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The effect of the anion on the absorption of tetracycline from the rat stomach

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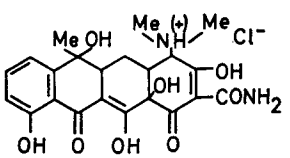
The absorption of tetracycline from the rat stomach has been investigated at acid pH values. The absorption is dependent on the anion of the buffer and appears to be related to the surface activity of the buffer.

As a result of the "pH partition hypothesis" (Brodie, 1964) it is widely believed that amines and certain other compounds can only be absorbed in appreciable quantities from the gut when the pH is such that a significant fraction of the drug is in the non-ionized and therefore lipid-soluble form. In such cases the absorption process is thought to be that of passive diffusion. Some drugs are absorbed as charged species; for example, Levine (1959) has suggested an active transport process for the absorption of quaternary ammonium amines. Fiese & Perrin (1968–1969) have shown that dextromethorphan can be absorbed from the rat stomach as the protonated species in an apparently passive diffusion process. Here the absorption was linked to the surface



Tetracycline HCl

$pK_{a1} = 3.30$
$pK_{a2} = 7.68$
$pK_{a3} = 9.69$

(Stepenhs, Murai & others, 1956)

activity of the species rather than the lipid solubilities. Tetracycline is known to exist as the charged species at all pH's of the alimentary tract; it is also therapeutically active when given orally, and so it must be absorbed as a charged species. In the investigations reported here, the effect of the anion on the absorption from acid conditions is reported, using the rat as the test animal.

EXPERIMENTAL

Materials

Tetracycline HCl and tetracycline base were supplied by Lederle (Lederle Labs. Division, American Cyanamid Co., Pearl River, N.Y.), the hydrochloride being for parenteral use and the base being 92·42% pure. Trichloroacetic acid was Baker Analyzed reagent grade (J. T. Baker Chemical Co., Phillipsburg, N.J.). Radioactive tetracyline was tetracycline-7-^{3}H (Amersham-Searle Corp., Des Plaines, Ill.) and the

TCA was trichloroacetic acid-1-^{14}C (Amersham-Searle Corp., Des Plaines, Ill.). PPO and Dimethyl POPOP (Packard Instrument Co., Downer's Grove, Ill.) were used in the Bray solution (Bray, 1960) for scintillation counting. The dioxane used in the Bray solution was redistilled over sodium metal. Naphthalene, sodium chloride, sodium nitrate, and sodium carbonate were all analytical reagent grade. Perfluoropropionic acid and perfluorobutyric acid were obtained from the Pierce Chemical Company (Rockford, Ill.). All aqueous solutions were prepared in deionized water, made isotonic, and adjusted to the pH required.

Investigation of absorption of tetracycline from various buffers

Isotonic hydrochloride, trichloroacetate (TCA), nitrate, perfluoropropionate (PFP), and perfluorobutyrate (PFB) buffers at pH 2·0, each containing 400 mg/litre tetracycline base, were used. Female Holtzman (Madison, Wis.) rats, 200 to 250 g, were fasted 18 to 24 h before the experiments but water was freely allowed. The animals were anaesthetized with urethane (1·25 g/kg) given intraperitoneally. The stomach was then exposed, tied off, care being taken not to injure or occlude major blood vessels, and then washed with distilled water or stock drug solution warmed to approximately 37°. Finally, 4 ml of labelled drug solution (previously warmed to 37°) was introduced by a blunt needle into the stomach. The ligature was tightened to prevent any backflow or leakage. Immediately the stomach was removed from the rat by cutting anterior to the ligature on the oesophagus and posterior to that on the small intestine. It was then briefly rinsed in warmed physiological phosphate buffer and subsequently put into the *in vitro* apparatus. This consisted of a large, jacketed, test tube-like container which allowed oxygen in from the bottom, and a constant temperature of 37° was maintained by circulation from a heater pump. All stomachs were surrounded by 50 ml of physiological phosphate buffer. At 30-min intervals two 0·5 ml samples were withdrawn for assay in a Tri-Carb Liquid Scintillation Spectrometer (Model 3002, Packard Instrument Co., Downer's Grove, Ill.). This 1 ml of buffer which was removed for assay purposes was replaced by 1 ml of fresh buffer, and the necessary adjustments were made to subsequent concentration determinations. At least four animals were used for each determination. After each set of experiments the stomach was examined both macroscopically and microscopically for gross effects; however, no untoward effects were seen on the membrane.

Investigation of absorption of trichloroacetate

It was also necessary to examine the absorption of TCA alone from the isotonic buffers. Isotonic TCA buffers of pH 2·0 and pH 3·0 were prepared and labelled with active TCA. The same assay procedure as above was used except that the assay was for radioactive carbon instead of tritium. In one set of experiments a dual analysis of the absorption from the stomach of both tetracycline-7-^{3}H and trichloroacetic acid-1-^{14}C was made. This was possible since the ratio of the beta energies for the nuclides ^{14}C and ^{3}H does not exceed four [the method and necessary equations can be found in the Packard Operation Manual (Gibbs, 1967) or Liquid Scintillation Counting (Nuclear Chicago Corporation, 1966)].

Surface tension measurements

Surface tensions of the drug solutions were determined with a du Nouy tensiometer at 30 $\pm$ 0·5°. Care was taken to keep the surfaces clean, and new solutions were prepared and the readings repeated four times.

RESULTS AND DISCUSSION

Tetracycline studies in which appearance of the drug in the physiological phosphate buffer surrounding the stomach containing chloride or other anionic buffers at various drug concentrations and pH's indicate that the drug is absorbed by a passive diffusional process (Table 1). The low absorption from chloride buffer (approximately 1%) in a 150 min test period and the passive diffusion process are in agreement with the observations of Pindell, Cull & others (1959). Using vascularly intact intestinal loops in dogs and assaying according to effluent blood from each segment, Pindell & others found absorption to be greater from the ileum and duodenum than from the stomach; however, only 3% of an administered dose of 334 mg was absorbed from the small intestine in an 80 min test period. The results of Table 1 show that the amount of tetracycline absorbed is directly dependent upon the amount in the stomach at any one time, suggesting a passive diffusional process; however, the role for the anion is not clearly determined.

Table 1. *Absorption of tetracycline in isotonic chloride buffers at pH 2·0 from the rat stomach*

Initial dose (mg)	Rate constant $\times 10^5$/min	Drug absorbed after 150 min (mg)	Drug absorbed %
0·80	7·21	0·0085	1·06
1·60	7·22	0·0165	1·03
2·40	6·74	0·0226	0·94
3·20	6·81	0·0293	0·91

To determine the effect of the anion on the absorption process, isotonic buffers containing only the anion under test were placed in the rat stomach, and the absorption of a constant dose of tetracycline from the various buffers determined. The use of buffers containing a single anion avoids any complex equilibria problem. The results of these experiments are seen in Table 2 and Fig. 1A, with the absorption data being treated as a first-order process. It can be seen that the anion exerts a definite influence on the amount of tetracycline absorbed, but does not show whether or not the anion is absorbed in association with the drug. To check whether or not the anion actually was involved in the absorption process, isotonic trichloroacetate buffers were made as before but were labelled with TCA-1-^{14}C, which enabled easy assay of the TCA transported. Experiments were made using two different pH's and two different solutions at each pH, one containing only TCA, the other TCA and tetracycline. Since TCA (pK_a 0·66) is probably absorbed as the undissociated acid, then significant quantities should be absorbed at pH's 2 and 3, with more being

Table 2. *Absorption of tetracycline (1·6 mg dose) in various pH 2·0 isotonic buffers from the rat stomach*

Buffer	% Drug absorbed (after 150 min)	Rate constant $\times 10^5$/min	Surface tension*
Hydrochloride	1·03	7·22	61·8
Trichloroacetate	1·39	9·83	56·0
Perfluoropropionate	2·10	14·43	52·9
Perfluorobutyrate	2·57	17·04	37·1
Nitrate	1·00	6·60	70·7

* Dyne cm^{-1} or mN m^{-1}.

absorbed at pH 2; this is confirmed by Fig. 1B. It can also be seen from this Figure that less TCA is absorbed in the presence of drug at the pH's involved. If tetracycline is being absorbed as an ion pair (in combination with an anion) then addition of it should have resulted in an increased amount of TCA absorption. Since there is no increase in TCA absorption (in fact, a slight decrease is seen), the conclusion may be made that tetracycline is not being absorbed in conjunction with trichloroacetate. The reason TCA was chosen as an appropriate anion for these investigations is that it is reported to have no physiological effect and it has an extended plasma half-life (Butler, 1948).

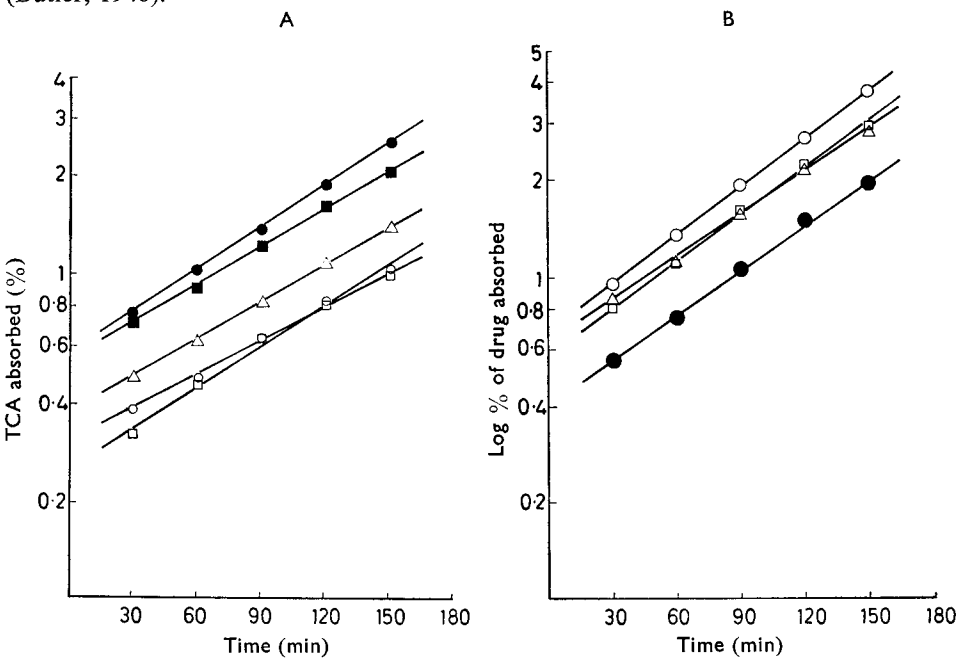


FIG. 1. A. Absorption of tetracycline (400 mg/litre) at pH 2·0 from various buffers in the rat stomach. ● Perfluorobutyrate buffer. ■ Perfluoroproprionate buffer. △ TCA buffer. ○ Chloride buffer. □ Nitrate buffer.

B. Absorption from the stomach of labelled trichloroacetate with and without tetracycline. ○ pH 2·0 no drug. △ pH 2·0 with drug. □ pH 3·0 no drug. ● pH 3·0 with drug.

These results suggest that the lipid solubility of the tetracycline-anion pair is not the dominant factor in the absorption process, but the data of Fig. 1A and Table 2 show the absorption to be anion dependent. Fiese & Perrin (1969) have shown that the absorption of protonated dextromethorphan was related to the surface activity of the various salts. To check whether or not these same factors are involved in the tetracycline absorption process, the surface tensions of all the buffers used (at a fixed tetracycline concentration of 400 mg/litre) were measured and are shown in Table 2. A plot of absorption rate against surface tension for the various anions is shown in Fig. 2, and, as was the case with dextromethorphan, a reasonable correlation between surface activity and absorption was found.

These results suggest that positively charged tetracycline is transported across the gut wall by combining with some non-specific anionic site on the membrane, the non-specific nature of the site being suggested by the apparent passive diffusional process involved.

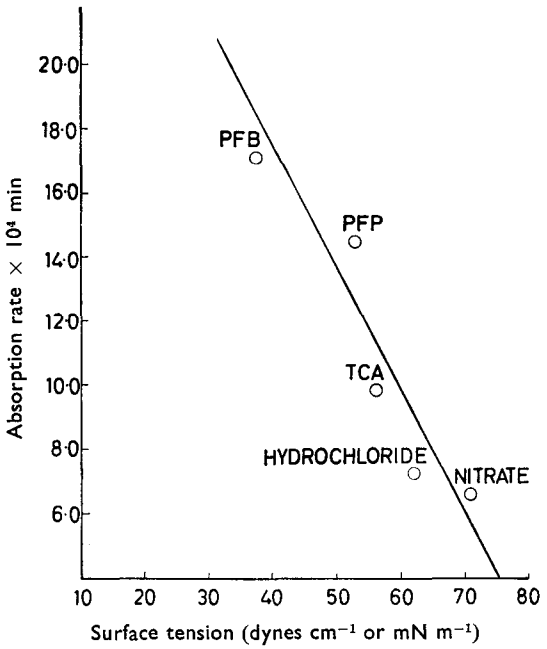


FIG. 2. Plot of surface tensions against absorption rate for various isotonic pH 2·0 buffers each containing 400 mg/litre of tetracycline base.

The measured surface activity of some of the salts indicates that their concentration is increased at the interface and therefore more drug is available for transfer at the absorption site. This is in agreement with the prediction of Ling (1964, 1965) that the absorption of a solute will be determined by its surface concentration and interaction at the membrane with fixed ionic and hydrogen bonding sites.

Preliminary investigations into the absorption of tetracycline from segments of the small intestine have shown that there is considerable absorption as the pH's are increased to 5·0 while keeping the anion constant. However, over the pH range from 2·0 to 5·0 the absorption from the small intestine is also anion dependent.

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