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SOME BIOLOGICAL PROPERTIES OF CEPHALOSPORIN C AND A DERIVATIVE

BY

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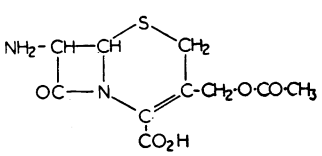
Some biological properties of cephalosporin C and of a pyridinium derivative, "cephalosporin C_A (pyridine)," were examined. Staphylococci, both penicillinase-producing and non-penicillinase-producing, and some other bacteria tested, were inhibited by 60 to 125 μg cephalosporin C/ml., and 5 to 20 μg cephalosporin C_A (pyridine)/ml. The ratio of the activity of the two antibiotics varied for different organisms. Resistance developed slowly on repeated subculture of penicillinase-producing staphylococci in presence of either antibiotic. The minimum inhibitory concentration of cephalosporin C_A (pyridine) upon penicillinase-producing staphylococci increased 4 to 8-fold with a 500-fold increase in inoculum size; with cephalosporin C there was a 2-fold increase. Their activity was not reduced by serum. Both substances were non-toxic. They were excreted quantitatively in the urine when given intravenously or subcutaneously to mice. After oral administration less than 5% of the dose was excreted. Cephalosporin C_A (pyridine) was about 8 times more active than cephalosporin C in protecting mice from an experimental streptococcal infection, nine doses of 6.25 mg/kg affording complete protection.

Cephalosporin C is an antibiotic which differs from the true penicillins in having as nucleus 7-aminocephalosporanic acid (I) (Abraham & Newton, 1961; Loder, Newton & Abraham, 1961) in place of 6-aminopenicillanic acid (II) (Sheehan, Henery-Logan & Johnson, 1953; Batchelor, Doyle, Nayler & Rolinson, 1959), the nucleus of the true penicillins, including cephalosporin N or synnematin B (Newton & Abraham, 1954; Abraham, Olson, Newton, Schuurmans, Schenck, Hargie, Fisher & Fusari, 1955). Cephalosporins N and C both have a D-α-aminoadipoyl side-chain. In cephalosporin C_A (pyridine) the acetoxy group of I appears to have been replaced by a pyridinium group (Abraham & Newton, 1958).



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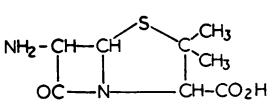

II

Cephalosporin C is much more resistant to penicillinase of *B. cereus* or *B. subtilis* than is benzylpenicillin (Abraham & Newton, 1956). It was decided to investigate the biological properties of cephalosporins C and C_A (pyridine), but the preliminary investigations reported here have been limited by lack of material.

METHODS

Antibiotic preparations. Cephalosporin C was obtained from the Medical Research Council Antibiotic Research Station, Clevedon, and was substantially pure. The pyridine derivative of cephalosporin C was prepared by Drs. Abraham and Newton (Hale, Abraham & Newton, 1961). It has been named "cephalosporin C_A (pyridine)" by these workers and was about 60% pure. Cephalosporin N was approximately 23% pure. The penicillin used was crystalline benzylpenicillin sodium (Boots Pure Drug Co.). Solutions were made in water and sterilized where necessary by passage through a sintered glass filter. In all cases results are expressed *in terms of the pure substances.*

Assay. This was carried out by the hole-plate or cylinder-plate method, with *Staphylococcus aureus* NCTC 6571 as test organism. A preparation of cephalosporin C of known purity was used as standard, not only for C but for the C_A (pyridine); though we were fully aware of the dangers of attempting to assay one substance in terms of another, this was felt at the time to be the least unsatisfactory course to follow.

The minimum inhibitory concentration was determined by the tube dilution method using 0.5 ml. vol. of Difco heart infusion broth (unless otherwise stated) and two-fold dilutions throughout. Tubes were read for the presence of visible growth after 24 hr (unless otherwise stated) and thereafter daily for 6 days.

Effect of inoculum size. The minimum inhibitory concentration was determined using as inoculum two drops (0.04 ml.) of (a) a 1 in 40, and (b) a 1 in 20,000 dilution in broth of a 24 hr broth culture.

Effect of serum on antibiotic activity. Two methods were used: (a) Solutions of antibiotic in water and in 50% horse serum were incubated at 37° C for 3 hr, then assayed by the plate method. (b) Two-fold dilutions of antibiotic in broth (containing 10% serum in the case of streptococci) and in broth containing 50% horse serum were inoculated with *Staphylococcus aureus* (strain R1) or *Streptococcus pyogenes* (CN10) and the minimum inhibitory concentration read after 24 hr incubation at 37°.

Acquisition of resistance. From the highest concentration showing growth in a dilution series, a transfer was made after 24 or 48 hr to a further range of tubes. This was repeated fourteen times, the end point being noted each time. The resistant organisms were then subcultured thirteen times in antibiotic-free broth and the minimum inhibitory concentration determined at the 5th, 9th, and 13th transfers.

Qualitative test for production by staphylococci of a penicillinase or of enzymes destroying cephalosporins C or C_A (pyridine). Equal volumes of antibiotic solution and 24 hr broth culture of the organism were incubated together for 1 hr at 37° C. The mixture was then assayed by the cylinder-plate method, using as controls (a) a solution of antibiotic in broth, and (b) antibiotic solution incubated for 1 hr with the non-penicillinase-producing *Staphylococcus aureus* NCTC 6571. Absence of inhibition zones round the cylinders containing test solution and their presence round those containing the controls was taken to indicate destruction of the antibiotic.

Serum concentration and rate of excretion in mice. The mice were catheterized and restrained as described by Heatley (1959) and antibiotic was given by various routes. Urine was assayed by the cylinder-plate method and serum by a vertical diffusion method (Heatley & Florey, 1953). *Staphylococcus aureus* NCTC 6571 was test organism for both assays. Standards were almost always prepared from a sample of the actual solution given to the animals, and for the serum assays they were made up in serum. Each urine sample was assayed in quadruplicate, but for serum often only single assays could be done, so that these values are only semi-quantitative. None of the animals was starved. Those given the antibiotic by the oral route were killed by coal gas after 2 or 4 hr. The stomach and small intestine were rinsed slowly with M/50 phosphate buffer *p*H 6.8, and the caecum and large intestine were likewise extracted; when the stomach or caecum could not easily be washed out, they were minced with scissors and extracted with the appropriate gut washings. These extracts were centrifuged and the supernatants assayed.

TABLE 1

COMPARATIVE ANTIBACTERIAL ACTIVITY OF BENZYLPENICILLIN AND OF CEPHALOSPORINS C, C_A (PYRIDINE), AND N

The inoculum was 2 drops of an overnight broth culture of the organism, diluted as indicated, per 0·5 ml. of medium, unless otherwise stated. The inoculum for *Myco. tuberculosis* was 0·1 ml. of a 6-day culture in Dubos medium, per 5 ml. HB=heart infusion broth; S=serum. The activity ratio C_A/C in the last column was calculated by dividing the min. inhibitory concentration of cephalosporin C by the min. inhibitory concentration of cephalosporin C_A (pyridine)

Organism	Inoculum broth culture diluted 1 in	Medium	Min. inhibitory concentration (μg/ml.) after 24 hr growth				Approx. ratio of activity
			C	C_A	N	Penicillin	C_A/C
Strep. pyogenes, CN10	40	HB+10% S	31	2·1	1·2	<0·1	15
Strep. viridans, NCTC 3165	40	HB+10% S	125	62·5	2·3	0·2	2
Pneumococcus, type I, CN33	40	HB+10% S	31	2·1	2·3	0·2	15
B. anthracis, avirulent	4,000	HB	31	7·8	1·1	0·1	4
Cl. welchii, NCTC 6125	Undil.	HB+5% S	125	40	1·1	0·2	3·1
Cl. septique	Undil.	HB+5% S	7·8	0·6	1·1	0·2	13
Cl. tetani, NCTC 279	Undil.	HB+5% S	3·9	1·2	1·1	<0·1	3·2
C. diphtheriae, gravis	4,000	HB	15·6	7·8	1·1	0·2	2
N. meningitidis	Undil.	HB+10% S	3·9	1·0	1·1	<0·1	4
N. gonorrhoeae							
NCTC 8676	Undil.	HB+10% S	7·8	1·0		—	8
NCTC 8375	Undil.	HB+10% S	1·9	1·0	1·1	0·2	2
NCTC 7129	Undil.	HB+10% S	1·9	2·0	—	—	1
Freshly isolated 1	Undil.	HB+10% S	3·9	1·0	—	—	4
Freshly isolated 2	Undil.	HB+10% S	15·6	2·5	—	0·1	6
Freshly isolated 3	Undil.	HB+10% S	15·6	1·2	—	0·1	13
Salm. typhi							
Rough	4,000	HB	15·6	15·6	2·3	4·8	1
Smooth	4,000	HB	15·6	27·4	1·1	—	1·8
Bact. friedländeri							
NCTC 5054	4,000	HB	31	31	4·6	19·2	1
Sh. shigae	4,000	HB	125	7·8	9·3	>6	16
Sh. sonnei	4,000	HB	125	7·8	—	>6	16
Escherichia coli, Type I							
Smooth	4,000	HB	125	13·7	9·3	>6	9
Rough	4,000	HB	125	6·9		—	18
Proteus vulgaris							
Non-spreading	4,000	HB	62·5	125	4·6	>6	0·5
Spreading	4,000	HB	31	110		—	0·3
Brucella abortus	40	HB+10% S	31	15·6	1·2	>6	2
Br. melitensis	40	HB+10% S	15·6	7·8		—	2
Haemophilus pertussis (incubated 2 days)	Undil.	Wheeler's	31	31	1·2	0·75	1
H. influenzae (incubated 2 days)	Undil.	Fildes'	31	31	9·3	6	1
Pseudomonas pyocyanea	4,000	HB	>1,000	>500	—	—	—
Vibrio cholerae							
Laboratory strain	4,000	HB	0·8	15	—	—	0·05
Strain 188	4,000	HB	2·0	13·7	—	—	0·14
Strain 189	4,000	HB	1·0	13·7	—	—	0·7
Mycobacterium tuberculosis							
H37Rv (human) (incubated 6 days)	Undil.	Dubos'	>200	>100	>9	>6	—
Branch (bovine) (incubated 6 days)	Undil.	Dubos'	>200	>100	>9	>6	—
Actinobovis israeli	Undil.	HB	>1,000	>500	>9	6	—
Cryptococcus neoformans	Undil.	Sabouraud's	>1,000	440		—	>0·5

RESULTS

Antibacterial properties against various organisms. Twenty-five different organisms were tested in parallel for sensitivity to cephalosporins C and C_A (pyridine). Table 1 gives the minimum inhibitory concentration after 24 hr incubation. After a further 5 days' incubation it had increased 2-fold for cephalosporin C and 2- to 4-fold for cephalosporin C_A (pyridine). The minimum inhibitory concentration for cephalosporin N and for penicillin, as determined by Heatley & Florey (1953), but quoted here in terms of pure substance, are also given for comparison.

Antibacterial activity against Staphylococcus aureus. Table 2 gives the min. inhibitory concentration after 24 hr incubation of cephalosporins C, C_A (pyridine) and N and also benzylpenicillin against 10 penicillinase-producing and 7 non-penicillinase-producing strains of *Staphylococcus aureus.* After 6 days the titre had decreased 2-fold for C and 4-fold for C_A (pyridine). That of penicillin had decreased about 2-fold for the non-penicillinase-producing strains and at least 8- to 64-fold for the penicillinase-producing strains.

Tables 1 and 2 show that *Staphylococcus aureus* is amongst the organisms more sensitive to cephalosporins C and C_A (pyridine) and, as with most other Gram-positive organisms, the latter is 6 to 30 times as effective as the former. But about twice as much C_A (pyridine) is needed to inhibit growth of the penicillinase-producing as of the non-penicillinase-producing strains. This was confirmed with 36 further strains of *Staphylococcus aureus*; 18 were penicillinase- and 18 non-penicillinase-producers, their growth being inhibited by 13.6 and 6.8 μg C_A (pyridine)/ml. respectively on agar ditch plates.

TABLE 2

COMPARATIVE ACTIVITY OF CEPHALOSPORINS C, N, AND C_A (PYRIDINE) AND BENZYLPENICILLIN AGAINST *STAPHYLOCOCCUS AUREUS*

Two-fold dilutions (0·5 ml.) in heart broth were inoculated with 0·04 ml. of a 1 in 40 dilution of 24-hr broth culture. Tubes were read after 24 hr incubation. All R and RD strains had been recently isolated from patients

	Min. inhibitory concentration (μg/ml.)					Ratio of activity				
Strain	C	C_A	Pen.	N	C_A/C	Pen/C	N/C	Pen/C_A	N/C_A	Pen/N
Penicillinase-producing strains										
G2	125	5·2	500	230	24	0·25	0·54	0·01	0·023	0·46
R1	125	7·8	500	230	16	0·25	0·54	0·016	0·034	0·46
R2	62	7·8	500	230	8	0·125	0·27	0·016	0·034	0·46
R3	62	7·8	500	230	8	0·125	0·27	0·016	0·034	0·46
R4	125	15·6	500	230	8	0·25	0·54	0·03	0·068	0·46
R5	125	7·8	500	230	16	0·25	0·54	0·016	0·034	0·46
R6	125	7·8	500	230	16	0·25	0·54	0·016	0·034	0·46
D3R	125	3·9	31	29	32	4	4·3	0·12	0·13	0·93
Carol	100	16·7	125	230	6	0·8	0·43	0·13	0·073	1·8
Bartlett	100	16·7	250	120	6	0·4	0·83	0·067	0·14	0·48
Non-penicillinase-producing strains										
RD21	62	4·9	0·03	3·6	13	2,100	17	160	1·4	120
RD23	62	4·9	0·03	1·8	13	2,100	35	160	2·7	60
RD24	125	19·8	0·06	1·8	6·3	2,100	70	330	11	30
RD25	62	4·9	0·03	1·8	13	2,100	35	160	2·7	60
RD26	62	4·9	0·03	1·8	13	2,100	35	160	2·7	60
RD27	62	4·9	0·01	1·8	13	6,200	35	490	2·7	180
NCTC 6571	125	4·2	0·015	1·	30	8,300	70	280	2·3	120

TABLE 3

DEVELOPMENT OF RESISTANCE TO CEPHALOSPORINS C AND C_A (PYRIDINE) IN PENICILLINASE-PRODUCING STRAINS OF *STAPHYLOCOCCUS AUREUS*

Figures represent -fold increase of min. inhibitory concentration over original

Strain	Subculture no. in presence of antibiotic														In plain broth		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	5	9	13
								Cephalosporin C_A (pyridine)									
R1	4	8	8	4	16	16	16	16	16	16	32	32	32	32	8	4	4
R2	8	8	4	8	16	16	8	8	8	16	32	16	32	32	8	8	4
R3	4	16	8	16	16	16	8	8	32	32	32	32	32	32	4	4	4
R4	4	8	8	8	16	16	8	8	8	16	32	16	32	32	8	4	2
R5	4	8	4	8	16	16	8	8	8	16	32	16	32	32	4	4	4
R6	4	8	8	16	16	32	16	16	16	16	32	32	16	32	16	16	16
D3R	2	2	8	8	16	16	32	64	32	32	32	64	32	64	—	—	32
B7	2	8	4	8	16	16	16	8	8	16	32	16	32	64	8	4	4
B8	4	8	8	16	16	16	16	8	16	16	32	32	32	64	8	4	4
B9	2	4	4	4	4	8	8	8	4	8	16	8	8	8	4	4	4
B10	8	16	16	16	16	32	16	32	16	16	64	64	32	64	16	8	8
B11	4	16	8	16	32	32	16	16	16	32	64	32	32	32	8	8	4
B12	4	8	8	8	16	32	16	16	16	32	32	32	32	32	16	16	16
								Cephalosporin C									
R1	0	0	4	2	8	4	4	8	4	8	4	8	8	8	—	—	2
D3R	2	2	4	2	8	8	8	16	8	8	8	8	16	16	—	—	16

Effect of inoculum size. The effect on min. inhibitory concentration of cephalosporins C and C_A (pyridine) and of penicillin of a 500-fold change in inoculum size was tested against 10 penicillinase-producing *Staphylococcus aureus* strains, and against the non-penicillinase-producing *Staphylococcus aureus* NCTC 6571. The heavier inoculum caused an average increase of 2-fold for cephalosporin C and of 4-fold for C_A (pyridine); for penicillin there was no change for NCTC 6571, but an increase of 256 to 2,000-fold for the penicillinase-producing strains.

The decrease in titre which took place when the incubation was prolonged for 3 days was slightly greater for C_A (pyridine) and penicillin when a large, than when a small, inoculum had been used. For cephalosporin C there was no difference.

Effect of serum. The activities of cephalosporins C and C_A (pyridine) were not appreciably altered by the presence of 50% horse serum in either solid or liquid medium.

Induction of resistance in penicillinase-producing strains of Staphylococcus aureus. Table 3 summarizes the changes in titre when 13 penicillinase-producing strains of *Staphylococcus aureus* were made resistant to cephalosporin C_A (pyridine) and 2 strains to cephalosporin C, and during their subsequent repeated subculture in antibiotic-free medium.

The resistant organisms, which grew somewhat more slowly than the sensitive parent strains, were unstable on repeated subculture in antibiotic-free medium, although no strain had reverted to its original sensitivity after 13 such transfers. Resistance to C_A (pyridine) developed more readily than to cephalosporin C.

Cross resistance. Six of the thirteen strains made resistant to cephalosporin C_A (pyridine) were tested for penicillin sensitivity. It was found not to have changed. Only two strains, R1 and D3R, were studied for cross resistance between cephalosporins C and C_A (pyridine). Whereas organisms made resistant to cephalosporin C became almost equally resistant to cephalosporin C_A (pyridine), those made resistant to cephalosporin C_A (pyridine) showed a relatively smaller increase to cephalosporin C. Clearly cross resistance between the antibiotics develops, but the pattern shown by the strains studied may not be typical.

Penicillinase and cephalosporinase activity of Staphylococcus aureus. By the qualitative method used 7 of the 10 penicillin-resistant strains were shown to be powerful penicillinase producers. Two strains (Carol & Bartlett) were less active and strain D3R was relatively feeble. There was scarcely detectable destruction of 1 mg or 0.5 mg/ml. of cephalosporins C or C_A (pyridine) by any of the strains, though the method was not sensitive enough to detect weak enzyme action with certainty.

Acute toxicity. The acute toxicity of cephalosporin C has already been briefly reported by Florey (1955, 1956), who found that a dose of 100 mg of material now known to be about 75% pure had no effect when injected intravenously into a 20 g mouse.

Solutions of cephalosporin C_A (pyridine) in 0.45 ml. distilled water were given intravenously to 20 g mice. Three mice were unaffected by 30 mg of 67% pure material and a fourth by 50 mg of the same product (equivalent to 1,000 and 1,670

mg of pure cephalosporin C_A (pyridine)/kg). A fifth mouse died at once when 100 mg of a different preparation, 50% pure, was injected slowly (2,500 mg of pure C_A (pyridine)/kg). No macroscopic or microscopic lesions were found in liver, spleen or kidneys of any of these mice.

Local toxicity. The injection of 0.1 ml. of a 10% solution of cephalosporin C_A (pyridine) in saline subcutaneously into 3 mice, and of 0.05 ml. intramuscularly into another 3 mice, caused no local reaction visible at post-mortem examination 5 days later, apart from a pin-point area of pigmentation at the site of the subcutaneous injection.

Serum concentration and excretion in mice. The limited number of experiments and the great scatter between replicates enables only tentative conclusions to be drawn. Table 4 shows that after intravenous or subcutaneous injection both cephalosporins C and C_A (pyridine) are excreted rapidly in the urine, largely within the first 2 hr. In this respect they resemble benzylpenicillin, but whereas total urinary

TABLE 4

SERUM CONCENTRATION AND URINARY EXCRETION OF CEPHALOSPORINS C AND C_A (PYRIDINE) AFTER INTRAVENOUS AND SUBCUTANEOUS ADMINISTRATION TO 20 G MICE

	Serum concn. (μg/ml.) after			% of dose excreted in urine in period (hr) after dose				
Route	1 hr	2 hr	3 hr	0–1	1–2	2–4	4–6	Total
Cephalosporin C (dose, 250 mg/kg)								
Intraven.	—	—	—	93		<6·3	<3·8	≮93
Intraven.	50	—	—	91		<8·2	<4·7	≮91
Intraven.	27	<20	<20	130		<7·5	<4·7	≮130
Subcut.	140	—	—	70·2	38·1	<4·3	<1·1	≮108
Subcut.	—	—	—	88·5	32·8	<4·3	<1·1	≮121
Subcut.	190	42	11	62·8	37·2	<4·0		≮100
Subcut.	145	27	<12	33·1	27·6	23·8	6·3	91
Cephalosporin C_A (pyridine) (dose, 200 mg/kg)								
Intraven.	80	<16	—	111		4·7	0·2	116
Intraven.	43	<16	—	135		1·2	0·2	136
Intraven.	69	<16	—	—		16·0	0·2	—
Intraven.	50	<14	—	99		8·8	<2·5	≮108
Intraven.	41	<12	—	99·5		4·5	<2·6	≮104
Subcut.	—	—	—	98·3		6·1	1·1	106
	—	<6	—	—		—	—	—
Benzylpenicillin (dose, 200 mg/kg)								
Intraven.	164	39	11	7·7	10·5	4·3	2·4	24·9
Intraven.	62	11	3·1	49·0	6·0	1·0	0·1	56·1
Intraven.	44	2	4	68·6	1·2	0·1	0	69·9
Intraven.	64	6	1	35·6	4·0	0·5	0·1	40·2
Intraven.	95	9	—	95·4	7·8	0·5	—	103·7
Intraven.	260	84	—	42·1	14·9	0·5	—	57·5
Intraven.	125	6	—	80·6	5·2	0·1	—	85·9
Intraven.	105	6·4	4	47·0	13·6	1·6	0·1	62·3
Subcut.	—	—	—	34·0	21·2	6·4	0·4	62·0
Subcut.	52·5	4·2	0·7	10·8	11·9	2·3	0·02	25·0
Subcut.	400	16	2·7	40·0	24·0	2·5	0·2	66·7

excretion of the latter averaged 60% of the dose, the whole of the cephalosporins appeared in the urine—indeed the urinary output was rather more than 100% of the administered dose.

After oral administration (see Fig. 1) the urinary excretion of both substances averaged less than 5% of the dose and was much the same whether the mouse was killed after 2 or 4 hr. This, together with the fact that the stomach and small intestine contained much less antibiotic at 4 than at 2 hr whereas the caecum and

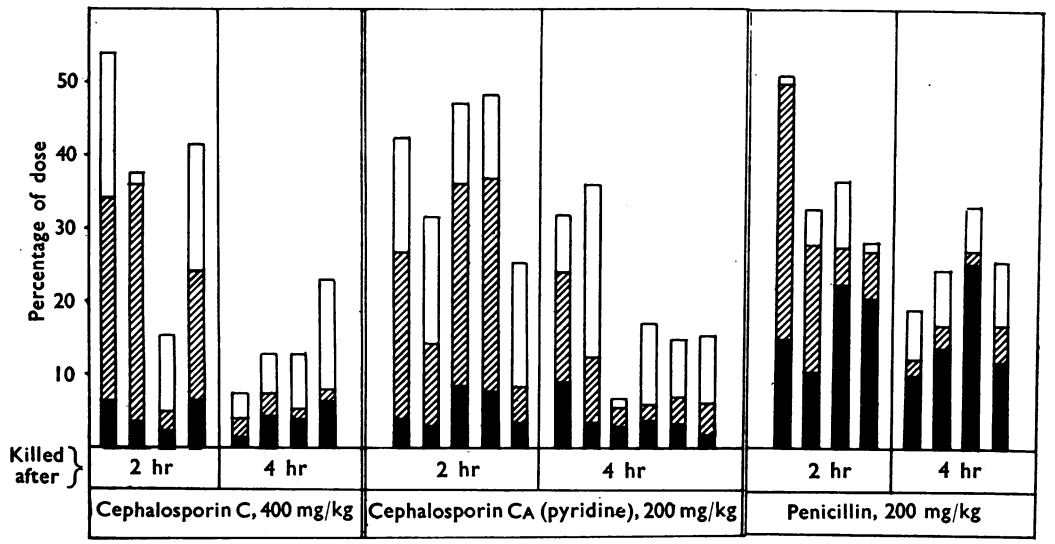

Fig. 1. Percentage of oral dose of various antibiotics given to mice, recovered 2 or 4 hr after administration; in urine ■; from stomach+small intestine ▨; from caecum+large intestine □.

large intestine contained about the same amount, suggests that little absorption takes place from the lower parts of the gut. That considerable destruction takes place is shown by the percentage of dose unaccounted for:

	Percentage of dose unaccounted for after	
	2 hr	4 hr
Cephalosporin C	46–85	77–93
Cephalosporin C_A (pyridine)	52–75	64–93
(Benzylpenicillin	49–72	67–81)

Since both substances appear to be quantitatively excreted after parenteral administration, it follows that no detectable destruction takes place in the body tissues; such loss as there is presumably occurs in the gut. Absorption from the gut is slight.

Mouse protection experiments. Florey (1955, 1956) reported that eight 3-hourly doses of 1 mg of cephalosporin C completely protected, 0.5 mg and 0.25 mg prolonged life and 0.125 mg had no effect in mice infected intraperitoneally with *Streptococcus pyogenes* (CN10) which produced 100% mortality of controls in 12 hr.

The experiment was repeated using cephalosporin C_A (pyridine). The results (Table 5, 1) show that 9 doses of 0.125 mg (6.25 mg/kg) or more completely protected the mice. Table 5, 2, shows the results of a milder infection with the same organism which gave 80% mortality of the controls after 2 weeks. Nine doses of 0.0625 mg (3.12 mg/kg) or more completely protected, and of 0.0312 mg (1.6 mg/kg) partially protected, the mice. In both experiments all surviving mice gave negative blood cultures.

TABLE 5

SURVIVAL OF MICE TREATED WITH CEPHALOSPORIN C_A (PYRIDINE) AFTER INFECTION WITH *STREP. PYOGENES*

Mice were infected intraperitoneally with 0·5 ml. of a 1 in 10 dilution of 24-hr broth culture of *Streptococcus pyogenes* CN10. The test groups received 9 doses subcutaneously of cephalosporin C_A (pyridine) in 0·3 ml. saline every 3 hr, starting 1 hr after infection

Expt. no.	Time after infection	Dose (mg) 0·5	0·25	0·125	0·0625	0·031	0 (control)
	25 hr	5/5	5/5	5/5	4/5	1/5	0/25
1	4 days	5/5	5/5	5/5	0/5	1/5	0/25
	4 weeks	5/5	5/5	5/5	0/5	0/5	0/25
	24 hr	5/5	5/5	5/5	5/5	5/5	13/25
2	2 weeks	5/5	5/5	5/5	5/5	4/5	5/25
	3 weeks	5/5	5/5	5/5	5/5	3/5	5/25

DISCUSSION

The mode of action of cephalosporins C and C_A (pyridine) upon bacteria is essentially the same as that of cephalosporin N and the common penicillins, all being powerfully bactericidal and bringing about the lysis of growing bacteria (Crawford, Loder, Abrahams & Newton, as quoted by Abraham & Newton, 1958). Comparisons of activities based on 2-fold dilution tests may have an error of up to 4-fold ; for this reason, and because relatively few strains have been examined, generalizations about antibacterial range, etc., must be only tentative. Cephalosporin C_A (pyridine) was 2 to 32 times more active than cephalosporin C against most Gram-positive organisms, including all staphylococci tested, although against several Gram-negative organisms there was little difference ; against shigellae and *Escherichia coli* it was slightly more active, but against three strains of *Vibrio cholerae* cephalosporin C was 7 to 18 times more active than cephalosporin C_A (pyridine) and more active than either benzylpenicillin or cephalosporin N. Although the penicillin was the most active of the four antibiotics against certain Gram-positive pathogens and Neisseria, cephalosporins C and C_A (pyridine) were both more active against the penicillinase-producing staphylococci ; furthermore, the activity of cephalosporin C was virtually independent of inoculum size and that of cephalosporin C_A (pyridine) almost so.

On repeated subculture in medium containing antibiotic, penicillinase-producing staphylococci gradually became resistant to cephalosporins C and C_A (pyridine)—somewhat more easily to the latter—but this resistance was gradually lost on cultivation in the absence of antibiotic. There was partial cross resistance between cephalosporins C and C_A (pyridine) but none to benzylpenicillin in the few strains tested.

Like cephalosporin C, cephalosporin C_A (pyridine) was not inactivated by serum, nor had it appreciable toxicity. It appeared to be about 8 times as effective as cephalosporin C in protecting mice from experimental streptococcal infection.

Both substances seemed to be excreted quantitatively in the urine after intravenous or subcutaneous administration to mice, though less than 5% of an oral dose was absorbed into the blood stream in spite of their acid stability; for the acid-labile benzylpenicillin the urinary recoveries in mice were 60% after intravenous or subcutaneous administration, and 16% after an oral dose. The amount of an oral dose unaccounted for was much the same for penicillin and the two cephalosporins in spite of the greater stability of the latter to acid and to penicillinase. The poor absorption from the gut and the high urinary excretion of cephalosporins C and C_A (pyridine) may well be a consequence of the polar nature of the side-chain, rather than a property associated with the nucleus.

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