Experiment 6.—For comparative purposes, 10 subjects received two 250-mg. capsules of tetracycline and a similar procedure was adopted as in the previous experiments except that no half-hour specimen was taken. The results are set out in Table VII. Serum

TABLE VII.—Serum Concentrations and Urinary Excretion of Tetracycline After a Single 500-mg. Dose

Subject	Dose mg.	Serum Concentration ($\mu\text{g./ml.}$)				Urine mg. in 6 hr.
		1 hr.	2 hr.	4 hr.	6 hr.	
A	500	2.0	2.8	2.5	1.8	101
C	500	1.7	1.9	2.4	0.8	66
D	500	0.9	0.8	0.9	0.3	17
G	500	1.5	2.0	2.0	1.1	31
H	500	0.7	0.9	0.9	0.3	30
K	500	0.8	1.9	1.9	1.3	72
L	500	1.0	1.9	2.4	1.3	27
M	500	1.3	1.8	2.1	1.2	85
N	500	0.8	1.6	2.0	1.1	72
P	500	2.1	2.8	2.4	1.5	82
Mean	..	1.3	1.8	2.0	1.1	58 (12%)

TABLE VIII.—Serum Concentration on Day 1 and Day 4 After the Administration of 500 mg. of Penbritin Every Eight Hours for Four Days

Subject	Dose mg.	Day	Serum Concentrations ($\mu\text{g./ml.}$)					
			$\frac{1}{2}$ hr.	1 hr.	2 hr.	4 hr.	6 hr.	8 hr.
B	500 t.d.s.	1	0.1	0.6	4.8	1.4	0.22	0.08
		4	0.12	1.0	3.2	0.7	0.23	0.06
D	500 t.d.s.	1	0.1	0.16	5.9	1.5	0.17	0.08
		4	0.27	1.7	3.3	0.8	0.19	0.06
K	500 t.d.s.	1	0.24	0.75	4.6	1.3	0.22	0.14
		4	0.17	2.6	2.0	0.5	0.21	0.06
M	500 t.d.s.	1	0.07	0.17	4.3	1.2	0.48	0.12
		4	1.05	3.7	2.3	0.8	0.21	0.05

concentrations obtained with 500 mg. of tetracycline are well below those of penbritin for the first two hours after dosing. The urine excretion of tetracycline over the six-hour period was only 12% of the administered dose; consequently the concentration of tetracycline in the urine (50–200 $\mu\text{g./ml.}$) was well below that obtained with an equivalent dose of penbritin (250–1,000 $\mu\text{g./ml.}$).

Experiment 7.—The data obtained so far relates only to single-dose studies, and in view of the rather slow absorption (peak serum concentrations at approximately two hours and significant levels at six hours) it was decided to investigate whether there was any accumulation of penbritin in the serum on a possible clinical regimen. Four subjects were given two 250-mg. capsules every eight hours for four days—that is, 12 doses. Serum concentrations were obtained on Day 1 and Day 4. No side-effects were reported in this small group. Results are shown in Table VIII. It will be seen that in this small number of subjects there was certainly no accumulation of penicillin on an eight-hourly regimen. In fact, the overall levels obtained on the fourth day were, on the whole, lower than those obtained on the first day.

Summary and Conclusions

Investigations were carried out on a new penicillin (penbritin; B.R.L. 1341) to determine a suitable scheme of dosage for therapeutic trials.

Consideration of serum concentrations in terms of $\mu\text{g./ml.}$, together with the minimum inhibitory concentrations of the antibiotic for various organisms, serves as a rough guide to the possible value of the penicillin *in vivo*, assuming that the presence of human serum does not markedly depress its antibiotic activity. Penbritin is little affected by serum (Rolinson and Stevens, 1961).

Penbritin is well absorbed orally, and some 30% of a given dose is excreted in the urine within six to eight hours of dosing.

The single- and multiple-dose studies described suggest that for therapeutic trials the following dosage regimen might be adopted:

250 mg. six-hourly for the treatment of infections due to Gram-positive organisms or those due to *H. influenzae*. 250–500 mg. six- or eight-hourly is suggested for infections of the urinary tract in view of the high concentrations of penbritin obtained in the urine. 750 mg. or more eight-hourly might well be required for the treatment of infections due to Gram-negative organisms other than those occurring in the urinary tract.

Our thanks are due to Miss J. Willacy and Miss E. Smith for the biological assays, to the volunteers who so willingly and regularly attended for their morning dose of penbritin, and to Miss J. E. Crawford for her help in the preparation of this paper.

REFERENCE

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"PENBRITIN": AN ORAL PENICILLIN WITH BROAD-SPECTRUM ACTIVITY

BY

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The isolation of 6-aminopenicillanic acid (Batchelor *et al.*, 1959) made possible the biosynthesis of many modifications of penicillin. Such modifications have already led to a compound with intensified anti-staphylococcal activity (methicillin) and to others with improved properties of absorption from the gut (pheneticillin, phenoxypropylpenicillin). To date, however, such compounds have shown a narrowing of antibacterial activity compared with penicillin G. An exception to this trend is represented by the derivative 6[D(–)- α -aminophenylacetamido]penicillanic acid ("penbritin"; B.R.L. 1341). This derivative was found to have a bactericidal effect at low concentrations against a variety of Gram-negative as well as Gram-positive organisms, and we are reporting here our microbiological and preliminary therapeutic results with it.

Methods

The laboratory and clinical methods followed closely those already described in our reports on methicillin (Stewart, 1960, 1961; Stewart *et al.*, 1960). The following modifications were introduced in this study.

Tests for Penicillinase.—In addition to conventional methods we now use a membrane filter technique for identifying organisms as producers of extracellular penicillinase. In this method (Holt and Stewart, 1961)

the unknown and control organisms are grown on top of a cellulose membrane laid on an agar plate containing the appropriate penicillin and already seeded with an indicator organism; on incubation, destruction of the penicillin is revealed by zonal growth of the indicator organism. This technique was found to be more sensitive for penicillinase detection than plate assays; it also enabled us to screen large numbers of organisms simultaneously against various penicillins. The defect is that it detects only extracellular forms of the enzyme, and we had to overcome this by re-examining whole-cell preparations, on assay plates, of organisms negative by the membrane test.

Penbritin was kept at 4° C. in powder form and made up weekly into a stock solution (50,000 µg./ml.) from which disks (10 µg.) for screening purposes and dilutions in agar, broth, and other media were all prepared. Patients were given the compound by mouth (50–100 mg./kg./day) in three or four daily doses. Specimens—for example, urine, pus—taken from patients receiving penbritin were cultured in media containing penicillinase.

Results

Penbritin is $C_{16}H_{19}N_3O_4S$, structurally

In the pure substance, the C* atom is in the D(–) configuration. We have tested a preparation (B.R.L. 1060), containing also the L(+) isomer, which shows *in vitro* slightly lower intrinsic activity but the same antibacterial range.

Penbritin was supplied to us as a nearly white, largely crystalline solid, 78% pure, melting and decomposing at 202° C. It dissolved sparingly in water (>20–40 mg./ml. at room temperature), giving a stable solution about pH 5. It dissolved to the extent of 33 mg./ml. in M/15 phosphate buffer at pH 7 and was stable for some hours in this solution at 37° C. The solubility was higher in alkaline solution.

Microbiological Results

Our results under this heading showed many similarities to those reported by Rolinson and Stevens (1961). We are therefore presenting here only a summary of essential findings, including some points of comparatively minor difference.

Like the other active members of the penicillin family, penbritin was active against all the major coccal pathogens (Table I). Streptococci of group A and pneumococci were all uniformly inhibited by low concentrations (<0.1 µg./ml.), comparable to those obtaining with penicillin G and lower than the minimal inhibitory concentrations of other synthetic penicillins or of tetracyclines. Streptococci of other groups required higher concentrations (1–5 µg./ml.), a higher proportion of these species and of *Haemophilus* being sensitive to penbritin and to *p*-aminobenzylpenicillin than to any other penicillin tested. *Staphylococcus aureus* showed variable behaviour, 77% of strains (in this series) being sensitive to 0.5 µg./ml. or less; the remainder were penicillin-G-resistant penicillinase-forming strains which were resistant also to penbritin.

Apart from the wider sensitivity of streptococci, the main difference between penbritin and the other major penicillins was the greater intrinsic activity of the new compound against many coliform organisms, including various types of *Escherichia coli*, *Shigella*, and *Salmonella*. All the salmonellae tested and a proportion of *E. coli*, *Proteus vulgaris*, and shigellae were inhibited by 1–5 µg./ml. Unlike Rolinson and Stevens, however, we found that many other organisms, including the majority of *E. coli*, were inhibited only by higher con-

TABLE I.—Sensitivity of Serially Isolated Pathogens to Penbritin and Other Penicillins in Concentrations up to 5 µg./ml.

	No. Sensitive/No. Tested at 5 µg./ml.					Effective Concentration of Penbritin (µg./ml.)
	Pen-britin	Pen. G	Methicillin	<i>p</i> -Amino-benzyl-pen.	Phenoxy-propyl-pen.	
<i>Staph. aureus</i> ..	216/282	220/282	*281/282	10/20	122/135	See text
Streptococcus (group A) ..	57/57	57/57	57/57	27/27	19/19	0.05–0.1
Streptococcus (group D) ..	22/24	12/24	0/24	10/12	4/6	1–5
Streptococcus (other groups)	19/19	17/19	11/19	18/19	9/12	0.1–5
Pneumococcus	61/61	61/61	61/61	27/27	22/22	0.05–0.1
Meningococcus	3/3	3/3	3/3	1/1	—	0.05
<i>Proteus</i> ..	29/36	20/36	0/9	12/16	0/11	1–> 100
<i>E. coli</i> ..	48/102	3/29	0/14	14/29	0/29	1–> 100
<i>Bact. aerogenes</i>	0/15	0/5	0/5	0/6	0/2	> 100
<i>Ps. pyocyanea</i> ..	0/13	0/2	0/2	0/6	0/2	> 100
<i>Sh. sonnei</i> ..	6/8	1/8	0/8	2/8	0/2	1–10
<i>Salm. typhi</i> ..	1/1	1/1	0/1	1/1	0/1	2–5
„ typhi-murium	8/8	0/8	0/3	8/8	0/8	1–5
„ others ..	5/5	0/5	0/5	5/5	0/5	1–5
<i>Haemophilus</i> ..	19/19	15/19	6/19	12/14	6/6	0.1–1
Fusiforms ..	2/2	2/2	0/2	1/1	1/1	1

* Out of over 1,300 examined, this is the only strain with natural resistance to methicillin (20 µg./ml.) identified in our laboratory.

centrations (5–50 µg./ml.) at which even penicillin G began to be effective. *Bact. aerogenes*, *Proteus morganii*, and *Pseudomonas pyocyanea* were uniformly resistant to the new compound, as to all other penicillins.

Against sensitive organisms, penbritin had a bactericidal action approaching that of penicillin G upon sensitive cocci, higher and more rapid than that of the other synthetic penicillins and of tetracyclines. It sterilized inocula of some group A streptococci and pneumococci in four hours; it killed 50–90% of inoculated cells of sensitive *Staph. aureus* in the same time; and it was also bactericidal, though not to this extent, against sensitive coliforms. With *Staph. aureus* the minimum inhibitory concentration was dependent upon the size of the inoculum, but this was not the case with sensitive coliforms, including salmonellae, against which the effect of a given concentration was maintained, even with steeply increasing inocula; conversely, reductions in the inocula of various coliforms did not enable a lower concentration of drug to be effective. The resistance of *Staph. aureus* to penbritin was invariably associated with penicillinase formation, for the drug was rapidly inactivated by both the extracellular and liberated intracellular forms of the staphylococcal enzyme. Penicillinases derived from *Bacillus cereus* and *B. licheniformis* were also able to inactivate the drug in a manner comparable on bio-assay to the destruction of equivalent amounts of penicillin G. Since penbritin is itself an acid, the liberation of small amounts of penicilloic acid was not demonstrable, so that it was impossible to say whether or not the enzymic destruction was an identical hydrolysis of the lactam ring.

When salmonellae and other sensitive coliforms were grown in sub-inhibitory concentrations of penbritin, no enzymic destruction of the drug occurred. Resistant

coliforms varied in their action; thus, some organisms, though completely resistant, caused little or no inactivation: *Ps. pyocyanea* and some strains of *E. coli* behaved in this way. Other resistant organisms, including *Bact. aerogenes*, inactivated concentrations up to 200 µg./ml. during their growth and produced a penicillinase-like substance capable of causing some destruction independently of the growth of the cells.

On the whole, Gram-negative organisms which were resistant on first isolation to 10 µg. or more of penbritin per ml. were resistant also to other forms of penicillin at this concentration; but there were some exceptions (Table II). Four strains of *Proteus* were inhibited by penicillin G and three by *p*-aminobenzylpenicillin. Two coliforms and one strain of *Bact. aerogenes* were inhibited by a preparation (B.R.L. 1060) containing the isomer of penbritin. Of the various penicillins examined, and excluding this isomer, *p*-aminobenzylpenicillin showed the closest similarity to penbritin in its action on Gram-negative bacteria.

Cross-resistance of *Staph. aureus* followed a different pattern: of 10 strains tested in this way three were inhibited by penicillin G, six by *p*-aminobenzylpenicillin, and all 10 by methicillin, despite their resistance to penbritin.

When pathogens from each group originally sensitive to penbritin were subjected to serial passage in media containing rising concentrations of the drug, a considerable degree of resistance was invariably produced. Thus, various salmonellae, *Proteus*, and coliforms were able to acquire fifteenfold to two-hundred-fold increase in resistance in 30–50 passages. *Sh. sonnei* acquired an eightfold increase in 40 passages. One strain of *Staph. aureus* (non-penicillinase forming) acquired a fortyfold increase in 25 passages, but two others required 40 passages for a similar increase. In acquiring resistance to penbritin these organisms also acquired cross-resistance to the other penicillins, in equal w/v concentrations (Table III).

Organisms thus rendered resistant to penbritin were tested, together with their sensitive parent-strains, for their power to inactivate penbritin during growth, and for penicillinase-formation. These tests showed that the compound was inactivated in varying degree, though never completely, by growing organisms, but that independently acting penicillinase was not formed.

TABLE II.—Cross-resistance to Other Penicillins of Organisms Naturally Resistant to Penbritin (>10 µg./ml.)

Resistant Organism	No. of Strains	No. Resistant to			
		Pen. G	Pen. T*	Methicillin	B.R.L. 1060
<i>E. coli</i> ..	35	11/11	21/21	11/11	9/11
<i>Bact. aerogenes</i> ..	11	4/4	5/5	4/4	3/4
<i>Ps. pyocyanea</i> ..	11	1/1	11/11	1/1	11/11
<i>Proteus</i> ..	6	0/4	2/6	1/1	6/6
<i>Streptococcus</i> (unclassified)	3	3	1/1	3	3
<i>Sh. sonnei</i> ..	2	2	1	1	2
<i>Staph. aureus</i> ..	10	3	6	0	10

* Penicillin "Toronto" (*p*-aminobenzylpenicillin).

TABLE III.—Cross-resistance to Other Penicillins of Organisms Rendered Resistant to Penbritin (>100 µg./ml.) by Passage

Organism	No. of Strains Passaged	No. Resistant to		
		Pen. G	Pen. T	Methicillin
<i>E. coli</i> ..	6	6		6
<i>Proteus</i> ..	1	1	1	1
<i>Sh. sonnei</i> ..	1	1	1	1
Salmonellae	3	3		3
<i>Staph. aureus</i> ..	2	2	2	0

The microbiological investigation detailed above was the preliminary part of our investigation of penbritin. We also knew at this stage, as a result of preliminary studies by Knudsen *et al.* (1961), that the new compound was well tolerated when given to adult volunteers and that inhibitory levels were produced in the serum by oral doses of 0.5–1 g. We therefore decided that, on the basis of its relatively wide range of antibacterial activity, penbritin should be tested for its therapeutic action in patients with Gram-negative and Gram-positive infections, selecting, in the first instance, those which had been proved refractory to treatment with other drugs. The results of this second part of the investigation, together with the relevant pharmacological observations, are described below.

Clinical Results

In the preliminary stages of this trial we selected patients with well-established urinary infections which had not responded to treatment with other drugs. In each case thus treated the responsible organism was shown in the laboratory to be highly sensitive to attainable concentrations of the drug, and it was felt that the high concentration normally attained in the urine would provide a condition whereby the efficacy or otherwise of the drug could rapidly be assessed. The first patient (Case 1) had a congenital (bifid) abnormality of the left renal pelvis and a chronic coliform pyelonephritis suppressed but not cured by maintenance therapy with nitrofurantoin (Table IV). Renal output was good and the blood urea normal. An acute relapse with frank pus in the urine was treated for five days (100 mg./kg./day) with the new compound. On the third day of treatment the urine was normal except for a trace of protein, and sterile on culture in media containing penicillinase; on the sixth day, and for a month thereafter, all signs of infection subsided. After a month a subacute relapse occurred which was treated at home with maintenance doses of nitrofurantoin as before. Renal output remained satisfactory and blood urea low (26 mg./100 ml.). There were no signs of intolerance.

A further series of children (Cases 2–8) with refractory urinary infections were then similarly treated with penbritin with similar satisfactory results (Table IV) showing that an antibacterial effect *in vivo*, comparable to that observed *in vitro*, was obtainable against sensitive coliforms and streptococci of group D. During therapy all these patients also showed pronounced suppression of the coliform and streptococcal flora of the faeces, lasting until about 48 hours after the last dose.

We then conducted a further trial in cases and carriers of infection due to *Salm. typhi-murium*, *Sh. sonnei*, and serotypes of *E. coli* (Table V). During treatment the expected suppressive action on the faecal flora was again observed, with disappearance of the pathogen as well as the coliform and streptococcal flora. This effect was short-lived, however, and mainly unselective, for a high proportion of the patients, including all six infected with *Salm. typhi-murium*, showed profuse reappearance of the pathogen on the second to the tenth days after the last dose of a five-day course, when the rest of the faecal flora began to emerge from the tetracycline-like repressive action of the drug. The proportion of successes in this part of the trial was too small to be conclusive, but mention should be made of an impressive clinical

result: this boy (Case 14) aged 5 years had been operated upon for acute appendicitis and peritonitis; the free pus in the peritoneum yielded, on culture, *Salm. typhi-murium*, as did his stools; post-operative recovery at this stage was uncertain, for the boy looked extremely ill, was leaking pus through his abdominal wound, was vomiting frequently, and had some diarrhoea; within 12 hours of receiving his first dose of penbritin he looked well, vomiting and diarrhoea ceased, the exudate dried, and he made a rapid uninterrupted recovery, though the stools remained positive for *Salm. typhi-murium*.

One chronic carrier of *Sh. sonnei* was treated with penbritin (Case 18). The stools were cleared of the pathogen during treatment and remained negative thereafter. One child carrying *E. coli* type 026 was also cleared of infection, but another child with this infection and a child carrying *E. coli* type 0111 were unaffected (Cases 15, 16, and 17).

We also treated four children (R.H., H.E., K.T., S.K.) with miscellaneous other infections, with satisfactory clinical and bacteriological results. K.T. was the most interesting case in this group. This hydrocephalic child, aged 9 months, originally had a severe urinary infection due to a coliform and streptococcus (group D). This infection was responding quickly to penbritin, but on the fifth and last day of treatment she showed signs of meningitis: the C.S.F. contained an inflammatory exudate and yielded, in culture, the urinary strain of streptococcus, the quantity of drug in the C.S.F. being insufficient to inhibit this organism. Since the organism was relatively resistant to other antibacterial agents, we treated the meningitis by injecting penbritin intraventricularly (2-4 mg. daily for four days) and continuing the oral administration. The signs of infec-

tion subsided in five days, and the C.S.F. was rapidly sterilized and remained so.

The remaining cases were treated later, when we felt convinced that penbritin had a strong antibacterial effect, comparable to that of other eligible antibiotics. H.E., aged 7 days, had an ileostomy for volvulus, and was gravely ill, with pus (coliform and streptococcus group D) leaking from her abdominal wound. After three days' treatment with penbritin her condition was greatly improved: a small amount of exudate was leaking from her wound, which was sterile on culture; in five days the wound was dry. Treatment was then stopped and convalescence seemed satisfactory. Two other children with staphylococcal infections were also treated—one with a respiratory infection and one with enteritis—with good clinical results and rapid disappearance of the organism during treatment and thereafter. Six other cases with mixed respiratory infections were also treated, but we are not proposing to assess these at present; one of these cases is referred to in a subsequent paragraph, as the re-isolated organisms appeared to have acquired some resistance to the drug.

Pharmacology

Assays of urine (Fig. 1) showed that penbritin was rapidly excreted in concentrations (500-4,000 µg./ml.) greatly in excess of those required for the inhibition of sensitive organisms. This excretion began within three hours of an oral dose and increased steeply during the next three to four hours. In cases in which 12- or 24-hour excretion rates were studied, urinary excretions accounted for 20-60% of the daily dose. This high rate of excretion continued for 12 hours after the last dose in patients receiving the drug for 5-10 days and then fell

TABLE IV.—Results of Treatment of Urinary Infections with Penbritin (50-100 mg./kg./day)

Case No.	Age	Organism	Previous* Therapy	Penbritin: Urine Examinations			Comments
				Before Treatment	Normal on (Day)	Sterile on (Day)	
1	2 yrs.	<i>E. coli</i>	Nitrofurantoin	Purulent	3rd	3rd	Bifid renal pelvis. Relapse of infection 1 month after treatment. Previous (left) nephrectomy. Chronic (right) pyelonephritis.
2	3 "	" "	Tetracycline.	"	6th (trace of protein)	4th	
3	5 "	Streptococcus (group D)	Nitrofurantoin	"	4th	2nd	Paralytic poliomyelitis. Recurrent urinary infections for 1 year.
4	6 "	Streptococcus (group D)	Penicillin G	"	6th	3rd	Reinfection with coliform 3 weeks after treatment. Chronic pyelonephritis. No recurrence of infection in 3 months.
5	7 wks	<i>E. coli</i> and <i>Pr. vulgaris</i>	Nil	"	5th	3rd	Spina bifida with hydrocephalus. No recurrence of urinary infection in 4 weeks.
6	11 yrs.	<i>E. coli</i>	"	"	5th	2nd	Uncomplicated case. No recurrence of infection in 4 weeks.
7	2 "	" "	"	Pus cells present	4th	4th	Spina bifida with cauda equina. No recurrence of infection in 3 weeks.
8	9 mths	Coliform and streptococcus (group D)	"	Purulent	6th	3rd	Hydrocephalus. Coliform eradicated from urine, but meningitis (Strep. group D) developed during treatment. See text.

* This refers only to therapy of a urinary infection immediately before giving penbritin.

TABLE V.—Treatment of Cases and Carriers of Intestinal Pathogens with Penbritin

Case No.	Description	Organism	Previous Therapy	Penbritin: Bacteriological Results	Comments
9	Convalescent carrier	<i>Salm. typhi-murium</i>	Nil	Organism reappeared on 3rd day after treatment	Not cleared by neomycin subsequently
10	"	" "	Streptomycin. Paromomycin	Organism present throughout treatment	
11	"	" "	Nil	Organism reappeared on 8th day after treatment	Cleared after a second course of penbritin
12	"	" "	"	Organism reappeared on 10th day after treatment	Not cleared by paromomycin subsequently
13	"	" "	Chloramphenicol	Organism present throughout treatment	Cleared after paromomycin subsequently
14	Case	" "	"	Organism reappeared on 3rd day after treatment	Appendicitis with peritonitis. <i>Salm. typhi-murium</i> in peritoneal pus. Good clinical response
15	Carrier	<i>E. coli</i> 0111	Nil	Organism reappeared on 4th day after treatment	Not cleared by neomycin subsequently
16	"	" " 026	Paromomycin	Remained clear of infection	Stools negative for <i>E. coli</i> 026 8 weeks later
17	"	" " 026	Paromomycin. Neomycin	Organism reappeared on 3rd day after treatment	Not cleared by a subsequent course of streptomycin and paromomycin
18	"	<i>Sh. sonnei</i>	Paromomycin. Streptomycin	Remained clear of infection	Stools negative for <i>Sh. sonnei</i> 8 weeks later

sharply (Fig. 2). In all the patients studied, including some in whom renal function may not have been perfect, excretion of the drug ceased after 48 hours. Assays of cumulative excretion showed that 30–50% of the total dose given over a period of five to seven days was excreted. Chromatographic bio-assays of the excretion product in the urine suggested that it was largely the original compound and not a metabolite.

Assays of plasma or serum (Fig. 3) showed that inhibitory levels (0.5–5 $\mu\text{g./ml.}$) of the compound were attained and maintained between one and half and seven hours after oral doses at the rate of 100 mg./kg./day; with lower doses (50 mg./kg./day) inhibitory concentrations were maintained between one and a half and five

FIG. 1.—Concentration of penbritin in the urines of four patients treated with 50 mg./kg./day.

FIG. 2.—Assays of approximate 24-hour excretion of penbritin in a patient receiving 50 mg./kg./day.

hours. Peak values occurred at one and a half to three hours, with variation in this respect between individuals. The active drug was detectable readily in the plasma (or serum) but not in corresponding samples of lysed red cells in which, even at peak plasma levels, trace or zero levels only were obtained.

Faecal excretion was not determined quantitatively, since traces only, or none, of the drug were detected in individual samples; this despite the fact that in the samples assayed the full suppressive effect on the bacterial flora, as described in preceding paragraphs, was demonstrable.

Penbritin was detected only in minute amounts ($<0.2 \mu\text{g./ml.}$) in normal C.S.F., two to three hours after an oral dose. One patient (K. T.) actually developed meningitis due to streptococcus (group D) during treatment for a mixed urinary infection caused by this organism and a coliform. The drug was detectable in the C.S.F., but the concentration therein ($0.7 \mu\text{g./ml.}$)

FIG. 3.—Concentrations obtained in serum or plasma of patients receiving penbritin orally.

resulting from oral dosage was clearly insufficient to inhibit this organism. This patient—a hydrocephalic—was then treated by intraventricular injections of the drug, which sterilized the infection. This method of administration produced high concentrations in the C.S.F. ($>20 \mu\text{g./ml.}$ at 6 hours) which persisted at inhibitory levels (2–5 $\mu\text{g./ml.}$) for 24 hours or more.

Toxicity

Like its precursors in the penicillin family of drugs, penbritin was well tolerated alike by well and ill subjects. In powder form, unadulterated, it had a transient unpleasant taste, but it was readily accepted in milk or fruit-juice, causing no alimentary disturbance, except possibly in three infants, who passed a few loose stools. During therapy the growth of natural coliforms, enterococci, and other organisms in cultures of the faeces was suppressed—often to the extent of producing sterile cultures aerobically—though mucoid coliforms and some strains of *Proteus* evaded this effect, and, in a few instances, superseded the natural flora. *Candida* appeared, in small numbers, in several cases, and in three became profuse. In all cases studied the natural flora reappeared within three to four days of stopping the drug, and no serious bacterial superinfections occurred.

Two children and one adult (each a carrier of *Salm. typhi-murium*) developed urticarial rashes during treatment. In the two children the rash disappeared when the drug was withdrawn. The adult retained the rash for four days, despite treatment with an antihistamine agent. Intradermal tests with 10 μ g. of penbritin, and, separately, with 10 μ g. of penicillin G, gave negative results. Another subject, known to be hypersensitive to penicillin G, gave a typical immediate urticarial response when tested intradermally with penbritin.

Haemoglobin and granulocyte levels were checked at least twice in 12 subjects receiving the drug and were unaffected. No adverse effects attributable to the drug were noted in repeated samples of urine. Some of the renal cases already had proteinuria due to their infections, and in all of them this protein diminished or disappeared during treatment and renal output did not decrease. Some of these patients were followed for periods of five to seven months, without obvious evidence of delayed toxicity.

Penbritin was also well tolerated when given intraventricularly in doses of 2-4 mg. daily in the hydrocephalic child (K. T.) with meningitis. The cell count and protein in the C.S.F. decreased rapidly. There were no signs, immediate or delayed, of neurological disturbance or cerebral irritation.

Drug Resistance

Organisms re-isolated at any stage during and after treatment in these patients were re-titrated along with the parent cultures for evidence of alteration in sensitivity. Six strains of *Salm. typhi-murium*, three coliforms, one streptococcus (group D), and one *Staph. aureus* were thus tested: the salmonellae and streptococcus showed no change, but one coliform and a staphylococcus—isolated together from a child with a mixed infection—showed a twofold increment in resistance. In all the urinary infections treated the organisms disappeared from the urine within 24 hours, so that there was no opportunity or need to check the possibility of resistance. One child (Case 1) had a subacute relapse of her pyelonephritis some weeks later; the responsible organism was her original faecal strain of *E. coli*, but its sensitivity to penbritin was unchanged.

Five patients were nasal carriers of staphylococci at the beginning of systemic treatment; these children remained carriers of the original organisms without change in sensitivity during and after treatment.

Discussion

The microbiological results reported here show that penbritin possesses bactericidal activity against a wide range of organisms, Gram-negative as well as Gram-positive. In its intensity of bactericidal action this new derivative resembles penicillin G, but its range, in terms of therapeutically attainable concentrations, is much wider. The range is, in fact, comparable to that of the "broad-spectrum" antibiotics such as the tetracyclines and chloramphenicol, which are essentially bacteriostatic and have a lower degree of intrinsic activity, weight for weight, against sensitive organisms than either penbritin or penicillin G. In our hands, the only other penicillin so far examined with comparable activity toward Gram-negative rods is *p*-aminobenzylpenicillin (penicillin T), which has the disadvantage of acid-lability and cannot be given orally.

In its action on the pyogenic cocci, penbritin is effective against not only group A streptococci, pneumococci, and neisseriae, which are uniformly sensitive to penicillin G, but also a wider range of miscellaneous streptococci, including group D strains, which are not infrequently resistant to other penicillins and other antibiotics. Like all the penicillins except methicillin, penbritin is susceptible to the action of various forms of penicillinase and is therefore ineffective against resistant strains of *Staph. aureus*. This susceptibility—which is shared by *p*-aminobenzylpenicillin—explains also the resistance of some coliforms to the drug, though our results again confirm an earlier finding (Stewart, 1947; Florey *et al.*, 1949) that coliform resistance is by no means wholly explained by the formation of penicillinase or other anti-penicillin factors: it is an interesting fact that many members of this "hard core" of penicillin-resistant organisms can grow freely in the presence of penicillin without inactivating it.

Our clinical results show that the activity of penbritin against sensitive organisms *in vitro* is also obtainable *in vivo* with certain forms of infection. In urinary infections, where a high concentration of the drug can readily and rapidly be attained, all eight patients showed an immediate clinical and bacteriological response, which was maintained, so far as sterility can be maintained among patients some of whom had anatomical deformities predisposing to reinfection. In the treatment of alimentary infections, however, the results were much less impressive; for, despite the clearance of two chronic infections—one with *Sh. sonnei* and one with a serotype of *E. coli*—six out of six cases of infection with *Salm. typhi-murium* were not cleared. Chloramphenicol, tetracyclines, and other drugs inhibitory to salmonellae *in vitro* have a similar shortcoming *in vivo* in this respect, but in view of its bactericidal action and of its temporary suppressive effect on the aerobic faecal flora, this result in the case of penbritin seems incongruous. The explanation may lie in a property of the drug reported in detail elsewhere (Harrison and Stewart, 1961): when given orally to rats, penbritin is rapidly absorbed and excreted, within one to three hours, in high concentration in the bile; the excreted moiety is then reabsorbed and re-excreted in the bile, still in a bactericidal concentration. This cycle, repeated in a diminishingly in the upper part of the jejunum, obviously prevents the drug reaching the ileum in fully effective concentration, so that organisms which reside only there, like the salmonellae in the cases treated, can presumably escape its action. By the same argument, this cycle of excretion and re-excretion serves to prolong a bactericidal concentration in the blood and bile, and suggests that penbritin might be unusually effective against infections localized to the biliary tract; in our series, no biliary carriers of salmonellae were available to test this hypothesis.

If these cases of salmonella infection be excluded, the clinical series as a whole suggests that there is a fairly close parallel between the efficacy of penbritin in the test-tube and in the body against sensitive organisms. Effective blood levels are readily attained with oral doses of 50-100 mg./kg./day and are maintained, possibly by virtue of reabsorption, for periods up to seven hours after an oral dose. The drug is rapidly concentrated and excreted in active form in the urine, and there is no evidence of undue retention. It is stable in acid and relatively unaffected by physiological changes in pH. There is no evidence in our series of any toxicity,

though—as with other penicillins so far tested—hypersensitivity to penicillin G means hypersensitivity also to this derivative. Drug-resistance can be induced easily *in vitro* but did not account for relapses *in vivo*, though there was evidence in two cases that it could occur in minor degree.

In our present series we have deliberately excluded some respiratory infections in which a clinical response was observed; the bacteriology of such cases is often obscure and we are in these trials guided by the belief that two criteria are essential: disappearance of the target pathogens in cases and carriers, and parallel clinical improvement in cases. Without these criteria a therapeutic trial on an antibacterial agent is questionable. On this basis, we consider that the results reported here indicate a place for penbritin as a non-toxic penicillin of high bactericidal broad-spectrum activity in the oral treatment of urinary and possibly in systemic infections due to pyogenic cocci and sensitive Gram-negative bacilli. It warrants trial also in infections of the biliary tract and in some alimentary infections, peritonitis, and pre-operative intestinal surgery; it cannot at present be recommended for the treatment of intestinal carriers of salmonellae, despite the high sensitivity of these organisms *in vitro*. In view of the variation in sensitivity of typical coliforms toward the drug, its use in urinary and abdominal infections should be contingent upon adequate bacteriological control, which should include tests at concentrations attainable in the affected tissue.

In assessing the novel features of this new compound we should not forget the fact that penicillin G itself has an inhibitory effect upon many coliforms (Abraham *et al.*, 1941; Schwartzman, 1946), sometimes bactericidal and strong enough to enable it to be used therapeutically (Helmholtz and Sung, 1944; Stewart, 1945). Methicillin, phenethicillin, and phenoxypropylpenicillin lack such activity toward coliforms, so that penbritin represents a valuable chemical advance in thus enhancing a specific property within the penicillin family of substances.

Summary

"Penbritin" has a bactericidal action, comparable to that of penicillin G upon pyogenic cocci, upon a wide range of coccal and bacillary pathogens.

It is affected by penicillinase and is therefore inactive against penicillinase-forming strains of *Staph. aureus* and certain coliforms.

Given orally in doses of 50–100 mg./kg./day, the new compound is freely absorbed and maintains a bactericidal level in the plasma for four hours or more. It is rapidly excreted, in very high concentration, in the urine.

Apart from transient rashes in three carriers of salmonellae, no toxic effects were observed in 28 patients who received the drug for five days or more.

Eight children with severe urinary infection due to coliforms and/or streptococci responded rapidly to treatment, as did six children with other infections; six carriers of *Salm. typhi-murium* and two of *E. coli* serotypes were not cleared of infection.

Penbritin warrants further trial as a bactericidal penicillin-like drug with the antibacterial range of the tetracyclines.

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DIURETIC RESPONSE TO CHLORTHALIDONE

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Chlorthalidone ("hygroton") is a new diuretic introduced in Switzerland in 1959 and in this country in 1960. It is a sulphamyl benzophenone derivative (see Formula) synthesized in the Geigy Laboratories (Graf *et al.*, 1959). Pharmacological studies on laboratory animals have been reported (Stenger, 1959; Stenger *et al.*, 1959; Pulver *et al.*, 1959). The drug is only slightly soluble in water; absorption from the gut is