and ampicillin were given to subjects who fasted for 12 hours before receiving the compound until after the one-hour blood specimens were taken. With both the 250-mg. dose and the 500-mg. dose the serum concentrations obtained after the administration of hetacillin rose more slowly than those obtained with ampicillin, reaching peak levels at two hours which were substantially lower than the ampicillin peak serum concentrations which also occurred two hours after administration. The serum concentrations obtained with hetacillin also fell more slowly than those of ampicillin, and from four hours onwards after administration the levels obtained with hetacillin

FIG. 3.—Mean serum concentrations in 24 fasting subjects after single 250-mg. oral dose of hetacillin and ampicillin. ● = Hetacillin. ○ = Ampicillin.

FIG. 4.—Mean serum concentrations in 26 fasting subjects after single 500-mg. oral dose of hetacillin and ampicillin. ● = Hetacillin. ○ = Ampicillin.

were of the same order as those of ampicillin. The mean urinary excretion after a 250-mg. dose was 28% in the case of hetacillin and 51% with ampicillin, and after a 500-mg. dose was 32% for hetacillin and 52% for ampicillin.

Study E (Fig. 5).—This study was carried out to compare hetacillin and ampicillin under conditions similar to those described by Bunn *et al.* (1966) in their studies on the absorption and excretion of these substances. A single 500-mg. oral dose of hetacillin or ampicillin was administered to fasting subjects who abstained from food for 12 hours before dosing until three and a half hours after. In addition, the subjects

FIG. 5.—Mean serum concentrations of 12 fasting subjects after single 500-mg. oral dose of hetacillin and ampicillin. ● = Hetacillin. ○ = Ampicillin.

were given 400 ml. of water at the time of dosing as described by Bunn *et al.* (1966) and were allowed water during the experiment. Under these conditions the absorption and excretion of both compounds was greater than was observed in the fasting studies previously described (C and D), but the rise and fall of the serum concentrations followed essentially the same pattern as before. Thus the ampicillin serum concentrations were from two to eight times higher than those obtained with hetacillin in the three-hour period after dosing, and were similar for both substances four to eight hours after administration. The mean urinary excretion of ampicillin in the six-hour period after dosing was 71% and that after hetacillin 48%.

Study F—Non-fasting (Fig. 6).—A single 500-mg. dose of hetacillin and ampicillin was administered immediately before the subjects had a standard breakfast of bacon and egg, toast, and coffee. The serum concentrations obtained in the non-fasting subjects were lower than those observed in the fasting subjects, the presence of food having a particularly marked effect on hetacillin, the serum concentrations remaining at about 1 µg./ml. from one to four hours after administration of the compound. The ampicillin serum concentrations were not depressed to anything like the same extent, and were from 2 to 10 times higher than those obtained with hetacillin for the first

FIG. 6.—Mean serum concentrations in 12 non-fasting subjects after a single 500-mg. oral dose of hetacillin and ampicillin. ● = Hetacillin. ○ = Ampicillin.

three hours after dosing. The levels obtained with hetacillin fell more slowly than with ampicillin, and the serum concentrations obtained with hetacillin were higher than those of ampicillin from six to eight hours after administration.

Intramuscular Studies (Figs. 7 and 8)

The compounds were dissolved in 2 ml. of sterile water and injected immediately by deep intramuscular injection into the upper and outer quadrant of the buttock. Hetacillin was administered as the potassium salt and ampicillin as the sodium salt, as doses equivalent to 250 mg. and 500 mg. of ampicillin

FIG. 7.—Mean serum concentrations in eight subjects after a single 250-mg. intramuscular dose of hetacillin and ampicillin. ● = Hetacillin. ○ = Ampicillin.

FIG. 8.—Mean serum concentrations in eight subjects after a single 500-mg. intramuscular dose of hetacillin and ampicillin. ● = Hetacillin. ○ = Ampicillin.

pure free acid. Ampicillin was absorbed more rapidly and the peak serum concentrations obtained one hour after administration were about twice as high as the peak serum concentrations obtained two hours after administration of hetacillin. The serum levels measured in subjects receiving hetacillin fell more slowly and were higher than those of the ampicillin group four hours and six hours after administration of the substances. After administration of hetacillin urinary recovery over a six-hour period was slightly less than that of ampicillin.

Measurement of Antibacterial Activities of Sera

The antibacterial activities of pooled sera from fasting subjects who received single 500-mg. oral doses of hetacillin and ampicillin (Study E) were determined by serial dilution of the sera in 5% blood agar. The serum-agar mixtures were poured into petri dishes and inoculated with a drop of an overnight broth culture of the test organisms—namely, *Staphylococcus aureus*,

β-haemolytic streptococcus, and *Streptococcus pneumoniae*. After overnight incubation at 37° C. the maximum dilutions of sera inhibiting growth of the test organisms were determined. The results of this experiment are shown in Fig. 9, where it can be seen that in general the antibacterial activities of pooled sera taken half, one, two, and three hours after dosing from

FIG. 9.—Antibacterial activity of sera of fasting human subjects after a single 500-mg. oral dose of hetacillin or ampicillin. * Antibacterial activity of serum is expressed as the reciprocal of the highest dilution of serum inhibiting growth of the test organism. † Serum concentrations measured by agar-diffusion assay using *Sarcina lutea* as assay organism. ● = Hetacillin. ○ = Ampicillin.

subjects who received ampicillin were greater than those of sera from subjects receiving hetacillin. At later time intervals—namely, four, six, and eight hours after administration of the compounds—the antibacterial activities of sera from both groups of subjects were the same.

Discussion

Hetacillin is prepared by the condensation of ampicillin with acetone, the reaction taking place in the presence of an excess of acetone. In the presence of water the reverse reaction takes place and hetacillin hydrolyses liberating ampicillin and acetone. The rate of hydrolysis of hetacillin in aqueous solution is rapid at neutral pH, and as tests for the measurement of antibacterial activity are normally performed in neutral aqueous media it is not surprising that the in-vitro antibacterial activities of hetacillin and ampicillin are identical. In tests with analogous compounds—namely, condensation products of ampicillin with formaldehyde—the activities demonstrated by these substances corresponded very closely with the rates to which these compounds hydrolysed to ampicillin. It is quite possible, therefore, that hetacillin has no intrinsic activity and that the activity demonstrated in antibacterial tests is that of its hydrolysis product, ampicillin; but it would be difficult to confirm this supposition, as any test envisaged would involve the use of aqueous media in which there would be hydrolysis of hetacillin to ampicillin.

The conversion of hetacillin to ampicillin also takes place in the body, and the administration of hetacillin to human subjects results in the appearance of ampicillin in the blood

stream, in which form it is mainly absorbed. After oral and intramuscular administration the amounts of unchanged hetacillin found in the blood are small compared with the quantities of ampicillin present, and, after oral administration in particular, represent an insignificant amount of the circulating antibiotic. The therapeutic significance of hetacillin will therefore depend upon to what extent the serum concentrations found in man after administration of the compound differ from the serum concentrations produced by equivalent doses of ampicillin.

In the sole published report in the literature comparing the absorption and excretion of ampicillin and hetacillin in man after administration by the oral and intramuscular routes, Bunn *et al.* (1966) found that the peak levels obtained with oral doses of hetacillin were as high as, or slightly higher than, those obtained with equivalent doses of ampicillin. Our findings are at variance with those of Bunn *et al.*, as we find that the serum concentrations produced by ampicillin are markedly higher than those obtained with hetacillin during the first three hours after dosing, the peak serum levels of ampicillin being generally about twice as high. In these studies the serum levels were measured by conventional agar-diffusion microbiological assay technique, but measurement of the antibacterial activity of the sera by a serial-dilution technique also showed that sera from subjects given ampicillin were more active than corresponding sera from subjects receiving hetacillin. This would be expected if the antibiotic serum concentrations of the ampicillin group were greater than those of the hetacillin group. Like Bunn *et al.* (1966) we find that the decline in serum antibiotic concentrations from peak levels is slower in subjects receiving hetacillin, but whereas these workers thought that this might have clinical implications, we feel that at the later periods the antibiotic serum concentrations are so low that any difference in levels is unlikely to be of therapeutic significance.

The presence of food in the stomach depresses the absorption of hetacillin to a relatively greater extent than that of ampicillin. In the study reported here the mean serum concentrations produced with a 500-mg. dose of hetacillin barely exceeded 1 $\mu\text{g./ml.}$ at any time after dosing.

The results of our studies of the serum concentrations obtained after intramuscular administration of hetacillin and ampicillin are in general agreement with those reported by Bunn *et al.* (1966). Thus the peak levels obtained with ampicillin occur earlier and are impressively higher than those obtained with hetacillin, and then decline to levels which are lower than those obtained with hetacillin from the fourth hour onwards. In comparing the intramuscular preparations of hetacillin and ampicillin we believe that the high serum concentrations achieved with ampicillin are likely to be of greater therapeutic significance than the maintenance of the relatively low prolonged levels observed with hetacillin.

The results of electrophoretic studies show that after oral administration trace quantities of unchanged hetacillin are present for short periods and that practically all the circulating antibiotic is in the form of ampicillin. This indicates that hetacillin is poorly or less rapidly absorbed than ampicillin, and

the slow rise to relatively low serum concentrations and the relatively low urinary excretion over a six-hour period can be attributed to delay in absorption while hetacillin is hydrolysing to ampicillin in the gut. Similarly, the slow rise and delayed fall of serum concentrations after intramuscular doses of hetacillin indicates delayed absorption from the injection site. The results of electrophoretic studies show only small quantities of unchanged hetacillin persisting in the blood stream for up to 150 minutes after intramuscular administration. These facts indicate that hetacillin is poorly or slowly absorbed after injection and that there is delay in hydrolysis to ampicillin, in which form it is mainly absorbed.

There is every reason to believe that the therapeutic value of hetacillin is due to its hydrolysis product, ampicillin, and, in view of the lower blood levels obtained with hetacillin compared with ampicillin at equivalent doses, hetacillin would be expected to possess inferior therapeutic efficiency.

Summary

Hetacillin, a condensation product of ampicillin and acetone, hydrolysed rapidly in aqueous media to ampicillin and acetone. The antibacterial spectra and in vitro activities of hetacillin and ampicillin were identical and it is probable that the antibacterial activity of hetacillin is that of its hydrolysis product ampicillin. Electrophoretic studies of serum after the administration of hetacillin to volunteers indicated the majority of circulating antibiotic to be in the form of ampicillin, and cross-over studies after oral and intramuscular doses of hetacillin and ampicillin showed ampicillin to produce consistently and substantially higher peak blood levels.

The amount of antibiotic excreted over a six-hour period in the urine was considerably less in the case of hetacillin, and this together with the lower serum concentrations obtained would indicate that hetacillin is absorbed less readily than ampicillin. It is concluded from these studies that hetacillin would be expected to possess inferior therapeutic efficiency compared with ampicillin.

We are indebted to Dr. J. H. C. Nayler for providing the hetacillin used in certain of the studies, to Dr. M. Cole for the data in Fig. 2, and to Mr. P. Langley and Mr. D. Hannan for the results of the electrophoresis studies. We also thank Mrs. E. Fairclough and Miss M. Jones for skilled technical assistance.

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