for skeletal muscle is responsible for its accumulation in the peripheral compartment.

The ratio of k_{21}/k_{12} ranged from 1.4 to 3.0, with a mean of 2.2, indicating that the drug returns rapidly from distribution sites for elimination from the body. However, the low k_{el}, with a mean of 0.46, indicates that the elimination of triamterene would not be as rapid as the ratio predicts. This situation could be related to the binding of the drug to tissue substances in the central compartment and to the ability of the rat to metabolize a limited amount of the drug (6). According to the plasma half-life of the slow component obtained in the present experiments, after intravenous administration of triamterene there will be a negligible concentration of the drug in the plasma within about five half-lives (13.9 hr).

In conclusion, the present observations illustrate the tissue binding property of triamterene in the rat. Although these data provide limited information about the nature of this property, they reveal the possibility of the effect of unchanged triamterene on the tissue content of sodium. A pharmacokinetic interpretation of the present study appears to be well correlated with the experimental results. Apparently, the tissue binding property of triamterene is an important factor in controlling its rate of distribution and elimination in the rat.

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Effect of Sodium Salicylate on Renal Elimination of a Quaternary Ammonium Compound

KISHOR A. DANDEKAR [*] and H. B. KOSTENBAUDER [x]

Abstract □ Administration of sodium salicylate inhibited elimination of the tetraethylammonium ion in rats. A two-compartment open model was used to describe plasma decline of tetraethylammonium bromide administered intravenously. The rate constant describing elimination from the central compartment was decreased by 25 and 40% in rats predosed with sodium salicylate at 30 and 50 mg/kg, respectively. Salicylate also enhanced the distribution of the tetraethylammonium ion to the peripheral compartment. Urinary excretion is the principal route of elimination for the tetraethylammonium ion, and studies of the effect of sodium salicylate on the uptake of the tetraethylammonium ion by rat renal cortical tissue indicated noncompetitive inhibition by salicylate.

Keyphrases □ Sodium salicylate—effect on renal elimination of tetraethylammonium bromide, rats □ Tetraethylammonium bromide—pharmacokinetic model, effect of sodium salicylate on renal elimination, rats □ Elimination, renal—tetraethylammonium bromide, effect of sodium salicylate, rats □ Pharmacokinetics—two-compartment open model for tetraethylammonium bromide, rats □ Quaternary ammonium compounds—tetraethylammonium bromide, pharmacokinetic model, effect of sodium salicylate on renal elimination, rats

Quaternary ammonium and other strongly basic compounds are actively secreted by the renal tubule. The secretory pathway for the basic compounds is similar, but not identical, to the anion transport mechanism. Both mechanisms are known to be saturable, specific, and subject to inhibition by structural analogs and antimetabolic compounds and to require energy for their operation. In general, tubular transport of basic compounds is not known to be inhibited by acidic compounds; conversely, the transport of acidic compounds is not known to be inhibited by basic compounds (1).

The recently reported, partly noncompetitive nature of the inhibition of *p*-aminohippurate tubular transport by salicylate (2) prompted investigation of the effect of salicylate on the renal tubular transport of a quaternary ammonium compound. The effect of sodium salicylate on renal elimination kinetics of the tetraethylammonium cation was investigated in *in vitro* and *in vivo* models. Rat renal cortical slice studies were used to determine the nature of the inhibitory effect of salicylate on the active uptake of the tetraethylammonium ion by cortical tissue. The significance of the salicylate effect on renal elimination of the ^{14}C-tetraethylammonium ion was then evaluated in pharmacokinetic studies in anesthetized rats.

EXPERIMENTAL

Renal Cortical Slice Study—Male Sprague–Dawley rats, 200–300 g, were killed by decapitation. Kidneys were removed quickly by abdominal laparotomy. The kidneys were decapsulated and stored briefly (not more than 10 min) under ice-cold normal saline bubbled with 100% oxygen. After discarding the two surface slices, three cortical slices, approximately 0.3–0.4 mm in thickness, were cut from dorsal and ventral sides using a tissue slicer[1]. In each experiment, two or three animals were killed and the slices were pooled.

About 60–80 mg of tissue (two to three slices) was equilibrated with 3 ml of incubation medium under 100% oxygen for 5 min. ^{14}C-Tetraethylammonium bromide[2] (100 μCi/19.2 mg) was then added in a volume of 100 μl, and the flasks were incubated for 30 min with continuous shaking at 60 cpm. The period between animal decapitation and the beginning of the incubation was never more than 30 min. At the end of the incubation period, the flasks were chilled in an ice bath, the tissue slices were blotted lightly and weighed, and the ^{14}C-tetraethylammonium-ion content of the slices was determined by liquid scintillation counting.

The incubation medium was prepared as suggested by Robinson (3), except for the addition of 11 m*M* glucose. The optimum incubation period was determined by observing the uptake of the tetraethylammonium ion at the end of 5, 10, 20, 30, 50, and 60 min. This uptake was determined for initial tetraethylammonium bromide concentrations of 0.125, 0.250, 0.500, 1.00, and 2.00 m*M* in the presence and absence of 1 m*M* sodium salicylate. Each solution contained 0.075 μCi of the ^{14}C-tetraethylammonium ion.

Passive uptake of the tetraethylammonium ion by the renal tissue was determined in a separate experiment. Sodium cyanide (1 m*M*) was added to the salicylate-free medium, and nitrogen gas was used in place of oxygen. Ten flasks were used in this experiment.

Pharmacokinetic Studies—Male Sprague-Dawley rats, 420–580 g, were acclimatized to laboratory conditions for at least 2 days. The animals had free access to food and water until the experiments began. Rats were anesthetized with pentobarbital sodium[3], 50 mg/kg ip. The bile duct was cannulated after a small incision (about 1 cm) was made 1 cm below the sternum on the abdominal wall. The wound was sutured and kept covered with 4 × 4 gauze soaked in normal saline. The femoral vein was cannulated and provided with a three-way stopcock and a 5-ml disposable syringe filled with heparinized (10 units/ml) normal saline.

The surgical procedure was completed within 10 min after anesthesia, and 30 min was allowed between its completion and the administration of the intravenous dose of tetraethylammonium bromide. The rats were watched for uniform breathing, proper level of anesthesia, and unobstructed bile flow during this period. In the salicylate treatment experiments, 30 or 50 mg/kg iv of sodium salicylate was given immediately after completion of the surgical procedure. A 0.4-ml blood sample and a 10-min bile sample were collected during this period to serve as a blank or background determination in the analytical procedure.

At the end of 30 min, ^{14}C-tetraethylammonium bromide solution, 10 mg/kg iv, was administered over 20 sec. This injection contained 5 mg of total drug/ml and 3.0 μCi of ^{14}C-tetraethylammonium bromide/ml. The timer was started at the midpoint of the injection period. The dose was flushed into the circulation with about 0.4 ml of normal saline. The three-way stopcock and the 5-ml syringe were discarded, and a new 5-ml syringe containing fresh heparinized normal saline was connected directly to the venous cannula. This procedure prevented contamination of the first blood sample with the very high radioactivity of the dose.

Blood samples, 0.4 ml each, were withdrawn every 5 min for the first 40 min and then every 20 min for a total of 2 hr. The blood volume withdrawn was replaced by injecting 0.4 ml of heparinized normal saline after each withdrawal. Blood clotting in the cannula was avoided by maintaining heparinized normal saline in the cannula tubing between blood samples. The plasma was separated by centrifuging, and the plasma and bile samples were analyzed for carbon-14 radioactivity by scintillation counting immediately after the experiment.

Tetraethylammonium-Ion Partition Study—Five solutions, 5 ml each, containing 0.05 m*M* tetraethylammonium bromide and 0.5, 1.0, 1.5, 2.0, or 2.5 m*M* sodium salicylate were prepared in 0.1 *M* phosphate buffer (pH 7.4). Exactly 0.03 μCi of ^{14}C-tetraethylammonium bromide was added to each tube. Then 5 ml of chloroform was added to each tube, and the tubes were closed and gently rocked for 30 min. The tubes were then centrifuged to separate the two phases. Radioactivity in the aqueous phase, prior to and after partitioning, was determined by scintillation counting.

Table I—Results of Renal Cortical Slice Study

Tetraethylammonium Ion in Medium, m*M*	Micromoles of Tetraethylammonium Ion per Gram of Tissue per 30 min × 10[a]		
	Control (n = 4)	With Sodium Salicylate (1 m*M*) (n = 4)	With Sodium Cyanide (1 m*M*) under Nitrogen (n = 2)
0.125	3.8872 ± 0.7147	2.2731 ± 0.6674	0.9587 ± 0.0143
0.250	5.5133 ± 0.2124	3.9493 ± 0.4507	1.9150 ± 0.0640
0.500	9.4887 ± 1.4737	6.3751 ± 0.2024	3.8604 ± 0.0944
1.00	13.8346 ± 0.0648	10.1238 ± 1.2987	7.4922 ± 0.0371
2.00	20.3419 ± 3.3068	17.1054 ± 0.7102	14.8463[b]

[a] Mean ± *SD*. [b] n = 1.

Radioactivity in Renal Cortical Slices—After determination of tissue weight, renal cortical slices were dissolved in 15 ml of a toluene base scintillation cocktail with the aid of 1 ml of tissue solubilizer[4].

Each vial was counted for 10 min in a liquid scintillation counter[5] to determine the carbon-14 radioactivity. An appropriate quench curve was made by adding tissue to a constant amount of ^{14}C-tetraethylammonium bromide (5323 dpm/vial).

Radioactivity in Plasma and Bile—Exactly 100 μl of plasma was placed in the glass scintillation vials and mixed with 15 ml of scintillation fluid. Each vial was counted for 10 min in a scintillation counter. The quench curve made by adding various volumes (20–150 μl) of plasma but the same known amount of ^{14}C-tetraethylammonium bromide (2200 dpm/vial) allowed the exact determination of counting efficiency of the plasma samples.

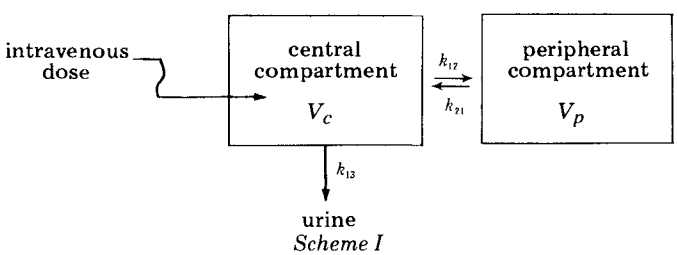
Entire 10-min bile samples were used for determining the ^{14}C-tetraethylammonium ion in the bile. Addition of 2 ml of methanol to bile prior to mixing it with 15 ml of scintillation fluid improved the sample appearance and the counting efficiency.

Tetraethylammonium-Ion Uptake Calculations—The amount of tetraethylammonium ion in the tissue was expressed in terms of micromoles per gram of tissue. The active uptake of the tetraethylammonium ion was determined by subtracting the uptake in the presence of 1 m*M* potassium cyanide and a nitrogen atmosphere from the aerobic uptake. The active uptake was treated according to Michaelis–Menten kinetics.

Pharmacokinetic Calculations—The plasma tetraethylammonium-ion *versus* time profiles in all experiments were biexponential, suggesting that pharmacokinetic analysis would require minimally a two-compartment open model (4). The average plasma level–time profiles of four experiments done in each category, *i.e.*, control, with 30-mg/kg salicylate dose, and with 50-mg/kg salicylate dose, were determined. The mean plasma level–time data of each category of experiments were fitted to the following biexponential equation using the SAAM-23 digital computer program (5):

$$C = Ae^{-\alpha t} + Be^{-\beta t} \qquad \text{(Eq. 1)}$$

The constants A, B, α, and β, estimated by the computer fit, were then used to determine the individual rate constants and related volume parameters of the two-compartment open model (Scheme I) (4).



Scheme I

[1] Harvard Apparatus Co., Millis, MA 02054.
[2] New England Nuclear, Boston, Mass.
[3] Nembutal sodium, 50 mg/ml, Abbott Laboratories, North Chicago, Ill.
[4] Soluene-100, Packard Instrument Co., Downers Grove, Ill.
[5] Packard Tri-Carb scintillation spectrometer, model 3375, Packard Instrument Co., Downers Grove, Ill.

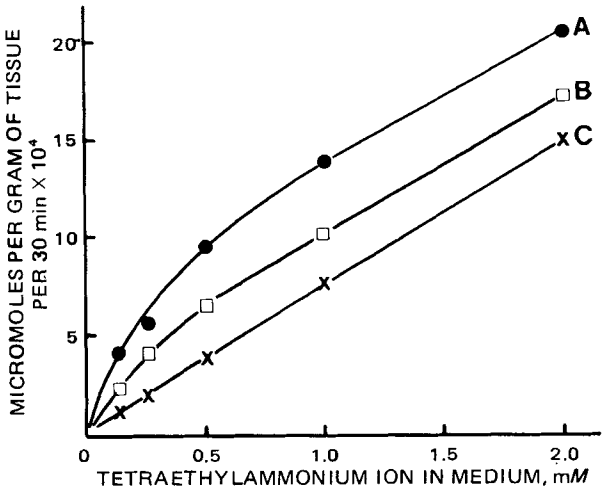


Figure 1—*Uptake of the tetraethylammonium ion by renal cortical slices. Key: A, control; B, in presence of 1 mM sodium salicylate; and C, under anaerobic conditions (nitrogen atmosphere) and with 1 mM sodium cyanide added to medium. Each data point represents the mean for the number of studies indicated in Table I.*

RESULTS AND DISCUSSION

Renal Cortical Slice Study—Data for the uptake of the tetraethylammonium ion by renal cortical tissue are presented in Table I. Maximum uptake occurred after 30 min of incubation. Tetraethylammonium-ion uptake in three categories of the experiments, *i.e.*, control, with 1 m*M* salicylate under proper physiological conditions, and in anaerobic conditions under nitrogen in the presence of 1 m*M* sodium cyanide, is illustrated in Fig. 1. The active uptake of the tetraethylammonium ion, with and without salicylate, is shown as a Woolf plot in Fig. 2.

Figure 1 clearly shows that the tetraethylammonium-ion active uptake by the cortical slices was a saturable process and obeyed Michaelis–Menten kinetics. The uptake of the tetraethylammonium ion in the presence of cyanide and nitrogen was the measure of passive diffusion of the tetraethylammonium ion into the cortical tissue and was a linear function of the concentration in the medium. The active oxygen and enzyme-dependent uptake, therefore, was determined by subtracting the passive uptake from the uptake determined in the presence of oxygen under proper physiological conditions. The Woolf plot of the active tetraethylammonium-ion uptake data (Fig. 2) indicates noncompetitive inhibition of the active uptake of the tetraethylammonium ion by salicylate. This result is in agreement with a generally accepted theory that an anionic drug does not compete with a cationic drug for tubular

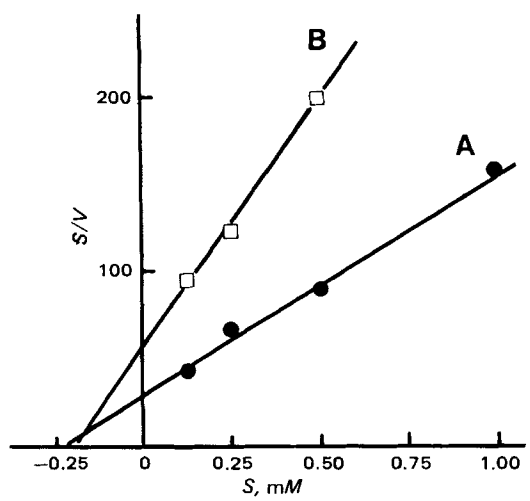


Figure 2—*Woolf plot of the active uptake of the tetraethylammonium ion by renal cortical slices; S is substrate concentration, and V is rate of uptake. Key: A, control, $V_{max} = 7.93 \times 10^{-4}$ mmole/g of tissue/30 min, and $K_m = 0.242$ mM; and B, in presence of 1 mM sodium salicylate, $V_{max} = 3.57 \times 10^{-4}$ mmole/g of tissue/30 min, and $K_m = 0.204$ mM. Data were derived from mean uptake reported in Table I.*

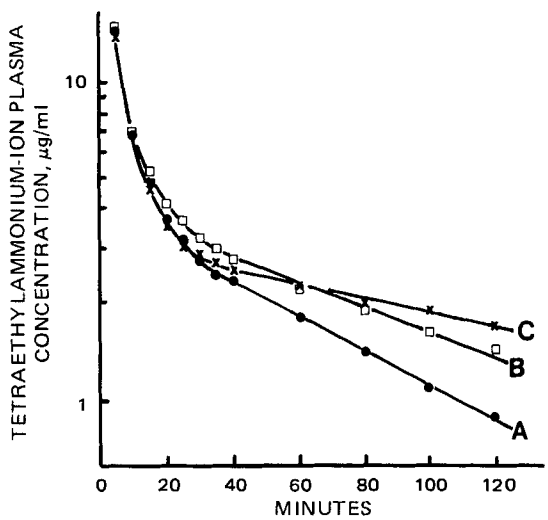


Figure 3—*Influence of salicylate on plasma decline of the ^{14}C-tetraethylammonium ion in rats following rapid intravenous injection of 10 mg of tetraethylammonium bromide/kg. Key: A, control; B, rats pretreated with 30 mg of sodium salicylate/kg; and C, rats pretreated with 50 mg of sodium salicylate/kg. Each data point is the mean for four animals in A and B and for three animals in C. The solid line is computer fit to Eq. 1.*

transport. Salicylate apparently did not cause the inhibition by acting as a competitive substrate but must have influenced tetraethylammonium-ion uptake by another mechanism.

Salicylate has been shown to be an uncoupler of oxidative phosphorylation, a subcellular biochemical process responsible for the synthesis of adenosine triphosphate (6). The exact mechanism of the salicylate effect on oxidative phosphorylation is not yet clearly defined (6). Salicylate also affects protein and amino acid metabolism in the body (7), which results in altered permeability of the cellular structure. Alteration of permeability of the cellular structure that constitutes a selective barrier could be the reason for the reduced uptake of the tetraethylammonium ion in the presence of salicylate.

Pharmacokinetic Study in Rats—After the inhibition of active tetraethylammonium-ion uptake by rat renal cortical slices was observed, the significance of the salicylate inhibition of tubular transport was assessed in single-injection pharmacokinetic studies in rats.

Biliary excretion of radioactive drug was examined as a possible route of extrarenal elimination of the tetraethylammonium ion. Biliary excretion of the tetraethylammonium ion was quantitated, but kinetic analysis suggested that less than 1% of the total elimination was taking place in bile.

The plasma level *versus* time data in each category of experiments, *i.e.*,

Table II—Pharmacokinetic Study of Tetraethylammonium Bromide (10 mg/kg iv) in Rats

	Plasma Tetraethylammonium Ion, μg/ml[a]		
Minutes	No Pretreatment ($n = 4$)	Pretreatment with 30 mg/kg iv Sodium Salicylate[b] ($n = 4$)	Pretreatment with 50 mg/kg iv Sodium Salicylate[c] ($n = 3$)
5	13.58 ± 1.22	14.15 ± 2.18	13.45 ± 1.05
10	7.00 ± 0.49	7.25 ± 0.93	6.63 ± 1.42
15	4.65 ± 0.38	5.02 ± 0.69	4.49 ± 0.69
20	3.57 ± 0.43	3.97 ± 0.58	3.43 ± 0.61
25	3.04 ± 0.42	3.54 ± 0.43	2.96 ± 0.61
30	2.64 ± 0.41	3.10 ± 0.54	2.76 ± 0.79
35	2.38 ± 0.40	2.89 ± 0.44	2.64 ± 0.83
40	2.32 ± 0.38	2.66 ± 0.52	2.44 ± 0.84
60	1.79 ± 0.25	2.17 ± 0.30	2.19 ± 0.75
80	1.40 ± 0.22	1.88 ± 0.30	1.96 ± 0.69
100	1.09 ± 0.21	1.60 ± 0.30	1.91 ± 0.65
120	0.89 ± 0.15	1.43 ± 0.17	1.69 ± 0.58

[a] Mean ± *SD*. [b] Plasma salicylate concentration of 10–12 mg %. [c] Plasma salicylate concentration of 16–18 mg %.

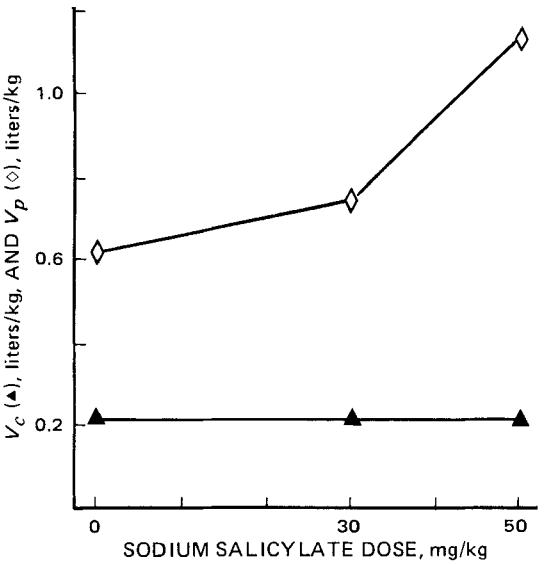


Figure 4—*Effect of sodium salicylate on the apparent volume of the central* (V_c) *and peripheral* (V_p) *compartments for the tetraethylammonium ion.*

control (four rats) and salicylate treatment at 30 (four rats) and 50 (three rats) mg/kg, are presented in Table II. The digital computer fit to mean plasma levels is illustrated in Fig. 3, and the pharmacokinetic parameters are given in Table III.

Salicylate treatments resulted in a significant decrease in the elimination rate constant, k_{13} (25 and 40% decrease compared to k_{13} in control experiments). This observation supports the results of the cortical slice study. Although it cannot be concluded whether tubular secretion, glomerular filtration, or tubular reabsorption was altered by salicylate, it appears that salicylate probably inhibited the active tubular secretion.

Besides the decrease in k_{13}, both k_{12} and k_{21} changed in the presence of salicylate. Increases in the volume of distribution and the ratio of k_{12}/k_{21} suggest enhanced distribution of the tetraethylammonium ion in the peripheral compartment. The apparent volume of the central compartment was unchanged by the salicylate treatment (Fig. 4).

Thus, in the present study, both elimination and distribution of the drug were altered by the salicylate treatment.

The actual amounts of the tetraethylammonium ion present in the peripheral compartment at various times in the three categories of experiments can be calculated by (8):

$$X_p = \frac{k_{12}(\text{dose})}{(\alpha - \beta)}(e^{-\beta t} - e^{-\alpha t}) \qquad \text{(Eq. 2)}$$

or they can be simulated using the CSMP (9) digital computer program. The amount of the tetraethylammonium ion in the peripheral compartment *versus* time data calculated by CSMP are shown graphically in Fig. 5.

Table III—Pharmacokinetic Parameters of Tetraethylammonium Ion

Parameter	Control	30 mg of Salicylate/kg	50 mg of Salicylate/kg
A, μg/ml	24.970 ±1.21	24.813 ±2.16	25.742 ±1.68
B, μg/ml	3.664 ±0.07	3.799 ±0.13	3.041 ±0.08
α, min^{-1}	0.1845 ±0.006	0.1796 ±0.011	0.1854 ±0.008
β, min^{-1}	0.0120 ±0.0003	0.0085 ±0.0005	0.0050 ±0.0004
k_{13}, min^{-1}	0.0649	0.0490	0.0385
k_{21}, min^{-1}	0.0340	0.0312	0.0240
k_{12}, min^{-1}	0.0975	0.1078	0.1278
k_{12}/k_{21}	2.8677	3.4551	5.3250
V_c, ml/kg	216.44	216.61	215.46
V_p, ml/kg	619.80	747.74	1144.72
V_{ss}, ml/kg	836.24	964.34	1360.18
V_{area}, ml/kg	1172.01	1245.09	1658.12

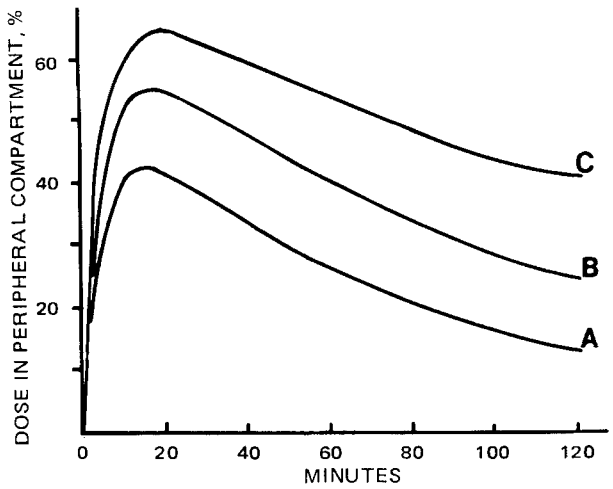


Figure 5—*Influence of sodium salicylate on the fraction of the tetraethylammonium dose in the peripheral compartment following rapid intravenous injection of tetraethylammonium bromide in rats. Key: A, control; B, pretreatment with 30 mg of sodium salicylate/kg; and C, pretreatment with 50 mg of sodium salicylate/kg.*

Tetraethylammonium ions are known to distribute only in extracellular space (10); penetration of the blood–brain barrier is limited. Pretreatment with salicylate resulted in enhanced distribution to peripheral tissues. An increased distribution could be taken as an opening of some barrier to tetraethylammonium-ion distribution somewhere outside the central compartment. Salicylate has various biochemical effects such as interference with protein and amino acid metabolism (7) and uncoupling of oxidative phosphorylation (11). Uncoupling agents are known to alter the selective barrier of the cellular membrane. Whether this alteration results primarily from the uncoupling action or its effects on protein metabolism is not clearly understood (12).

The results of a partition study suggest that there is no ion-pair formation between salicylate and the tetraethylammonium ion that would account for a change in *in vivo* distribution. The apparent partition coefficient of the tetraethylammonium ion between chloroform and pH 7.4 phosphate buffer in the presence of a 10–50 M excess of salicylate remained unchanged at 1.02×10^{-2}.

The most significant influence of the coadministration of salicylate on tetraethylammonium-ion pharmacokinetics was the increase in the amount of the tetraethylammonium ion in the peripheral compartment. This increase was a result of a decrease in k_{13} and an increase in the distribution of drug into the peripheral compartment. The amount of drug in the central compartment was essentially unchanged by salicylate administration. Therefore, the clinical significance of coadministration of salicylate with a quaternary ammonium drug such as tetraethylammonium bromide depends on whether pharmacological response to the drug most closely parallels the amount of drug in the central compartment or that in the peripheral compartment.

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Simultaneous Determination of 4-Nitroanisole, 4-Nitrophenol, and 4-Nitrocatechol by Phase-Sensitive ac Polarography

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Abstract □ Phase-sensitive ac polarography was applied to the simultaneous quantitative determination of 4-nitroanisole, 4-nitrophenol, and 4-nitrocatechol in alkaline solutions. Certain experimental precautions are necessary to determine each compound in the presence of the other two. Thus, 4-nitrocatechol is determined indirectly by forming a yellow ratio chelate with cupric ions, whereas 4-nitroanisole is determined directly by the reduction waves of the nitro group. For the determination of 4-nitrophenol, the interference by the simultaneously present 4-nitrocatechol must be eliminated by masking it by the addition of magnesium ions. The method described permits a qualitative and quantitative analysis of all three compounds in one solution since linear calibration curves are obtained.

Keyphrases ■ 4-Nitroanisole—polarographic analysis in presence of 4-nitrophenol and 4-nitrocatechol, alkaline solutions ■ 4-Nitrophenol—polarographic analysis in presence of 4-nitroanisole and 4-nitrocatechol, alkaline solutions ■ 4-Nitrocatechol—polarographic analysis in presence of 4-nitroanisole and 4-nitrocatechol, alkaline solutions □ Polarography—simultaneous analyses, 4-nitrophenol, 4-nitroanisole, and 4-nitrocatechol, alkaline solutions

Polarography is a common procedure in the analysis of aromatic nitro compounds of different character (1–3), because these compounds are easily reduced at the dropping mercury electrode. The mechanism of the electrode reaction was studied in aqueous solutions (4), and the mechanism of nitro derivative reduction was investigated in aprotic solvents (5, 6). More detailed studies with nitrophenol derivatives also were reported (7, 8).

From these studies, it follows that the nitro compounds can be quantitatively determined when analyzed independently. However, conditions become more complex when several nitro compounds are present. As expected (9), the peak potentials of such compounds are so close to each other that their peaks often overlap, and it is difficult to obtain qualitative and quantitative results without prior separation. Because of the inaccuracy of the results obtained by dc polarography (9), use of phase-sensitive ac polarography was suggested (10). This method allows a better interpretation of neighboring peaks. The logarithmic analysis for overlapping waves (11) is not feasible routinely.

In drug metabolism studies, 4-nitroanisole (I) was used as a model drug for demonstrating oxidative *O*-demethylation activity (12). More recently, the production of 4-nitrocatechol (III) was observed in microsomal suspensions after incubations of I or its main metabolic product, 4-nitrophenol (II) (13, 14). Compound III has an absorption maximum slightly different from that of II, the formation of which is usually recorded spectrophotometrically for 5–10 min at pH 7.85 as a routine kinetic method (15) to measure the initial velocity of the oxidative *O*-demethylation of I to II. Thus, III might interfere with the spectral recording of II formation during prolonged incubations[1].

Pharmacologically, it is desirable to determine these three compounds with the greatest possible simplicity and rapidity. The described experiments resulted in the development of a rapid ac polarographic method, allowing the simultaneous determination of these compounds[2]. In this connection, special emphasis is placed on the effects of pH variation in the polarographic base solution.

EXPERIMENTAL

Measurements were carried out using a phase-sensitive ac polarograph, as described previously (16), with an additional phase-sensitive rectifier.

An internal low frequency oscillator creates a frequency of 12 Hz, which is superimposed on the polarographic cell potential. The variable phase of the phase-sensitive rectifier was adjusted so that the capacitance current was largely eliminated. To avoid beat phenomena, a synchronization of the increase in drop size with the alternating current was necessary. Therefore, the internal low frequency was applied to the drop controller in the electrode stand[3]. The amplitude of the alternating current was 30 mv for all experiments.

The polarograms were recorded with an *xy*-recorder[4] after bubbling purified nitrogen through the solution for 2.5 min. The flow rate of mercury dropping into 0.1 *N* NaOH at a potential of −1.0 v was 3.24 mg/sec, and the drop time was 2.72 sec. With automatic drop control, it amounted to 0.69 sec at a reservoir height of 67 cm. All measurements were recorded *versus* a normal calomel electrode at 21 ± 1°. A commercially available pH meter[5] with a glass electrode was used after calibration

[1] P. Bergheim and K. J. Netter, unpublished results.
[2] The application of the polarographic determination to kinetic analyses in biological samples will be described later.
[3] Metrohm E 354, Herisau, Switzerland.
[4] Metrawatt AG, Nürnberg, Germany.
[5] Knick, Berlin, Germany.