cardia may well lead to the development of a hiatus hernia and account for the high incidence of this condition in the Billroth I group.

A further possible explanation of the findings is that there is a reduction in tone of the intrinsic sphincter mechanism of the lower oesophagus after partial gastrectomy. Clark and Vane (1961) showed that in animals the presence of alkali in the lower oesophagus reduces the tone in this sphincter, and it is thought that alkali in the oesophagus of post-gastrectomy patients reduces the efficiency of the gastro-oesophageal closing mechanism still further.

Stammers and Williams (1963) have pointed out that iron absorption after partial gastrectomy is adequate for normal needs provided there is no occult loss. An attempt was made to correlate anaemia with radiological reflux. Iron deficiency and anaemia were present in as many patients with reflux as in those without, and no significant conclusions could be drawn from this small series. A more detailed study is planned to clarify this point. It is suggested, however, that oesophagitis after partial gastrectomy is a much more common condition than hitherto recognized and is a possible cause of loss of blood in the post-gastrectomy patient.

Summary

Sixty-one unselected patients were investigated after partial gastrectomy, and heartburn was found to be a complaint in 16 of them. Radiological reflux was induced in 24 patients—50% of the Billroth I and 27% of the Polya cases. Hiatus hernia was present in only six patients. It is suggested that the symptoms are due to alkaline reflux with tryptic digestion of the oesophageal mucosa, and a mechanism is suggested for its causation.

An attempt to correlate the symptoms of oesophagitis with post-gastrectomy anaemia was unsuccessful, though this is thought to be a likely association.

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Laboratory Evaluation of a New Antibiotic—Cephaloridine (Ceporin)

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Cephaloridine (Ceporin: Glaxo Ltd.) is a semisynthetic antibiotic, 7-[(2-thienyl)acetamido]-3-(1-pyridylmethyl)-3-cephem-4-carboxylic acid betaine. It is produced chemically from cephalosporin C by replacing the α -aminoadipic side-chain at position 7 by 2-thienylacetic acid and the acetoxy group at position 3 by pyridine. It has the following structure:

The natural antibiotic, cephalosporin C, from which cephaloridine is derived, is produced by a strain of the mould *Cephalosporium acremonium*, which was isolated by Brotzu (1948); its structure was elucidated by Abraham and Newton

(1961). Though it is stable to acid and to penicillinase and is equally active against Gram-positive and Gram-negative organisms, its intrinsic activity is relatively low.

Replacement of the 7- α -aminoadipic acid side-chain of cephalosporin C with various substituted acetic acids gives compounds showing a marked increase in activity against *Staphylococcus aureus* and other Gram-positive organisms, but this type of substitution in general renders the acetoxy group at position 3 far more labile in the mammalian body (O'Callaghan and Muggleton, 1963).

When the acetoxy group of cephalosporin C is replaced with pyridine or its analogues the resulting compounds have increased antibacterial activity (Hale, Newton, and Abraham, 1961) and are, moreover, stable in the body (O'Callaghan and Muggleton, 1963).

Many analogues of cephalosporin C have been prepared in which both the α -aminoadipic acid side-chain and the acetoxy group have been replaced. They have been examined for their possible therapeutic use. One with considerable potential usefulness is cephaloridine; its properties are reported here.

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Physical Properties and Stability

Cephaloridine is a white crystalline solid with a molecular weight of 415.5. It is very soluble in water, and a solution containing 250 to 500 mg. can easily be prepared in 2 ml. of water for injection.

Aqueous solutions (20% w/v) are stable for at least four weeks in the refrigerator (4° C.) in the dark. Less than 10% decomposition occurred when such 20% w/v solutions were stored at room temperature for one week. Solutions of cephaloridine are slightly acid (about pH 4.5 to 5). They are more stable at this pH than are solutions made more alkaline.

The solid is stable at 4° C. in the dark, and cephaloridine in phials is stable for at least twelve months if kept in the refrigerator. Exposure to sunlight causes some discoloration.

Toxicology

An extensive study of the toxicity of cephaloridine will be reported elsewhere (Currie and Tomich, 1964). The results have shown it to have a low degree of toxicity, and sizable doses can be injected repeatedly over long periods without giving rise to troublesome side-effects.

We have been unable to induce hypersensitivity to cephaloridine in rabbits or guinea-pigs injected repeatedly over long periods (three months). Patients who were found to be hypersensitive to penicillin have received injections of cephaloridine without showing untoward reactions. It would appear that cephaloridine does not readily cause hypersensitivity and, moreover, that it is not cross-allergenic with the penicillins.

Experimental Methods

(a) *In-vitro* Antibacterial Activity.—A wide range of micro-organisms were tested for sensitivity to cephaloridine using the twofold serial dilution technique in appropriate nutrient media. The results are expressed as the lowest concentration in µg./ml. which prevented visible growth after incubation. One drop of broth or saline suspension was used to inoculate the tubes, which

contained 2-ml. amounts of culture to give a final concentration of about 0.5×10^8 orgs./ml.

(b) *Mouse-protection Tests*.—Protection tests with cephaloridine were carried out on mice experimentally infected with *Staph. aureus*, *Escherichia coli*, *Proteus mirabilis*, *Salmonella typhi*, *S. typhimurium*, *S. paratyphi*, and *Pseudomonas pyocyanea*. The challenge dose of organisms (approximately $LD_{100} \times 5$) was sufficient to kill control untreated mice in 24 hours when injected into the peritoneum in 0.2 ml. of saline. Treatment doses in 0.2 ml. were given by subcutaneous injection to groups of three mice at each dose level at $\frac{1}{2}$, 4, 8, 24, and 32 hours after challenge.

(c) *Serum and Urine Levels*.—Levels of cephaloridine in experimental animals and in human volunteers were determined by the cup-plate method on large plates with *Staph. aureus* strain N.C.T.C. 7447 in Difco tryptose agar. Serum samples from experimental animals were assayed against standards prepared in horse serum; samples from human volunteers were assayed against standards prepared in human serum. Urine samples were diluted for assay with phosphate buffer pH 7 (1.76% $Na_2HPO_4 \cdot 12H_2O$ and 0.2% KH_2PO_4) and the standards were also prepared in this buffer.

(d) *Chromatography and Bioautographs*.—Samples for chromatographic analysis were applied to Whatman No. 1 paper, buffered at pH 6 with 0.05 M phosphate buffer (0.7% Na_2HPO_4 adjusted to pH 6 with phosphoric acid), and run downflow overnight in *n*-butanol:ethanol:water 4:1:5, upper layer. The dried paper was placed on large agar plates seeded with *Staph. aureus*. The paper was removed after 15 minutes and the plates were then incubated for 18 hours at 37° C. The zones of antibiotic activity were then seen by the inhibition zones produced.

Results and Discussion

Antimicrobial Activity in vitro (Table I)

Cephaloridine has a broad spectrum of activity, and sensitive organisms include *Staph. aureus* (including penicillin-resistant

TABLE I.—Antimicrobial Activity of Cephaloridine in Vitro

Organism	Medium*	Incubation Temperature (°C.)	Incubation Time (Days)	No. of Organisms with Minimum Inhibitory Concentrations of (µg./ml.):					No. of Strains Tested
				0.05-1	1-8	8-25	25-100	> 100	
<i>Staph. aureus</i> (penicillin-sensitive)	1	37	1	18					18
<i>Staph. aureus</i> (penicillin-resistant)	1	37	1	21	8				29
<i>Str. pyogenes</i>	2	37	1	3					3
<i>Str. pneumoniae</i>	2	37	1	2					2
<i>Str. viridans</i>	2	37	1	1					1
<i>Str. faecalis</i>	2	37	1					1	1
<i>Str. agalactiae</i>	2	37	1	2					2
<i>Str. dysgalactiae</i>	2	37	1	1					1
<i>Str. uberis</i>	2	37	1	1					1
<i>C. diphtheriae</i>	2	37	1	2					2
<i>E. rhusiopathiae</i>	2	37	1	1					1
<i>Cl. septicum</i>	3	37	1	1					1
<i>Cl. welchii</i>	3	37	1	1					1
<i>E. coli</i>	1	37	1		21	4			25
<i>A. aerogenes</i>	1	37	1		1		1	3	5
<i>Kl. pneumoniae</i>	1	37	1		1				1
<i>S. typhi</i>	1	37	1		4	1			5
<i>S. paratyphi</i>	1	37	1		5				5
<i>Salmonella</i> spp.	1	37	1		6				7
<i>Shigella</i> spp.	1	37	1		8	10			6
<i>Pr. mirabilis</i>	1	37	1					4	18
<i>Pr. vulgaris</i>	1	37	1					3	4
<i>Pr. morgani</i>	1	37	1					1	3
<i>Pr. rettgeri</i>	1	37	1					4	1
<i>Ps. pyocyanea</i>	1	37	1						4
<i>N. gonorrhoeae</i>	4	37	2		2	2			4
<i>N. catarrhalis</i>	2	37	1						1
<i>Bordetella pertussis</i>	5	37	2	1	2				2
<i>H. influenzae</i>	6	37	2		3				3
<i>Brucella abortus</i>	4	37	2					1	1
<i>Pasteurella</i> spp.	4	37	1	1	2				3
<i>Vibrio cholerae</i>	2	37	1		1				1
<i>M. tuberculosis</i>	7	37	8			1			2
<i>Leptospira</i> spp.	8	37	4	3					3
<i>Candida albicans</i>	9	37	1					1	1
<i>Trichophyton mentagrophytes</i>	10	28	3					1	1
<i>Microsporum canis</i>	10	28	3					1	1

* Media used: 1, Nutrient broth. 2, Brain-heart infusion broth. 3, Brain-heart infusion containing N/400 thioglycollate. 4, Brain-heart infusion containing 5% horse serum. 5, Cohen and Wheeler's liquid medium. 6, Brain-heart infusion containing 10% blood extract. 7, Dubos' liquid medium. 8, Korthof's medium. 9, Nutrient broth containing 1% glucose. 10, Sabouraud's liquid medium.

strains), *Streptococcus pneumoniae*, *Str. pyogenes*, *Str. viridans*, *Corynebacterium diphtheriae*, *Neisseria catarrhalis* and *N. gonorrhoeae*, *Clostridium septicum*, *Cl. welchii*, *E. coli*, *S. typhi*, *S. typhimurium*, *S. paratyphi*, various *Shigella* spp., *Proteus mirabilis*, *Bordetella pertussis*, *Haemophilus influenzae*, *Leptospira icterohaemorrhagiae*, *L. pomona*, and *L. canicola*. Most strains of *Pr. morgani*, *Pr. vulgaris*, and *Pseudomonas pyocyanea* are insensitive, and cephaloridine had no activity against dermatophytes or *Candida albicans*. The sensitivities of strains of *Aerobacter aerogenes* were variable.

Cephaloridine is highly bactericidal in action, particularly against Gram-negative organisms, and addition at the minimum inhibitory concentration to a growing culture causes a rapid kill of the cells and then their lysis.

The development of resistance by strains of *Staph. aureus* takes place slowly, in small steps, and is independent of the sensitivity of the strains to penicillin. Cephaloridine is about 1,000 times more resistant to staphylococcal penicillinase than is penicillin V.

Antimicrobial Activity in vivo

Cephaloridine is highly effective in protecting mice against experimental *Staph. aureus* infections (Table II). Six of the strains tested were highly resistant to penicillin.

TABLE II.—Activity in Experimentally Infected Mice

Organism	Strain	ED ₅₀ in mg./kg./Dose				
		Cephaloridine	Tetracycline	Ampicillin	Chloramphenicol	Methicillin
<i>Staph. aureus</i>	663	2.0	10	10	150	100
	11092*	2.5	>200	>50	75	40
	11127*	<0.75	ca25	ea50	20	50
	11171*	<0.75	>200	ca50	25	15
	11071*	1.0	75	>50	40	—
	11146*	0.75	>200	25	>200	—
	11152*	2.5	100	>50	25	—
<i>E. coli</i>	573	<6.25	30	12.5	31	—
	4	12.5	10	37.5	10	—
	6	6.25	6	37.5	15	—
	10	ca25	>100	50	20	—
	086	<3	12	—	12	—
<i>Pr. mirabilis</i>	431	15	200	—	>200	—
	2	10	>100	>100	75	—
	14	3	>100	7.2	<7.2	—
	17	3	75	25	10	—
<i>S. typhi</i>	481	10	20	25	12.5	—
<i>S. typhimurium</i>	478	15	4	15	125	—
<i>S. paratyphi B</i>	804	25	>200	—	200	—
	805	15	—	—	—	—
<i>Ps. pyocyanea</i>	150	>200	>200	>100	>200	—
	MA	>200	—	>100	—	—
	MB	>200	—	>100	—	—
	MD	>200	—	>100	—	—

* All penicillinase producers.

— Not tested.

Cephaloridine is also active against a range of experimental infections with Gram-negative organisms in mice and protected the mice against all strains of *E. coli*, *Pr. mirabilis*, and *Salmonella* spp. tested at a median effective dose (ED₅₀) of 25 mg./kg./dose or less. It has no activity *in vivo* against *Ps. pyocyanea*.

In general, cephaloridine is somewhat more active against Gram-negative organisms in this test than ampicillin and is consistently much better than tetracycline or chloramphenicol. It is also much more active against *Staph. aureus* than ampicillin, tetracycline, chloramphenicol, or methicillin.

Serum Levels and Urinary Excretion in Experimental Animals

Good serum levels, lasting for up to six hours, were obtained by intramuscular injection into rabbits, dogs, and monkeys, with peaks at about half an hour after injection. The urinary recovery, as measured in the rabbit, is good; on average 54% of the dose given is recovered in 24 hours (see Table III). No evidence of serum binding was observed and the assays of cephaloridine were unaffected by the presence of serum.

TABLE III.—Serum Levels and Urinary Recoveries in Animals

Animal	Dose (mg./kg.)	No. of Animals Tested	Average Serum Level (μg./ml.) at Time After Injection (Hours):					% Urinary Recovery
			½	1	2	4	6	
Rabbit	5	3	3.2	2.6	2.2	~0.2	Nil	50
	10	5	9.4	5.4	2.9	0.2	Nil	50
	20	6	13.8	8.0	3.5	tr	Nil	64
Dog	5	2	22.0	17.3	2.7	1.5	0.4	38
	10	2	19.8	13.7	2.5	1.4	0.14	37
Monkey (Cynomolgus)	20	3	24.0	23.0	10.0	1.4	0.7	NT

NT = Not tested.

Serum Levels and Urinary Excretion in Human Volunteers

Serum levels and urinary excretion of cephaloridine have been studied in human volunteers. Eight adult males received 250 mg. and 13 received 500 mg. by the intramuscular route in about 2 ml. of water for injection. Five received 1 g. by oral administration in rice-paper cachets.

The volunteers had a normal diet and a normal fluid intake during the tests. Those who received the substance orally did so about two and a half hours after a normal breakfast.

The injections were singularly painless. There were no complaints of pain either immediately after the injection or subsequently. There were no signs of general malaise. Similarly, those who received 1 g. orally reported no toxic side-effects. Urine samples taken from the volunteers before, during, and after the experiments were examined for the presence of albumin, sugar, and abnormal cellular or crystalline deposits (microscopical examination). No abnormal findings were reported.

FIG. 1.—Average serum levels in eight human volunteers after one 250-mg. dose intramuscularly.

FIG. 2.—Average serum levels in 13 human volunteers after one 500-mg. dose intramuscularly.

Serum samples, obtained by venepuncture at $\frac{1}{2}$, 1, 2, 4, 6, and 8 hours after administration, were separated and kept at 4° C. until they were assayed 4 to 16 hours later (Figs. 1 and 2).

Urine samples were collected over the periods 0–2, 2–4, 4–6, 6–12, 12–24 hours after administration. The results are given in Table IV.

TABLE IV.—*Urinary Levels and Recoveries in Human Volunteers After Intramuscular Injection*

Dose	No. Tested	Average Urine Levels (μ g./ml.) at (hours):					Total	% Recovery
		0–2	2–4	4–6	6–12	12–24		
250 mg.	8	769	804	359	49	6	265	106
500 mg.	13	1,016	1,197	439	77	15	442	88

Dose	No. Tested	Average Urine Recoveries (mg.) at (hours):					Total	% Recovery
		0–2	2–4	4–6	6–12	12–24		
250 mg.	8	120	79	51	13	2	265	106
500 mg.	13	204	145	55	32	6	442	88

The urine samples were examined by chromatographs and bioautographs as well as by plate assay. No other micro-biologically active metabolite of cephaloridine was observed, and it would appear that the substance is excreted unchanged.

Good levels of antibacterial activity were obtained in the serum after intramuscular administration of cephaloridine. The levels and their persistence agreed well with those obtained in rabbits, monkeys, and dogs. At a dose of 250 mg., the peak serum levels found at half to one hour cover the minimum inhibitory concentrations of most *Staph. aureus* strains 10 to 100 times. This dose also gave excellent antibiotic levels in the urine (400 to 1,000 μ g./ml.) which would be expected to be effective against many Gram-negative organisms in the urinary tract. Higher serum levels were obtained after the 500-mg. dose. These were high enough to cover easily the minimum inhibitory concentrations against the more resistant staphylococci and also to cover a great many of the Gram-negative organisms. The recovery of the injected dose was at least 80%. Serum levels and urinary recovery after oral administration of a 1-g. dose were low, only about 1% of the dose given being recovered in the urine. It was concluded that cephaloridine is only poorly absorbed when given by mouth.

Conclusions

Cephaloridine has a broad spectrum of activity *in vitro* and is highly effective in protecting experimentally infected mice against both penicillin-sensitive and penicillin-resistant strains of *Staph. aureus*, and against *E. coli*, *Pr. mirabilis*, and

Salmonella spp. Protection is obtained at practicable dose levels (2 to 25 mg./kg./dose by subcutaneous injection), and in general the substance is more active than tetracycline, ampicillin, or chloramphenicol. It is very rapidly bactericidal.

Good serum levels are obtained when cephaloridine is injected intramuscularly into dogs, rabbits, or monkeys, with a peak at half to one hour. The substance is excreted efficiently by the kidney; from rabbits an average of 54% is recovered in the urine. No evidence of serum-binding was observed with either human or animal sera.

When cephaloridine is given intramuscularly to human volunteers, good peak serum levels are obtained at half to one hour, with activity lasting for eight hours. The peak serum concentrations are sufficient to cover the minimum inhibitory concentrations against most *Staph. aureus* strains 10 to 100 times. There was good urinary recovery of the dose given; also high concentrations were achieved in the urine (200 to 2,000 μ g./ml.). This, coupled with the highly bactericidal action of the antibiotic, suggests that treatment by it of Gram-negative infections in the urinary tract would be effective. The injections were well tolerated and were stated by the volunteers to be singularly free from pain; none of the volunteers experienced any untoward reactions.

Summary

Cephaloridine (Ceporin) is a new semisynthetic antibiotic derived from cephalosporin C.

It has a broad spectrum of activity *in vitro*, and is active against both penicillin-sensitive and penicillin-resistant strains of *Staph. aureus*. It is bactericidal in action.

It is highly effective in protecting experimentally infected mice against *Staph. aureus*, *E. coli*, *Pr. mirabilis*, and *Salmonella* spp.

Good blood levels are attained in experimental animals after intramuscular injection.

Intramuscular injection of 250 and 500 mg. doses into human volunteers gave good serum levels, high urinary levels, good urinary recovery, and complete freedom from undesirable side-effects.

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