plasma since aglycones of the drug and metabolites are not well resolved and elute near the solvent front, where there are high levels of nonspecific fluorescence due to plasma constituents.

Aglycone separation is not a problem in the *in vitro* assays since there are few interfering substances. Separation of aglycones from deoxyaglycones can be accomplished for most anthracyclines using the aminocyanosilica column if a less polar mobile phase is used. The parent compound, however, will have a longer retention time under these conditions.

Marcellomycin is an anthracycline with a three-sugar chain linked by a glycosidic bond to pyrromycione (22). Mussettamycin and pyrromycin are the di- and monosaccharides in this series, respectively. As can be seen from Table II, these four anthracyclines can be separated easily by this system.

The relative peak heights of test compounds compared to doxorubicin in this HPLC system depend on three major factors: (*a*) the molar fluorescence of the compounds at the excitation–emission bandwidths used in the fluorescence detector, (*b*) the extraction efficiency of the chloroform–isopropanol solvent, and (*c*) the retention time of the compound. Since retention times of the compounds are short, there is not much spreading of peak widths so the third factor does not influence peak heights substantially. However, the other two factors are important since the relative fluorescence of carubicin is ~2.5–3 times that of doxorubicin in this system. For example, 50 ng of carubicin/ml produces a peak height that is ~33% of that due to 400 ng of doxorubicin/ml. This result means that the fluorescence due to 50 ng of carubicin is equivalent to that due to 132 ng of doxorubicin/ml. Since the standard curve for carubicin is linear, it is not necessary to determine if this greater relative fluorescence is due to differences in molar fluorescence or to extraction efficiency. The relative fluorescence of doxorubicin, daunorubicin, and marcellomycin appears to be comparable when these drugs are extracted from pooled plasma.

The examples reported here demonstrate the usefulness and versatility of HPLC to anthracycline research. There is good evidence that doxorubicin toxicity can be predicted on the basis of elevated drug exposure or the area under the plasma concentration curve (23). If this observation holds for anthracycline analogs, then drug monitoring could warn against toxicity during early clinical trials. This HPLC system would then be useful in obtaining concentrations within a reasonable time with accuracy and reproducibility.

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ADDENDUM TO ANTHRACYCLINE ASSAY BY HIGH-PRESSURE LIQUID CHROMATOGRAPHY: MODIFICATION OF MOBILE PHASE FOR AMINOCYANOSILICA COLUMNS USED FOR ANTHRACYCLINE ASSAY

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High-pressure liquid chromatography (HPLC) is an efficient technique for the separation and quantification of anthracycline antibiotics. Various assay procedures have been described, including several that employ an aminocyanosilica column[3] (1–3). Users of this column should be aware that a modification in the manufacturing process for the packing material has changed the properties of this column with respect to anthracycline assay. Although the change improved the uniformity of the columns without significantly altering retention times of standard test compounds or the number of theoretical plates, the affinity of the packing for anthracyclines was decreased. No other problems secondary to the change have been reported to the manufacturer.

Table AI—Retention Times for Several Compounds [a]

Compound	Retention Time, min
Solvent front	1.3
Acridine	1.3
Aclacinomycin A	1.3
Carubicin	3.0
Quinidine sulfate	3.3
Daunorubicin	3.6
Puromycin	3.6
Quinine sulfate	3.8
Carminomycinol	4.0
Proflavin	4.0
Daunorubicinol	4.8
Doxorubicin	6.3

[a] The column was Whatman Partisil PXS 10/25 PAC (lot 100320), the mobile phase was hexane–ethylene chloride–methanol–acetic acid–water (40:40:10:4:1), and the flow rate was 3.0 ml/min.

Those columns[3] manufactured prior to May 24, 1979, separate doxorubicin (adriamycin), adriamycinol, adriamycin aglycones, daunorubicin, and daunorubicinol with a mobile phase of choloform–methanol–acetic acid–water (80:20:2:3). Columns manufactured after May 1979 (lots 100302 and higher) require a modification in the mobile phase to separate these compounds; hexane–ethylene chloride–methanol–acetic acid–water (40:40:10:4:1) at a flow rate of 3.0 ml/min can be used without loss of separation or sensitivity compared to the old system.

To document that the new system can be used for assay of doxorubicin and other anthracyclines, standard curves for doxorubicin and carubicin (carminomycin) were prepared in plasma. Daunorubicin (50 ng/ml) and

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doxorubicin (150 ng/ml) were used as the internal standards, respectively. Samples were assayed according to the procedure of Averbuch *et al.* (3), except that new columns (lot 100302) were used with the hexane–ethylene chloride–methanol–acetic acid–water mobile phase system. The assay sensitivity (signal strength of >2.5 times baseline noise) was 5 and 1 ng/ml, respectively. In both cases, the response was linear to 200 ng/ml (highest concentration tested). The correlation coefficient for six points was >0.99 for both assays. Retention times for several anthracyclines and other fluorescent compounds were determined (Table AI).

The aminocyanosilica column is effective in separating highly polar compounds and is almost free of solvent memory. It is unfortunate that a change in silica gel manufacture caused a substantial change in operating characteristics with respect to the anthracycline assay. However, if the appropriate mobile phase is used, depending on lot number, then this column continues to be useful to anthracycline research.

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Effect of Surfactant on Tetracycline Absorption across Everted Rat Intestine

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Abstract □ Absorption of tetracycline hydrochloride (500 μg/ml) from oxygenated modified Krebs buffer in randomized everted rat jejunal segments was determined alone and in the presence of calcium, polysorbate 80, and calcium plus polysorbate 80. Surfactant increased absorption of tetracycline in the presence and absence of calcium, with 0.01% (w/v) polysorbate 80 increasing transfer to the greatest extent of the concentrations examined (0.005, 0.01, 0.05, 0.1, and 1%); tetracycline hydrochloride + 12.5 m*M* $CaCl_2$, 143 ± 45 μg/ml; tetracycline hydrochloride + polysorbate 80, 389 ± 18 μg/ml; tetracycline hydrochloride + 12.5 m*M* $CaCl_2$ + polysorbate 80, 255 ± 31 μg/ml. On the premise that the effective surfactant concentration is similar to the critical micelle concentration, an absorption mechanism based on micellar solubilization is postulated.

Keyphrases □ Tetracycline—absorption, effect of polysorbate 80, everted rat intestine □ Polysorbate 80—effect on tetracycline absorption across everted rat intestine □ Surfactants—effect of polysorbate 80 on tetracycline absorption across everted rat intestine

Tetracycline is a widely used antibiotic. Its serum level should not fall below the minimum inhibitory concentration (MIC), usually between 0.1 and 1.5 μg/ml, for most bacterial strains. Occasionally, this concentration is difficult to maintain due to decreased absorption caused by other drugs or divalent and trivalent cations present in the GI tract (1–3). The intestinal absorption of most tetracyclines is rapid but incomplete, and the absorption mechanism is poorly understood (4, 5).

Physicochemical interactions in the gut are not uncommon and, at least pharmacokinetically, seem to be the most important form of interaction affecting the absorption of tetracycline derivatives. Chelation of tetracycline with polyvalent cations, which has been well documented, is the most common reaction that decreases tetracycline absorption. In addition to forming direct tetracycline–metal complexes, tetracycline binds to both nucleic acids and proteins, with the binding mediated by divalent cations such as zinc, calcium, and magnesium.

Recently, the role of endogenous and exogenous surfactants in drug absorption experiments was explored (6, 7). In some instances, the addition of surfactants enhanced drug absorption (8).

Since decreased tetracycline absorption presents a clinical problem, often requiring alteration of food and medications, a method of optimizing tetracycline absorption to avoid the present restrictions would be valuable. Surfactants may enhance tetracycline absorption to an acceptable level, even in the presence of divalent ions; if so, they may prove to be a valuable additive to oral dosage forms.

This investigation determined the effects of surfactants on tetracycline absorption from the rat everted gut sac to obtain information on the enhancement of tetracycline transport and the tetracycline transport mechanism.

EXPERIMENTAL

Intestine Preparation—An everted intestinal sac technique (9, 10) was used. The solution on the inside of the sac is referred to as the serosal solution, and the solution in which it is incubated is called the mucosal solution.

Seven male white Holtzman rats, 175–200 g, were fasted overnight (20–24 hr), but water was not withheld prior to the experiment. The animals were anesthetized with ether and then killed by stunning and cervical fracture. The jejunum was removed and rinsed immediately with several portions of cold normal saline and everted on a thin glass rod. After eversion, the jejunum was washed in cold normal saline and cut into seven segments of 5-cm length. Segments of the rat intestine were selected randomly for the different treatment groups.

Sacs of everted intestine were prepared by tying one end tightly and the other end loosely with fine thread. Then 0.5 ml of buffer was introduced inside the sac with a 1-ml syringe fitted with a blunt needle. The loose ligature was tightened, and the buffer-filled sacs then were transferred immediately to 25-ml erlenmeyer flasks containing 15 ml of buffer mixed with tetracycline hydrochloride[1], 0.5 g/liter with or without the additives. Control sacs were placed in buffer alone. The other five sacs were incubated in buffer to which 0.005, 0.01, 0.05, 0.1, or 1% (w/v) pol-

[1] Lot 6 X090-71000, Pfizer Laboratories, New York, N.Y.

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