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UPTAKE OF TRIMETHOPRIM BY RENAL CORTEX

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Abstract—The purpose of this study was to examine the mechanisms involved in the uptake of the urinary antibacterial drug trimethoprim by incubated slices of rat renal cortex. Concentration-dependent studies of the uptake process demonstrated that a saturable component was involved. The results of inhibitor studies as well as the time-course pattern support the conclusion that at least two processes are involved in the uptake of trimethoprim. These include active transport via the organic cation system, accounting for about 40% of the total uptake, and a second component that continues to operate under conditions of inhibited cellular metabolism. Chromatographic examination of post-incubation bathing medium and slice extracts failed to demonstrate renal cortex metabolism of trimethoprim.

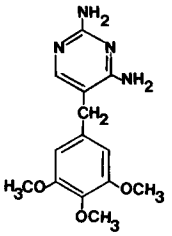

Fig. 1. Structure of trimethoprim [2,4-diamino-5(3,4,5-trimethoxybenzyl)pyrimidine].

Trimethoprim (TMP; Fig. 1) is a synthetic antibacterial drug used commonly to treat urinary tract infections, an application which takes advantage of the fact that this agent accumulates within the kidney [1, 2]. The nature of the accumulation process has yet to be clearly defined. Although several studies of the pharmacokinetics of TMP have been published, fewer have addressed the cellular mechanisms of the interaction of the drug with its target organ cells. Some renal clearance data suggest that TMP may undergo tubular secretion, although the contribution of active transport to this process is uncertain [3, 4]. Hepatic and total body metabolism of TMP have been reported previously [5]. Renal metabolism, however, has yet to be addressed. It is important to clarify this since some metabolites are apparently antimicrobial while others are not [6]. The current study, using renal cortex slices, was designed to determine the relative contributions of active and passive processes to the uptake of TMP by the renal cortex under controlled *in vitro* conditions, and to assess the ability of *in vitro* cortex slices to metabolize TMP.

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MATERIALS AND METHODS

Reagents. Unlabeled trimethoprim was purchased from the Sigma Chemical Co. (St. Louis, MO), while [^{3}H]trimethoprim (60.3 mCi/mmole) was supplied by Hoffman-LaRoche, Inc. (Nutley, NJ). Purity was periodically checked chromatographically as described below. Both [^{14}C]tetraethylammonium and [^{14}C]*p*-aminohippuric acid were purchased from the New England Nuclear Corp. (Boston, MA). Ringer's solution constituents and transport inhibitors were obtained from various major suppliers and were all of at least reagent grade.

Slice preparation and incubation. Mature Sprague–Dawley rats of both sexes were killed by decapitation, and the kidneys were rapidly removed, decapsulated and placed in ice-cold Ringer's solution (see composition below). Thin (0.3 to 0.5 mm) slices were cut from the cortical surface of the kidney by means of a Stadie–Riggs microtome. The slices, approximately 100 mg wet wt each, were incubated individually in 50-ml beakers containing 5 ml of phosphate-buffered (0.1 M) Ringer's solution (pH 7.4) of the following composition (mM): NaCl, 104.4; KCl, 43.2; $CaCl_2$, 0.79. In those experiments in which incubations were carried out under a nitrogen atmosphere, the Ringer's solution was de-gassed under vacuum, bubbled with nitrogen for 20 min and the process repeated to purge the solution of as much dissolved oxygen as possible. Incubations were carried out at 25° in a metabolic shaker bath (Blue M. Electric Co., Blue Island, IL) fitted with an atmospheric hood into which oxygen or nitrogen was continuously delivered at 4 l/min. In a typical experiment, slices from a single animal were incubated individually, with each treatment performed in triplicate. Slices were preincubated alone or in the presence of a metabolic or transport inhibitor for 15 min after which the [^{3}H]TMP was added and the timed incubation begun. After the desired time, slices were rapidly separated from medium by fil-

tration through gauze, blotted, and placed into preweighed vials containing 3 ml of 0.5 N NaOH. The vial was reweighed to obtain the slice weight. Tissue was dissolved in an oven at 60° with occasional shaking (2–3 hr).

Analysis and expression of uptake. Radioactivity in each medium and slice solution (acidified to reduce chemiluminescence) was determined by counting aliquots in a Beckman LS 7000 scintillation spectrometer using 3a70B (RPI, Inc., Mount Prospect, IL) as cocktail. Degree of quench was monitored by comparing experimental samples to radioactive standards. Uptake was expressed either as nmoles/g wet tissue weight or as a slice-to-medium concentration ratio (S/M) with S = nmoles/g wet weight and M = nmoles/ml medium. Statistical significance was determined using Student's *t*-test with $P < 0.05$ considered significant.

Extraction and chromatography. Each post-incubation medium and slice solution was subjected to chloroform extraction at pH 10.6 to 10.8 as described by Schwartz *et al.* [5] to determine the identity of the ^{3}H-label. This step served to separate TMP and its free metabolites from conjugated metabolites which remain in the aqueous layer. An aliquot of the aqueous layer was sampled for scintillation counting before and after the extraction procedure. The chloroform phase was evaporated at room temperature under reduced pressure, and reconstituted in a small volume of chloroform. Ten to twenty microliters of this was streaked across a 3 × 20 cm silica gel thin-layer plate (Eastman Chromagram sheet No. 13179) which was then developed in a mobile phase of chloroform–1-propanol–25% aq. ammonia (80:20:1) [5]. Radioactive spots were located by serially cutting the developed plate into 1 cm strips and counting each strip individually in a liquid scintillation spectrometer.

RESULTS

Time-dependence of uptake. The time course of the uptake of TMP is shown in Fig. 2. The 0.01 mM concentration used in these experiments was chosen because it is within the range of therapeutically effective plasma levels in man. Accumulation (S/M > 1) of the drug was evident within 10 min of incubation, with a S/M value of 3.9 achieved after 2 hr. Though no steady-state was achieved within the time-frame of the experiment, the shape of the curve indicates that the uptake was not a simple zero-order process. Plotting the same data on semilogarithmic coordinates (log uptake vs time) yielded a curved line of similar shape.

To ascertain the chemical form of ^{3}H-label, the post-incubation media and slice solutions from the 120-min samples were subjected to chloroform extraction and thin-layer chromatography. Virtually all of the ^{3}H-label (>96%) was chloroform extractable, indicating absence of appreciable amounts of polar metabolites. TLC analysis of the chloroform extract demonstrated the presence of a single spot with an R_f value (0.84) corresponding to that of authentic [^{3}H]TMP standards. Therefore, there was no evidence of metabolism of TMP during the incubation procedure.

Influence of concentration on uptake. The S/M value for [^{3}H]TMP was determined in the presence of increasing initial medium concentrations of unlabeled TMP. The results (Fig. 3) showed a progressive decline in the relative uptake of ^{3}H-labeled TMP as the concentrations of competing unlabeled TMP were raised. This is a pattern that indicates the operation of a limited capacity component in the uptake process.

Dependence of uptake in cellular metabolism. A definitive property of an "active" uptake process is

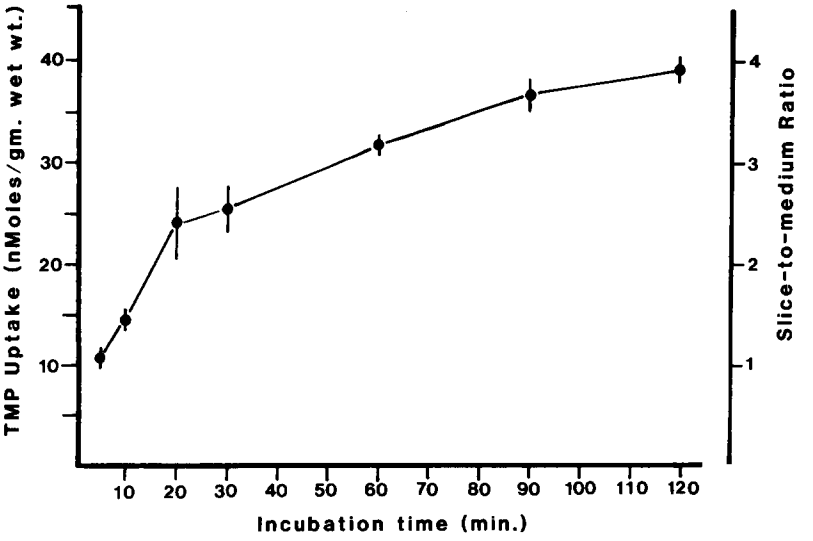

Fig. 2. Time course of uptake of [^{3}H]trimethoprim by rat renal cortex slices. Each point is the mean ± S.D. of nine to twelve individually incubated slices. S/M = slice-to-medium ratio with S = nmoles/g wet wt and M = nmoles/ml medium. Conditions: 0.01 mM [^{3}H]trimethoprim at 25° under a 100% O_2 atmosphere, pH = 7.4.

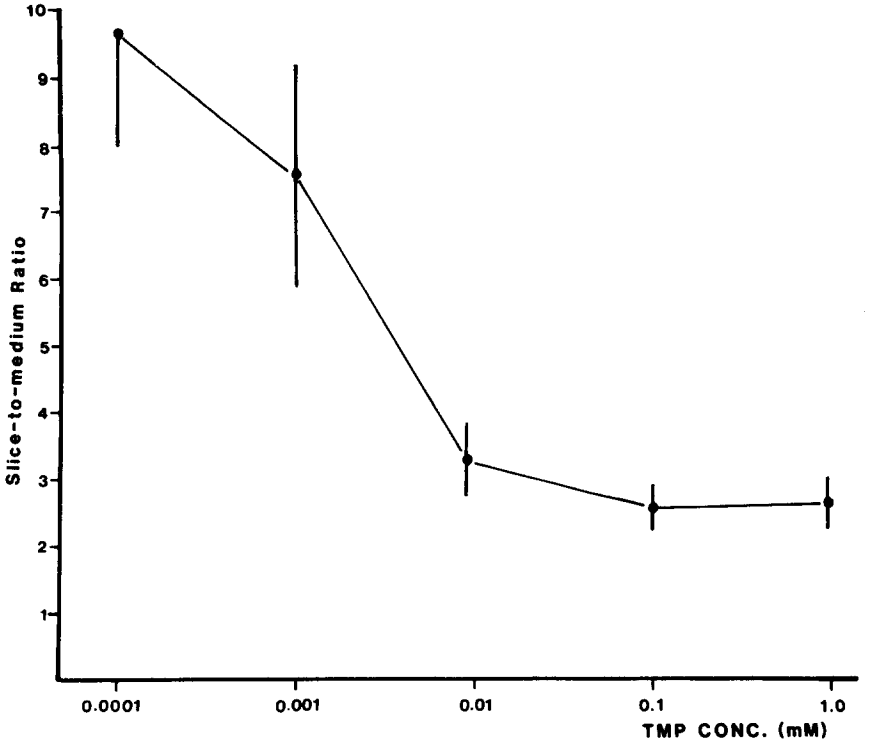

Fig. 3. Concentration dependence of the uptake of trimethoprim (TMP) by rat renal cortex slices. Each point is the mean ± S.D. of eleven to sixteen individually incubated slices. A trace amount of [^{3}H]-trimethoprim was combined with an appropriate amount of unlabeled drug to yield the desired concentration. Incubation time was 90 min.

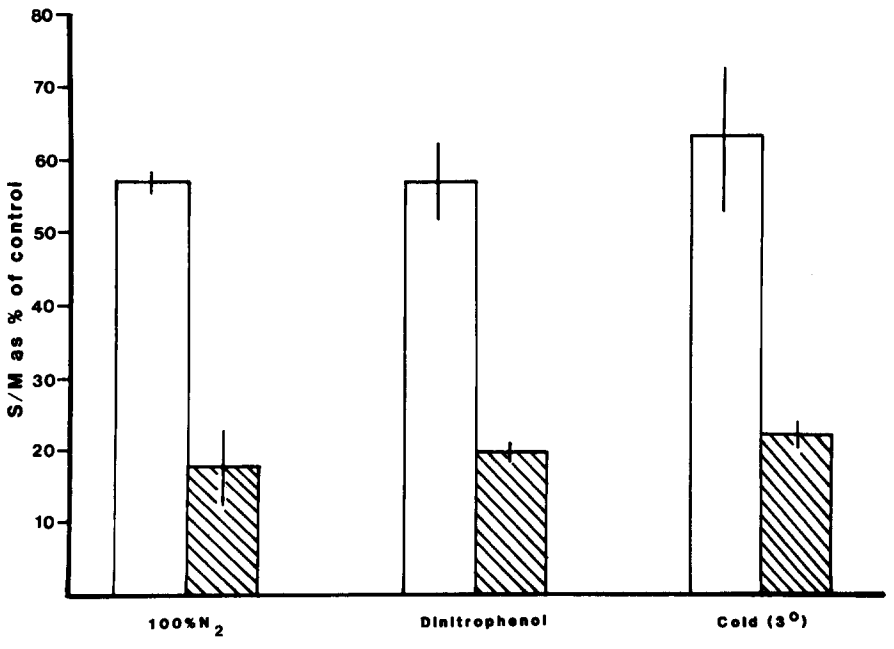

Fig. 4. Effects of metabolic inhibitors on uptake of [^{3}H]trimethoprim (TMP) and [^{14}C]tetraethylammonium (TEA) by rat renal cortex slices. Each point is the mean ± S.D. of at least six individually incubated slices. Incubation time was 90 min. Clear bars = 0.01 mM TMP, hatched bars = 0.01 mM TEA. All bars represent a statistically significant difference from control ($P < 0.05$).

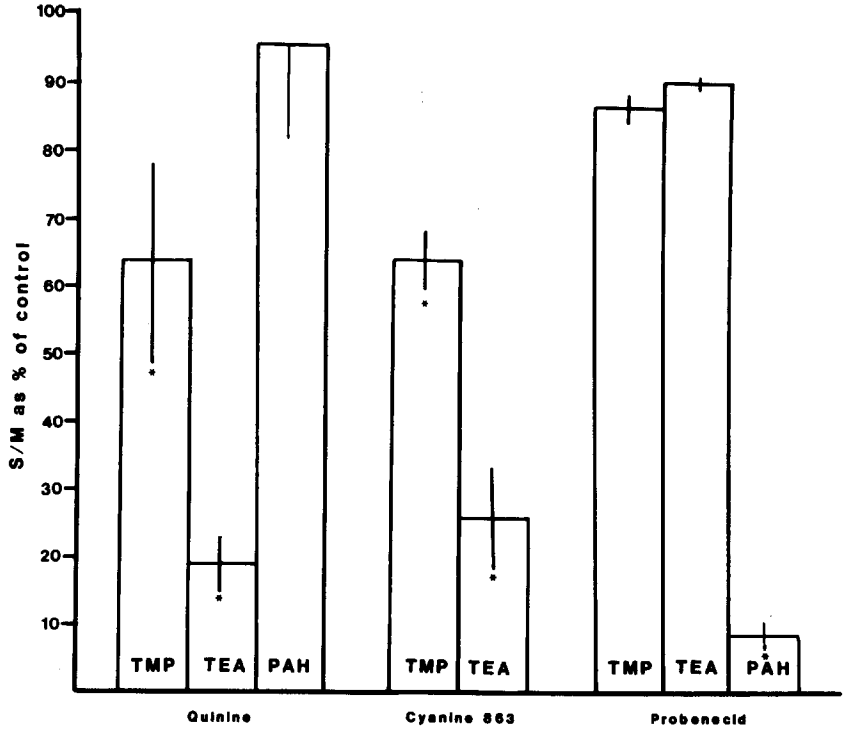

Fig. 5. Effects of organic ion transport inhibitors on uptake of [^{3}H]trimethoprim (TMP), [^{14}C]tetraethylammonium (TEA) and [^{14}C]*p*-aminohippuric acid (PAH) by rat renal cortex slices. Each point is the mean ± S.D. of at least nine individually incubated slices. Incubation time was 90 min. All substrates = 0.01 mM. An asterisk indicates a significant difference from control ($P < 0.05$).

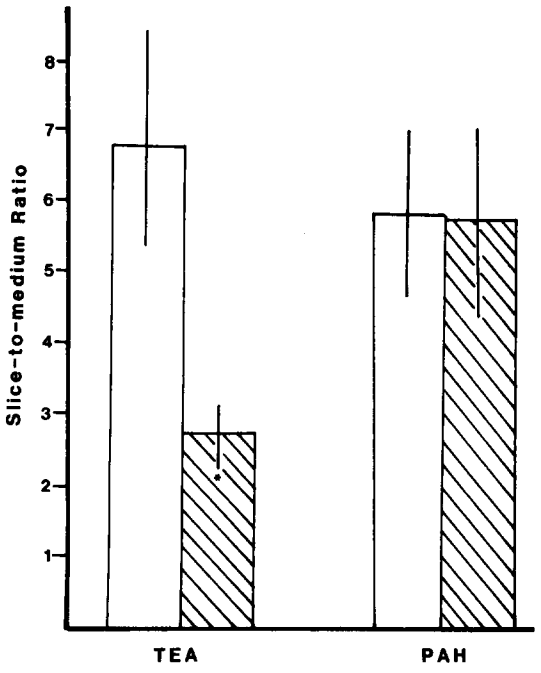

Fig. 6. Effects of trimethoprim on uptake of [^{14}C]tetraethylammonium (TEA) and [^{14}C]*p*-aminohippuric acid (PAH) by rat renal cortex slices. Each value is the mean ± S.D. of nine individually incubated slices. Time of incubation was 90 min. Concentrations (mM): TMP 1.0; TEA, 0.01; and PAH, 0.01. Clear bars = substrate alone; hatched bars = substrate plus TMP. An asterisk indicates a significant difference from control ($P < 0.05$).

its dependence on cellular energy expenditure. To assess the importance of energy to the overall uptake process from TMP, S/M for 0.01 mM TMP was determined after 90 min of incubation under various conditions designed to depress cellular energy use: 100% nitrogen atmosphere, presence of 0.05 mM dinitrophenol (an uncoupling agent), and cold temperature (3°). Controls for each experiment were parallel incubations of slices from the same rat with TMP at 25° under an O_2 atmosphere. The results are summarized in Fig. 4. Also presented are the effects of these three treatments on uptake of [^{14}C]-tetraethylammonium (TEA), a reference organic base known to be actively transported by renal cortex slices [7]. All treatments produced statistically significant decreases in S/M values for both TMP and TEA. The mean percent decreases in the S/M value for TMP under the three conditions were quite close, these being 42.6% (N_2), 42.7% (DNP) and 36.0% (cold). It should be noted, however, that even under the inhibitory conditions TMP S/M values ranged from 1.9 to 3.3, indicating accumulation within the tissue. S/M values for TEA were depressed 77–82% under these conditions. This corresponds to S/M values of less than 1.6 for TEA and indicates that these conditions effectively inhibited energy-dependent transport by the cortical tissue.

Effect of competing organic ions on uptake. TMP uptake was compared in the presence and absence (control) of two organic cation transport inhibitors, cyanine 863 and quinine, and the organic anion transport inhibitor probenecid. The results are summarized in Fig. 5. As an index of the effectiveness and selectivity of the inhibitors at the concentrations employed, their effects on uptake of a standard organic cation (TEA) and organic anion [^{14}C]*p*-aminohippuric acid (PAH) are also presented. Quinine significantly inhibited uptake of both TMP (−36%) and TEA (−82%) without significantly affecting that of PAH (−5%). Similarly, cyanine 863 significantly depressed S/M for both TMP (−36%) and TEA (−74%). Probenecid, on the other hand, showed only a slight effect on TMP (−15%) and TEA (−10%) uptake at a concentration that virtually eliminated accumulation of the reference anion PAH (−92%). The apparent slight inhibitory effect of probenecid on TMP uptake cannot be clearly attributed to a specific action since, at this concentration, probenecid also slightly inhibited S/M for TEA with which it shares no transport mechanism. These results suggest that TMP is transported by an organic cation process.

Effect of TMP on uptake of TEA and PAH. In view of the transport inhibitor results, it was decided to test the effect of TMP on uptake of TEA and PAH (Fig. 6). TMP selectively inhibited S/M TEA (−59%) without significant action on PAH. These results are consistent with the idea that TMP transport is cationic.

DISCUSSION

Despite more than a decade of clinical use of trimethoprim to treat urinary tract infections, a clear definition of the mechanisms responsible for uptake of the drug into the kidney has not been established. That the drug accumulates within the kidney is well known [1, 2]. The results of the TMP distribution study in rats of Trottier *et al.* [2] point to the cortex as the intrarenal site that most avidly concentrates the drug following i.p. injection. In the current study, we have attempted to define better the process involved in the uptake of TMP by the cortex. Our use of *in vitro* slice incubation principally measures movement of chemicals across the basolateral surface of the tubule cells, thus quantifying a net uptake/retention process. Since the lumens of the tubules in such a preparation are collapsed, movement across the luminal membrane is generally considered to be minimal.

The current study has demonstrated that the renal cortex concentrates trimethoprim to various degrees over a wide concentration range (0.0001 to 1.0 mM). Although the experiments were not specifically designed to determine the actual limits of the process, at the lowest concentration used the tissue: medium ratio reached 9.6:1. At the concentration commonly achieved in the plasma in therapeutic applications (0.01 mM), the ratio was 3.5:1, which reflects a tissue content of 10.2 μg/g. Although such *in vitro* data cannot be considered directly analogous in a quantitative sense to *in vivo* uptake, this tissue value is close to the 15–25 μg/g of whole kidney (cortex and medulla) measured after sacrifice of Rhesus monkeys in whom an equilibrium plasma trimethoprim concentration of approximately 0.01 mM had been maintained [1].

The apparent lack of metabolism by the cortex deserves some comment. The major metabolites of trimethoprim are oxidation products and their glucuronide conjugates [5]. The rat, in fact, is especially active in the metabolism of this drug, excreting only about 20% of an administered dose unchanged. This contrasts to man who excretes 50–80% as unmetabolized trimethoprim. Although there was no apparent metabolism of trimethoprim during our incubations, we would hesitate to cite this as convincing evidence that the kidney plays no role in the biotransformation of the drug *in vivo*. The kidney is known to carry out both oxidative and conjugative reactions of the type involved in the biotransformation of trimethoprim [8]. Incubation of tissue slices at 25° does not represent an ideal model system for metabolism studies. Nevertheless, lack of metabolites in our preparation simplifies interpretation of the uptake data which, accordingly, quantifies disposition of the unchanged drug only.

The renal uptake of TMP in rats clearly seems to involve more than a single process. This is supported by both the rate data and the results of the inhibitor experiments. Lack of linearity in the relationship of incubation time to corresponding tissue concentrations or their logarithms argues against the operation of a single zero or first-order process. In addition, only about 40% of the uptake was dependent upon cellular energy. Under conditions which almost eliminated accumulation of the reference substrate TEA, the renal cortex continued to accumulate trimethoprim to a concentration in excess of twice that of the medium. Our results do not allow a firm conclusion as to the mechanism of this accumulation process, but unionized TMP is lipid soluble and, as

a consequence, is able to penetrate many biological membranes [9]. TMP also binds to renal tissue homogenates [10]. As a weak base with a pK_a of 7.3, TMP exists in cationic form to the extent of about 44% at pH 7.4. The ionic form would be a potential substrate for the renal organic cation transport process. The selective inhibition of TMP uptake by cyanine 863 and quinine, and the observation that TMP selectively reduced uptake of TEA strongly suggest that cationic active transport contributes to uptake and presumably excretion of TMP *in vivo*. That the cation transport was the only active process involved in the uptake of trimethoprim by the renal cortex is suggested by a comparison of the inhibitor effects summarized in Figs. 4 and 5. If it is accepted that the transport blockade produced by the organic bases was specific for the cation system while that produced by the metabolic inhibitors was a general depression of all active transport, then the observation that both types of inhibitors caused virtually the same degree of depression of TMP S/M leads to the conclusion that the cation process accounted for all of the active transport.

The successful use of TMP against urinary bacterial infections requires that it enter not only kidney tissue but also the urine by which it can be carried to the lower urinary tract. The initial step is extraction of the drug from extracellular fluid by the renal cells. This study indicates that both active cationic transport and passive processes play important roles in trimethoprim uptake by kidney cortex cells. The participation of TMP in the cationic transport system suggests that a wide variety of therapeutic agents are probable competitors for renal uptake of TMP. The clinical significance of this potential interaction has yet to be determined.

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REFERENCES

1. W. A. Craig and C. M. Kunin, *J. infect. Dis.* **128**, S575 (1973).
2. S. Trottier, M. G. Bergeron and C. Lessard, *Antimicrob. Agents Chemother.* **17**, 383 (1980).
3. T. Bergan and E. K. Brodwall, *Chemotherapy* **17**, 320 (1972).
4. P. Nielsen and F. Rasmussen, *Acta pharmac. toxic.* **36**, 123 (1975).
5. D. E. Schwartz, W. Vetter and G. Engleri, *Arzneimittel-Forsch.* **20**, 1867 (1970).
6. J. Reider, *J. infect. Dis.* **128**, S567 (1973).
7. J. Holm, *Acta pharmac. tox.* **31**, 129 (1972).
8. M. W. Anders, *Kidney Int.* **18**, 636 (1980).
9. P. J. Wilkinson and D. S. Reeves, *J. Antimicrob. Chemother.* **5**, (Suppl. B), 159 (1979).
10. A. Gerber, N. Frimodt-Moller and W. A. Craig, *Infection* **8**, S276 (1980).