Table VI—Rate Constants, Half-Lives, and Sticking Probability Factors of Platelet Aggregation in the Presence of Arachidonic Acid and Aspirin

Arachidonic Acid[a], μg	Aspirin[a], μg	Rate Constant[b], $K_{ss} \times 10^{-11}$, cm^3/sec	Half-Life, $t_{1/2}$, sec	Sticking Probability Factor, $\gamma \times 10^6$/cm/Platelet
540	0.0	3.18	95.90	0.0222
720	0.0	18.90	16.10	0.1321
540	2.25	−0.24	—	−0.0016
720	2.25	−4.40	—	−0.0307

[a] Amount injected into 1 ml of platelet-rich plasma. [b] Rate constant is zero for control experiments.

$\times 10^{-9}$ cm^2/sec is obtained, which is rather small compared to molecular diffusivities which are usually about 10^{-4}–10^{-6} cm^2/sec. Platelets physically approaching each other may be repulsed or aggregated or aggregates may disperse, depending on the reactivity of their surfaces, which is modulated by the molecular species in the solution.

The surface-barrier process is further supported by calculated values of the stability constant, W, from Eq. 9 showing numbers smaller than 1. Values of the sticking probability constant, γ, were calculated and are reported in Tables I–VI. As reported previously (10), adenosine diphosphate produces an alteration in the platelet membrane surface. Therefore, larger γ values in the presence of adenosine diphosphate probably are the result of a specific modification of the membrane surface reactivity by that compound. More work is needed regarding the biosynthesis of prostaglandins and surface-barrier effects.

The experimental results with prostaglandins and aspirin suggest the following with respect to therapeutics. If it is assumed that the blood volume in an average individual is 5 liters and that a person receiving aspirin or prostaglandin has complete systemic availability of the dose with little or no tissue distribution, the doses of aspirin and prostaglandin E_1 needed to block platelet aggregation completely will be around 10 and 1 mg, respectively. Thus, a combined dose of aspirin and prostaglandin E_1 will have a dual effect in blocking platelet aggregation and possibly thrombus formation.

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Possible Ion-Pair-Mediated Absorption of Mixidine I: Partitioning and Lethality Studies

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Received August 12, 1977, from the *Research Division, McNeil Laboratories, Inc., Fort Washington, PA 19034.* Accepted for publication October 28, 1977.

Abstract □ Mixidine, a very soluble base which is completely ionized in all physiological fluids, was found to form ion-pairs as demonstrated by its ability to partition into 1-butanol from acidic solutions. A similar relationship was observed for the effect of acids on the absorption of intraduodenally and orally administered solutions of mixidine in rats (as determined by lethality). Studies also demonstrated that the pH-lethality effects were not specific for a particular counterion. Mixidine was more lethal when administered intraduodenally than when administered orally, and the counterions *per se* were not lethal in the doses used.

Keyphrases □ Mixidine—ion-pair formation *in vitro* determined by partitioning, absorption in rats determined by lethality □ Ion-pair formation—mixidine *in vitro*, determined by partitioning □ Absorption—mixidine in rats, determined by lethality □ Vasodilators, coronary—mixidine, ion-pair formation *in vitro* determined by partitioning, absorption in rats determined by lethality

The utilization of ion-pairs, neutral species formed by electrostatic attraction between oppositely charged ions in solution, has been studied as a method for improving drug absorption. The duration of rabbit corneal anesthesia was related to the chloroform–water distribution of dibucaine combined with 10 counterion species (1). The enhanced pharmacological effect of an orally administered quaternary ammonium compound by trichloroacetic acid was related to increases in partition between chloroform or 1-octanol and pH 6 buffer (2).

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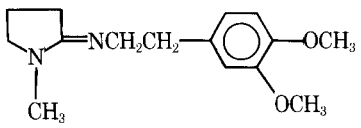

I

The enhancement of the absorption of quaternary ammonium compounds in rat preparations was reported using trichloroacetic acid (3), salicylic acid (3), and decylsulfuric acid (4) as the counterion species.

BACKGROUND

Absorption experiments with ion-pairs have not always resulted in clear pictures of the absorption-enhancing mechanism. One study (5) related apparent ion-pair-mediated absorption more to mucosal binding or mucosal erosion. Apparent site specificity was demonstrated for ion-pair-mediated absorption in rats, *i.e.*, absorption enhancement through the stomach and rectal walls but not through the intestinal wall (6).

Ion-pair-enhanced absorption has become controversial. The absorption of protonated dextromethorphan from the rat stomach was counterion dependent, but dextromethorphan was not absorbed in direct association with the counterion (7). An interfacial tension lowering effect on the gut wall by counterions was proposed as the mechanism for absorption enhancement. Another study (8) supported these conclusions (7) by demonstrating a tetracycline absorption enhancement relationship to surface tension lowering by counterions. However, in a later study (9), bile salts that enhanced isopropamide partitioning failed to increase isopropamide absorption from the rat ileum *in situ*, even when the bile salts were tested above and below their critical micelle concentrations.

Subsequently, Klink and Colaizzi (10) challenged the surface tension lowering theory of Perrin and Vallner (8) by demonstrating increases in the partitioning of several tetracyclines by trichloroacetic acid in acid media.

Mroszczak *et al.* (11) proposed that absorption enhancement, purportedly through ion-pair formation, actually may be caused by the minimization of protein binding of the drug by the counterion. The feasibility of this concept was demonstrated with trichloroacetic acid, a counterion frequently used in ion-pair studies.

More recently, various effects of chloride-ion concentration on the solubility, partitioning, dissolution, and GI absorption of haloperidol, droperidol, and pimozide were reported (12). These observations were explained as interactions among solubilization, precipitation, and ion-pair formation.

A unique aspect of the role of ion-pair formation on the biological activity of methantheline bromide was reported (13); an increase was observed in the lethal intravenous dose of the methantheline bromide solution upon the addition of neutralized trichloroacetic acid. This result was attributed to an increase in drug distribution in the body because of the increased lipophilicity of the ion-pairs.

Although the role of ion-pairs in drug absorption is controversial, the idea of increasing the absorption of a fully ionized, water-soluble drug by forming a neutral lipid soluble complex with a counterion remains intriguing.

Mixidine, 3,4-dimethoxy-*N*-(1-methyl-2-pyrrolidinylidene)benzeneethanamine (I), is a very soluble, surface-active compound and is completely ionized in all physiological fluids. The pKa of mixidine is greater than 12. It is poorly absorbed following oral administration to rats. Consequently, mixidine is an ideal candidate for testing the ion-pair-mediated absorption hypothesis.

This report deals with some preliminary experiments to determine under what conditions a mixidine ion-pair would form and if absorption would be enhanced. Partitioning studies were performed to assess the existence of ion pairs. Rat lethality studies permitted an evaluation of absorption without the intricacies of a quantitative assay in biological fluids.

Originally, mixidine was crystallized as the fumarate salt. Arylsulfonate salts were supplied in the hope that the extremely high ionization of arylsulfonic acids would permit them to function more effectively as ion-pair formers.

EXPERIMENTAL

Partitioning Studies—Citrate–phosphate buffers, with potassium chloride added to adjust ionic strength, were prepared at $\mu = 0.077$ (pH 2.2–3.0), $\mu = 0.157$ (pH 2.2–3.8), and $\mu = 0.344$ (pH 2.2–5.8) according to the method of Elving *et al.* (14). Several organic solvents were screened for their ability to extract mixidine from aqueous solutions, and 1-butanol was the most satisfactory. The buffers were saturated with 1-butanol and vice versa. The concentration of mixidine as mixidine fumarate in the aqueous phase was 5 mg/ml. The phases were combined and agitated for 30 min. The aqueous phase was assayed spectrophotometrically at 275 nm for remaining mixidine.

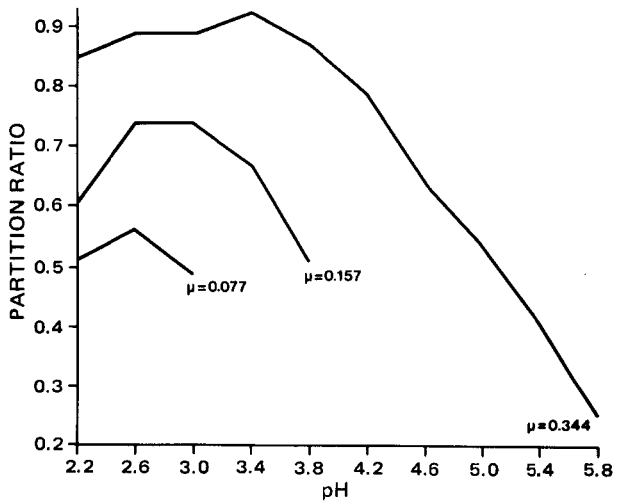

Figure 1—*Effect of pH and ionic strength of citrate–phosphate buffers on mixidine partition ratios between 1-butanol and water.*

A partitioning study with simulated gastric fluid USP without pepsin as the aqueous phase also was performed.

Lethality Studies—Female Sprague–Dawley rats, 150–200 g, were fasted for 24 hr and then lightly anesthetized with ether. For intraduodenal administration, a midline incision was made and the duodenum was identified. The test preparation was administered into the lumen of the duodenum, approximately 1 cm distal to the pylorus, through a 26-gauge needle fitted with a syringe. Reflux of the test preparation into the stomach was prevented by digital occlusion between the injection site and the pylorus.

The incision site was closed with metal wound clips, and the rats were allowed to recover in individual cages. Water was allowed *ad libitum;* food was allowed 4 hr after drug administration. The rats were observed continuously for the 1st hr for signs of drug effects or lethality and then periodically for an additional 5 hr.

For oral administration, unanesthetized rats were used except where noted. Administration of the test preparation was by the standard oral administration technique.

Solution Preparation—Solutions of 75 mg of mixidine/ml for intraduodenal administration and of 100 mg of mixidine/ml for oral administration were prepared by dissolving the mixidine salt or an equivalent mixture of mixidine with an acid in distilled water. To obtain preparations within a wide range of pH values, the pH of the solutions was adjusted with additional amounts of acid. Solutions of pH 5.0 and higher were prepared similarly, except that simulated intestinal fluid USP without pancreatin was used as the solvent.

Doses—Preliminary experiments determined that very acidic solutions

Table I—Mixidine (75 mg/ml) Lethality in Rats following Intraduodenal Administration [a]

Counterion	pH	Dose, mg/kg 1500	750	375	188
Hydrochloride	1.6	3/3	3/3	0/3	0/3
Fumarate	5.0	—	0/3	—	—
Besylate	1.3	—	3/3	—	—
Besylate	2.4	—	2/3	—	—
Besylate	4.0	—	0/3	—	—
Besylate	5.6	—	0/3	—	—
Tosylate	1.3	—	3/3	—	—
Tosylate	2.5	—	3/3	—	—
Tosylate	3.0	—	2/3	—	—
Tosylate	3.6	—	0/3	—	—
Tosylate	4.0	—	0/3	—	—
Tosylate	5.6	—	0/3	—	—

[a] Values are number of deaths per number of rats.

Table II—Mixidine (100 mg/ml) Lethality in Rats following Oral Administration [a]

Counterion	pH	Dose, mg/kg 4000	2000	1000	500
Besylate	1.2	3/3	2/3	0/3	0/3
Besylate	6.2	—	0/3	0/3	0/3
Tosylate	1.3	—	2/3	0/3	0/3
Tosylate	2.1	—	3/3	0/3	0/3
Tosylate	2.5	3/3	2/3	0/3	—
Tosylate	5.0	—	0/3	0/3	0/3
Napsylate	1.2	—	3/3	0/3	0/3
Napsylate	2.5	3/3	3/3	0/3	—

[a] Values are number of deaths per number of rats.

of mixidine administered intraduodenally were always lethal at 750 mg/kg. Similar experiments with oral administration determined that 4000 mg/kg was always lethal. Consequently, studies of the effect of counterion concentrations (as measured by pH) on mixidine lethality were initiated at these doses. For proprietary reasons, more lethality tests were conducted at doses of 2000, 1000, and 500 mg/kg in the oral dose study. All doses were tested on three rats.

Effect of Route and Anesthesia—To ascertain more fully the effects of the route of administration and of anesthesia–surgery on mixidine lethality, a solution containing 150 mg of mixidine/ml as the fumarate at pH 3.7 was administered at 1500 mg of mixidine/kg to four groups of 20 rats each. The groups represented rats dosed orally with mixidine, rats dosed orally with mixidine and receiving the anesthesia–surgery as in intraduodenal administration, rats dosed intraduodenally with mixidine and receiving anesthesia–surgery, and rats dosed intraduodenally with distilled water and receiving anesthesia–surgery.

Effect of Counterions—The lethality of the counterions *per se* was determined with solutions of acids equivalent to 150 mg of mixidine/ml. Rats were dosed intraduodenally with the equivalent of 750 mg of mixidine/kg and orally with the equivalent of 4000 mg of mixidine/kg. All doses were tested on three rats.

RESULTS AND DISCUSSION

Figure 1 illustrates the influence of pH and ionic strength on the 1-butanol–water partitioning of mixidine from pH 2.2 to 5.8. In the citrate–phosphate buffer, counterions facilitated the partition of mixidine into the organic layer. The partition from the citrate–phosphate buffer was maximum in the pH 2.6–3.4 range. Partitioning also depended on ionic strength: the greater the ionic strength, the greater the partitioning. Consequently, partitioning would be expected to change with buffer composition because it would be a function of buffer species, pKa values and concentrations. Figure 1 illustrates that ion-pair-mediated partitioning of mixidine into 1-butanol can occur and that it is influenced by the aqueous phase.

The partition ratio of mixidine (supplied as the fumarate) from simulated gastric fluid USP without pepsin into 1-butanol was 0.381. The simulated gastric fluid USP without pepsin had a pH of 1.26 and an ionic strength of $\mu = 0.697$. At that pH and ionic strength, the partitioning ratio agrees with those reported in Fig. 1.

Rat lethality, following intraduodenal administration, was selected as a rapid screening method for mixidine absorption. The onset of mixidine lethality (respiratory depression) was rapid, often occurring a few minutes after administration. Table I relates the influence of dose, salt form, and pH on rat lethality following intraduodenal administration.

Included in Table I is the response of four dose levels of intraduodenally administered mixidine as a solution in simulated gastric fluid USP without pepsin (pH 1.6). The data show the anticipated response to increasing doses in the rat, *i.e.*, lethality at the higher doses, and no lethality at the lower doses. The minimum lethal dose administered was 750 mg/kg.

Table III—Mixidine Lethality in Rats as a Function of the Route of Administration and the Condition of the Rats

Route	Dose[a]	Anesthesia	Surgery	Lethality[b]
Oral	1500	No	No	4/20
Oral	1500	Yes	Yes	2/20
Intraduodenal	1500	Yes	Yes	20/20
Intraduodenal	0[c]	Yes	Yes	0/20

[a] Milligrams of mixidine per kilogram as the fumarate (pH 3.7). [b] Number of deaths per number of rats. [c] Vehicle only (distilled water).

Table IV—Counterion Lethality in Rats

Counterion[a]	pH	Lethality[b]
Intraduodenal Administration (Equivalent to 750 mg of Mixidine/kg)		
Fumarate[c]	2.3	0/3
Besylate	0.5	0/3
Tosylate	0.5	0/3
Oral Administration (Equivalent to 4000 mg of Mixidine/kg)		
Besylate	0.5	0/3
Tosylate	0.5	0/3
Napsylate	0.5	0/3

[a] Solutions equivalent to 150 mg of mixidine/ml. [b] Number of deaths per number of rats. [c] Suspension.

With the minimum lethal dose as a standard, the influences of salt form and pH were evaluated. The form of mixidine had no measurable effect on lethality. What was important was the effect of pH (counterion concentration) on lethality: the lower the pH, the higher the lethality. To an extent, this pH–lethality relationship follows the pH–partition relationship in 1-butanol where, within limits, a lower pH resulted in greater partitioning. But, unlike the pH–partition relationship which peaked at pH 2.6, lethality increased continuously to the lowest pH tested, 1.3. At the lowest pH values, the increase in lethality was not anticipated from the partitioning ratio. Apparently, the relative affinity for the mixidine ion-pairs is different for the rat duodenum as compared to 1-butanol.

Lethality following oral administration required three to four times more mixidine than lethality following intraduodenal administration. Otherwise, the effects of dose, salt form, and pH were the same as following intraduodenal administration (Table II).

A further examination of the effect of the administration route on the response to mixidine is given in Table III. Orally administered mixidine, whether administered with or without the anesthesia and the surgery necessary for intraduodenal administration, was much less lethal than intraduodenally administered mixidine. Also, a sham operation with the surgery and the anesthesia but without the mixidine was not lethal. Thus, the results demonstrate that intraduodenally administered mixidine does not owe its lethality to anesthesia–surgery and that it is possible that the duodenum is a better absorption site for mixidine in rats.

Although the lethality of the counterions *per se* was not expected to be a problem, solutions of all counterions in distilled water, equivalent to 4000 mg of mixidine/kg po and 750 mg of mixidine/kg id, were tested (Table IV). None of the counterions was lethal. Consequently, the lethalities reported in Tables I–III were due to respiratory depression caused by mixidine and not from any overt property of the counterions.

SUMMARY

If it is assumed that the absorption (cause) and lethality (effect) premise upon which the absorption studies were founded is correct, it appears that mixidine absorption can be facilitated by increasing the counterion concentration, *i.e.*, decreasing the vehicle pH. Consequently, the ion-pair-mediated absorption hypothesis appears to operate, *i.e.*, increased partitioning into a lipophilic phase, 1-butanol, or rat duodenum, with an increased counterion concentration. However, the absorption hypothesis obviously does not operate in the exact manner suggested by ion-pair-mediated partitioning into 1-butanol. The lack of a perfectly parallel relation between 1-butanol partitioning and lethality exists at the highest counterion concentration (lowest pH).

Plasma level studies and pathological examinations are underway and will be reported later.

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Possible Ion-Pair-Mediated Absorption of Mixidine II: Plasma Levels and Histology

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Abstract □ Plasma intact ^{14}C-mixidine levels in rats increased when the drug was administered intraduodenally with 1:3 and 1:5 molar ratios of 2-naphthalenesulfonic acid. Upon histological examination of the duodenums, similar doses of mixidine combined with 2-naphthalenesulfonic acid produced no dose-related lesions. These and previous observations demonstrate that mixidine absorption may be enhanced by ion-pair formation.

Keyphrases □ Mixidine—absorption, effect of intraduodenal administration with various ratios of 2-naphthalenesulfonic acid as counterion □ Absorption—mixidine, effect of intraduodenal administration with various ratios of 2-naphthalenesulfonic acid as counterion □ Ion-pair formation—effect on mixidine absorption, intraduodenal administration with various ratios of 2-naphthalenesulfonic acid as counterion □ Vasodilators, coronary—mixidine, absorption, effect of intraduodenal administration with various ratios of 2-naphthalenesulfonic acid as counterion

The influence of counterions (acids) on the 1-butanol–water partitioning and the lethality in rats of mixidine, a completely ionized base, was reported (1). When the counterion concentration in an aqueous solution of mixidine was increased, mixidine partitioning into 1-butanol increased, as did the lethality of the solution in rats. This concomitant increase in partitioning with an increase in lethality led to the conclusion that mixidine absorption, as measured by lethality, was enhanced by ion-pair formation.

Also reported were the observations that the ion-pair-mediated absorption of mixidine was most easily demonstrated following intraduodenal administration, that counterions *per se* were not lethal, and that the counterions were equivalent in their ability to mediate mixidine absorption at a given pH (1).

In this study, plasma ^{14}C-mixidine levels were followed to determine if a parallel relationship to the effect of counterion concentration on partitioning and lethality occurred. In addition, rats dosed similarly to those in the plasma level study were examined histologically in an effort to show an absence of counterion-related pathology.

EXPERIMENTAL

Plasma Level Studies—*Preparations*—^{14}C-Mixidine fumarate, specific activity of 5.1 μCi/mg, was dissolved in distilled water to yield a salt concentration of 12.6 mg/ml. Rats were dosed with the equivalent of 25 mg of ^{14}C-mixidine/kg (~0.5 ml/rat).

The labeled mixidine was administered alone and in 1:1, 1:3, and 1:5 mixidine to 2-naphthalenesulfonic acid molar ratios. In experiments with 2-naphthalenesulfonic acid, the volumes of the solutions never exceeded 0.5 ml. In one experiment, 2-naphthalenesulfonic acid in a 1:3 drug to adjuvant ratio was neutralized by adding sodium hydroxide equimolar to the 2-naphthalenesulfonic acid.

Administration—Male CFN rats, 180–200 g, were fasted for approximately 17 hr. For oral administration, the rats were dosed *via* stomach tube followed by a 0.5-ml water wash. For intraduodenal administration, the rats were anesthetized with ether and dosed according to the procedure described previously (1).

Three rats were employed for each dose-route combination, except for the mixidine–2-naphthalenesulfonic acid (1:3) combination for intraduodenal administration where four rats were employed.

Analysis—The assay for intact drug was one in which the specificity had been established (2). A 100-μl blood sample was taken from the tail vein and rinsed into 0.5 ml of distilled water. Ten milliliters of chloroform, 1.0 ml of 1.0 *N* NaOH, and 1.0 ml of 0.096 mg of mixidine/ml as the fumarate were added. Then the mixture was agitated and centrifuged.

Table I—Plasma Intact Mixidine Levels (Nanograms per Milliliter) following Oral Administration of ^{14}C-Mixidine (25 mg/kg)

	1:0 Drug–Adjuvant (pH 3.7)				1:1 Drug–Adjuvant (pH 2.5)				1:3 Drug–Adjuvant (pH 1.6)			
Minutes	A	B	C	Mean	A	B	C	Mean	A	B	C	Mean
20	36	31	76	48	33	20	31	28	25	19	27	24
40	32	32	43	36	42	32	27	34	21	30	42	31
60	28	23	40	30	31	30	19	27	30	—	34	32
120	26	163	14	68	31	17	19	22	19	21	21	20
180	49	34	60	48	55	8	59	41	15	18	19	17
240	66	12	28	35	25	15	127	56	12	—	17	14
360	7	1	14	7	8	18	8	11	12	17	18	16

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