[8]. Although no complexes of josamycin with cytochrome P-450 have been described [10], a nitroso derivative may be formed which reacts with GSH but not with the iron of cytochrome P-450 because of steric hindrance. It is conceivable that a labile adduct with GSH may be split by free GSH to the hydroxylamine and GSSG [17]. Since the capacity of the hepatocyte to reduce GSSG is limited and excess GSSG is actively extruded from the cell, this mechanism could result in the depletion of intracellular GSH. If the adduct of troleandomycin with GSH was somewhat less labile, the re-utilization of the constituent amino acids would be less efficient, this accounting for the more severe depletion of GSH in fed rats administered troleandomycin. Alternatively, josamycin and troleandomycin might increase the efflux of GSG from the liver. Additional studies are required to clarify the mechanism of the increased consumption of hepatic GSH.

In summary, our study demonstrates that josamycin which does not form cytochrome P-450-nitroso complexes *in vitro* or *in vivo* nevertheless decreases hepatic GSH and markedly increases hepatic GSH turnover like troleandomycin which is known to form a cytochrome P-450-metabolite complex. In spite of an increased utilization following the administration of josamycin and troleandomycin the synthesis of GSH is maintained for a prolonged period of time suggesting that the constituent amino acids are re-utilized for the synthesis of GSH. Thus, a substantial portion of the increased consumption of hepatic GSH may be due to the loss of free GSH or GSSG from the liver rather than a loss by formation of a stable GSH-adduct with the macrolide antibiotic.

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REFERENCES

1. J. P. Miguet, D. Vuitton, D. Pessayre, H. Allemand, J. M. Metreau, R. Poupon, J. P. Capron and F. Blanc. *Ann. intern. Med.* **92**, 434 (1980).
2. A. C. Hayton, *N.Z. Med. J.* **69**, 42 (1969).
3. M. Weinberger, D. Hudgel, S. Spector and C. Chidsey, *J. Allergy clin. Immunol.* **59** 228 (1977).
4. C. Dravet, E. Mesdjian, B. Cenraud and J. Roger, *Lancet* **i**, 810 (1977).
5. D. Pessayre, D. Larrey, J. Vitaux, P. Breil, J. Belghiti and J. P. Benhamou, *Biochem. Pharmac.* **31**, 1699 (1982).
6. S. J. Szefler, J. Q. Rose, E. F. Ellis, S. L. Spector, A. W. Green and W. J. Jusko, *J. Allergy clin. Immunol.* **66**, 447 (1980).
7. D. Pessayre, V. Descatoire, M. Konstantinova-Mithcheva, J. C. Wandscheer, B. Cobert, R. Level, J. P. Benhamou, M. Jaouen and D. Mansuy, *Biochem. Pharmac.* **30**, 553 (1981).
8. D. Pessayre, M. Tinel, D., Larrey, B. Cobert, C. Funck-Bretano and G. Babany, *J. Pharmac. exp. Ther.* **224**, 685 (1983).
9. D. Larrey, M. Tinel and D. Pessayre, *Biochem. Pharmac.* **32**, 1487 (1983).
10. J. L. Brazier, J. Kofman, G. Faucon, M. Perrin-Fayolle, A. Lepape and R. Lanoue, *Therapie* **35**, 545 (1980).
11. G. L. Ellman, *Archs Biochem. Biophys.* **82**, 70 (1959).
12. B. H. Lauterburg, Y. Vaishnav, W. G. Stillwell and J. R. Mitchell, *J. Pharmac. exp. Ther.* **213**, 54 (1980).
13. B. H. Lauterburg and J. R. Mitchell, *J. clin. Invest.* 67, 1415 (1981).
14. G. L. Newton, R. Dorian and R. C. Fahey, *Analyt. Biochem.* **114**, 383 (1981).
15. F. Tietze, *Analyt. Biochem.* **27**, 502 (1969).
16. B. H. Lauterburg and J. R. Mitchell, *Hepatology* **2**, 8 (1982).
17. D. Dölle, W., Töpner and H. G. Neumann, *Xenobiotica* **10**, 527 (1980).

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In vivo renal tubular secretion of trimethoprim without metabolism

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Because it is potent and concentrates in the renal cortex, the anti-folate agent trimethoprim (TMP) is commonly used as a urinary tract anti-infective drug. There is evidence that this drug enters the urine via a secretory process [1, 2]. Recently, we examined the mechanisms by which TMP is taken up by incubated slices of rat renal cortex [3]. The results indicated the participation of active transport, probably via the organic cation system.

Although TMP is metabolized by the liver [4] and some metabolites are reported active as antibacterials [5], no metabolism of TMP by the slices was evident. The experiments reported here were designed to extend these *in vitro* results by quantifying TMP excretion *in vivo* using the Sperber chicken preparation, a model for the study of tubular transport and metabolism in an unanesthetized animal.

Materials and methods

The theoretical details of the Sperber method have been described previously [6, 7]. Briefly, the method takes advantage of two anatomical peculiarities of birds: lack of a urinary bladder (each ureter draining separately to the outside) and a functional venous portal circulation supplying the peritubular circulation but not the arterial glomerular vessels. Since the renal portal vessels to each kidney are supplied separately with blood by veins draining the ipsilateral leg, it is possible to infuse a test substance into the saphenous vein of one leg and expose that substance to the tubules but not the glomeruli of the same-side kidney before it enters the general circulation. First-pass tubular extraction of the test substance by the infused-side kidney can be quantified as an "apparent tubular excretion fraction" (ATEF), calculated as: $^{I-C}/_{Inf}$ where I = total amount excreted in infused side urine/min, C = amount excreted in control side urine/min and Inf = amount infused/min. In practice, the ATEF of the test substance must be corrected for variable shunting of leg blood away from the portal veins which reduces the amount of infused substance actually entering the kidney. This is accomplished by factoring the ATEF of the test substance by the ATEF of a co-infused blood flow marker such as the anion *p*-aminohippuric acid (PAH) or the cation tetraethylammonium (TEA), both of which are nearly completely extracted in a single pass through the portal vessels. The resulting corrected ATEF

value is termed the tubular excretion ratio (TER). For theoretical reasons, passive diffusion can account for a TER of no greater than about 0.08 [6].

The chickens used in the current study were mature Rhode Island Red laying hens of about 2.5 kg body weight. They were awake but lightly restrained with a harness during the experiments. In a typical experiment, unlabeled TMP (Sigma Chemical Co., St. Louis, MO), [^{3}H]TMP (60.3 mCi/mmol, a gift of Hoffmann-LaRoche, Nutley, NJ) and a ^{14}C-labeled blood flow marker (PAH, 57.6 mCi/mmol or TEA, 4.5 mCi/mmol, NEN Research Products, Boston, MA) were infused via size 50 polyethylene tubing inserted into the saphenous vein. In some experiments, unlabeled PAH was infused, and this was analyzed colorimetrically [8]. Thirty minutes after the start of the infusion, a series of six 5-min urine samples was collected separately from each kidney. This was facilitated by sewing small plastic funnels over each ureteral opening [6]. In some experiments, a co-infusion of a potential transport inhibitor was then introduced via a separate line as a supplement to the continuing [^{3}H]TMP/[^{14}C]marker infusion. After 30 min, a second series of urine samples was collected. Radioactivity in urine samples and infusion solution was quantified by standard methods of liquid scintillation spectrometry including corrections for quench. To test for the presence of TMP metabolites, chloroform extracts of the urines were analyzed by thin-layer chromatography as previously described [4]. Statistical significance ($P < 0.05$, $N = 4$–5) was assessed with Student's t-test.

Results and discussion

Extent of TMP tubular transport and metabolism. The mean (± SD) TER value for TMP during simultaneous infusion of TMP and a blood flow marker (TEA or PAH) into thirteen chickens was 0.301 ± 0.093. This value indicates that the tubules of the infused-side kidney extracted TMP from portal blood at a mean rate of about 30%. This is considerably in excess of the rate that can be accounted for by diffusion and clearly suggests the operation of an active tubular excretory transport process. About 38 ± 8% of the TMP infused/min was recovered in the total urine. This typically resulted in 4000–5000 cpm of ^{3}H excreted. Ninety-eight percent of the tritium activity in urine was chloroform extractable, indicating a lack of significant amounts of polar (conjugated) metabolites. Analysis of chloroform extracts by thin-layer chromatography showed that all tritium activity was concentrated in a single spot at R_f 0.83 which corresponds to authentic TMP. It is possible that metabolites were formed but in quantities that could not be detected. However, the scintillation counting method would have detected any metabolite in excess of 5% of the total (i.e. 200–250 cpm; 8–10 times background). Thus, the tubular cells did not seem to metabolize TMP as it passed from portal blood to tubular lumen. This evidence parallels our previous observations that TMP is accumulated in unaltered form by rat renal cortical slices via an energy-dependent process [3].

Effect of organic ion transport blockers on tubular excretion of TMP. Tables 1 and 2 summarize the effects of the organic anion transport system inhibitor probenecid and the organic cation transport system inhibitor quinine on excretory transport of simultaneously infused TMP and the reference organic ions PAH and TEA. Probenecid and quinine are commonly utilized as definitive inhibitors of the renal organic anion and cation active transport systems respectively [9, 10]. In these inhibitor experiments, the reference compound of opposite charge served as the blood flow marker, with the tubular excretion (TER value) of the other serving as an indicator of the inhibitory effectiveness of the probenecid or quinine infusion. Probenecid, at an infusion rate sufficient to reduce transport of the anion PAH by 88.5%, had no significant inhibitory effect on tubular excretion of either TMP or TEA (Table 1). By contrast, quinine reduced the TER value for TMP by 78.1% at an infusion rate that had no significant inhibitory action on PAH excretion but which reduced tubular excretion of the cation TEA by about 95% (Table 2). The selective inhibition of the tubular excretion of TMP by quinine indicates that this drug is transported *in vivo* by the organic cation active transport system.

Effect of TMP on excretion of organic ions. If TMP were indeed transported by the organic cation process, it should selectively inhibit transport of TEA without affecting that of PAH. Table 3 summarizes the results of three experiments in which unlabeled TMP infused at 0.1 μmol/min was used to challenge the tubular excretion of simultaneously infused [^{14}C]TEA and unlabeled PAH. The selectivity of the effect is quite clear. During the period of TMP infusion, the ATEF

Table 1. Effect of probenecid on simultaneous renal tubular excretion of trimethoprim (TMP), *p*-aminohippuric acid (PAH) and tetraethylammonium (TEA)

Infusion	TER of substrates TMP	PAH	ATEF of marker TEA
Substrates alone (control)	0.280 ± 0.090	1.135 ± 0.071	0.519 ± 0.175
Substrates + probenecid	0.236 ± 0.072	0.130 ± 0.214*	0.482 ± 0.181

Each value is the mean ± SD from five experiments. Infusion rates (μmol/min): TMP, PAH, and TEA, 0.01; probenecid 15. TER = tubular excretion ratio; ATEF = apparent tubular excretion fraction.

* Significantly different from control ($P < 0.05$, Student's t-test).

Table 2. Effect of quinine on simultaneous renal tubular excretion of trimethoprim (TMP), tetraethylammonium (TEA) and *p*-aminohippuric acid (PAH)

Infusion	TER of substrates TMP	TEA	ATEF of marker PAH
Substrates alone (control)	0.3013 ± 0.1156	0.917 ± 0.088	0.446 ± 0.0813
Substrates + quinine	0.0661 ± 0.0841*	0.184 ± 0.103*	0.396 ± 0.106

Each value is the mean ± SD from four experiments. Infusion rates (μmol/min): TMP, TEA and PAH, 0.01; quinine 1.0. TER = tubular excretion ratio; ATEF = apparent tubular excretion fraction.

* Significantly different from control ($P < 0.05$, Student's t-test).

Table 3. Effect of trimethoprim (TMP) infusion on simultaneous renal tubular excretion of tetraethylammonium (TEA) and *p*-aminohippuric acid (PAH)

Infusion	Tetraethylammonium ATEF	Tetraethylammonium % Recovery/min	*p*-Aminohippuric acid ATEF	*p*-Aminohippuric acid % Recovery/min
Substrates alone (control)	0.536 ± 0.147	92.0 ± 5.9	0.59 ± 0.04	106.8 ± 17.7
Substrates + TMP	0.115 ± 0.044*	63.2 ± 3.4*	0.55 ± 0.07	92.7 ± 7.4

Each value is the mean ± SD from three experiments. Recovery is defined as the total amount excreted/min from both kidneys ÷ total amount infused/min. Infusion rates (μmol/min): TEA, 0.01; PAH, 0.01; and TMP, 0.10. ATEF = apparent tubular excretion fraction.
* Significantly different ($P < 0.05$) from control.

value of TEA was reduced by 78.6%, whereas that of PAH was not changed significantly (−6.3%). Additionally, TMP selectively reduced the urinary recovery of TEA without significantly affecting that of the PAH. These data are consistent with the conclusion that TMP is secreted by the organic cation transport system.

In summary, this study has produced the following three principal conclusions: (1) TMP was actively transported *in vivo* in the excretory direction by the renal tubules, (2) the renal tubular cells did not metabolize TMP as it crossed from peritubular blood to tubular lumen, and (3) the active transport occurred via the organic cation system. Thus, a number of cationic drugs and endogenous chemicals are potential competitors for the active tubular excretion of TMP.

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REFERENCES

1. P. Sharpstone, *Post-grad. med. J.* **45**, (Suppl), 32 (1969).
2. P. Nielsen and F. Rasmussen, *Acta pharmac. tox.* **36**, 123 (1975).
3. W. Cacini and S. A. Myre, *Biochem. Pharmac.* **34**. 3483 (1985).
4. D. E. Schwartz, W. Vetter and G. Engleri, *Arzneimittel-Forsch.* **20**, 1867 (1970).
5. J. Reider, *J. infect. Dis.* **128**, S567 (1973).
6. I. Sperber, *Ann. R. Agr. Coll. Sweden* **16**, 49 (1948).
7. W. Cacini and A. J. Quebbemann, *J. Pharmac. exp. Ther.* **207**, 574 (1978).
8. H. W. Smith, N. Finkelstein, L. Aliminosa, B. Crawford and M. Graber, *J. clin. Invest.* **24**, 388 (1945).
9. I. M. Weiner, in *The Kidney, Physiology and Pathophysiology* (Eds. D. W. Seldin and G. Giebisch), Vol. 2, p. 1703. Raven Press, New York (1985).
10. B. R. Rennick, *Am. J. Physiol.* **240**, F83 (1981).

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Postictal refractoriness associated with reduction of glutamic acid decarboxylase in discrete brain regions in epilepsy-prone gerbils

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It has been known for a long time that experimentally-induced seizures results in a refractory state during which the animal will not respond to previously effective seizure-precipitating stimuli [1–5]. It is possible that this postictal refractoriness represents an adaptation of the brain to diminish or prevent the spread of further seizure activity [4]. The mechanisms involved in producing this postictal state are not known, but numerous studies suggest that the inhibitory neurotransmitter, γ-aminobutyric acid (GABA), may be involved. Thus, transient increases in GABA concentrations have been observed in different brain regions of rats following a seizure induced by electroshock or bicuculline [6]. The increase observed in the hippocampus was closely related temporally to the increase in seizure threshold following the initial convulsion. Marked increases of GABA in hippocampus and other brain regions were also observed after seizure activity induced by kainic acid and L-allylglycine in rats [7]. An indication of increased GABAergic function was also reported by Ross and Craig [8], who found an enhanced GABA receptor binding in the cortex after an electroshock seizure in rat, and by Shin *et al.* [9], who found increases in GABA binding in rat hippocampus following an amygdala-kindled seizure. Furthermore, several groups observed increases in the binding of benzodiazepines at the GABA/benzodiazepine receptor complex following a seizure induced by electroshock or pentylenetetrazol in rats [10], by amygdala-stimulation in kindled rats [11, 12] and by handling in epilepsy-prone gerbils [13, 14]. The increase in benzodiazepine receptor binding in the postictal period was shown to be accompanied by increased inhibition using electrophysiological measurements [15]. However, although attractive, the relationship between postictal refractoriness and increased GABAergic inhibition is not straightforward, because biochemical indices for altered GABAergic function are not found after all types of generalized seizures and some workers [6] could not reproduce the postictal increase in benzodiazepine binding reported by others (see above). Furthermore, more recently Green and co-workers [16–18] found decreases in GABA synthesis and release following an electroshock- or flurothyl-induced seizure, which seems to contradict the GABA hypothesis of postictal refractoriness. In the present study, we investigated the time course of postictal depression in seizure-prone Mongolian gerbils, a genetically predisposed species in which seizures can be initiated by different sensory stimuli