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NEPHRON TRANSPORT AND RENAL TUBULAR EFFECTS OF CEPHALORIDINE IN ANIMALS

BY

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Renal tubular secretion by hen kidney of the semi-synthetic antibiotic cephaloridine, 7-[(2′-thienyl)acetamido]-3-(1″-pyridylmethyl)-3-cephem-4-carboxylic acid betain (Ceporin, Glaxo) has been described. Although the extent of this renal tubular transport was small, it was inhibited by probenecid administration. No evidence of renal tubular secretion was obtained in other species (cat, dog, rabbit and monkey) and it was concluded that in these animals cephaloridine was eliminated in the urine solely by glomerular filtration (Child & Dodds, 1966). Welles, Gibson, Harris, Small & Anderson (1966) have since reported similar results in dogs and have shown that probenecid treatment failed to modify the renal clearance of cephaloridine in this species.

Large doses of the antibiotic produce proximal renal tubular necrosis in a number of laboratory animal species (Atkinson, Currie, Davis, Pratt, Sharpe & Tomich, 1966; Welles *et al.*, 1966), and preliminary experiments in the mouse, hen and monkey have indicated that this necrosis is completely prevented by previous administration of probenecid (Child & Dodds, 1966).

These findings, first, that cephaloridine was secreted to a small extent by hen kidney and that probenecid could both block the tubular transport of the antibiotic and prevent its nephrotoxic action, and second, that probenecid could also protect against cephaloridine nephrotoxicity in the mouse, led us to suggest that cephaloridine-induced necrosis might result from the high intracellular concentrations that would occur during renal tubular secretion (Child & Dodds, 1966).

Although renal clearance studies had not provided evidence of renal tubular secretion of cephaloridine in the dog or rabbit, it was possible that bi-directional tubular transport of the antibiotic was occurring. Nephron transport of cephaloridine has therefore been re-examined by the method of " stop-flow " analysis both in dogs, which are not sensitive to the nephrotoxic effects of cephaloridine, and in rabbits, which are sensitive. The protective effect of probenecid and a number of other agents, some of which are known to interfere in renal tubular secretory mechanisms, has also been investigated in mice.

Some of the results presented in this paper were communicated at the III International Congress of Nephrology, Washington, D.C., September 25 to 30, 1966.

METHODS

Stop-flow analysis experiments in dogs

The method used was essentially that first described by Malvin, Wilde & Sullivan (1958). Female dogs weighing between 10.3 and 14.7 kg were anaesthetized by intravenous injection of pentobarbitone sodium (30 mg/kg) and allowed to breathe spontaneously through a tracheal cannula or endotracheal tube. The abdomen was opened by midline incision and both ureters were cannulated with No. 2 Sterivac polyethylene tubing. The left kidney only was used for occlusion and the catheter on that side was advanced and tied securely in place in the region of the renal pelvis. The abdomen was closed with sutures. Stainless steel cannulae were introduced into the right external jugular vein and the right femoral artery for the administration of infusion fluids and for the withdrawal of blood samples (5 ml.) respectively. The left carotid artery was connected to a mercury manometer for blood pressure recording and the left or right cephalic vein was cannulated for the injection of drugs. Heparin was placed in the carotid cannula but was not administered systemically.

A 10% mannitol solution was first infused intravenously at 1 ml./kg/min by means of a Sigmamotor pump to produce an osmotic diuresis. After 15 to 45 min, during which time the urine flow from each kidney stabilized at more than 6 ml./min, the required plasma concentrations of test substances were rapidly produced by intravenous injections of priming doses followed by standard intravenous infusions (mannitol 10%, NaCl 0.9%, creatinine 0.2%, inulin 0.2%, p-aminohippurate (PAH) 40 mg%, cephaloridine 30 mg%) at 1.0 ml./kg/min. After a stabilization period of about 30 min the ureteral catheter on the left side was clamped for exactly 6 min, during which time the infusion rate was halved to reduce changes in the plasma concentrations of test substances to a minimum. A blood sample (4 to 5 ml.) was withdrawn at the mid-point of the occlusion period. On release of the ureteral clamp some 20 to 30 serial urine samples of approximately 0.6 to 1 ml. were collected over a 2 or 2½ min period in pre-weighed sample pots arranged in a tray moved manually under the ureteral catheter. Urine samples and mid-point blood samples were also collected during " free-flow " clearance periods before and after ureteral occlusion.

When the effect of probenecid or other drugs was being investigated, the drugs were injected a few minutes after the control period sampling and allowed to circulate for about 20 min before the test period clearance and occlusion were started.

Stop-flow analysis experiments in rabbits

The procedure was modified in the rabbit because of its small size and poor viability. New Zealand White rabbits of either sex weighing between 2.05 and 4.61 kg were anaesthetized by intravenous injection of urethane (1.25 g/kg) and pentobarbitone sodium (12.5 mg/kg). Blood pressure recording was omitted and arterial blood samples (4 ml.) were withdrawn from the left carotid artery. The mannitol infusion (10 or 20%) and the standard infusion (mannitol 10 or 20%, NaCl 0.9% inulin 0.1%, PAH 25 mg%, cephaloridine 25 or 40 mg%) were infused intravenously into an external jugular vein or marginal ear vein at 2 ml./kg/min. After the 6 min occlusion period about 20 to 30 serial urine samples of 0.2 to 0.5 ml. were collected over a 2 or 2½ min period. The procedure was otherwise similar to that outlined above for dogs, although it was not always possible to perform more than one occlusion experiment in the same animal as the preparation deteriorated.

Analytical methods

Blood samples were centrifuged for 5 min and the plasma was separated. The serial urine sample volumes were determined by weighing. Plasma and urine concentrations of the test substances and of cephaloridine were determined by the methods already described (Child & Dodds, 1966), except that inulin was determined by the automated method of Dawborn (1965).

Experiments on the nephrotoxic action of cephaloridine and the protective effect of probenecid and other drugs

Hens. Light Sussex hens (body weight range 2.1 to 3.1 kg) were used in these experiments. Physiological saline or a 30% aqueous solution of cephaloridine was injected intramuscularly into the

right leg. When the dose volume exceeded 5 ml. it was divided and injected at two sites. Forty-eight hours after injection the hens were killed by intravenous injection of pentobarbitone sodium and their kidneys removed and fixed in buffered formol saline for histological examination.

In some hens, probenecid, dissolved with the minimum amount of N-sodium hydroxide and diluted with physiological saline to a concentration of 10 mg/ml., was injected slowly into a wing vein 30 min before the intramuscular injection of saline or cephaloridine.

The kidney sections were examined histologically without knowing the treatment the hens had received. The sections were scored as follows: no abnormalities −, isolated degenerating cells +, scattered tubular necrosis ++, massive tubular necrosis +++.

Mice. Groups of five female mice (A2G strain, body weight 15 to 20 g) were used, the mice in each group being injected subcutaneously with solutions of cephaloridine. A dose of 0.2 ml./20 g of a 10% solution of cephaloridine in normal saline was administered to mice receiving a 1 g/kg dose; the dose volume and/or solution concentration was increased as appropriate for mice receiving the 2, 4, 8 and 12 g/kg doses. After cephaloridine treatment the groups of mice were kept under standard conditions for 48 hr with free access to food and water. They were then killed by cervical dislocation and their kidneys were removed and fixed in buffered formol saline. Each kidney was given an individual code number and the kidneys were randomized before histological examination.

Probenecid was administered orally as an aqueous suspension; other drugs were administered orally or intraperitoneally as appropriate. The particular drugs and the dosages and schedules of treatment employed in these experiments are included in the Results (Table 7).

The results of the histological examinations were expressed as:

(1) $\frac{\text{Number of kidneys with tubular necrosis}}{\text{Number of kidneys examined}}$

(2) the total "score" (obtained by adding up the individual "necrosis scores" of the kidneys in each treatment group). The "score" for any particular kidney, according to the extent of histological damage, was designated as 0, 5, 10, 20, 30 or 40 units.

RESULTS

Localization of nephron transport of cephaloridine in the dog and rabbit by the method of stop-flow analysis

Dogs. The interpretation of results obtained with the stop-flow technique and the limitations of the method have been discussed by Malvin *et al.* (1958) and Pitts, Gurd, Kessler & Hierholzer (1958). The results presented here have been calculated and displayed according to the conventions established in these first reports.

The urine to plasma concentration ratios (U/P) for Na^+, p-aminohippurate (PAH) and cephaloridine have been corrected for water absorption effects by dividing their observed U/P ratios by the U/P ratio for inulin. The active tubular reabsorption of Na^+ is demonstrated in the stop-flow pattern by a marked reduction in the U/P Na^+// U/P inulin ratio and the secretion of PAH by a marked increase in the U/P PAH//U/P inulin ratio. The Na^+ concentration minimum and the PAH maximum therefore serve to localize the distal and proximal parts of the nephron (Fig. 1). In two early experiments creatinine instead of inulin U/P values were used to correct for water absorption. This practice was discontinued when it was found that creatinine, particularly under the exaggerated conditions of stopped-flow, is itself secreted to a small extent by the proximal tubules of dog kidney. This weak secretory mechanism has been fully described by Swanson & Hakim (1962) and O'Connell, Romeo & Mudge (1962). The

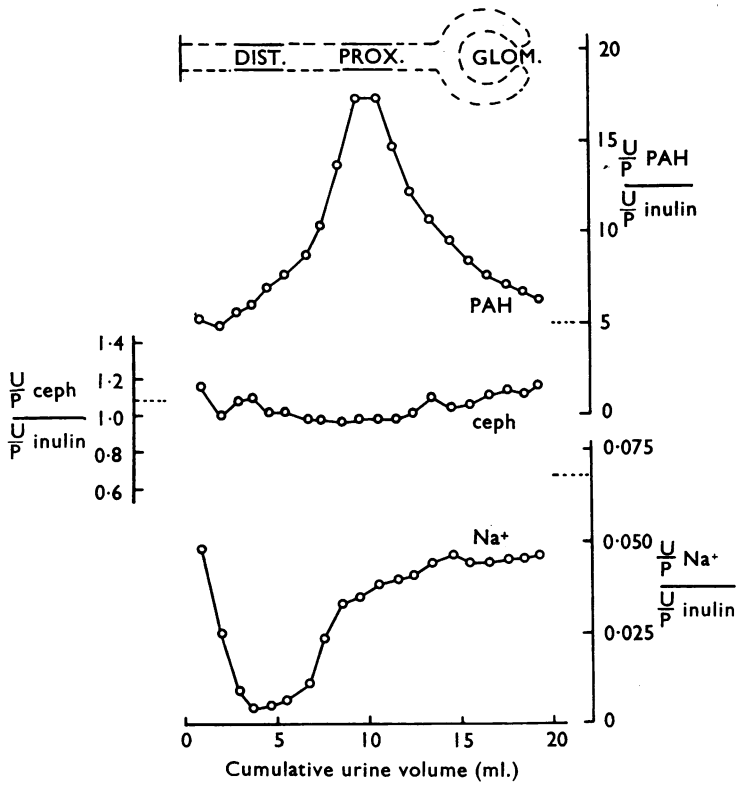


Fig. 1. Stop-flow concentration patterns for p-aminohippurate (PAH), Na+ and cephaloridine (ceph). Dog No. 4, female, 11.3 kg. Dotted lines indicate the free flow values obtained in the pre-occlusion clearance period.

experiments in which creatinine U/P values were used involved only females, in which sex there is very little tubular secretion of creatinine (O'Connell *et al.*, 1962), and consequently the evaluation of cephaloridine stop-flow patterns was probably not materially affected.

Cephaloridine U/P ratios were similar to the inulin U/P values throughout the period of stopped-flow which indicated that neither reabsorption nor secretion was occurring in this species (Fig. 1). The results from nine dogs are summarized in Table 1. In the table the cephaloridine U/P ratios (divided by the inulin U/P ratios) are given both during the " free-flow " pre-occlusion period and during the " stop-flow " period. The stop-flow values were obtained from those points during the stop-flow pattern that coincided with the distal Na^+ reabsorptive minimum and the proximal PAH secretory maximum. During stopped-flow the proximal tubules of dog kidney develop a two- to seven-fold increase in concentration of the organic acid PAH, but at the same proximal site the cephaloridine U/P values do not differ by more than about 0.1 or 0.2 unit from the free-flow values.

Probenecid (an inhibitor of the proximal tubular organic acid secretory mechanism), injected into dog 3 after a control occlusion period, was without effect on the cephaloridine

TABLE 1

STOP-FLOW ANALYSIS EXPERIMENTS IN DOGS

Creatinine was used instead of inulin in dogs 1 and 2. * Treatment administered after control occlusion. Dosage: probenecid 30 mg/kg; 2:4 dinitrophenol 5 mg/kg; mepiperphenidol 10 mg/kg; acetazoleamide 25 mg/kg (+ 0·695% $NaHCO_3$ incorporated in the mannitol infusion). Plasma concentrations of cephaloridine at the mid-point of the control occlusion period were in the range 18·5 to 50·3 μg/ml.

Dog No.	Sex	Body weight (kg)	Treatment*	U/P Na^+ / U/P inulin		U/P PAH / U/P inulin		U/P cephaloridine / U/P inulin		
				Free flow	Stop flow minimum i.e. distal tubule site	Free flow	Stop flow maximum i.e. proximal tubule site	Free flow	Distal tubule site	Proximal tubule site
1	F	11·4		0·247	0·028	3·64	8·90	0·98	0·90	0·86
2	F	14·25		0·270	0·033	3·15	8·88	0·91	0·84	0·71
3	F	12·7	Control period	0·155	0·016	3·78	11·86	1·00	0·93	0·93
			Probenecid	0·198	0·023	1·08	1·26	0·95	0·81	0·83
4	F	11·3		0·068	0·004	4·99	17·32	1·08	1·09	0·97
5	F	10·3		0·177	0·023	2·34	15·70	0·65	0·95	1·00
6	F	13·15	Control period	0·196	0·017	2·11	12·60	0·89	0·79	0·92
			2:4 Dinitrophenol	0·111	0·014	1·62	5·04	0·92	0·80	0·74
7	F	13·1	Control period	0·125	0·011	3·83	11·60	0·97	0·93	0·76
			Mepiperphenidol	0·125	0·012	3·55	9·82	0·84	0·72	0·74
8	F	14·7	Control period	0·167	0·009	3·53	12·70	0·68	0·95	1·02
			Acetazoleamide	0·170	0·020	3·84	9·36	0·68	0·63	0·64

or Na^+ stop-flow patterns, whereas tubular secretion of PAH was reduced (Fig. 2). Similarly, cephaloridine stop-flow patterns were unaltered by mepiperphenidol (an inhibitor of the proximal tubular organic base secretory mechanism), 2:4 dinitrophenol (a metabolic inhibitor) or metabolic alkalosis (induced by acetazoleamide and the infusion of bicarbonate) (Table 1).

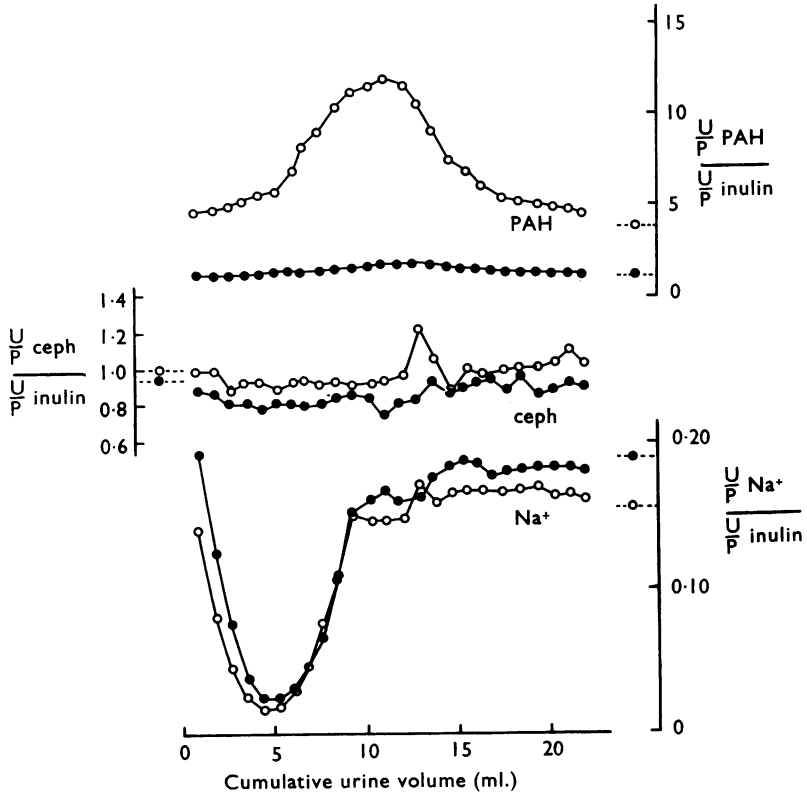


Fig. 2. Stop-flow concentration patterns for p-aminohippurate (PAH), Na+ and cephaloridine (ceph) before and after probenecid treatment. Dog No. 3, female, 12.7 kg. ○——○ =control. ●——● =after probenecid 30 mg/kg i.v. Dotted lines indicate the free flow values obtained in the pre-occlusion clearance periods.

Rabbits. Although stop-flow analysis has been chiefly employed in dogs, the method has been used also in the rat (Williamson, Skulan & Shideman, 1961), monkey (Vander & Cafruny, 1962) and rabbit (Beechwood, Berndt & Mudge, 1964). The stop-flow patterns for inulin, Na^+ and PAH observed in the rabbit experiments reported here were qualitatively similar to those obtained in dogs. The results from eight rabbits are given in Table 2. The U/P values for Na^+ (divided by the inulin U/P values) during "free-flow" and at the distal tubular stop-flow minimum were higher than the corresponding values observed in dogs. This quantitative difference in Na^+ concentration between rabbit and dog may reflect a species difference in the Na^+ reabsorptive mechanism, but

TABLE 2

STOP-FLOW ANALYSIS EXPERIMENTS IN RABBITS

Probenecid (30 mg/kg) was administered 30 min before the occlusion period in rabbit 6. Probenecid (30 mg/kg) was administered after the control occlusion in rabbits 7 and 8. Plasma concentrations of cephaloridine at the mid-point of the control occlusion period were in the range 16·7 to 97·6 μg/ml.

Rabbit No.	Sex	Body weight (kg)	Treatment	U/P Na^+ / U/P inulin		U/P PAH / U/P inulin		U/P cephaloridine / U/P inulin		
				Free flow	Stop flow minimum i.e. distal tubule site	Free flow	Stop flow maximum i.e. proximal tubule site	Free flow	Distal tubule site	Proximal tubule site
1	F	4·61		0·361	0·101	4·21	20·30	0·84	0·81	0·96
2	F	4·25		0·297	0·086	3·31	18·60	0·75	0·73	1·00
3	F			0·161	0·034	3·48	11·90	0·83	0·73	0·96
4	F	4·32		0·377	0·127	3·64	16·50	0·56	0·64	0·62
5	M	2·40		0·291	0·052	3·62	17·60	0·56	0·76	1·24
6	M	2·05	Probenecid	0·167	0·061	2·57	6·06	0·81	0·68	0·73
7	M	2·62	Control period	0·366	0·144	3·37	19·90	0·72	0·64	0·96
			Probenecid	0·365	0·142	1·66	6·10	0·74	0·89	0·82
8	M	2·13	Control period	0·263	0·066	2·50	9·76	0·69	0·90	0·95
			Probenecid	0·320	0·096	1·36	3·54	0·73	0·71	0·76

it might also result from differences in transit time and mixing of the tubular fluid. Williamson *et al.* (1961) noted similar high concentrations of Na^+ in the rat compared with the dog.

Cephaloridine stop-flow patterns in all but one of the rabbits were similar to those obtained in dogs, the cephaloridine U/P values at the proximal site not differing from the free flow values by more than about 0.1 or 0.2 unit (Table 2). During stopped-flow, rabbit kidney developed at the proximal site an increase in concentration of the organic acid PAH similar to that reported in dogs. In one male rabbit (No. 5) a proximal secretory peak was observed in the cephaloridine stop-flow pattern (Fig. 3). The stop-flow value of 1.24 at the proximal site was about twice that existing under free-flow conditions. This rabbit was not treated with probenecid, but administration of probenecid to three other rabbits did not alter the stop-flow patterns appreciably (Fig. 4 and Table 2).

Nephrotoxic action of cephaloridine in the hen and the protective effect of probenecid

The histological grading of the kidneys 48 hr after the various treatments is summarized in Table 3.

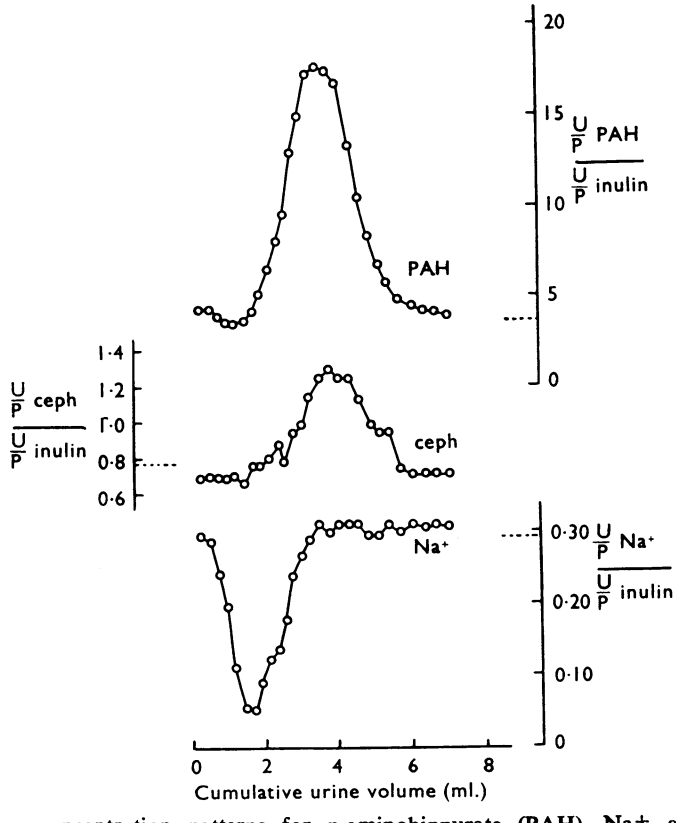


Fig. 3. Stop-flow concentration patterns for p-aminohippurate (PAH), Na^+ and cephaloridine (ceph). Rabbit No. 5, male, 2.40 kg. Dotted lines indicate the free-flow values obtained in the pre-occlusion clearance period.

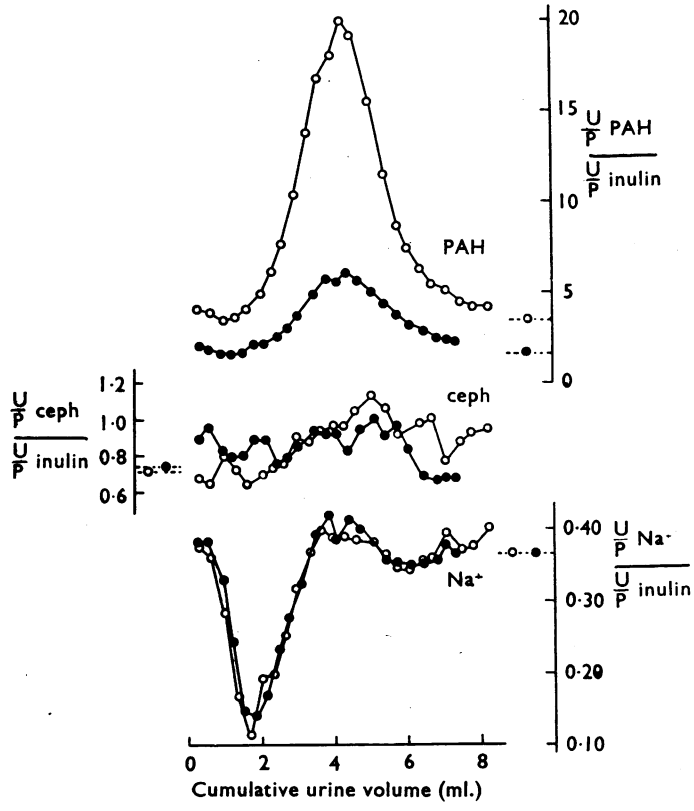


Fig. 4. Stop-flow concentration patterns for p-aminohippurate (PAH), Na^+ and cephaloridine (ceph) before and after probenecid treatment. Rabbit No. 7, male, 2.62 kg. ○——○ = control. ●——● = after probenecid 30 mg/kg i.v. Dotted lines indicate the free-flow values obtained in the pre-occlusion clearance periods.

Isolated degenerating cells were present in the kidneys of the hens that had received saline treatment alone, and little significance was attached to kidneys showing such abnormalities. About half of the kidneys from hens injected with 125, 250 or 500 mg/kg doses of cephaloridine had necrotic tubules. Nephrotoxicity was most severe at the 1000 mg/kg dose level, and two of the four hens that had received this dose died within 30 hr, their kidneys exhibiting massive tubular necrosis.

A 100 mg/kg dose of probenecid, administered intravenously 30 min before cephaloridine injection, prevented the development of nephrotoxicity; furthermore all three probenecid-treated hens survived a 1,000 mg/kg dose of the antibiotic.

Effect of probenecid on the production of renal tubular necrosis in mice

The histological appearance and time course of the proximal renal tubular necrosis produced in mice by cephaloridine and the finding that female mice of the A2G strain were much more sensitive than males to the nephrotoxic effects of cephaloridine have been reported by Atkinson *et al.* (1966).

TABLE 3

NEPHROTOXIC ACTION OF CEPHALORIDINE IN THE HEN AND THE PROTECTIVE EFFECT OF PROBENECID

Saline or cephaloridine was injected intramuscularly into the right leg. Probenecid was administered intravenously into the wing vein 30 min before intramuscular injection of saline or cephaloridine. The hens were killed 48 hr after injection, except hens 11 and 12 which died within 30 hr of cephaloridine treatment. The right kidney of hen 13 was absent. All doses are in mg/kg.

Hen No.	Wt. (kg)	Probenecid dose	Intramuscular treatment	Histology score Left	Right
1	2·5		Saline	−	+
2	2·4		Saline	−	+
3	2·9		Cephaloridine 125	+	−
4	2·6		Cephaloridine 125	−	++
5	2·7		Cephaloridine 250	++	+++
6	2·9		Cephaloridine 250	−	+
7	2·7		Cephaloridine 500	+	+
8	2·8		Cephaloridine 500	++	++
9	2·7		Cephaloridine 1000	++	++
10	2·6		Cephaloridine 1000	++	++
11	2·6		Cephaloridine 1000	+++	+++
12	2·2		Cephaloridine 1000	+++	+++
13	2·5	100	Saline	−	
14	2·1	100	Saline	−	−
15	3·1	100	Saline	+	++
16	2·8	100	Saline	−	+
17	2·2	100	Cephaloridine 250	−	−
18	2·4	100	Cephaloridine 500	+	+
19	2·9	100	Cephaloridine 1000	+	−
20	2·4	100	Cephaloridine 1000	−	+
21	3·0	100	Cephaloridine 1000	−	+

In the present series of experiments cephaloridine (1 g/kg) administered subcutaneously to female mice produced tubular necrosis in about 90% of kidneys. In a preliminary experiment probenecid (100 mg/kg orally) administered at the same time as the cephaloridine completely prevented the development of these histological changes. The time relationship of probenecid protection with respect to cephaloridine treatment is given in Table 4. Probenecid (100 mg/kg) was completely protective when given 6 hr

TABLE 4

PROTECTIVE EFFECT OF A SINGLE DOSE OF PROBENECID AT VARIOUS TIMES BEFORE OR AFTER CEPHALORIDINE TREATMENT

Probenecid (100 mg/kg) was administered to female A2G mice as a single oral dose before or after the subcutaneous injection of cephaloridine (1·0 g/kg).

Treatment	Time of probenecid treatment; before (−) or after (+) cephaloridine (hr)	Renal tubular necrosis No. positive / No. kidneys	Score
Cephaloridine alone	−	20/20	580
Probenecid and cephaloridine	−6	0/10	0
Probenecid and cephaloridine	−4	0/10	0
Probenecid and cephaloridine	−2	0/10	0
Probenecid and cephaloridine	−1	0/10	0
Probenecid and cephaloridine	−½	0/10	0
Probenecid and cephaloridine	0	0/10	0
Probenecid and cephaloridine	+½	6/10	70
Probenecid and cephaloridine	+1	10/10	130
Probenecid and cephaloridine	+2	10/10	250
Probenecid and cephaloridine	+4	10/10	240
Probenecid and cephaloridine	+6	10/10	270

TABLE 5

PROTECTIVE EFFECT OF PROBENECID AGAINST THE RENAL TUBULAR NECROTIZING ACTION OF CEPHALORIDINE

Probenecid was administered to female A2G mice as a single oral dose 30 min before injection of a single subcutaneous dose of cephaloridine. * Eight mice were treated but four died within 48 hr: kidneys were removed from the four survivors. † Eighteen mice were treated but four died within 48 hr: kidneys were taken from 12 of the 14 survivors

	Probenecid (mg/kg p.o.)											
	0		6·25		12·5		25		50		100	
Cephaloridine (g/kg s.c.)	No. positive / No. kidneys	Score	No. positive / No. kidneys	Score	No. positive / No. kidneys	Score	No. positive / No. kidneys	Score	No. positive / No. kidneys	Score	No. positive / No. kidneys	Score
1·0	47/60	990	0/10	0	0/30	0	0/10	0	0/10	0	0/20	0
2·0	9/10	250			6/20	120						
4·0	10/10	290			14/20	250						
8·0	10/10	260			6/20	150						
12·0	8/8*	180			5/24†	30					0/10	0

TABLE 6

EFFECT OF PROBENECID ON URINE PRODUCTION AND CEPHALORIDINE TUBULAR NECROSIS IN HYDRATED MICE

Five mice per group. The probenecid suspension was administered in a dose of 1 ml./20 g = 100 mg/kg. The subcutaneous injection was given immediately after the oral fluid load

Group No.	Oral treatment (1·0 ml./20 g)	Subcutaneous treatment (0·8 ml./20 g)	Cumulative urine volume (ml.)						Renal tubular necrosis	
			1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	No. positive / No. kidneys	Score
1	Distilled water	Saline	0·9	4·5	5·3	5·8	6·0	6·2	0/10	0
2	Distilled water	Cephaloridine 1·0 g/kg	0·4	4·2	6·0	6·1	7·4	7·6	8/10	135
3	Distilled water	Cephaloridine 4·0 g/kg	0·2	0·8	1·6	3·3	4·8	5·4	9/10	270
4	Distilled water	Cephaloridine 12·0 g/kg	0·5	0·8	0·8	1·6	2·2	2·3	10/10	205
5	Probenecid susp.	Saline	0·5	4·2	5·8	7·2	7·6	8·2	0/10	0
6	Probenecid susp.	Cephaloridine 1·0 g/kg	0·2	3·8	5·9	6·7	7·1	7·3	0/10	0
7	Probenecid susp.	Cephaloridine 4·0 g/kg	0	0·6	4·0	4·7	5·8	6·8	0/10	0
8	Probenecid susp.	Cephaloridine 12·0 g/kg	0·2	0·2	0·8	0·8	1·0	1·1	0/10	0

before or at the time of cephaloridine administration. It was only partially protective when administered half an hour after cephaloridine and completely ineffective in reducing the incidence of renal tubular necrosis when administered 1 hr after cephaloridine; the damage "score" at this time was, however, reduced.

The protective effect of probenecid against various doses of cephaloridine is given in Table 5. Tubular necrosis produced by 1 g cephaloridine/kg was prevented by as little as 6.25 mg probenecid/kg and the action of 12 g cephaloridine/kg was completely prevented by 100 mg probenecid/kg. Fifty per cent of the mice treated only with 12 g cephaloridine/kg died within 48 hr, but in the group pre-treated with 12.5 mg probenecid/kg both the tubular necrosis and the number of deaths were reduced. In the group receiving 100 mg probenecid/kg tubular necrosis was completely prevented and all the mice survived.

In an experiment in which groups of mice were hydrated with an oral fluid load of 1 ml./20 g (distilled water or probenecid suspension) and a subcutaneous fluid load of 0.8 ml./20 g (saline or cephaloridine solution), all mice receiving 12 g cephaloridine/kg survived, with or without probenecid treatment (Table 6); only the probenecid-treated groups were, however, completely protected from the tubular necrotising action of cephaloridine. Urine volume after cephaloridine (1 g/kg) or saline was similar, but it was slightly reduced after 4 g cephaloridine/kg and markedly reduced after 12 g cephaloridine/kg. The protective effect of probenecid was not however affected by the oliguria observed in mice that had received 4 or 12 g cephaloridine/kg.

TABLE 7

DRUGS AFFECTING THE RENAL TUBULAR NECROTIZING ACTION OF CEPHALORIDINE IN FEMALE A2G MICE

Doses are in mg/kg. Drugs were administered orally unless otherwise indicated. Each drug was given three times: 6 hr before, 1 hr before and 1 hr after the subcutaneous injection of cephaloridine (1·0 g/kg)

Protective drugs	*Doses*
Probenecid	
p-Carboxybenzenesulphonamide	750
p-Carboxybenzenesulphondichloroamide	200
Benzenesulphonic acid	500
p-Aminobenzenesulphonic acid	500
Benezenesulphonamide	100
p-Aminobenzoic acid	500
o-Aminobenzoic acid	500
Salicylic acid	100
Phenylbutazone	25
Oxyphenylbutazone	25
Sulphinpyrazole	25
Acetazoleamide	100
2:4-Dinitrophenol	10
Benzylpenicillin i.p.	600
Sodium acetrizoate	1000
Pyrazinamide	100
Cinchophen	200

Not protective drugs	*Doses*
p-Aminobenzenesulphonamide	750
Benzoic acid	500
Aniline i.p.	50
p-Aminosalicylic acid	500
p-Aminohippuric acid i.p.	500
Chlorothiazide	500
Hydrallazine	15
2:3-Dimercaptopropanol (BAL) i.p.	20
Acetic acid	500
Mannitol i.p.	500

Effect of other drugs on the renal tubular necrosis produced in mice by cephaloridine

Many agents, including compounds related to probenecid, a number of benzene sulphonic acid and benzoic acid derivatives, and a miscellany of other drugs, also protect female A2G mice against the nephrotoxic effects of cephaloridine administered as a single subcutaneous dose of 1 g/kg. These protective agents are listed in Table 7 together with a list of compounds that failed to protect.

DISCUSSION

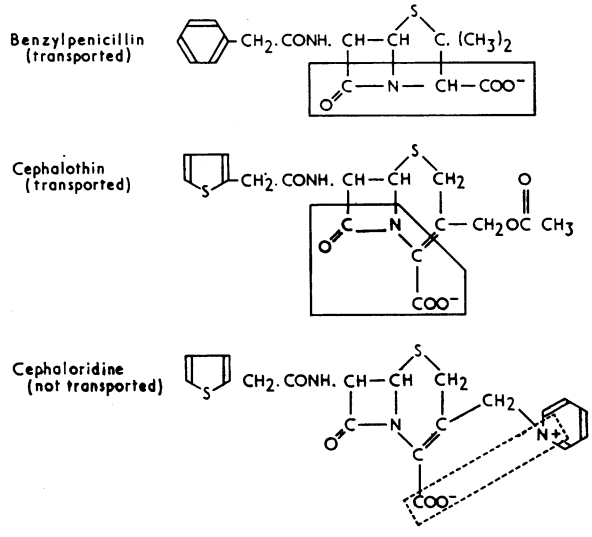
It is concluded that there is no evidence of active renal tubular secretion or reabsorption of cephaloridine in the rabbit or dog, and that urinary excretion in these species is not effected by different mechanisms. The stop-flow technique of Malvin *et al.* (1958) has found wide application in the localization of the nephron transport of solutes whose net urinary excretion may be the resultant of the concomitant processes of glomerular filtration, tubular secretion and tubular reabsorption. However, the cephaloridine stop-flow patterns obtained in dogs are consistent with the conclusions already drawn from renal clearance procedures: that the antibiotic, which is completely filterable, is eliminated solely by glomerular filtration (Child & Dodds, 1966; Welles *et al.*, 1966). Stop-flow analysis failed to reveal trans-tubular movement of cephaloridine in either proximal or distal regions, and the administration of inhibitors of proximal secretory mechanisms failed to alter the concentration patterns. Stop-flow analysis in the rabbit, a species that has received little previous study by this method (Beechwood *et al.*, 1964; Møller, 1965). indicated that the nephron transport of cephaloridine was similar to that in the dog (with the possible exception of one rabbit in the series), and probenecid treatment failed to reveal any component of proximal tubular secretion.

Despopoulos (1965) has reviewed the structural requirements for carboxylic substrates of the renal hippurate transport system. The general structure common to all substrates that are transported is:

$$R\text{-}CO\text{-}NX\text{-}(CHR')_n\text{-}COOH$$

(in which R and R′ may be either aliphatic or aromatic; X is either H or CH_3; when R′=H, "n" may vary from 1 to 5). The carbonyl and carboxyl groups are indispensable for interaction with the receptor.

Benzylpenicillin (Smith, 1951) and the 3-acetoxy analogue of cephaloridine, cephalothin (Lee, Herr & Anderson, 1963), are both avidly secreted by the proximal tubules of dog and other mammals including man. Cephaloridine is cleared by the human kidney more slowly than are the penicillins or cephalothin and probenecid has little effect on serum levels of the antibiotic (Kislak, Steinhauer & Finland, 1966; Tuano, Brodie & Kirby, 1966). During the intravenous infusion of cephaloridine into volunteers Tuano *et al.* (1966) obtained cephaloridine: creatinine clearance ratios of between 0.78 and 1.01 (average 0.89); probenecid treatment lowered clearance ratios to an average value of 0.70 and the authors concluded that a small amount of renal tubular secretion was occurring. Failure to clearly demonstrate renal tubular secretion of cephaloridine in animals and man, despite its close structural relationship to benzylpenicillin and cephalothin, may be explained if in cephaloridine the $-COOH$ group is not available (through interaction with the pyridyl nitrogen) to form a reinforced ionic bond with a locus on the receptor, a requisite for transport by this system.



It has been shown previously using the Sperber technique that the renal tubules of hen kidneys secrete cephaloridine to a small extent and that probenecid blocks this tubular transport (Child & Dodds, 1966). The results recorded here indicate that probenecid is also effective in preventing the development of cephaloridine tubular necrosis in hens. Since the hen possesses a renal portal circulation (Sperber, 1960), the injection of a substance into the leg causes that substance to perfuse the renal tubules before it reaches the systemic circulation, and if that substance is secreted by the renal tubules its rate of excretion will be higher on the injected side. Cephaloridine is differentially excreted in this way. In these experiments cephaloridine was injected into the right leg muscle and, if the hypothesis was valid that necrosis resulted from the intracellular accumulation of cephaloridine during tubular secretion, it was at least theoretically possible that the ipsilateral kidney would exhibit more necrosis than the contralateral kidney. Support for this hypothesis was not obtained in these experiments as necrosis was usually bilateral and of similar degree.

Many diverse chemical agents have been shown to produce a proximal renal tubular necrosis; these include mercuric chloride, uranyl nitrate, potassium dichromate (Smith, 1951; Allen, 1962), potassium tartrate, potassium tetrathionate (Smith, 1951), bacitracin (Genkins, Uhr & Bryer, 1954), chloroform (Hewitt, 1956). The mechanism of action of these nephrotoxic agents is complex and in most cases little understood. Necrosis induced by mercuric chloride may be prevented by treatment with 2,3-dimercaptopropanol (BAL), which is presumed to act by freeing protein sulphydryl groups from combination with mercury (Smith, 1951; Allen, 1962). It is significant that cephaloridine necrosis in mice, which is histologically similar to that produced by mercuric chloride treatment, cannot be prevented by administration of 2,3-dimercaptopropanol. Mannitol, which is protective in certain forms of experimental acute renal failure (Mason, Bowler & Brown, 1961; Parry, Schaefer & Mueller, 1963; Teschan & Lawson, 1966) was also ineffective.

The suggestion that renal tubular secretion of cephaloridine might be responsible for the development of nephrotoxicity, tentatively put forward to explain the protective effect of probenecid in the mouse and hen, is not fully consistent with the results obtained in the mouse experiments described above. A number of compounds including sulphonic acids, sulphonamides, phenylbutazone, acetazoleamide, pyrazinamide and penicillin, which are transported by (Weiner & Mudge, 1964; Despopoulos, 1965) and can, therefore, behave as competitive inhibitors in the system were protective against cephaloridine tubular necrosis. Chlorothiazide, PAH and p-amino-salicylic acid, compounds which are also secreted by the tubules (Weiner & Mudge, 1964), failed to protect mice even when administered in large doses. Ortho- and para-aminobenzoic acids which are not actively excreted by the tubules (Knoefel, Huang & Despopoulos, 1959) were protective; benzoic acid which is not secreted by the tubules (Knoefel & Huang, 1956) was not protective.

Findings inconsistent with the suggestion that probenecid and other drugs protect through inhibition of the renal tubular secretion of cephaloridine might be explained as resulting from inadequate dosage or from species differences in metabolism or renal transport. The anomalous results in mouse protection experiments, taken in conjunction with failure to observe renal tubular secretion of cephaloridine in the rabbit, the most sensitive species, indicate that the nephrotoxic action of cephaloridine and the properties of protective drugs require different explanations.

SUMMARY

1. Urinary concentration patterns for cephaloridine have been obtained in dogs and rabbits by the method of stop-flow analysis.

2. No evidence of proximal or distal tubular sites of reabsorption or secretion was obtained in the dog. No consistent evidence of proximal renal tubular secretion was obtained in the rabbit, and the possibility that such tubular transport might be responsible for the development of cephaloridine nephrotoxicity in this species is therefore unlikely.

3. The structural requirements for substrates of the proximal renal tubular organic acid transport system have been discussed in relation to the cephaloridine molecule.

4. In the hen probenecid treatment can both inhibit the renal tubular transport of cephaloridine and prevent the development of renal tubular necrosis.

5. Mice may be protected against the renal tubular necrotizing action of cephaloridine by oral treatment with probenecid. Treatment with a number of other drugs affords similar protection to mice. The possible role of renal tubular secretion in the development of cephaloridine nephrotoxicity is considered in relation to the action of these compounds.

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