Further Investigations into the Absorption of Dextromethorphan from the Rat's Stomach

G. FIESE* and J. H. PERRIN

Abstract □ Dextromethorphan has been shown to be absorbed by a passive diffusion mechanism from the rat's stomach as a protonated species. Using buffers of a constant pH 2.0, it was found that the absorption rate depended on the anionic species of the buffer, but experiments with ^{14}C-labeled trichloroacetate buffers suggested that the drug was not absorbed as the lipid-soluble ion pair. Good correlation between the surface activity of the drug solutions and the corresponding absorption rates was observed.

Keyphrases □ Dextromethorphan absorption—rat stomach □ Passive diffusion—dextromethorphan absorption □ Interfacial tension, dextromethorphan solutions—absorption relationship □ Colorimetric analysis—spectrophotometer

The absorption, excretion, and metabolism of *d*-3-methoxy-*N*-methylmorphinan (dextromethorphan) or its analogs have been investigated by several authors (1–6). pK_a's of 7.97 (7) and 8.25 (8) have been quoted for dextromethorphan, which, assuming the pH-partition hypothesis to be the all-important factor in the absorption process (9), suggests that no absorption should be observed until the drug reaches the pH's of the small intestine. In a paper advancing the pH-partition hypothesis, Shanker *et al.* (2) found in preliminary experiments that they obtained some absorption of the analog dextrorphan (*d*-3-hydroxy-*N*-methylmorphinan) from a 0.1 *M* hydrochloric acid solution in the rat's stomach, but they were unable to confirm these findings. However, they found considerable absorption from a buffer of pH 8.0, a pH at which a significant fraction of the drug was in the unionized form. Salts of dextromethorphan have considerable lipid solubility (7, 10, 11), and if the lipid solubility is the all-important factor in the absorption process, then some absorption of dextromethorphan should be noticed at pH's where the drug is in the ionized state. In an earlier investigation, the authors (5) found some absorption of dextromethorphan from an isotonic chloride buffer of pH 2.0 in the rat's stomach. Assuming a first-order passive diffusion process, the data can be summarized by Fig. 1 and Table I.

Table I—Absorption of Dextromethorphan from Isotonic Chloride Buffers of pH 2.0 (from *Reference 5*)

Initial Dose, mg.	Rate Constant × 10^4/min.	Drug in Stomach after 2 hr. (mg.)	Drug Absorbed, mg.	Drug Absorbed, %
0.15	23.03	0.112	0.038	25.3
0.30	22.8	0.211	0.089	29.7
0.60	27.1	0.428	0.172	28.7
0.90	26.2	0.650	0.250	27.8
1.50	22.8	1.140	0.360	24.0

The data clearly showed absorption of the drug at a pH at which the concentration of the unionized species is negligible, but does not show whether or not the anion is involved in the absorption process. In the work reported here the role of the anion is investigated.

EXPERIMENTAL

Materials—The *d*-3-methoxy-*N*-methylmorphinan was an analytical sample of dextromethorphan base.[1] Tropaeolin OO dye (sodium *p*-diphenylamineazobenzenesulfonate, Eastman) was recrystallized four times from water. Normal heptane[2] was redistilled and the fraction collected at 93°. All buffer materials and solvent were analytical grade. 2,5-Diphenyloxazole (PPO)[3] and 2-ethoxyethanol[4] were used as supplied by the manufacturers. Trichloroacetic acid-1-C-14[5] and mineral oil USP[6] were also used.

Investigation of Absorption of Various Buffers—Isotonic hydrochloride, trichloroacetate, trifluoroacetate, perfluoropropionate, and nitrate buffers of pH 2.0, each containing 200 mg./l. dextromethorphan base, were used in the investigations. Sprague-Dawley female

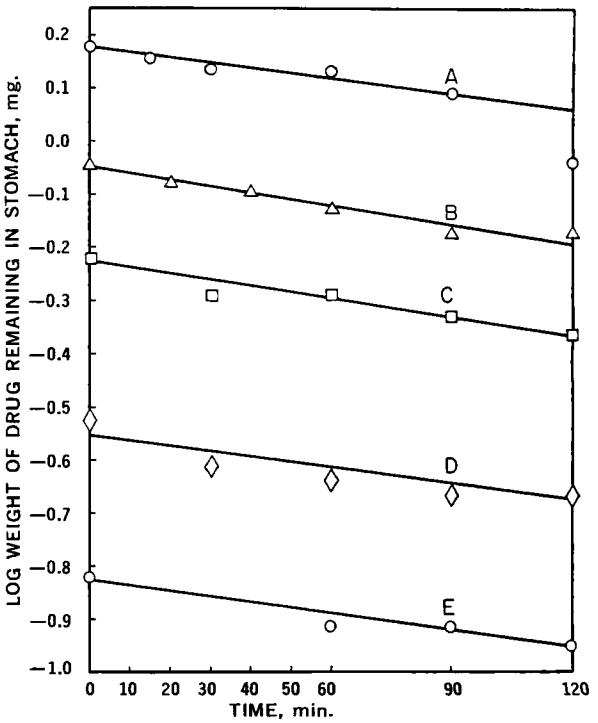

Figure 1—*Plots of the disappearance of dextromethorphan from isotonic chloride buffers of pH 2.0 in the rat's stomach. Initial doses are on the curves (taken from* Reference 5*). Key: A, 1.5 mg.; B, 0.9 mg.; C, 0.6 mg.; D, 0.3 mg.; E, 0.15 mg.*

[1] Vick Research, Mount Vernon, N. Y.
[2] Phillips Petroleum, Oklahoma.
[3] Packard Instrument Co., Downer's Grove, Ill.
[4] Cellusolve, Eastman Organic Chemicals.
[5] Amersham-Searle Corp., Des Plaines, Ill.
[6] American white oil No. 35, American Oil Co., Chicago, Ill.

Table II—Absorption of Dextromethorphan from Various pH 2.0 Isotonic Buffers

Buffer	Drug Absorbed, %	Rate Constant $\times 10^4$ min.$^{-1}$	Extraction Constant	No. of Animals
Hydrochloride	30.0	29.7	6×10^{-4}	6
Trichloroacetate	38.4	39.2	7.0	5
Trifluoroacetate	31.6	31.6	—	3
Perfluoropropionate	40.8	43.7	—	4
Nitrate	5.2	4.4	4×10^{-4}	7

rats (150 g.) were fasted for 24 hr. and then anesthetized with urethan, their stomachs exposed, tied off, and then washed with distilled water. Three milliliters of the isotonic drug solution was injected *via* the pyloric sphincter. After 3 hr. the animals were sacrificed and the stomach contents extracted and assayed by measuring the absorbance of the drug-tropaeolin OO complex in chloroform at 410 mμ in a spectrophotometer.[7] At least six animals were used for each determination. The stomachs were homogenized, extracted, and assayed in a similar manner and found to contain no measurable quantity of drug. Full experimental details are given in *Reference 5.*

Investigation of Absorption of Trichloroacetate—An isotonic trichloroacetate buffer of pH 2.0 containing 200 mg./l. dextromethorphan was prepared and labeled with trichloroacetic acid-^{14}C. A similar buffer without the drug was used as a blank. Three milliliters of these solutions was placed in the stomachs of 250-g. rats as above. Two-milliliter blood samples were withdrawn from a cannulated artery at various time intervals and replaced with 2 ml. blood from a donor animal. Heparin and an ice bath were used to prevent coagulation. The larger animals were used to facilitate the cannulation. The blood samples were centrifuged for 30 min. and the plasma samples so obtained were digested and then assayed by liquid scintillation as described by Mahin and Lofberg (12).

Confirmation of Passive Diffusion Mechanism—*In vitro* experiments were carried out in a 500-ml. wide-mouth bottle set up in a manner similar to that described by Crane and Wilson (13). A gas-dispersion tube introduced the 95% O_2 and 5% CO_2 gas, which also provided the agitation. Sorensen phosphate buffers adjusted to the desired pH were used, and the apparatus was immersed in a constant-temperature bath at 37° and allowed to equilibrate for 1 hr. before attachment of the inverted stomach. These experiments were run for 1 hr., and then the solutions were extracted and assayed as above.

Measurement of Interfacial Tensions—Surface tensions of the drug solutions and interfacial tensions between the drug solutions and mineral oil USP were measured using a du Nouy tensiometer at 22 ± 0.3°. Repetitive measurements were made to assure that equilibrium had been reached.

In other preliminary experiments dextromethorphan was found to have satisfactory stability in the acid solutions, with and without stomach mucous, over a 9-hr. period at 37°. The absorption of dextromethorphan hydrochloride before and after exposure to the various organic anions was used to check the stability of the stomach membrane. No change in the membrane permeability was noticed after 2 hr. of exposure to these acid solutions.

RESULTS AND DISCUSSION

Figure 1 and Table I summarize the findings of the authors' earlier paper (5) in which a passive diffusion process was postulated for the absorption of dextromethorphan hydrochloride from the rat's stomach. *In vitro* experiments using the inverted stomach technique of Crane and Wilson (13) confirmed this lack of an active transport mechanism. In the first experiments a high concentration of drug in an isotonic buffer of pH 7.4 was placed on the external side of the membrane and a lower concentration of drug in a pH 2.0 buffer on the inside; the drug was unable to move against a concentration gradient. Using the same technique with no drug in the pH 7.4 buffer and using phosphate buffers of pH 7.4 and pH 4.4 containing the drug as the internal phase, it was found that three times as much drug was transferred through the membrane from the pH 7.4 as from the pH 4.4 solution, suggesting that although absorption of the ionized species does occur, the absorption of the unionized species is the preferred process.

The earlier paper did not attempt to show the role of the anion in this absorption process; however, in view of the varying lipid solubilities of the different salts of dextromethorphan, the loss of dextromethorphan from buffers of various anions in the rat's stomach was investigated. To avoid complex equilibria problems only the anion under investigation was included in the buffer; these nonphysiological buffers had been found to have no untoward effects on the membranes in preliminary experiments. The absorption data treated as a first-order process are summarized in Table II.

The absorption data is compared with an extraction constant for the various salts of dextromethorphan from aqueous buffers into a cyclohexane-chloroform mixture as determined by Higuchi *et al.* (10) to whom the reader is referred for full definition of this extraction constant. It can be seen from the limited data presented here that there is no correlation between the extraction constant and the extent or rate of absorption. These extraction constants are for the ion-pair species of the amine, the absorption data are for loss of drug, but no determination of the loss of anion could be made in these experiments. To check whether or not the anion was involved in the absorption process, trichloroacetate buffers were made as before but were labeled with trichloroacetic-^{14}C acid before being injected into the stomach. The appearance of the radioactivity in the plasma was then determined by liquid scintillation after various time intervals. If the drug is being absorbed as an ion pair, then the addition of dextromethorphan to the buffer should cause an increased transfer of the labeled anion. The appearance of the ^{14}C activity in the plasma can be treated as a first-order process as shown in Fig. 2. There is no significant difference in the appearance of labeled trichloroacetate, with or without the addition of dextromethorphan, suggesting that the dextromethorphan is not being absorbed in association with the trichloroacetate. The trichloroacetic acid (pKa 0.66) is probably absorbed as the undissociated acid, explaining the higher absorption levels at pH 2.0 rather than pH 3.0. Trichloroacetic acid was chosen for these investigations because of its extended plasma half-life (14) and the availability of the labeled material. These observations seem to confirm that the

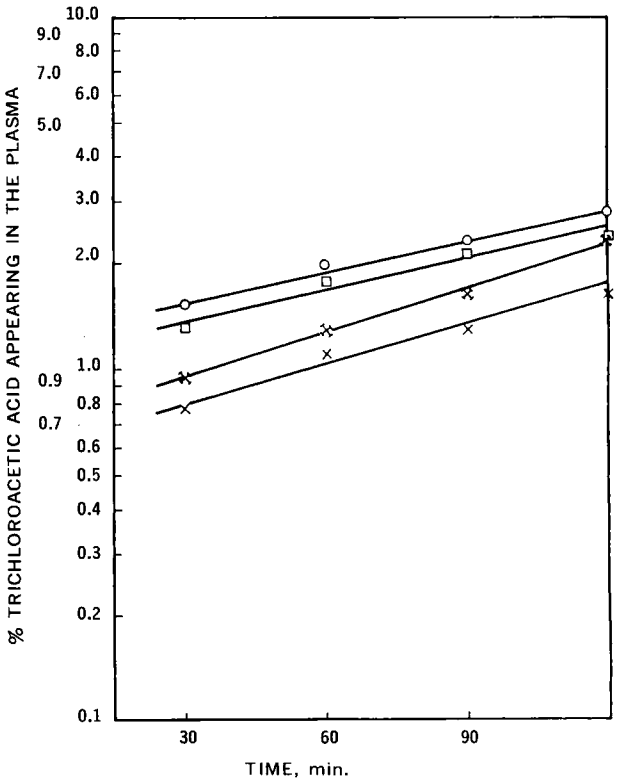

Figure 2—*Plots of the appearance of ^{14}C-labeled trichloroacetic acid in the plasma. Key:* ○, *pH 2.0 without drug;* □, *pH 2.0 with drug;* X, *pH 3.0 with drug;* ×, *pH 3.0 without drug.*

[7] Cary model 14.

lipid solubility of the ion pair is not the significant factor in the absorption processes reported in Table II. The surface tensions of the isotonic buffers containing 200 mg./l. dextromethorphan base and the interfacial tension between the drug solutions and mineral oil USP were measured and are shown in Table III. In other experiments it was found that as the drug concentration increased in a given buffer, the surface tension decreased, suggesting that the dextromethorphan is the surface-active species.

This change of surface activity with the buffer (or salt) species is similar to that observed by Zografi and Zarenda (15), who investigated the surface activity of some phenothiazine salts.

Figure 3 shows the absorption rates for the various anions plotted against the surface and interfacial tensions, and it can be seen that there is good correlation between the absorption rate and the surface activity of the salts.

It appears that in the absorption process the surface-active species, in this case the protonated dextromethorphan, concentrates at the interface (the gut wall or membrane), thus causing an increased local concentration at the site of absorption. Ling (16, 17) has predicted that the absorption of a solute would be controlled by its surface concentration; at the surface the solute then interacts with the fixed ionic and hydrogen bonding sites on the membrane. Transport of inorganic ions (18–21) is thought to involve a fixed charge at the membrane surface which chemically reacts with the ion from solution and then diffuses as an uncharged complex across the membrane. The data reported here suggest that the protonated dextromethorphan may become attached to an anionic site on a mobile carrier, possibly a phospholipid, and be transported across the membrane. Passive diffusion is suspected and, therefore a nonspecific carrier may be involved, having no other requirement than the negative charge. Previous work by Shanes and Gershfeld (22) and Skou (23) have shown good correlation between surface activity and potency and toxicity for several local anesthetics.

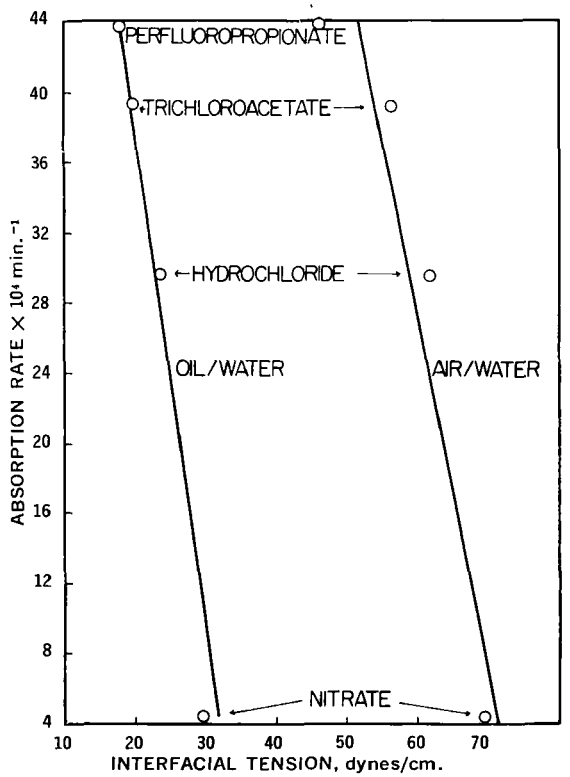

Figure 3—*Plot of interfacial tensions against absorption rate for various isotonic pH 2.0 buffers.*

Table III—Surface and Interfacial Tensions (dynes/cm.) of pH 2.0 Isotonic Buffers Containing 200 mg./l. Dextromethorphan

Buffer	Surface Tension	Interfacial Tension
Water	72.4	47.3
Hydrochloride	61.8	23.5
Trichloroacetate	56.2	19.6
Nitrate	69.5	29.3
Perfluoropropionate	45.8	17.9

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