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Effect of Piperonyl Butoxide on Organic Anion and Cation Transport in Rabbit Kidneys (41720)

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Abstract. Piperonyl butoxide has been shown to reduce accumulation of cephaloridine in rabbit renal cortex; however, the mechanism responsible for this effect remains unclear. Cephaloridine is a zwitterion and its accumulation in renal cortex has been suggested to be regulated by both organic anion and cation transport systems. Thus, it was of interest to determine the effect of piperonyl butoxide on renal transport of *p*-aminohippurate (PAH, an organic anion) and tetraethylammonium (TEA, an organic cation). Although pretreatment with piperonyl butoxide markedly inhibited renal cortical uptake of cephaloridine, the same treatment had less inhibitory effect on either PAH or TEA uptake. Efflux of PAH from preloaded renal cortical slices was enhanced by pretreatment with piperonyl butoxide; however, TEA efflux was unaffected. Thus, piperonyl butoxide appears to have effects on renal membrane functions which result in differential effects on PAH, TEA, and cephaloridine transport.

Cephaloridine, a prototype of the commonly used semisynthetic cephalosporin antibiotics, is known to accumulate selectively in the renal cortex (1) and produce necrosis of renal proximal tubular cells (2–4). Accumulation of cephaloridine in the renal cortex occurs by active transport (1) and this process can be inhibited by other organic anions such as probenecid and *p*-aminohippurate (5, 6). However, unlike *p*-aminohippurate, cephaloridine is a zwitterion; it has a positive charge on the quarternary nitrogen of the pyridinium substituent on the β-lactam ring in addition to the anionic carboxyl group. Therefore, cephaloridine transport may also be influenced by organic cations in the proximal tubular cells. Wold and Turnipseed (7) reported that cyanine (an organic cation transport inhibitor) decreased the efflux of cephaloridine from renal cortical cells. This observation, accompanied with the findings described previously (1, 5, 6), indicates that renal cortical accumulation of cephaloridine may be regulated by both organic anion and cation transport systems.

McMurty and Mitchell (8) reported that cephaloridine nephrotoxicity in rats was reduced by piperonyl butoxide, a mixed-function oxidase inhibitor (9). Similarly, Tune *et al.* (10) demonstrated that piperonyl butoxide decreased cephaloridine nephrotoxicity in rabbits. However, this compound also decreased accumulation of cephaloridine in renal cortical slices and in the renal cortex *in vivo* (10). Thus, it is possible that the blunting effect of piperonyl butoxide on cephaloridine nephrotoxicity could result from decreased renal cortical concentration of cephaloridine. Since cephaloridine accumulation in renal cortex has been suggested to be regulated by both organic anion and cation transport, it was of interest to determine the effects of piperonyl butoxide on the renal uptake and efflux of *p*-aminohippurate (an organic anion) and tetraethylammonium (an organic cation), to gain a better understanding of the renal handling of cephaloridine.

Methods. *Animals.* Female New Zealand white rabbits (1.5–2.5 kg) were purchased from a local breeder, housed in a light- (12 hr light:12 hr dark) and temperature-controlled room and allowed free access to water and food until use.

In vitro effect of piperonyl butoxide on PAH and TEA transport. Renal cortical slices were prepared from untreated animals and incubated in 4.0 ml of phosphate-buffered medium (11) for 90 min at 25°C under 100% O_2 in a Dubnoff metabolic incubator. Incubation media contained 7.4×10^{-5} *M* PAH, 1.0×10^{-5} *M* [^{14}C]TEA, and various concentrations of piperonyl butoxide (suspended in 1%

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TABLE I. EFFECT OF PIPERONYL BUTOXIDE AND TWEEN 80 ON ACCUMULATION OF *p*-AMINOHIPPURATE AND TETRAETHYLAMMONIUM IN RABBIT RENAL CORTEX SLICES[a]

Tween 80[b] (μg/ml)	Piperonyl butoxide[b] (mg/ml)	PAH S/M		TEA S/M	
		−PB	+PB	−PB	+PB
0	0	14.18 ± 0.92	—	13.55 ± 0.90	—
0.31	0.012	13.40 ± 1.00	9.99 ± 1.15[c]	12.41 ± 0.75	10.04 ± 0.80
3.1	0.12	13.92 ± 1.15	7.53 ± 0.92[c]	13.28 ± 0.63	7.75 ± 0.22[c]
31	1.2	13.76 ± 0.86	6.22 ± 0.84[c]	13.71 ± 1.00	6.75 ± 0.35[c]
310	12	13.84 ± 0.31	3.97 ± 0.56[c]	15.69 ± 0.51	4.76 ± 0.51[c]

[a] Renal cortical slices prepared from nontreated rabbits were incubated in 4.0 ml of phosphate-buffered medium containing 7.4×10^{-5} *M* PAH and 1.0×10^{-5} *M* TEA under 100% O_2 at 25°C for 90 min. Different concentrations of piperonyl butoxide were present in the incubation media. Each value represents mean ± SE of six animals.

[b] Piperonyl butoxide was suspended in 1% Tween 80 and different amounts of the piperonyl butoxide suspension (375 mg/ml) were added to incubation media (+PB). In the control media, only equivalent amounts of 1% Tween 80 were added (−PB).

[c] Significantly different from slices incubated in the medium containing an equivalent concentration of Tween 80 but without piperonyl butoxide ($P < 0.05$).

Tween 80). After incubation, slices were removed, blotted, weighed, homogenized in 3.0 ml of 10% TCA, and brought to a final volume of 10 ml with water. Two milliliters of incubation medium was treated similarly. The samples were centrifuged; 1.0 ml of slice or medium supernatant was added to 10 ml of ACS counting cocktail (Amersham, Arlington Heights, Ill.) and radioactivity was determined in a Searle Delta 300 liquid scintillation spectrometer. PAH was determined by the method of Smith *et al.* (12). The accumulation of PAH and TEA in renal cortical slices was expressed as a slice-to-medium (S/M) concentration ratio, where S represents milligrams PAH or TEA per gram of tissue and M represents milligrams of PAH or TEA per milliliter of medium.

In vivo effect of piperonyl butoxide on PAH and TEA transport. Animals received a single ip injection of 750 mg/kg of piperonyl butoxide in 1% Tween 80 (vehicle). Control animals received the vehicle alone. Forty-five or ninety minutes later animals were killed and renal cortical slices were prepared for determination of uptake and runout of PAH and TEA. For the determination of PAH and TEA uptake, renal cortical slices were incubated in 4.0 ml of phosphate-buffered medium containing 7.4×10^{-5} *M* PAH and 1.0×10^{-5} *M* [^{14}C]TEA at 25°C under 100% O_2 for 15, 30, 60, or 90 min. After incubation, concentrations of PAH and TEA in the slices and media were determined as described previously. PAH and TEA runout from control and piperonyl butoxide-pretreated renal cor-

TABLE II. EFFECT OF *IN VIVO* PIPERONYL BUTOXIDE PRETREATMENT (45 min) ON *p*-AMINOHIPPURATE AND TETRAETHYLAMMONIUM ACCUMULATION IN RABBIT RENAL CORTICAL SLICES[a]

Incubation time (min)	PAH S/M		TEA S/M	
	Control	Treated	Control	Treated
15	4.63 ± 0.50	3.50 ± 0.46	4.76 ± 0.64	3.83 ± 0.45
30	6.91 ± 0.38	4.71 ± 0.65[b]	6.84 ± 0.29	5.15 ± 0.49[b]
60	12.32 ± 1.58	9.48 ± 0.65	11.97 ± 1.40	9.01 ± 0.57
90	17.34 ± 2.41	13.10 ± 0.56	16.16 ± 1.69	11.94 ± 0.84[b]

[a] Female New Zealand rabbits received a single ip injection of 750 mg/kg of piperonyl butoxide in 1% Tween 80. Control animals received 1% Tween 80 (vehicle) alone. Animals were killed 45 min later. Each value represents mean ± SE of four animals.

[b] Significantly different from control animals ($P < 0.05$).

TABLE III. EFFECT OF *IN VIVO* PIPERONYL BUTOXIDE PRETREATMENT (90 min) ON *p*-AMINOHIPPURATE AND TETRAETHYLAMMONIUM ACCUMULATION IN RABBIT RENAL CORTICAL SLICES[a]

Incubation time (min)	PAH S/M[b]		TEA S/M[b]	
	Control	Treated	Control	Treated
15	4.21 ± 0.34	3.51 ± 0.32	4.58 ± 0.34	3.94 ± 0.41
30	6.54 ± 0.44	6.42 ± 0.73	6.88 ± 0.31	6.07 ± 0.37
60	10.25 ± 0.56	9.84 ± 0.49	10.64 ± 0.58	10.04 ± 0.54
90	11.96 ± 0.77	12.76 ± 1.05	13.31 ± 0.67	12.10 ± 1.17

[a] Female New Zealand rabbits received a single ip injection of 750 mg/kg of piperonyl butoxide in 1% Tween 80. Control animals received 1% Tween 80 (vehicle) alone. Animals were killed 90 min later and renal cortical slices were prepared and incubated in a phosphate-buffered medium containing 7.4×10^{-5} *M* PAH and 1.0×10^{-5} *M* TEA under 100% O_2 for 15, 30, 60, and 90 min.

[b] Accumulation of PAH or TEA in renal cortical slices is expressed as the concentration of PAH or TEA in slices to the concentration in the medium ratio. Each value represents mean ± SE of seven or more animals.

tical slices also was determined according to the method of Farah *et al.* (13). Measurements of PAH or TEA runout were performed with a pair of animals (one control and one treated) each time under the same incubation condition for at least four times. Differences between the control and treated were compared with Student's paired *t* test. Slices (approximately 500 mg) were preloaded with PAH or TEA by incubating in 6 ml of medium with 6.0×10^{-4} *M* [^{3}H]PAH (sp act, 1.0 Ci/mole) or 6.0×10^{-4} *M* [^{14}C]TEA (sp act, 1.0 Ci/mole) for 90 min. Slices were removed, rinsed, and transferred at 1-min intervals through a series of beakers containing 4.0 ml of PAH and TEA-free medium. The media in the runout beakers and the slices were counted for [^{3}H]PAH or [^{14}C]TEA and the results were expressed as disintegrations per minute PAH or TEA per microgram of tissue. The efflux rate constants were calculated following the methods of Farah *et al.* (13).

In the second series of experiments, animals received piperonyl butoxide (750 mg/kg) 30 or 90 min prior to infusion with a solution containing PAH and inulin according to the method of Tune and Fernholt (1). Rabbits were given a priming dose of 17.5 mg/kg of PAH and 2.0 ml of 10% inulin solution via an ear vein. A solution containing 10% inulin and 20 mg/ml PAH was then infused iv at a rate of 0.11 ml/min. After 1 hr of infusion the animals were killed by cervical dislocation. Blood was collected and allowed to clot for 1 hr at room temperature and serum was prepared by centrifugation at 2000*g* for 10 min. A 0.25-ml aliquot of serum was mixed with 0.6 ml 10% TCA, brought to a final volume of 2.0 ml with distilled water, and centrifuged at 2000*g* for 10 min and the precipitate dis-

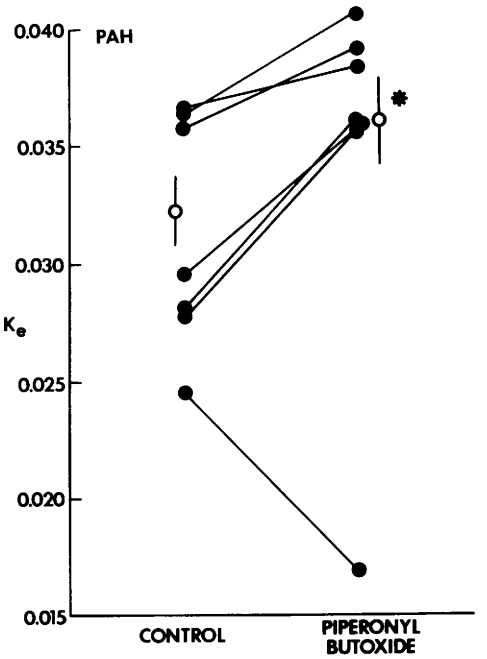

FIG. 1. Effect of *in vivo* treatment with piperonyl butoxide (90 min) on PAH runout in rabbit renal cortical slices. Rabbits received a single ip injection of 750 mg/kg piperonyl butoxide or 1% Tween 80 alone (control) and were killed 90 min later. Open circles and vertical bars represent means ± SE of seven animals. The runout rate constants (K_e) for control and piperonyl butoxide treated are 0.0322 ± 0.0015 and 0.0360 ± 0.0019, respectively.

carded. Portions of renal cortex and liver were homogenized with 20 vol of 3% TCA and centrifuged at 2000*g* for 10 min. The resulting supernatants from tissue and serum samples were then used to determine PAH concentrations by the method of Smith *et al.* (12) and the tissue supernatants were also used to measure inulin concentrations following the method of Schreiner (14). In addition, a 0.1-ml aliquot of serum was mixed with 0.2 ml of 0.75 *N* NaOH, 0.2 ml of $ZnSO_4$ in H_2SO_4 (100 g of $ZnSO_4 \cdot 7H_2O$ and 40 ml of 6 *N* H_2SO_4 diluted to 1000 ml with water) and 1.5 ml distilled water and centrifuged at 2000*g* for 20 min. The supernatants were then analyzed for inulin concentrations according to the method of Walser *et al.* (15).

Statistics. The data were analyzed by Student's *t* test or analysis of variance, completely randomized design, and treatment means were compared with the least-significant-difference test (16). The 0.05 level of probability was used as the criterion of significance.

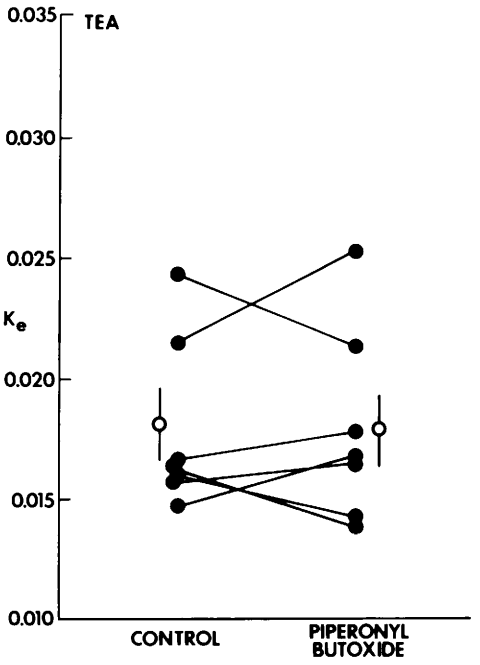

FIG. 2. Effect of *in vivo* treatment with piperonyl butoxide (90 min) on TEA runout in rabbit renal cortical slices. Animals were treated as described in Fig. 1. The runout rate constants (K_e) for control and piperonyl butoxide treated are 0.0181 ± 0.0014 and 0.0179 ± 0.0014, respectively.

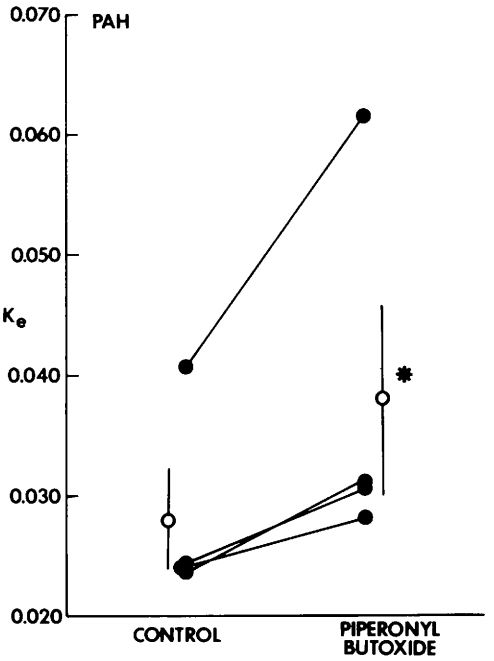

FIG. 3. Effect of *in vivo* treatment with piperonyl butoxide (45 min) on PAH runout in rabbit renal cortical slices. Rabbits received a single ip injection of 750 mg/kg of piperonyl butoxide or 1% Tween 80 alone and were killed 45 min later. Open circles and vertical bars represent means ± SE of four animals. The runout rate constants (K_e) for control and piperonyl butoxide treated are 0.0281 ± 0.0041 and 0.0378 ± 0.0079, respectively.

Results. Direct addition of piperonyl butoxide into the incubation medium decreased PAH and TEA accumulation by renal cortical slices in a dose-dependent manner; Tween 80 alone did not have any significant effect on PAH or TEA accumulation (Table I).

Systemic administration of piperonyl butoxide appeared to have different effects on PAH and TEA transport. Since concentrations of piperonyl butoxide in the kidneys will decline with time following administration, two time points (45 and 90 min after administration of piperonyl butoxide) were chosen for the determination of the effect of piperonyl butoxide on PAH and TEA transport. Slices from the animals treated with piperonyl butoxide 45 min prior to examination appeared to accumulate less PAH and TEA (Table II). However, slices from animals treated with piperonyl butoxide 90 min previously did not accumulate less PAH or TEA than slices from

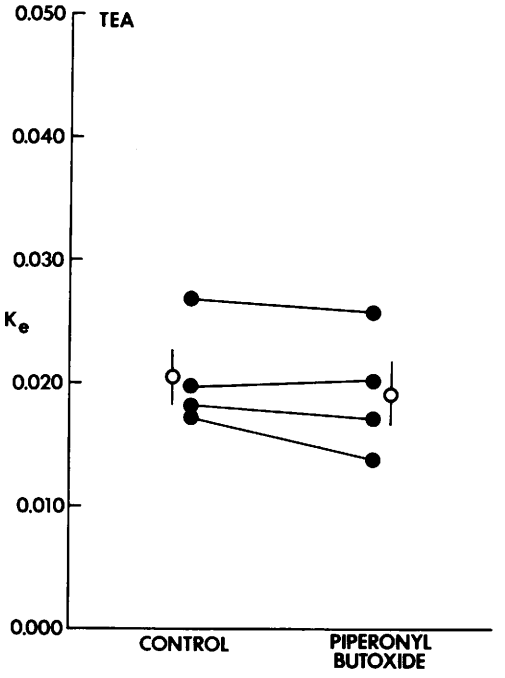

FIG. 4. Effect of *in vivo* treatment with piperonyl butoxide (45 min) on TEA runout. Animals were treated as described in Fig. 3. The runout rate constants (K_e) for control and piperonyl butoxide treated are 0.0204 ± 0.0022 and 0.0192 ± 0.0026, respectively.

controls (Table III). Similar treatment had differential effects on the efflux of PAH and TEA; pretreatment with piperonyl butoxide significantly increased the efflux of PAH from the slices (Figs. 1 and 3) but had no significant effect on TEA efflux (Figs. 2 and 4).

Systemic administration of piperonyl butoxide did not significantly alter accumulation of PAH and inulin in renal cortex *in vivo* (Tables IV and V).

Discussion. Piperonyl butoxide is known to inhibit microsomal mixed-function oxidase systems (9, 17, 18), but little information is available regarding its effect on renal transport. Piperonyl butoxide markedly inhibited both PAH and TEA accumulation by renal cortical slices when added directly to the incubation medium (Table I), but systemic administration of piperonyl butoxide had little effect on PAH and TEA transport (Tables II–V). This discrepancy between *in vitro* and *in vivo* treatment with piperonyl butoxide may be due to different concentrations of piperonyl butoxide in the renal cortical cells. Although a significant amount of piperonyl butoxide (750 mg/kg) was administered systemically, the compound is deposited in the fat and lung as well as being metabolized and excreted (8). Thus, its concentration in the renal cortex may be too low to express profound inhibitory effects on PAH and TEA accumulation. This is probably not the case, however, for the same treatment regimen significantly reduced cephaloridine accumulation. Furthermore, the limited inhibitory effect of piperonyl butoxide

TABLE IV. EFFECT OF PIPERONYL BUTOXIDE (90 min) ON PAH AND INULIN ACCUMULATION IN RABBIT RENAL CORTEX *IN VIVO*[a]

Pretreatment	Concentration (μg/ml)	Concentration (μg/g)	Cortex-to-serum ratio
	Serum PAH	Cortical PAH	
None[b]	36.5 ± 5.3	190.4 ± 14.5	5.76 ± 1.14
1% Tween 80	48.8 ± 2.5	247.6 ± 48.4	5.51 ± 0.92
Piperonyl butoxide	44.6 ± 5.6	242.5 ± 38.8	6.92 ± 1.05
	Serum inulin	Cortical inulin	
None[b]	1767 ± 293	1716 ± 158	1.03 ± 0.11
1% Tween 80	2096 ± 228	2700 ± 248	1.46 ± 0.19
Piperonyl butoxide	1876 ± 174	2223 ± 231	1.28 ± 0.29

[a] Animals received piperonyl butoxide (750 mg/kg) 90 min prior to a single iv injection of 17.5 mg/kg of PAH and 2 ml of 10% inulin, followed by a 1-hr infusion with 10% inulin and 20 mg/ml of PAH at an infusion rate of 0.11 ml/min. Control animals received 1% Tween 80 prior to infusion with inulin and PAH. Each value represents mean ± SE of four or more animals.

[b] Animals did not receive piperonyl butoxide or 1% Tween 80 before PAH and inulin infusion.

TABLE V. EFFECT OF PIPERONYL BUTOXIDE (30 min) ON PAH AND INULIN ACCUMULATION IN RABBIT RENAL CORTEX[a]

Pretreatment	Concentration (μg/ml)	Concentration (μg/g)	Cortex-to-serum ratio
	Serum PAH	Cortical PAH	
1% Tween 80	42.1 ± 15.1	377.6 ± 130.0	8.77 ± 1.32
Piperonyl butoxide	66.0 ± 12.7	518.9 ± 124.0	7.88 ± 0.77
	Serum inulin	Cortical inulin	
1% Tween 80	1367 ± 508	2471 ± 744	1.98 ± 0.17
Piperonyl butoxide	1532 ± 332	2572 ± 490	1.72 ± 0.10

[a] Animals received piperonyl butoxide (750 mg/kg) 30 min prior to a single iv injection of 17.5 mg/kg of PAH and 2 ml of 10% inulin, followed by a 1-hr infusion with 10% inulin and 20 mg/ml of PAH at an infusion rate of 0.11 ml/min. Control animals received 1% Tween 80 prior to infusion with inulin and PAH. Each value represents mean ± SE of four or more animals.

on PAH and TEA transport in kidney slices from animals killed 45 min following piperonyl butoxide administration was greater than that in animals killed 90 min after piperonyl butoxide suggesting a higher concentration of piperonyl butoxide was present in the kidney at 45 min than at 90 min.

The different magnitudes of inhibition of PAH and cephaloridine transport by piperonyl butoxide could be explained by several different mechanisms. First, cephaloridine and PAH may share the same transport mechanism but have different affinities for carriers. Thus, under the present treatments, piperonyl butoxide may inhibit the transport of PAH and cephaloridine to different degrees. Furthermore, in the present experiment, *in vivo* renal cortical accumulation of PAH was measured under steady state conditions. Thus, a constant high concentration of PAH was present in the blood, possibly masking the inhibitory effect of piperonyl butoxide on PAH accumulation. In contrast, renal cephaloridine accumulation was measured at selected time points following a single dose of cephaloridine (10). Under these conditions, serum concentration of cephaloridine was declining, thus a decreased accumulation of cephaloridine due to piperonyl butoxide would be detected more readily.

Although piperonyl butoxide had similar effects on PAH and TEA uptake, it had different effects on PAH and TEA runout. Piperonyl butoxide increased PAH efflux but not that of TEA. The reason for the lack of effect on organic base efflux is not known. Piperonyl butoxide may enhance the efflux of organic acids at the antiluminal membrane and leave the luminal membrane unaltered. Because the efflux of PAH in the kidney *in vivo* is far greater across the luminal than across the antiluminal membrane, the stimulatory effect on PAH efflux produced by piperonyl butoxide may not be seen in *in vivo* studies. In contrast, in kidney slices, the lumens of the tubules are frequently collapsed; thus, transport of organic compounds at the luminal membrane is highly restricted. Under this circumstance, efflux of PAH at the antiluminal membrane becomes significant for the determination of net accumulation of PAH in the slices. This may explain why piperonyl butoxide has a more profound effect on PAH transport in kidney slices than the kidney *in situ*. In contrast, cephaloridine, unlike PAH, does not readily cross the luminal membrane (19). Because of this luminal block, cephaloridine concentrations in the proximal tubular cells appear to be determined by the net balance between active transport into the cells at the antiluminal side and subsequent efflux from the cells also at the antiluminal side. Consequently, the presence (the kidney *in situ*) or absence (kidney slices) of transport of organic acids at the luminal membrane does not have a significant effect on cephaloridine concentration in the proximal tubular cells. Therefore, effects of piperonyl butoxide on

cephaloridine accumulation can be detected both in slices and *in vitro* and the kidney *in vivo* (10).

In summary, piperonyl butoxide has some inhibitory effects on renal organic anion and cation transport. These effects may partially explain the inhibitory effect of piperonyl butoxide on renal cortical accumulation of cephaloridine.

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