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Effect of Bile Salts on Partitioning Behavior and GI Absorption of a Quaternary Ammonium Compound, Isopropamide Iodide

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Abstract □ The effect of various bile salts and, in particular, sodium glycocholate upon the partitioning behavior of the quaternary ammonium compound isopropamide iodide was studied *in vitro*. Absorption of this compound from the rat ileum *in situ*, in the presence of various concentrations of bile salt, was also studied. The results indicate that sodium glycocholate progressively increases the partitioning of isopropamide from a physiological aqueous buffer into *n*-octanol below the CMC of the bile salt, but increased partitioning is inhibited above this value. Isopropamide did not partition in the absence of the bile salt counterion. The formation of a lipid-soluble ion-pair between the bile salt anion and the quaternary cation is suggested as the mechanism by which enhanced partitioning occurs, the decrease in maximal transport being related to mixed micelle formation or adsorption of ammonium ions to the outer surface of the bile salt aggregate. Absorption from the rat ileum *in situ* in the presence of sodium glycocholate below and above its CMC appears not to follow a similar pattern. It is suggested that the GI absorption of the isopropamide cation cannot be increased in the presence of bile salt molecules through ion-pair formation or mixed micelle formation. Above the CMC of bile salt, the absorptive process appears actually to be hindered through a decrease in the availability of the drug to the absorptive surface, either by a physicochemical interaction with the micellar phase or by decreased diffusivity of the drug in the presence of bile salt aggregates.

Keyphrases □ Isopropamide iodide—effects of bile salts on partitioning behavior (*n*-octanol–water) and GI absorption, possible ion-pair formation, rat ileum □ Bile salts—effects on partitioning behavior (*n*-octanol–water) and GI absorption of isopropamide iodide, possible ion-pair formation, rat ileum □ Ion-pair formation—isopropamide iodide–bile salts, isopropamide partitioning behavior (*n*-octanol–water) and GI absorption, rat ileum □ Partitioning—isopropamide iodide from buffer to *n*-octanol, effects of bile salts □ Absorption, GI—isopropamide iodide, effects of bile salts, possible ion-pair formation, rat ileum

The mucosal surface of the GI tract acts as a lipoidal barrier to nutrient and drug molecules. To characterize drug absorption through such a barrier, Schanker *et al.* (1) proposed the pH–partition hypothesis, relating the degree of absorption of weak acids and bases to their lipid solubility and degree of ionization. Quaternary ammonium compounds, being fully charged at physiological pH, cannot be characterized adequately using such criteria. However, many drugs of this class are known to be pharmacologically active in very small quantities when given *via* the enteral route.

Quaternary ammonium compounds may possibly be absorbed by combination with an endogenous substance in the lumen or wall of the gut (2). Combination with such a substance could conceivably enhance absorption by acting to facilitate diffusion or to initiate an active transport mechanism.

One might postulate the involvement of the bile salts in such an absorptive process. Bile salts, normally present within the lumen of the gut, form micellar solutions which enable fatty acids and monoglycerides to be absorbed (3). Drugs such as the quaternaries, many

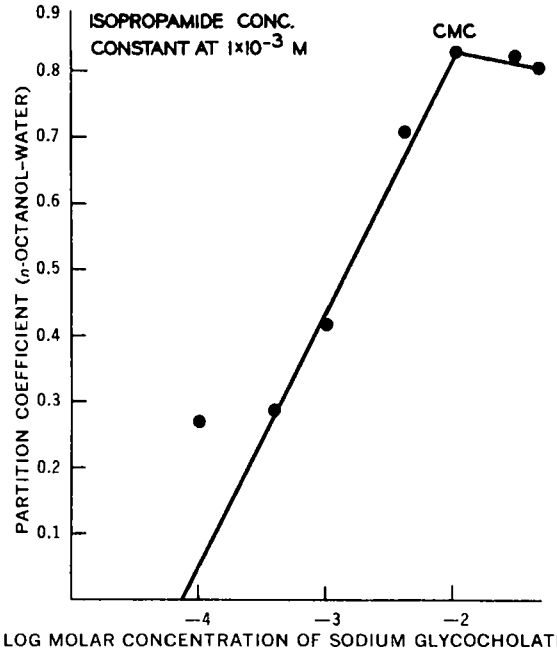

Figure 1—*Apparent partition coefficients of isopropamide iodide equilibrated between* n-*octanol and pH 7.4 potassium phosphate buffer in the presence of various concentrations of sodium glycocholate. Representative data from single experiments are shown. CMC refers to the reported CMC of sodium glycocholate.*

of which are surface active themselves, may incorporate into mixed micelles. Or ion-pairs may form between a bile salt anion and a quaternary cation at concentrations of bile salt below the CMC.

The purposes of this study were to determine if bile salts have the ability to enhance the partitioning of the monoquaternary compound isopropamide iodide across an organic lipoid barrier and to characterize the behavior of this compound in solutions of bile salt, both below and above the CMC. The *in situ* absorption of isopropamide iodide was also studied in the presence of various concentrations of sodium glycocholate.

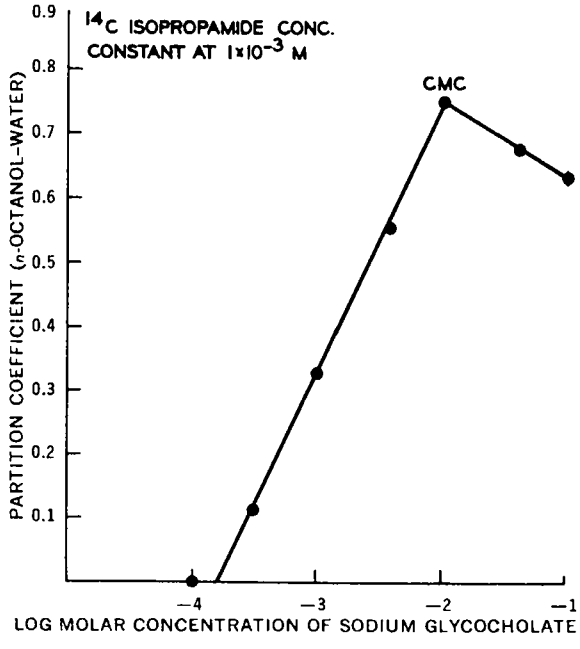

Figure 2—*Apparent partition coefficients of isopropamide iodide equilibrated for 24 hr. between* n-*octanol and pH 7.4 potassium phosphate buffer in the presence of various concentrations of sodium glycocholate. Each data point represents an average of four experimental assay values.*

EXPERIMENTAL

Materials—The following drugs were used as received: isopropamide iodide[1], ^{14}C-isopropamide iodide (specific activity 1.7 mc./mmole[2], sodium cholate[3], sodium glycocholate[4], sodium taurocholate[4], sodium taurodeoxycholate[3], sodium deoxycholate[3], and sodium dehydrocholate[3]. Methyl orange (analytical reagent grade) was used in the colorimetric assay for isopropamide iodide, and Bray's solution (4) was used as the scintillation cocktail in the assay for ^{14}C-isopropamide iodide in the partition studies. A premixed scintillation cocktail[5] was used for counting the ^{14}C-isopropamide iodide recovered from intestinal loops. Grade I (99% pure) *n*-octanol[3] was used as the organic phase in the partition studies. All other solvents and chemicals used in preparing buffers or assay solutions were of analytical reagent grade.

Procedure for *In Vitro* Partition Studies—Partitioning of isopropamide iodide from an aqueous buffer into *n*-octanol in the presence of various concentrations of bile salts was studied colorimetrically and with labeled material. In each case, the concentration of isopropamide iodide was 10^{-3} *M*, with only the bile salt concentration being varied between 10^{-4} and 10^{-1} *M*. At these concentrations of isopropamide iodide, the measured[6] specific conductance showed a linear dependence on concentration, suggesting no formation of large aggregates.

In each case, the isopropamide iodide and bile salts were dissolved together in the same volume of pH 7.4 potassium phosphate buffer[7], which was composed of 3.07 g. KH_2PO_4, 18.36 g. K_2HPO_4, and sufficient deionized water to make 1 l. All solutions were made fresh on the day of use. The pH of all buffer solutions was checked[8].

For the colorimetric assay, a modification of the method of Santoro (5) was employed. Fifteen milliliters of methyl orange buffer[9], pH 10.2, was added to a 1-ml. aliquot of the aqueous phase being assayed (containing at least 0.1 mg. of isopropamide iodide) in a 125-ml. separator. After mixing, this solution was extracted and filtered through a chloroform-saturated pledget of cotton into a 100-ml. volumetric flask, with three 25-ml. portions of chloroform. Ten milliliters of 0.5 *N* HCl in absolute ethanol was then added to develop the color. Volume was brought to a total of 100 ml. with chloroform, and a sample of the final solution was read on a spectrophotometer[10] at 525 nm. against a blank containing chloroform which had been extracted in the same manner. A standard curve was prepared with known concentrations of isopropamide iodide and was linear over the concentration range studied (0.1–0.6 mg./sample). Control experiments indicated that 98–103% of the isopropamide–indicator complex was extractable by the chloroform in the absence, or in the presence, of bile salts after vigorous shaking for 10 min.

Equilibration was with 25 ml. of each phase for 10 min. at 22 ± 1°. After separation by centrifugation, an aliquot (1.0 ml.) of the aqueous phase was assayed and a partition coefficient was calculated by difference. The organic phase, *n*-octanol, was chosen on the basis of its favorable nonpolar lipoid character as suggested by Hansch *et al.* (6).

For determination of partition coefficients using ^{14}C-isopropamide, solutions were prepared as previously described in pH 7.4

[1] Darbid, Smith Kline & French Laboratories, Philadelphia, Pa.
[2] Smith Kline & French Laboratories, Philadelphia, Pa.
[3] Sigma Chemical Co., St. Louis, Mo.
[4] Mann Research Laboratories, New York, N. Y.
[5] Insta-Gel, Packard Instrument Co., Downers Grove, Ill.
[6] Beckman conductivity bridge, model RC-18A, Beckman Instruments, Cedar Grove, N. J.
[7] At this pH the bile salts were all present primarily as ionized species, particularly taurocholic acid (pKa = 1.4) and glycocholic acid (pKa = 4.4).
[8] Beckman Zeromatic pH meter, Beckman Instruments, Cedar Grove, N. J.
[9] Prepared by saturating 1 l. of a solution containing 44 g. dipotassium hydrogen phosphate and 21 g. of sodium carbonate with approximately 2 g. of methyl orange. This solution was purified by extraction with three 50-ml. portions of chloroform and filtration through a Whatman No. 2 filter.
[10] Bausch & Lomb Spectronic-20.

potassium phosphate buffer and equilibrated against *n*-octanol for 24 hr. at 22 ± 1° by continuous shaking[11]. Equal phase volumes of 4 ml. each were placed in a 15-ml. glass centrifuge tube, and the aqueous phase was spiked with a sufficient quantity of ^{14}C-isopropamide. An aliquot (100 μl.) of the aqueous phase was taken before equilibration, counted in 15 ml. of Bray's solution, and considered as 100% of the radioactivity present before partitioning. After equilibration, 100 μl. of the aqueous phase was counted as above and partition coefficients, in the presence of each concentration of sodium glycocholate, were calculated by difference. Each partition coefficient was an average value based on four determinations.

Drug Solutions for Absorption Studies—Isopropamide iodide (10^{-3} *M*) was prepared in isotonic sodium phosphate buffer (pH 6.6) composed of 11.44 g. $NaH_2PO_4 \cdot H_2O$, 8.73 g. Na_2HPO_4, and enough deionized water to make 1 l. Ten-milliliter volumes of these solutions were prepared to contain no sodium glycocholate or 10^{-3}, 10^{-2}, or 10^{-1} *M* sodium glycocholate. Ten milliliters of each solution was spiked with 100 μl. of ^{14}C-isopropamide. A 0.5-ml. volume represented the amount of isotope present in the intestine at the beginning of the absorptive period and gave an average of 8.137×10^3 d.p.m.

Procedure for Absorption Studies—Male albino rats[12], weighing 300–350 g. and fasted overnight, were anesthesized with sodium pentobarbital (35 mg./kg. i.p.). A midline incision was made and the terminal ileum was removed from the abdominal cavity. Two ligations were made, using (00) silk thread, approximately 5 and 15 cm. proximal to the ileocecal junction. Care was taken not to tie off the mesenteric blood supply leading to the segment, and manipulation of the tissue was kept minimal. Following ligation, 0.5 ml. of the test solution was injected using a 27-gauge, 1.27-cm. (0.5-in.) needle. After injection, gentle digital pressure over the injection site for a few seconds prevented solution leakage. The ileal segment was replaced in the abdominal cavity and the incision was covered with a 5.1 × 5.1-cm. (2 × 2-in.) gauze pad saturated with 0.9% saline solution. Six periodic checks were made with an ophthalmoscope[13] to determine the presence and onset of mydriasis. Animals were randomly chosen for any given treatment (isopropamide-bile salt combination).

After a 60-min. absorption period, the animals were sacrificed with an overdose of sodium pentobarbital and the ileal segment was removed. The segment was placed in a scintillation vial and flushed with 4.5 ml. of pH 6.6 sodium phosphate buffer. After appropriate mixing, a 0.5-ml. sample of the wash was withdrawn and added to 15 ml. of premixed scintillation cocktail. The mixture was shaken and then allowed to stand several hours in the dark before counting.

After conversion of counts per minute to disintegrations per minute, the percent of ^{14}C-isopropamide still remaining in the gut was determined and the percent absorbed was calculated by difference.

To determine the extent of binding to the intestinal mucosa, tissue digests were prepared[14] by weighing a piece of the ileal segment (approximately 150 mg.) and digesting it[15] for several hours at 50°. When fully dissolved, 15 ml. of premixed scintillation cocktail was added. A separate efficiency calculation was necessary for each sample since slightly different quenching occurred due to differences in color present.

RESULTS AND DISCUSSION

Partition Experiments—Sodium glycocholate, in increasing concentration, enhanced the interphase transport of isopropamide from an aqueous buffer (pH 7.4) into *n*-octanol. Increased partitioning of isopropamide was observed until a concentration of 10^{-2} *M* bile salt was reached. This value is reported to be the approximate CMC of sodium glycocholate within the pH and counterion concentration used in this study (7). These partitioning data were obtained using both the methyl orange assay (Fig. 1) and ^{14}C-isopropamide (Fig. 2). No partitioning of isopropamide occurred in the absence of the bile salt. With ^{14}C-isopropamide at a 10-fold dilution (10^{-4} *M*), partitioning followed the same pattern (Fig. 3).

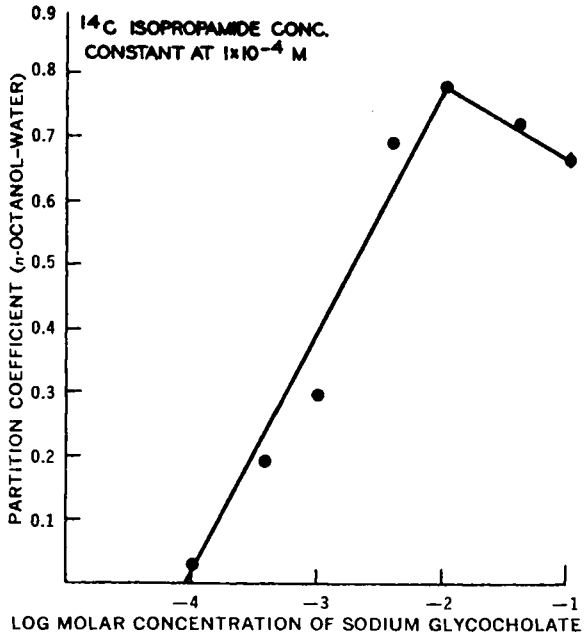

Figure 3—*Apparent partition coefficients of isopropamide iodide equilibrated for 24 hr. between* n-*octanol and pH 7.4 potassium phosphate buffer in the presence of various concentrations of sodium glycocholate.*

Several other bile salts were used in partitioning studies, but not all displayed the same behavior. Sodium taurodeoxycholate behaved very much the same as sodium glycocholate, increasing partitioning to its reported approximate CMC (8) and then exhibiting a plateau (Fig. 4). Other bile salts (Table I) showed increased partitioning of isopropamide at and above their respective CMC values; however, sodium dehydrocholate has been reported not to form micelles (9).

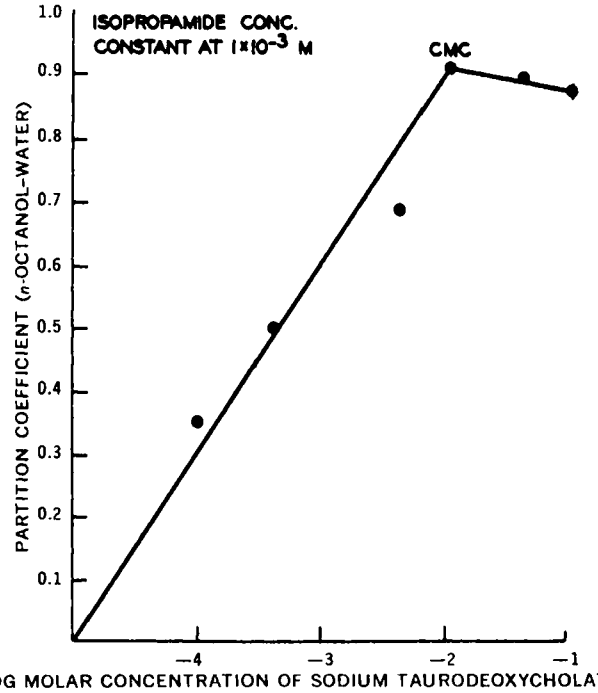

Figure 4—*Apparent partition coefficients of isopropamide iodide equilibrated between* n-*octanol and pH 7.4 potassium phosphate buffer in the presence of various concentrations of sodium taurodeoxycholate. Representative data from single experiments are shown.*

[11] Eberbach automatic shaker, Eberbach Co., Ann Arbor, Mich.
[12] Rolfsmeyer Farms, Madison, Wis.
[13] Welch Allyn, Co., Skaneateles Falls, N. Y.
[14] Only two randomly selected samples were taken from each treatment group.
[15] In 2 ml. of Soluene, Packard Instrument Co., Downers Grove, Ill.

Table I—Apparent Partition Coefficients for Isopropamide Iodide (10^{-3} *M*) in the Presence of Various Concentrations of Different Bile Salts[a]

Bile Salt	Concentration, *M*	Apparent Partition Coefficient (*n*-Octanol–Water)
Sodium cholate	1×10^{-4}	0.31
	1×10^{-3}	0.29
	1×10^{-2}	0.44
	5×10^{-2}	0.81
Sodium deoxycholate	1×10^{-4}	0.35
	1×10^{-3}	0.63
	1×10^{-2}	0.88
	5×10^{-2}	0.96
Sodium dehydrocholate	1×10^{-4}	0.29
	1×10^{-3}	0.38
	1×10^{-2}	0.52
	5×10^{-2}	0.75
Sodium taurocholate	1×10^{-4}	0.40
	1×10^{-3}	0.44
	1×10^{-2}	0.85
	5×10^{-2}	0.85

[a] Isopropamide concentration in the aqueous phase, determined after equilibration by the methyl orange assay method from duplicate extractions.

Studies by Levine and Clark (10) and by Schanker and Solomon (11) indicated that various other monoquaternary compounds can be extracted into an organic phase (ethylene dichloride) in the presence of sodium glycocholate below its CMC. Formation of ion pairs between quaternary ammonium ions and carboxylic acids (12), as well as sulfonates (13), is known to occur. Methyl orange (5) and bromthymol blue (14) also form ion-pairs with quaternary ammonium cations. Tetraheptylammonium chloride was used by Hofmann (15) to extract steriod sulfates quantitatively as well as glycine and taurine conjugates of bile acids from aqueous solvents.

Various possibilities exist for the interaction of bile salt anions *in vitro* among themselves and with quaternary cations in the aqueous phase.

Mixed micelles may form between short-chain alkyl amines and bile salts involving van der Waals' forces between the hydrocarbon chains and hydrophobic surface of the steroid nucleus (16); also, adsorption of the oppositely charged amine may occur onto the micellar surface at concentrations of bile salts above the CMC.

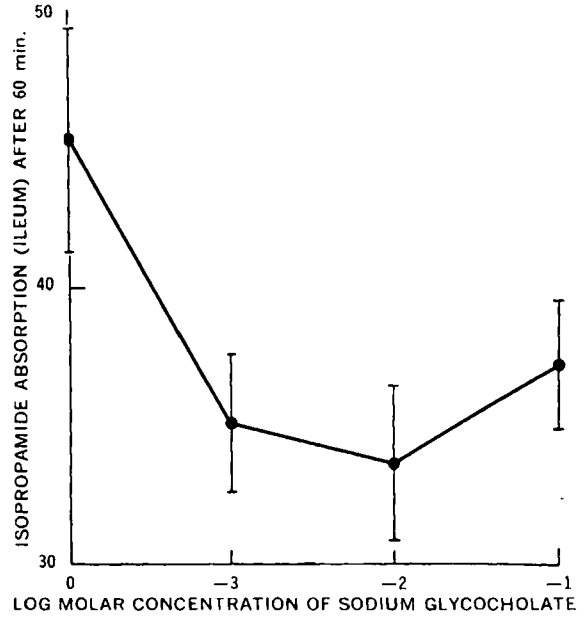

Figure 5—In vivo *absorption of isopropamide iodide from ligated segments of rat ileum. Each data point is the mean of 12 animals, and bars represent the standard error of the mean for each point. Points at zero* versus 10^{-3} *and* 10^{-2} M *were the only means significantly different* ($p < 0.05$) *from one another.*

Below the CMC, the most likely situation is the formation of ion-pairs and the partitioning of these into the organic phase. In the case of the ion-pair partitioning, the following interaction in the aqueous phase might be assumed:

$$B_a^- + Q_a^+ \overset{K_{BQ}}{\rightleftharpoons} (BQ)_a \overset{K_p}{\rightleftharpoons} (BQ)_o$$

Scheme I

Here K_{BQ} is the equilibrium constant for ion-pair formation between the bile salt anion (B_a^-) and quaternary cation (Q_a^+) in the aqueous phase, and K_p is the partition coefficient for partitioning of the neutral species $(BQ)_a$ into the organic phase.

According to the partitioning data, this would seem to be a possible mechanism for interphase transport of isopropamide below the CMC. Micellar transport probably would be inhibited by a dense negative charge surrounding the micelle.

Absorption Studies—The present studies indicate that the absorption of isopropamide iodide is not influenced by the presence of sodium glycocholate. Isopropamide iodide itself is capable of being absorbed from the rat ileum to the extent of 44% over a 60-min. absorptive period (Fig. 5). A decrease in absorption of this cation is seen in the presence of all concentrations of sodium glycocholate. Statistically, the mean value for isopropamide absorption in the absence of any bile salt was significantly different ($p < 0.05$) only from 10^{-3} and 10^{-2} *M* sodium glycocholate, as determined using analysis of variance and a Duncan's multiple-range test. Between 10^{-2} and 10^{-1} *M* bile salt concentration, there was an apparent increase in the amount of isopropamide absorbed, although this rise was still well below the value found in the absence of bile salt. Jejunal absorption followed the same pattern, with the corrected percentages for absorption being essentially the same.

Others (17) showed that above their respective CMC's, sodium glycocholate and sodium taurocholate at pH 6.5 inhibit the absorption of the amide, 2-allyloxy-4-chloro-*N*-(2-diethylaminoethyl)-benzamide hydrochloride, from the rat small intestine.

Digests of the intestinal tissue indicated that approximately 21.2 and 13.0% of the radioactivity remained in the ileal and jejunal gut wall, respectively. The value was subtracted from the graphical values (Fig. 5) and agrees well with a figure of 20% for a dose of anisotropine methylbromide reported recently (18). Corrections for volume changes due to secretion into the lumen were also made, since it appeared in early studies that 10^{-1} *M* sodium glycocholate was causing an intraluminal increase in drug solution volume. The nonabsorbable marker ^{14}C-inulin was used in control experiments involving all bile salt concentrations, and the only detectable effect was seen at the 10^{-1} *M* level. This amounted to a 15% dilution of the amount of drug remaining in the intestinal loop after 1 hr. The corrected value at this concentration of sodium glycocholate is the one expressed in Fig. 5.

During the absorptive period, observations were made to determine the onset and degree of mydriasis. This was not a good indicator of absorption, however, since only one or two animals in each group exhibited this response, and there was no relationship between time of onset of the response and bile salt concentration. Irwin *et al.* (19) suggested that ion-pair formation with trichloroacetate as the anion can enhance the partitioning of isopropamide iodide into chloroform and *n*-octanol, as well as increase the degree of mydriasis in mice given the drug orally with a large molar excess of trichloroacetate. Their conclusion that this anion increases absorption by ion-pair formation in the lumen has been criticized (20), however, since the concentrations of trichloroacetate administered could have decreased protein or tissue binding of the isopropamide, thus increasing the bioavailability of the small amount of drug that might have actually been absorbed *via* a different mechanism.

If an ion-pair or other type of complex is present in the gut at concentrations of bile salt below its CMC, several possibilities for the diffusion of this complex arise. Depending upon various equilibrium constants, the ion-pair may cross the mucosal cell barrier as the neutral species and remain unchanged within the cell, thus subsequently passing to the blood or lymph. It may, however, be disrupted by protein constituents of the membrane surface, as has been described for some dye complexes (21), and thus enter as the free cation. Furthermore, any amount of quaternary cation that is not part of an aggregate or ion-pair may complex within the intes-

tinal mucosa with anionic membrane components and be transported to the circulation as a different complex. In this regard, some related compounds (dialkyl propionamides) were recently shown to have the apparent ability to form a complex within the intestinal membrane itself and thus enhance the absorption of prednisone and prednisolone (22).

Some quaternary ammonium compounds have been found to be rapidly absorbed initially and then more slowly (23), indicating possible saturation of a carrier and a facilitated diffusion mechanism (24).

Tetracycline appears to be absorbed from the rat stomach as the charged species by such a mechanism (25), and Levine (26) postulated an endogenous carrier for quaternary compounds. Other effects, such as cooperative membrane reactions (27) or solvent drag (28), may also play a role in the absorption of quaternary drugs. Isopropamide, which possesses both some lipophilicity and hydrophilicity as do many other monoquaternary compounds, may possibly be taken up into globular micelles (29) of the absorptive cell membrane. This seems relevant in view of the ability of isopropamide to interact with bile salt micelles as reported here.

Isopropamide is absorbed to a greater extent than expected for a charged molecule, but the mechanism does not appear to involve bile salts. A facilitated transport process may be involved, but it is not proven by the data reported here.

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