Brit. J. Pharmacol. (1966), **26**, 108–119.

MECHANISM OF URINARY EXCRETION OF CEPHALORIDINE AND ITS EFFECTS ON RENAL FUNCTION IN ANIMALS

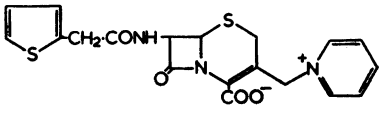
BY

K. J. CHILD AND M. G. DODDS

From Glaxo Research Ltd., Fulmer, Bucks.

(*Received April* 9, 1965)

A new semi-synthetic antibiotic, 7-[(2-thienyl)acetamido]-3-(1-pyridylmethyl)-3-cephem-4-carboxylic acid betaine (cephaloridine; Ceporin, Glaxo), has recently been prepared from cephalosporin C.



Cephaloridine is highly bactericidal against many Gram-positive and Gram-negative bacteria, including penicillin-resistant strains. It is virtually unaffected by penicillinase, and it shows no cross-resistance or cross-allergenicity with the penicillins. Clinical studies (Murdoch, Speirs, Geddes & Wallace, 1964; Stewart & Holt, 1964) indicate the antibiotic's usefulness in man, and preliminary accounts of the toxicology (Atkinson, Caisey, Currie, Middleton, Pratt, Sharpe & Tomich, 1966a; Atkinson, Currie, Davis, Pratt, Sharpe & Tomich, 1966b) and experimental chemotherapy (Muggleton, O'Callaghan & Stevens, 1964; Stewart & Holt, 1964) have already appeared.

Cephaloridine possesses low toxicity for most species of laboratory animals, but in some of them administration of the antibiotic results in the development of a characteristic proximal renal tubular necrosis (Atkinson *et al.*, 1966b). The study recorded here was designed to investigate the mechanism of urinary excretion of cephaloridine and to determine its effects on renal function in anaesthetized animals.

METHODS

Effects of cephaloridine on renal function in anaesthetized cats and dogs

Cats. Cats of each sex and weighing between 1.9 and 4.1 kg were anaesthetized by intraperitoneal injection of chloralose (80 mg/kg) and pentobarbitone sodium (10 mg/kg). A cannula was inserted in the trachea, and the left external jugular vein and right femoral artery were prepared for cannulation. A short midline incision was made in the lower abdomen and a urethral catheter was inserted for urine collection. After securing the cannula by ligatures, the abdomen was closed. The blood vessels were cannulated, and heparin (1,000 units/kg) was injected intravenously. Blood pressure was recorded from the carotid artery by a mercury manometer. Mannitol (300 mg/kg) was given intravenously, and 5% mannitol in 0.9% saline was infused intravenously at 0.75 ml./min throughout the experiment to ensure moderate diuresis. Control blood (from femoral artery) and urine samples were collected during the subsequent stabilization period of approximately 1 hr.

Conventional renal clearance procedures were used to assess glomerular filtration rate (estimated by the creatinine clearance), renal plasma flow (estimated by clearance of sodium *p*-aminohippurate), urea clearance, glucose reabsorptive capacity and the maximal tubular secretory capacity for *p*-aminohippurate. Drugs for clearance determinations were given as a priming dose and then added to the mannitol infusion in amounts sufficient to maintain plasma concentrations of: creatinine, 10 to 30 mg/100 ml.; *p*-aminohippurate, 0.5 to 5 mg/100 ml. for clearance estimation or 15 to 30 mg/100 ml. for maximal tubular secretory capacity estimation; urea, 25 to 40 mg/100 ml.; and glucose, 500 to 2,000 mg/100 ml. Cephaloridine was also given as a priming dose before intravenous infusion.

Blood samples (2 to 3 ml.) were withdrawn from the femoral artery at the beginning and end of each timed urine collection period of approximately 10 min. After each blood sample an equivalent volume of 0.9% saline was injected intra-arterially to help maintain fluid and electrolyte balance.

Dogs. Female mongrel dogs weighing between 6.8 and 9.0 kg were anaesthetized in the way described above for cats. The method of study of renal function was also similar to that used for cats, except that ureteral instead of urethral cannulation was performed and the rate of intravenous infusion of 5% mannitol was 2.0 ml./min. Only glomerular filtration rate and renal plasma flow were measured.

Renal clearance of cephaloridine in anaesthetized animals

Cats and dogs. The method of study in cats and dogs was the same as that outlined above.

Rabbits. Rabbits of each sex, weighing between 2.4 and 2.8 kg, were anaesthetized by intravenous injection of urethane (1.25 g/kg) and pentobarbitone sodium (12.5 mg/kg). Except that ureteral cannulation was performed and blood pressure recording was omitted, the method was similar to that outlined above for cats.

Monkeys. Male and female Patas monkeys (2.0 to 2.9 kg body weight) were anaesthetized by intramuscular injection of 5 mg of phencyclidine hydrochloride and subsequent intraperitoneal injection of pentobarbitone sodium (20 mg/kg). Heparin was not administered; blood pressure recording was accordingly omitted, and blood samples were withdrawn into heparinized syringes. Urine was collected from a bladder cannula, but in all other respects the method was similar to that outlined above for cats.

In the experiments on anaesthetized cats, dogs, rabbits and monkeys, a simultaneous estimate of glomerular filtration rate was also obtained. Glomerular filtration rate was estimated by the clearance of creatinine, which was maintained at plasma concentrations of 10 to 30 mg/100 ml. In some monkeys, inulin instead of creatinine was used to estimate glomerular filtration rate (plasma inulin concentration, 5 to 60 mg/100 ml.); the reason for this was that, although renal clearance of creatinine is a reliable measure of glomerular filtration rate in the cat (Eggleton & Habib, 1949, 1950, 1951; Gammeltoft & Kjerulf-Jensen, 1943) and in the rabbit and dog (Smith, 1951a) there is some evidence that creatinine may be secreted to a small extent by the primate (Smith & Clarke, 1938). Cephaloridine was introduced by a priming dose before intravenous infusion.

Urinary excretion of cephaloridine by the hen kidney

A modification of the method of Sperber (1948) was used. Light Sussex hens weighing between 2.1 and 3.1 kg were anaesthetized by intravenous injection of 60 mg of pentobarbitone sodium into the left wing vein; frequent smaller supplementary doses of anaesthetic were administered during the experiment. The right wing vein was cannulated for injections into the general circulation and a vein in the right leg was cannulated for infusions into the renal portal circulation. The hen, with its head supported, was then suspended in a standing position in a cloth sling. A 5% mannitol solution in 0.9% saline was infused intravenously into the wing vein at 1.0 ml./min throughout the experiment; this infusion produced a diuresis sufficient to ensure movement of the thick mucoid urine. The cloaca was partially everted and its outer margin securely fastened by sutures to a metal ring 5.5 cm in diameter. Further sutures were used to fasten the cloacal mucosa to the ring until the ureteral orifices were clearly exposed. The urine was collected as it appeared from the ureters by attaching two small glass funnels (0.8 cm diameter) to the mucosal surface. The funnels were attached by suction applied to glass cups (1.2 cm diameter) surrounding the funnels. The urine from the left and right kidneys was conveyed in polyethylene tubing to the collecting vessels. After a 30- to 60-min stabilization period, during which control urine samples were obtained, cephaloridine was infused intravenously or injected intramuscularly.

For the infusion experiments the terminology of Sperber (1948) has been used to express tubular secretion n terms of the "apparent tubular excretion factor." This equals:

$$\frac{\text{Rate of excretion on the infused (right) side} - \text{Rate of excretion on the opposite (left) side}}{\text{Infusion rate}} \times 100$$

To provide unequivocal evidence that the increased excretion of a substance by the kidney on the infused side is a result of active tubular secretion, and not due solely to diffusion processes, the apparent tubular excretion factor should be greater than 10 (Sperber, 1948). Aminohippurate is known to be actively secreted by the kidney tubules of the hen (apparent tubular excretion factor values usually lie between 40 and 90), and its excretion has therefore been measured in these experiments simultaneously with that of cephaloridine, to confirm that the leg vein blood is perfusing the tubules and is not being shunted directly into the general venous return, which can sometimes occur (Rennick & Gandia, 1954).

Analytical methods

Blood samples were centrifuged for 5 min and the plasma was separated. Plasma and urine concentrations of all substances except cephaloridine were determined on a Technicon Auto-Analyser. The methods, appropriately modified for use with the Auto-Analyser, were: creatinine (Folin & Wu, 1919); *p*-aminohippurate (Bratton & Marshall, 1939); urea (Marsh, Fingerhut & Kirsch, 1957); glucose (Hoffman, 1937); and inulin (Schreiner, 1950). Plasma and urine concentrations of cephaloridine were determined by the spectrophotofluorimetric method of Child & Stocker (unpublished), in which the antibiotic is estimated as pyridine after acid hydrolysis.

In all the clearance experiments midpoint plasma concentrations were calculated by averaging the values obtained at the beginning and end of each urine collection period.

RESULTS

Effects of cephaloridine on renal function in anaesthetized cats and dogs

Glomerular filtration rate. In untreated cats glomerular filtration rate was constant over a 4-hr period (Table 1,*A*). Infusion of cephaloridine slightly but significantly reduced

TABLE 1

EFFECT OF CEPHALORIDINE ON GLOMERULAR FILTRATION RATE IN CATS AND DOGS

The control and test phases each lasted 2 hr. Results shown are means and standard errors. Figures in parentheses indicate the number of urine collection periods. Means in bold type are significantly different from those of corresponding controls ($P<0.05$)

Animal No.	Sex	Body weight (kg)	Dosage of cephaloridine during test phase		Glomerular filtration rate (ml./min)	
			Priming dose (mg/kg)	Infusion rate (mg/kg/min)	Control phase	Test phase
A. Cats						
1	M	2·6	0	0	6·7±0·4 (9)	6·6±0·3 (11)
2	M	2·7	0	0	8·5±0·4 (11)	7·8±0·4 (11)
3	M	3·0	0	0	8·4±0·3 (10)	8·3±0·2 (11)
33	M	3·1	0·25	0·05	19·5±0·3 (11)	**15·0**±0·5 (11)
34	M	2·1	0·25	0·05	9·6±0·4 (11)	9·0±0·2 (10)
35	F	2·5	0·25	0·05	11·6±0·3 (9)	**8·8**±0·2 (11)
4	M	2·0	2·5	0·5	8·1±0·2 (8)	**6·4**±0·2 (11)
5	M	3·8	2·5	0·5	15·0±0·4 (11)	**12·3**±0·4 (12)
6	M	2·8	2·5	0·5	7·1±0·3 (11)	7·3±0·2 (12)
7	F	2·7	12·5	2·5	8·2±0·3 (10)	8·0±0·3 (12)
8	M	3·1	12·5	2·5	6·8±0·2 (10)	**4·9**±0·3 (10)
9	F	2·2	12·5	2·5	4·9±0·2 (10)	**3·9**±0·2 (11)
B. Dogs						
1	F	6·8	2·5	0·5	44·2±1·0 (5)	**40·0**±0·9 (8)
2	F	9·0	2·5	0·5	47·4±1·2 (7)	45·8±0·9 (8)
3	F	8·2	2·5	0·5	30·0±0·8 (5)	27·5±0·6 (9)

glomerular filtration rate in six out of nine cats. The effect was independent of cephaloridine dose and plasma concentration.

In two of the three dogs studied (Table 1,*B*) cephaloridine infusion likewise slightly but significantly reduced glomerular filtration rate.

Renal plasma flow. At the low cephaloridine infusion rate, the absolute value of renal plasma flow was increased in cats 36 and 38 (Table 2,*A*), but the clearance ratio (*p*-aminohippurate clearance divided by creatinine clearance) was reduced in cat 36 and increased in cat 38. At the higher infusion rate the clearance ratio was reduced in cats 12 and 23. The rise in clearance ratio in cat 13, associated with a marked reduction in glomerular filtration rate and renal plasma flow, may have resulted from an intra-abdominal haemorrhage that occurred.

TABLE 2

EFFECT OF CEPHALORIDINE ON *p*-AMINOHIPPURATE CLEARANCE IN CATS AND DOGS

Control and test phases each lasted 2 hr. All results are means. Clearance ratios are means with standard errors. Figures in parentheses indicate the number of urine collection periods. Means in bold type are significantly different from those of corresponding controls ($P<0.05$). PAH=*p*-aminohippurate; CTN=creatinine (glomerular filtration rate)

Animal No.	Sex	Body weight (kg)	Dosage of cephaloridine during test phase		Control phase			Test phase		
					Clearances (ml./min)		Clearance ratio	Clearances (ml./min)		Clearance ratio
			Priming dose (mg/kg)	Infusion rate (mg/kg/min)	PAH	CTN	PAH/CTN	PAH	CTN	PAH/CTN
A. Cats										
36	M	3·3	0·25	0·05	25·6	6·5	3·96±0·14 (9)	26·1	7·3	**3·58**±0·06 (10)
37	M	1·9	0·25	0·05	22·9	8·6	2·68±0·06 (8)	13·4	5·0	2·67±0·10 (7)
38	M	3·3	0·25	0·05	29·0	14·7	1·97±0·04 (8)	42·2	19·0	**2·21**±0·04 (9)
12	M	3·4	2·5	0·5	27·5	9·4	2·92±0·18 (8)	19·9	8·6	**2·34**±0·12 (11)
13	M	4·1	2·5	0·5	31·7	7·8	4·07±0·22 (7)	14·1	2·6	**5·49**±0·13 (12)
23	M	3·0	2·5	0·5	36·0	10·6	3·41±0·08 (10)	29·1	9·9	**2·91**±0·08 (11)
B. Dogs										
1	F	6·8	2·5	0·5	116·0	44·2	2·63±0·03 (5)	101·0	40·0	**2·53**±0·03 (7)
2	F	9·0	2·5	0·5	124·0	47·4	2·63±0·01 (7)	111·0	45·8	**2·42**±0·01 (8)
3	F	8·2	2·5	0·5	63·4	30·0	2·12±0·03 (5)	59·1	27·5	2·16±0·03 (9)

In two of the three dogs studied, a small but significant reduction in the clearance ratio occurred (Table 2,*B*).

Glucose reabsorptive capacity. Plasma glucose concentrations of 500 to 2,000 mg/100 ml. were used to produce glucosuria in cats. Intravenous infusion of cephaloridine did not significantly affect glucose reabsorption (Table 3). Glucose reabsorptive capacity was calculated as the percentage reabsorption of the filtered glucose load, for in the cat (unlike dog or man) no tubular reabsorptive maximum can be demonstrated (Eggleton & Shuster, 1954).

Urea clearance. Cephaloridine infusion did not affect the clearance ratio (urea clearance divided by creatinine clearance) (Table 4). The high values for this ratio of 0.72 and 0.90 during the control phases reflected the degree of diuresis induced by the mannitol infusion.

Maximum tubular secretory capacity for p-*aminohippurate.* This was not significantly altered by cephaloridine infusion (Table 5). The low clearance values for creatinine and

p-aminohippurate in these cats could have resulted from the depressant effect of the high plasma *p*-aminohippurate concentration (Eggleton & Habib, 1950) and were therefore probably unrelated to treatment with cephaloridine.

TABLE 3

EFFECT OF CEPHALORIDINE ON THE TUBULAR REABSORPTION OF GLUCOSE IN CATS

The control and test phases each lasted 2 hr: All results are means. Results for glucose reabsorption are means with standard errors. Figures in parentheses indicate the number of urine collection periods. * Values are not significantly different from those of corresponding controls. ($P>0{\cdot}05$). Gluc=glucose; CTN=creatinine (glomerular filtration rate)

Cat No.	Sex	Body weight (kg)	Dosage of cephaloridine during test phase		Control phase			Test phase		
			Priming dose (mg/kg)	Infusion rate (mg/kg/min)	Clearances (ml./min) Gluc	Clearances (ml./min) CTN	Reabsorption of glucose load (%)	Clearances (ml./min) Gluc	Clearances (ml./min) CTN	Reabsorption of glucose load (%)
14	M	2·7	2·5	0·5	4·3	6·3	31±5 (10)	4·2	6·3	*33±3 (11)
15	F	2·8	2·5	0·5	2·0	7·7	74±2 (11)	1·8	6·8	*74±1 (12)

TABLE 4

EFFECT OF CEPHALORIDINE ON THE UREA CLEARANCE IN CATS

The control and test phases each lasted 2 hr. All results are means. Clearance ratios are means with standard errors. Figures in parentheses indicate the number of urine collection periods. * Values are not significantly different from those of corresponding controls ($P>0{\cdot}05$). CTN=creatinine (glomerular filtration rate)

Cat No.	Sex	Body weight (kg)	Dosage of cephaloridine during test phase		Control phase			Test phase		
			Priming dose (mg/kg)	Infusion rate (mg/kg/min)	Clearances (ml./min) Urea	Clearances (ml./min) CTN	Clearance ratio Urea/CTN	Clearances (ml./min) Urea	Clearances (ml./min) CTN	Clearance ratio Urea/CTN
10	M	2·0	2·5	0·5	5·8	8·1	0·72±0·03 (8)	4·7	6·4	*0·72±0·02 (11)
11	M	3·6	2·5	0·5	6·7	7·4	0·90±0·05 (10)	5·1	5·9	*0·85±0·06 (11)

TABLE 5

EFFECT OF CEPHALORIDINE ON THE TUBULAR SECRETION OF *p*-AMINOHIPPURATE IN CATS

The control and test phases each lasted 2 hr. All results are means. Results for maximal tubular secretory capacity for *p*-aminohippurate (Tm_{PAH}) are means with standard errors. Figures in parentheses indicate the number of urine collection periods. * Values are not significantly different from those of corresponding controls ($P>0{\cdot}05$). PAH=*p*-aminohippurate. CTN=creatinine (glomerular filtration rate, GFR)

Cat No.	Sex	Body weight (kg)	Dosage of cephaloridine during test phase		Control phase			Test phase		
			Priming dose (mg/kg)	Infusion rate (mg/kg/min)	Clearances (ml./min) PAH	Clearances (ml./min) CTN	Tm_{PAH} (mg/100 ml. GFR)	Clearances (ml./min) PAH	Clearances (ml./min) CTN	Tm_{PAH} (mg/100 ml. GFR)
16	M	2·5	2·5	0·5	15·5	6·3	23±1 (10)	10·1	4·6	*25±1 (11)
17	F	2·6	2·5	0·5	11·0	6·5	16±1 (10)	8·6	5·6	*17±1 (11)

Renal clearance of cephaloridine in the cat, dog, rabbit and monkey

Cephaloridine was rapidly eliminated in the urine of anaesthetized cats, dogs, rabbits and monkeys. The renal clearance of cephaloridine was approximately 1 to 5 ml./kg/min and the ratio cephaloridine clearance divided by creatinine clearance ranged from 0.30 to 1.20. The results are summarized in Table 6.

TABLE 6

RENAL CLEARANCE OF CEPHALORIDINE IN THE CAT, DOG, RABBIT AND MONKEY

All clearance results are means. Plasma cephaloridine (Ceph) concentrations are ranges. Clearance ratios are means with standard errors. Figures in parentheses indicate the number of urine collection periods.
* Glomerular filtration rate (GFR) was estimated by inulin clearance

Species	Animal No.	Sex	Body weight (kg)	Cephaloridine dosage: Priming dose (mg/kg)	Cephaloridine dosage: Infusion rate (mg/kg/min)	Plasma cephaloridine concentrations (μg/ml.)	Clearance (ml./min): Ceph	Clearance (ml./min): GFR	Clearance ratio Ceph/GFR	Fiducial limits (P=0·05)
Cat	26	F	2·3	0·25	0·05	4·4–15·4	5·7	9·8	0·58±0·13 (10)	0·29–0·87
	28	M	2·8	0·25	0·05	5·0–219	3·5	8·6	0·39±0·16 (10)	0·08–0·70
	23	M	3·9	2·5	0·5	82–147	4·7	6·6	0·76±0·08 (9)	0·58–0·96
	24	F	2·5	2·5	0·5	36–113	5·1	11·5	0·59±0·10 (10)	0·37–0·81
	25	F	2·3	2·5	0·5	43–113	6·9	6·4	1·08±0·08 (11)	0·90–1·26
	27	F	2·5	12·5	2·5	286–930	6·2	11·8	0·53±0·03 (11)	0·46–0·60
	29	F	3·5	12·5	2·5	540–1,130	6·3	10·5	0·60±0·05 (11)	0·49–0·71
Dog	1	F	6·8	2·5	0·5	32·2–49·0	35·8	40·0	0·91±0·05 (6)	0·78–1·04
	2	F	9·0	2·5	0·5	30·8–61·4	34·0	45·9	0·74±0·07 (7)	0·57–0·91
	3	F	8·2	2·5	0·5	39·6–64·8	27·8	27·6	1·00±0·04 (7)	0·91–1·09
Rabbit	1	F	2·8	2·5	0·5	13·9–80·8	12·0	10·8	1·05±0·08 (6)	0·79–1·31
	2	M	2·4	2·5	0·5	42·4–77·2	10·6	12·2	0·87±0·04 (5)	0·76–0·98
	3	F	2·8	2·5	0·5	30·6–73·6	10·5	15·8	0·66±0·05 (5)	0·55–0·77
Monkey	1	M	2·9	0·25	0·05	5·6–14·5	8·8	12·3*	0·71±0·08 (5)	0·49–0·93
	2	M	2·5	1·0	0·1	4·4–14·5	9·4	29·3*	0·42±0·07 (10)	0·26–0·58
	3	F	2·0	1·0	0·1	1·9–9·9	32·9	35·3*	1·20±0·26 (5)	0·48–1·92
	6	F	2·6	1·0	0·1	33·0–56·0	2·1	8·6	0·30±0·08 (4)	0·04–0·56
				5·0	0·5	50·0–95·0	10·3	12·6	0·90±0·07 (4)	0·68–1·12
				25·0	2·5	244–337	9·1	10·7	0·89±0·01 (4)	0·86–0·92

Cephaloridine is not protein-bound to any significant extent (Barber & Waterworth, 1964; Muggleton *et al.*, 1964) and is hence completely filterable at the glomerulus; the observed clearance ratios of less than 1.0 indicate a small net reabsorption of the antibiotic. No evidence of secretion of cephaloridine was obtained. The clearance ratio was unaltered over a wide range of plasma cephaloridine concentrations in the cat (10 to 1,000 μg/ml.) and monkey (10 to 300 μg/ml.). No marked alteration in the clearance ratio was obtained by alteration of urine *p*H, for in two cats in which metabolic alkalosis was induced by intravenous infusion of sodium bicarbonate the ratios (means and standard errors) were 0.79±0.06 and 0.90±0.06 respectively, and in one cat rendered acidotic by infusion of dilute hydrochloric acid the ratio was 1.11±0.08.

Urinary excretion of cephaloridine by the hen kidney

Intravenous infusion experiments (*hens* 1, 2 *and* 3). In these experiments a solution containing *p*-aminohippurate and cephaloridine in 0.9% saline was infused continuously into the vein of the right leg at 0.187 ml./min (or 150 μg/min of each compound). Simul-

TABLE 7

EXCRETION OF *p*-AMINOHIPPURATE AND CEPHALORIDINE BY THE HEN KIDNEY

p-Aminohippurate and cephaloridine were each infused into a vein in the right leg at 150 μg/min. Urine was collected for 15 min periods from the right (infused) and left (opposite) sides. Tubular secretion has been calculated as the apparent tubular excretion factor (ATEF) (see Methods). All results are means. ATEF values are means with standard errors. Figures in parentheses indicate the number of urine collection periods

Hen No.	Body weight (kg)	*p*-Aminohippurate			Cephaloridine		
		Excretion rate (μg/min)			Excretion rate (μg/min)		
		Right	Left	ATEF	Right	Left	ATEF
1	2·7	115	13	68±3 (10)	61	39	15±2 (10)
2	2·5	108	30	52±6 (8)	63	44	13±3 (8)
3	2·8	131	10	81±3 (9)	81	35	30±2 (9)

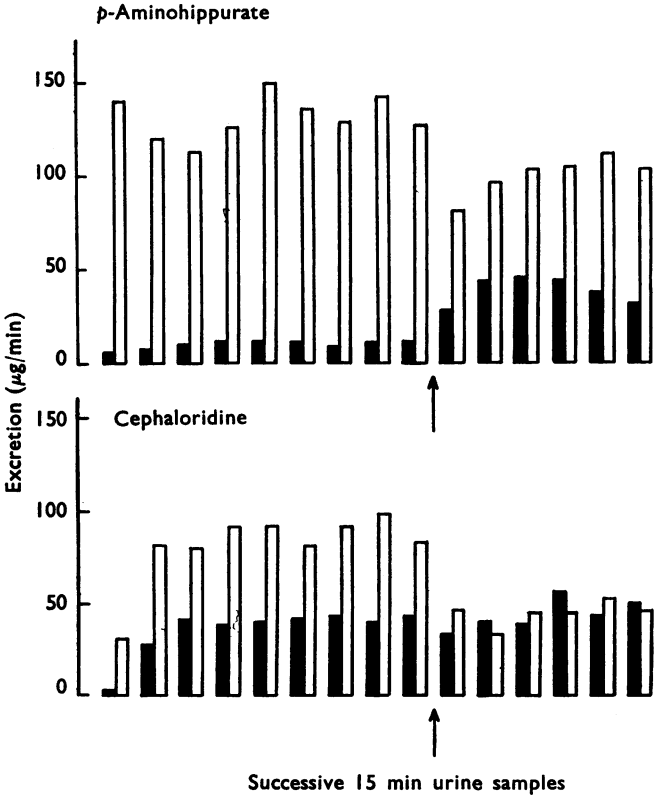


Fig. 1. Excretion of *p*-aminohippurate and cephaloridine by hen kidney during unilateral infusion into the renal portal circulation. Results from an experiment on a hen of 2.8 kg body weight. *p*-Aminohippurate (150 μg/min) and cephaloridine (150 μg/min) were infused throughout into a vein of the right leg. Open columns indicate excretion by the kidney on the right (infused) side, closed columns excretion by the kidney on the left (opposite) side. Pairs of columns refer to successive 15-min urine collection periods. At the arrows, probenecid (100 mg) was injected into the wing vein.

taneous collections of urine from the left and right kidneys were made for successive 15-min periods. Probenecid, dissolved with the minimum amount of N-sodium hydroxide and diluted with saline to a concentration of 10 mg/ml., was injected slowly into the wing vein in a single intravenous dose of 100 mg before the second part of the experiment.

In all experiments, the results of which are summarized in Table 7, *p*-aminohippurate excretion was markedly higher on the infused (right) side, apparent tubular excretion factor values averaging 70. The excretion of cephaloridine was also consistently higher on the infused side, and apparent tubular excretion factor values of 13 to 30 indicate that tubular secretion of the antibiotic was occurring to a small extent. The results of the experiment in which the secretion of cephaloridine was most marked are shown in Fig. 1. The intravenous injection of probenecid (100 mg) partially blocked the tubular secretion of *p*-aminohippurate and completely blocked the tubular secretion of cephaloridine.

Intramuscular injection experiments (*hens* 4, 5, 6 *and* 7). In two hens (4 and 5) a single 100-mg dose of cephaloridine (1.0 ml. of a 10% solution in 0.9% saline) was injected into the muscle of the right leg. Urine was collected simultaneously from the left and right kidneys for successive 1-hr periods for 4 hr.

In two further hens, a single 300-mg dose of probenecid was injected slowly into the wing vein 30 min before the intramuscular injection of 100 mg of cephaloridine into the right leg. The probenecid solution was prepared as before at a concentration of 30 mg/ml. Urine was collected as for hens 4 and 5.

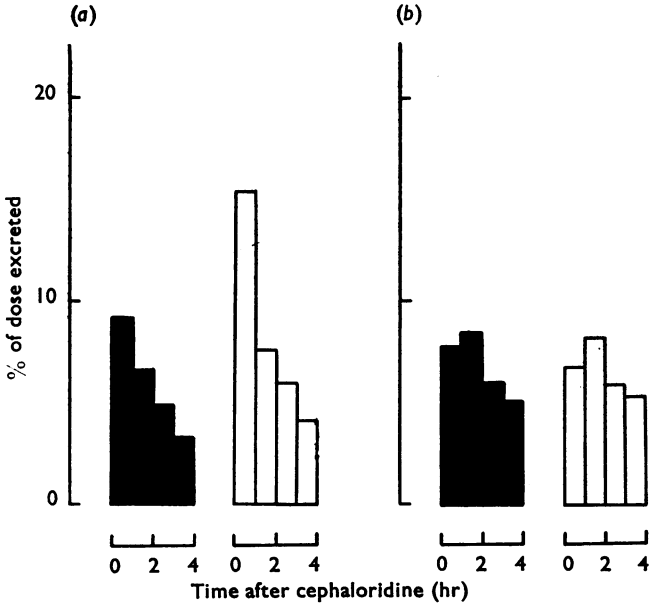


Fig. 2. Effect of probenecid on the urinary excretion of cephaloridine in the hen. Open columns indicate excretion by the kidney on the right (injected) side. Closed columns indicate excretion by the kidney on the left (opposite) side. Values are the averages for two hens. (*a*) Hens 4 and 5; cephaloridine (100 mg) was injected intramuscularly into the right leg. (*b*) Hens 6 and 7; probenecid (300 mg) was injected intravenously into the wing vein 30 min before the intramuscular injection of cephaloridine (100 mg) into the right leg.

In the two hens receiving a single intramuscular dose of 100 mg of cephaloridine injected into the right leg the average totals excreted in the urines from the right and left kidneys in 4 hr were 33.1 and 24.0% of the injected dose, respectively (Fig. 2). The excretion of cephaloridine was greater by 6.2% on the right side in the first 1-hr collection period. Subsequently excretions from the left and right sides were nearly identical.

In the two hens previously treated with a large dose of probenecid (300 mg), no differential excretion was obtained; the totals excreted were 26.1 (right) and 27.3% (left) in the 4-hr period.

The total antibiotic recovered in the urine in 4 hr was 57.1% from the untreated hens and 53.4% from the probenecid-treated hens.

DISCUSSION

In some cats and dogs, glomerular filtration rate and renal plasma flow have been altered to a small extent by the intravenous infusion of cephaloridine. Reduction of glomerular filtration rate in the cat was unrelated to plasma cephaloridine concentration and is therefore of doubtful importance. Anaesthesia depresses glomerular filtration rate (Pitts, 1963a; Smith, 1951b), and losses of red cells and plasma protein have been shown to reduce it in the cat (Eggleton & Shuster, 1954); renal plasma flow can be depressed by the same factors (Papper & Papper, 1964). The effects of prolonged anaesthesia and blood sampling may therefore have contributed to some of the observed changes, although in untreated cats glomerular filtration rate was constant during a 4-hr period. If the effects on glomerular filtration rate and renal plasma flow are attributed solely to cephaloridine infusion, then the influence of the antibiotic is small even at plasma concentrations up to 100-times those achieved in man injected intramuscularly with 0.5 g of cephaloridine (Muggleton *et al.*, 1964).

In cats glucose reabsorptive capacity and tubular secretory capacity for *p*-aminohippurate, both of which are proximal tubular functions particularly sensitive to nephrotoxic agents (Pitts, 1963b), and the ratio of urea clearance to creatinine clearance, which is reduced by agents damaging the tubules (Smith, 1951b), were not affected by cephaloridine infused at the rate of 0.5 mg/kg/min.

These studies of renal function were made while the toxicity experiments were proceeding, at which time the sensitivity of the cat and dog to the nephrotoxic action of cephaloridine had not been determined. The relevance of these tests of function may now be disputed, since the sensitivity to the nephrotoxic action of the antibiotic is in the descending order rabbit—monkey—mouse—hen—rat, no renal damage being demonstrable in the cat (1.0 g/kg, intramuscularly) or the dog (1.5 g/kg, intramuscularly). In the species in which nephrotoxicity has been demonstrated, it appears to be a gradual process, becoming histologically apparent 12 to 24 hr after administration of the antibiotic, reaching a maximum at 48 to 72 hr and then regressing (Atkinson *et al.*, 1966b). Mice can be completely protected against this nephrotoxic action of cephaloridine if probenecid is administered before or with the antibiotic, but not if the probenecid is administered as little as 30 min after the antibiotic (unpublished observations). It therefore seems likely that the renal lesion is initiated during the first hour or so after administration of cephaloridine, when no impairment of renal function would be demonstrable. Preliminary experiments on

anaesthetized monkeys have indicated that renal function is unchanged after 0.5 g/kg doses of cephaloridine; further, glomerular filtration rate was maintained during cephaloridine infusion in the rabbit and monkey (Table 6).

The renal clearance of cephaloridine was similar in all of the species examined and the ratio cephaloridine clearance to creatinine clearance was not altered by changes in plasma cephaloridine concentration or urinary *p*H. The average ratio of 0.6 to 0.7 indicates passive reabsorption of some 30 to 40% of the filtered cephaloridine, but a more complicated pattern of bidirectional tubular transport may be occurring, in which the urinary excretion rate is a little lower than the filtration rate. No evidence for active tubular secretion of cephaloridine was obtained, which distinguishes it not only from the penicillins (Smith, 1951d) but also from cephalothin (Lee, Herr & Anderson, 1963).

Evidence for the active tubular secretion of cephaloridine by the hen has, however, been obtained. The extent of this tubular transport is small, especially when compared with the marked secretion of benzylpenicillin and methicillin (Acred, Brown, Turner & Wright, 1961) and ampicillin (Acred, Brown, Turner & Wilson, 1962). The results with cephaloridine are closely similar to those obtained with cloxacillin by Acred & Brown (1963), who observed that the percentage excretion after a 100-mg dose was only 5.8% greater on the injected side during the first hour after administration and that thereafter there was no difference in the percentage excreted by the two kidneys. At 4 hr they had recovered 34.6% from the kidney on the injected side and 28.7% from the kidney on the opposite side; after probenecid the percentages excreted were almost identical.

The demonstration of renal tubular secretion of cephaloridine by hen kidney, and the effective inhibition of this tubular transport by probenecid, should be particularly noted in the light of preliminary experiments indicating that cephaloridine nephrotoxicity can be prevented by probenecid treatment. It is possible, therefore, that nephrotoxicity in the hen arises as a result of the high intracellular concentration of the antibiotic that occurs during tubular secretion. Treatment with probenecid can similarly protect mice and monkeys against cephaloridine nephrotoxicity (Child, Dodds, Pratt & Stocker, unpublished). Further work is in progress to investigate both the possible role of tubular secretion in the development of cephaloridine nephrotoxicity and the protective action of probenecid and other agents.

SUMMARY

1. The administration of cephaloridine, a new semi-synthetic antibiotic obtained from cephalosporin C, causes proximal renal tubular necrosis in several animal species. The mechanism of urinary excretion of the antibiotic and its effects on renal function in animals have therefore been investigated.

2. The effects of intravenously-infused cephaloridine on renal function in anaesthetized cats and dogs were determined by renal clearance procedures. Cephaloridine, infused for 2 hr, altered glomerular filtration rate and renal plasma flow to a small but statistically significant extent in some cats and dogs. Renal tubular function in cats, assessed by measurement of glucose reabsorptive capacity, urea clearance and *p*-aminohippurate secretory capacity, was unaffected by cephaloridine infusion.

3. Cephaloridine was rapidly eliminated in the urine of all the species examined. Clearance ratios (cephaloridine clearance to creatinine clearance) were similar in cats,

dogs, rabbits and monkeys, averaging 0.6 to 0.7. As the antibiotic is not protein-bound, and as the clearance ratios were not altered by changes in plasma cephaloridine concentration or urinary *p*H, it was concluded that some 30 to 40% of the filtered cephaloridine is passively reabsorbed.

4. Cephaloridine was secreted to a small extent by the kidney tubules of the hen, and this active tubular transport of the antibiotic was prevented by probenecid.

5. The mechanism of urinary excretion of cephaloridine is compared with that of other antibiotics, and it is tentatively suggested that tubular transport of the antibiotic may be associated with the development of nephrotoxicity.

We wish to thank Mrs G. Stocker who assayed the cephaloridine and Mr J. D. Caisey who performed the other chemical assays.

REFERENCES

ACRED, P. & BROWN, D. M. (1963). Further pharmacology and chemotherapy of cloxacillin. *Brit. J. Pharmacol.*, **22**, 339–354.

ACRED, P., BROWN, D. M., TURNER, D. H. & WILSON, M. J. (1962). Pharmacology and chemotherapy of ampicillin—a new broad-spectrum penicillin. *Brit. J. Pharmacol.*, **18**, 356–369.

ACRED, P., BROWN, D. M., TURNER, D. H. & WRIGHT, D. (1961). Pharmacology of methicillin. *Brit. J. Pharmacol.*, **17**, 70–81.

ATKINSON, R. M., CAISEY, J. D., CURRIE, J. P., MIDDLETON, T. R., PRATT, D. A. H., SHARPE, H. M. & TOMICH, E. G. (1966a). Subacute toxicity of cephaloridine to various animal species. *J. Toxicol. appl. Pharmacol.*, in the press.

ATKINSON, R. M., CURRIE, J. P., DAVIS, B., PRATT, D. A. H., SHARPE, H. M. & TOMICH, E. G. (1966b). Acute toxicity of cephaloridine, an antibiotic derived from cephalosporin C. *J. Toxicol. appl. Pharmacol.*, in the press.

BARBER, M. & WATERWORTH, P. M. (1964). Penicillinase-resistant penicillins and cephalosporins. *Brit. med. J.*, **ii**, 344–349.

BRATTON, A. C. & MARSHALL, E. K. (1939). A new coupling component for sulphanilamide determination. *J. biol. Chem.*, **128**, 537–550.

EGGLETON, M. G. & HABIB, Y. A. (1949). Sodium thiosulphate excretion in the cat. *J. Physiol. (Lond.)*, **110**, 98–109.

EGGLETON, M. G. & HABIB, Y. A. (1950). Excretion of para-aminohippurate by the kidney of the cat *J. Physiol. (Lond.)*, **110**, 458–467.

EGGLETON, M. G. & HABIB, Y. A. (1951). The mode of excretion of creatinine and inulin by the kidney of the cat. *J. Physiol. (Lond.)*, **112**, 191–200.

EGGLETON, M. G. & SHUSTER, S. (1954). The effect of insulin on the excretion of glucose and phosphate by the kidney of the cat. *J. Physiol. (Lond.)*, **125**, 623–630.

FOLIN, O. & WU, H. (1919). A system of blood analysis. *J. biol. Chem.*, **38**, 81–110.

GAMMELTOFT, A. & KJERULF-JENSEN, K. (1943). The mechanism of renal excretion of fructose and galactose in rabbit, cat, dog and man. *Acta physiol. scand.*, **6**, 368–384.

HOFFMAN, W. S. (1937). A rapid photoelectric method for the determination of glucose in blood and urine. *J. biol. Chem.*, **120**, 51–61.

LEE, C. C., HERR, E. G. & ANDERSON, R. C. (1963). Pharmacological and toxicological studies on cephalothin. *Clin. Sci.*, 1123–1138.

MARSH, W. H., FINGERHUT, B. & KIRSCH, E. (1957). Determination of urea nitrogen with the diacetyl method and an automatic dialyzing apparatus. *Amer. J. clin. Path.*, **28**, 681–688.

MUGGLETON, P. W., O'CALLAGHAN, C. H. & STEVENS, W. K. (1964). Laboratory evaluation of a new antibiotic—cephaloridine (Ceporin). *Brit. med. J.*, **ii**, 1234–1237.

MURDOCH, J. MCC., SPEIRS, C. F., GEDDES, A. M. & WALLACE, E. T. (1964). Clinical trial of cephaloridine (Ceporin), a new broad-spectrum antibiotic derived from cephalosporin C. *Brit. med. J.*, **ii**, 1238–1240.

PAPPER, S. & PAPPER, E. M. (1964). The effects of pre-anaesthetic, anaesthetic and post-operative drugs on renal function. *Clin. Pharmacol. Ther.*, **5**, 205–215.

PITTS, R. F. (1963). *Physiology of the Kidney and Body Fluids* (a), p. 137; (b) pp. 84–85. Chicago: Year Book Medical Publishers.

RENNICK, B. R. & GANDIA, H. (1954). Pharmacology of smooth muscle valve in renal portal circulation of birds. *Proc. Soc. exp. Biol. (N.Y.)*, **85**, 234–236.

SCHREINER, G. E. (1950). Determination of inulin by means of resorcinol. *Proc. Soc. exp. Biol. (N.Y.)*, **74**, 117–120.

SMITH, H. W. (1951). *The Kidney. Structure and Function in Health and Disease.* (a) Pp. 182–183; (b) p. 445; (c) p. 79; (d) pp. 196–197. Oxford: University Press.

SMITH, H. W. & CLARKE, R. W. (1938). The excretion of inulin and creatinine by the anthropoid apes and other infrahuman primates. *Amer. J. Physiol.*, **122**, 132–139.

SPERBER, I. (1948). The excretion of some glucuronic acid derivatives and phenol sulphuric esters in the chicken. *Ann. roy. agric. Coll., Sweden*, **15**, 317–349.

STEWART, G. T. & HOLT, R. J. (1964). Laboratory and clinical results with cephaloridine. *Lancet*, **ii**, 1305–1309.