Table I—Quantum Yields (Φ) of the Anaerobic Photoreduction of IIb (1×10^{-4} M) in the Presence of Acridan Derivatives (6.67×10^{-4} M)

Acridan Derivative	Φ	Solvent
Ia	0.012	pH 9 phosphate buffer, anaerobic
Ia	0.10[a]	pH 9 phosphate buffer, aerobic
Ib	0.039	Acetonitrile, anaerobic
If	0.018	Acetonitrile, anaerobic, $\alpha_{H/D} = 2.17$
Ic	0.18	Acetonitrile, anaerobic
Ig	0.12	Acetonitrile, anaerobic, $\alpha_{H/D} = 1.50$
Id	0.000	Acetonitrile, anaerobic
Ie	0.000	Acetonitrile, anaerobic

[a] Quantum yield for the IIb-sensitized oxidation of Ia.

compared to its deuterated analog (If). A primary isotope effect of 2.20 ± 0.10 was found, and the N-10 methylated derivatives (Ic and Ig) showed a smaller isotope effect of 1.50 (Table I).

Therefore, the oxidation of Ia by flavin, which proceeds not only on illumination but even very slowly (0.21 M^{-1} sec^{-1} at pH 4) in the dark, is likely to proceed *via* intermediate formation of an adduct of Structure V, which decomposes heterolytically as pointed out for acridan. This proposal is confirmed on repeating the isotope effect measurements of Digenis *et al.* (2). Compound Ih, prepared according to their method (2), showed a primary isotope effect of 2.1 ± 0.20 when compared to Ia. For inexplicable reasons, Digenis *et al.* (2) found no isotope effects.

Therefore, it can be concluded that Ia as well as other acridan derivatives is photooxidized by the flavin triplet *via* covalent intermediates, which split heterolytically when the N-10 position is not blocked by an alkyl group. If the N-10 position is blocked, *e.g.*, in Ic, the covalent intermediate of type V can be observed spectroscopically at low temperatures, splitting homolytically upon heating.

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Effect of Ionization on Absorption of Cephalosporins

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Abstract □ To explore the relative absorbabilities of different ionic forms of cephalosporins, the absorption rates of four compounds were measured in the pH 5–9 region using an *in situ* rat gut technique. Cephalexin, cephradine, and cephaloglycin have some oral activity, while 3-[(acetyloxy)methyl]-8-oxo-7-[[(4-oxo-1(4*H*)-pyridinyl)acetyl]-amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (I) has insignificant oral activity. The pH–species profiles calculated from their ionization constants showed that cephalexin, cephradine, and cephaloglycin have a large proportion of uncharged molecules plus zwitterions in the pH range of the small intestine, while I exists as the anion throughout this range. When the species profiles are compared with the pH–absorption rate profiles for cephalexin, cephradine, and I, the results are consistent with a model in which the zwitterionic and/or uncharged forms of the molecules are well absorbed, whereas the anions show little or no absorption. Although it has a pH profile for zwitterions plus uncharged molecules similar to cephalexin, cephaloglycin shows poor absorption, suggesting that the ratio of uncharged molecules to zwitterions may be important in absorption.

Keyphrases □ Absorption, GI—various cephalosporins, effect of ionization, rats □ Cephalosporins, various—GI absorption, effect of ionization, rats □ Ionization—effect on GI absorption of various cephalosporins, rats □ Antibacterials—various cephalosporins, GI absorption, effect of ionization, rats

There has been very little published concerning the mechanism of cephalosporin absorption. Some cephalosporins are known to be actively secreted into the renal tubules (1), suggesting that an active process could also be important in absorption. Penzotti and Poole (2) investigated this possibility using everted rat intestinal sacs; for some penicillins and cephalosporins, including cephalexin and cephaloglycin, they found no evidence for a specialized transport mechanism.

If these compounds are passively absorbed, the extent of their ionization in the pH region of the GI tract could determine partly the ease with which they are absorbed. Therefore, the absorption rates of cephalexin, cephradine, cephaloglycin, and 3-[(acetyloxy)methyl]-8-oxo-7-[[(4-

Table I—Composition of Buffer Solutions Used in Absorption Experiments

Buffer[a]	Components	pH[b]
A	0.095 *M* Na_2HPO_4 0.046 *M* Citric acid 0.003 *M* NaCl	5.1
B	0.072 *M* Na_2HPO_4 0.018 *M* Citric acid 0.049 *M* NaCl	6.0
C	0.012 *M* Na_2HPO_4 0.088 *M* NaH_2PO_4 0.048 *M* NaCl	6.0
D	0.020 *M* Na_2HPO_4 0.047 *M* NaH_2PO_4 0.084 *M* NaCl	6.5
E	0.087 *M* Na_2HPO_4 0.006 *M* Citric acid 0.039 *M* NaCl	7.0
F	0.061 *M* Na_2HPO_4 0.039 *M* NaH_2PO_4 0.030 *M* NaCl	7.0
G	0.171 *M* Tromethamine[c]	7.3
H	0.176 *M* Tromethamine	7.8
I	0.200 *M* Tromethamine 0.020 *M* NaCl	8.2
J	0.200 *M* Tromethamine 0.240 *M* NaCl	8.7

[a] All except C are based on published buffer systems (4) adjusted to isotonicity. When necessary, the pH was adjusted with sodium hydroxide or hydrochloric acid. [b] At 37°. [c] Tris(hydroxymethyl)aminomethane.

oxo-1(4*H*)-pyridinyl)acetyl]amino]-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylic acid[1] (I) were studied in the pH 5–9 region using the *in situ* rat gut technique described previously (3). The pH–rate profiles obtained were compared with pH–species profiles calculated from the ionization constants of the compounds.

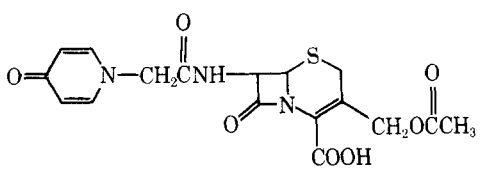
EXPERIMENTAL

Reagents and Solutions—All commercially available chemicals were reagent or spectrophotometric grade. Cephalexin monohydrate[2], cephaloglycin dihydrate[2], and cephradine[3] were used as received. Drug solutions for absorption experiments were prepared at concentrations of 1–3 mg/ml in the isotonic buffer solutions described in Table I or in isotonic saline.

Test Animals—Male Sprague–Dawley rats, 200–300 g, were fasted 12–14 hr before surgery. Drinking water was allowed.

Analytical Methods—Samples (0.1 ml) of cephalexin, cephradine, and I removed from the rat intestine were diluted with 2–10 ml of 0.1 *M* phosphate buffer, pH 7.5, the volume depending on the concentration of the starting solution and the absorptivity of the compound. The UV spectra of these solutions were measured using a recording spectrophotometer[4]. Rate constants for the disappearance of drug from the lumen were calculated using the wavelengths of maximum absorbance: cephalexin and cephradine, 262 nm; and I, 264 nm.

Because of instability, cephaloglycin solutions were assayed using a high-pressure liquid chromatographic (HPLC) method that separates starting material from degradation products. A 15-μl aliquot of undiluted



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[1] RMI 15,307.
[2] Eli Lilly and Co.
[3] E. R. Squibb and Sons.
[4] Beckman Acta III.

Table II—Experimental Conditions and Apparent First-Order Rate Constants for Loss of Cephaloglycin

pH[a]	Buffer[b]	$10^3 k_{deg}$[c], min^{-1}	$10^3 k_{obs}$[d], min^{-1}	$10^3 k_{ab}$, min^{-1}
5.1	A	1.25	2.9	1.6
6.0	B	3.3	2.9	−0.4
6.1[e]	Isotonic saline	~0[f]	~0[f]	0
7.3	G	4.4	6.2	1.8

[a] Initial pH, except 6.1. See text. [b] See Table I for composition. [c] Obtained *in vitro*. [d] Average of results from two rats. [e] See Table III. [f] No significant changes in concentration seen in at least 1 hr.

sample (1.3 mg/ml) from the gut or from a stability test was injected onto a reversed-phase, high efficiency column[5] and eluted[6] using a flow rate of 80 ml/hr. The elution solvent was acetonitrile–formate buffer. The effluent from the column was monitored at 254 nm. Cephaloglycin had a retention time of about 5.5 min. Peak areas and/or heights were used in the calculation of rate constants.

Elution Solvent—A solvent containing 15% acetonitrile in a 0.05 *M* formate buffer was prepared as follows. A solution of 26 ml of 88% formic acid in 800 ml of water was adjusted to pH 3.2 by the addition of triethylamine and then diluted to 1 liter. One hundred milliliters of the concentrate was mixed with 150 ml of acetonitrile and diluted to 1 liter with water to give the elution solvent.

Stability of Cephaloglycin Solutions—Solutions containing approximately 1.3 mg of cephaloglycin/ml in the buffers listed in Table I were held in a 37° water bath for 3–5 hr. They were sampled at appropriate times and assayed by HPLC.

Animal Preparation—The method of studying absorption from the small intestine was the same as that used by Doluisio *et al.* (3). The animals were anesthetized by 1.25 mg of urethan/g ip. Samples were removed every 10 or 15 min for 1–1.5 hr and assayed as described. The pH was measured at the beginning and end of an experiment and, in some instances, at intervals throughout the experiment.

RESULTS

Stability of Cephalosporin Solutions—The HPLC studies indicated that cephaloglycin degradation was occurring in all buffers. Only when cephaloglycin was dissolved in isotonic saline (pH 6) was there no significant degradation in the time period studied. Degradation rate constants were calculated from plots of:

$$\ln X = \ln X_0 - k_{deg}t \qquad \text{(Eq. 1)}$$

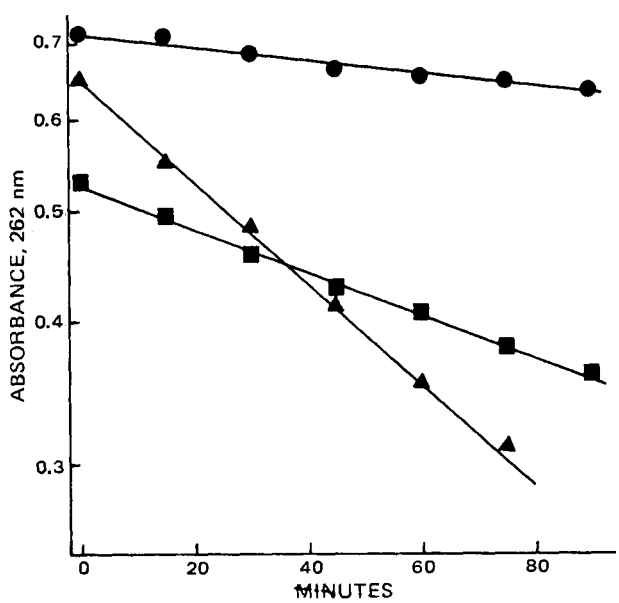

Figure 1—*First-order plots of data for cephalexin in the* in situ *rat gut model. Key:* ●, *pH 8.2;* ■, *pH 7.3; and* ▲, *pH 5.1.*

[5] μBondapak C_{18}, Waters Associates.
[6] Varian model 8500 liquid chromatograph equipped with a Variscan UV-visible detector.

Table III—Experimental Conditions and Apparent First-Order Rate Constants for Loss of Cephalexin from Rat Small Intestine

pH[a]	Buffer[b]	$10^3 k_{ab}$, min^{-1}
5.1	A	10.7[c]
6.0	C	9.0[d]
6.1[e]	Isotonic saline	11.4[c]
6.5	D	10.2[c]
7.0	E	9.1[f]
7.3	G	4.1[f]
7.8	H	3.2[f]
8.2	I	1.8[f]
8.7	J	0.5[f]

[a] Initial pH, except 6.1. See text. [b] See Table I for composition. [c] Average of results from three rats. [d] Average of results from four rats. [e] Final pH. Initial pH of ~5.3 changed to final value within 20 min. See text. [f] Average of results from two rats.

where X is either peak height or peak area and X_0 represents the values at time zero. Graphs of peak heights or peak areas obtained in the same experiment gave k_{deg} values in good agreement. The k_{deg} values obtained using least-squares linear regression on the data are given in Table II.

Previous stability experiments indicated that no significant I decomposition would occur in the pH range used in the time needed for a rat gut study. The adequate stability of cephalexin and cephradine was established using literature data (5, 6).

Loss of Cephalexin, Cephradine, and I from Small Intestine—Absorbance data gave linear plots when graphed according to:

$$\ln A = \ln A_0 - k_{ab}t \qquad \text{(Eq. 2)}$$

where A is the absorbance at time t at the wavelength used for the particular cephalosporin (see *Experimental*), A_0 is the initial absorbance, and k_{ab} is the apparent first-order rate constant for disappearance of drug from the lumen and is assumed to represent absorption. The k_{ab} values for cephalexin and cephradine are listed in Tables III and IV. Typical plots are shown in Fig. 1. Compound I gave no evidence of being absorbed in the pH 5.1–7.3 range. The pH values usually changed by less than 0.5 pH unit during an experiment, except at pH 5 and above pH 8, where changes of about 0.8 unit were seen. Even when a relatively large pH change took place, the first-order plots did not show a deviation from linearity.

As a check for buffer effects on absorption, several experiments were run using a solution of cephalexin in isotonic saline. Measurements at 10-min intervals showed that the pH adjusted rapidly in the rat to a stable value of about 6.1. The average rate constant obtained was not significantly different from the constants obtained in buffers in the same pH region (Table III).

Loss of Cephaloglycin from Small Intestine—HPLC data were plotted according to Eq. 1. The rate constants obtained, k_{obs}, are equal to the sum of the rate constants for loss due to absorption and degradation

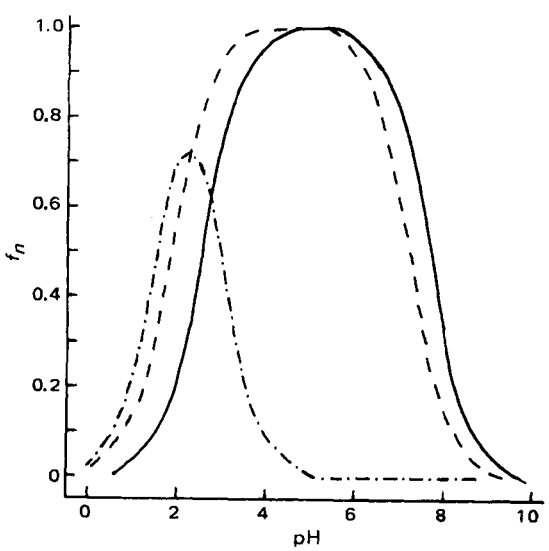

Figure 2—*Plots of* f_n, *fraction of molecules present as zwitterions plus uncharged molecules, as a function of pH for cephalexin (—), cephaloglycin (– –), and I (-·-).*

Table IV—Experimental Conditions and Apparent First-Order Rate Constants for Loss of Cephradine from Rat Small Intestine

pH[a]	Buffer[b]	$10^3 k_{ab}$, min^{-1} [c]
5.1	A	10.6
6.0	C	8.9
6.5	D	11.5
7.0	F	8.7
7.3	G	7.4
7.8	H	4.2
8.2	I	1.8
8.7	J	0.9

[a] Initial pH. [b] See Table I for composition. [c] Average of results from two rats, except at pH 6.5 when four rats were used.

(7). Therefore, k_{ab} values in Table II were calculated by subtracting k_{deg} from k_{obs}. Since the composition of the buffer undoubtedly changes while in the rat gut, the effective values for k_{deg} must be somewhat different from those found *in vitro*. For this reason, the k_{ab} values obtained should be viewed as approximations, indicating only that cephaloglycin has relatively poor absorption characteristics.

DISCUSSION

Work by Penzotti and Poole (2) suggests that, like most drugs, cephalosporins are absorbed by passive diffusion. A number of more or less complex models have been proposed to explain the behavior of passively absorbed drugs (8–13). In general, these theories agree that the neutral drug form will be absorbed more easily than charged species because of its greater ability to penetrate the lipoidal intestinal membranes.

In a series of related compounds, such as the cephalosporins, it is therefore reasonable to suppose that one criterion for a well-absorbed compound is a pH–species profile in which a large fraction of the molecules are present in a neutral form in the pH range of the intestinal tract. The importance of ionization in cephalosporin absorption was investigated using two well-absorbed cephalosporins, cephalexin and cephradine; a relatively poorly absorbed oral compound, cephaloglycin; and I, which has insignificant oral activity.

All of these compounds are amphoteric and can exist in four forms: the cation, zwitterion, uncharged molecule, and anion. Since the zwitterion

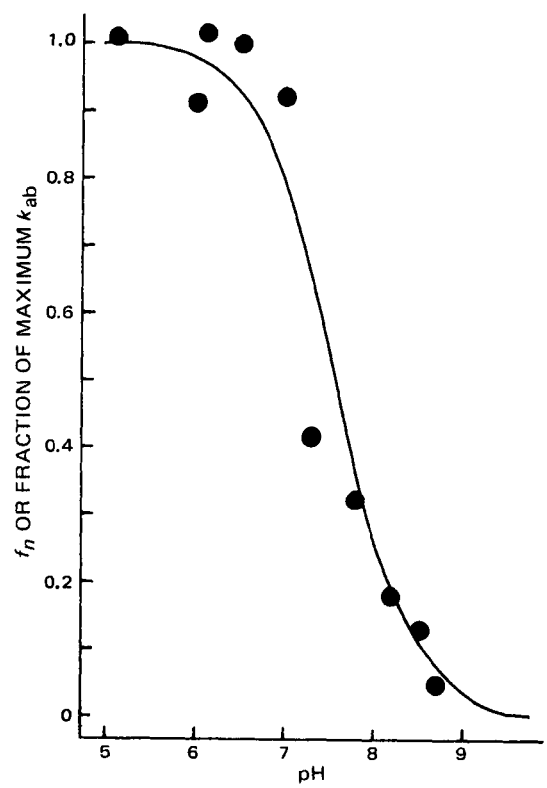

Figure 3—*Absorption rate constants for cephalexin (Table III) expressed as fractions of the average rate constant at pH 5 and 6 plotted on the same scale with* f_n. *(See Fig. 2.)*

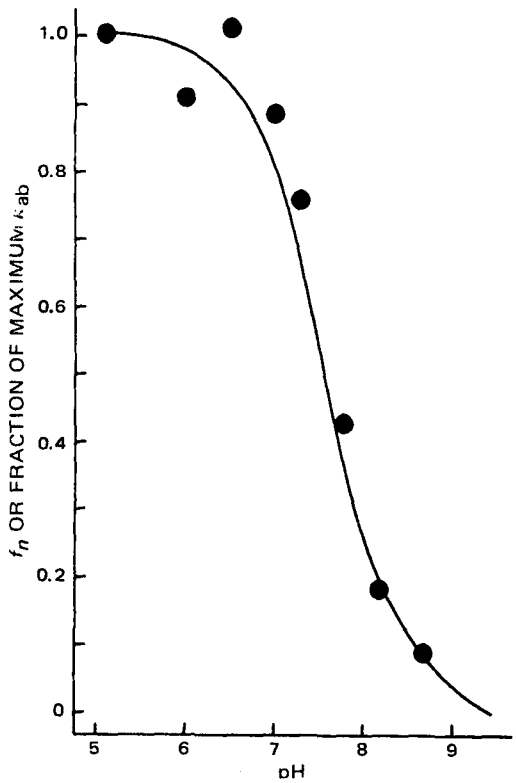

Figure 4—*Absorption rate constants for cephradine (Table IV) expressed as fractions of the average rate constant at pH 5 and 6 plotted on the same scale with f_n for cephalexin. (See Fig. 2.)*

and uncharged molecule, which exist together in a fixed ratio, have no net charge, they should be the best absorbed species. The ionization constants for I (14), cephalexin, and cephaloglycin were used to construct plots of zwitterions plus uncharged molecules as a function of pH[7] (Fig. 2). The pKa values for cephradine were assumed to be essentially the same as those for cephalexin because of their close structural similarity. Based on these profiles, cephalexin, cephradine, and cephaloglycin have significant numbers of molecules with no net charge present in the pH range of the small intestine (about pH 6–8). Compound I, however, is present only as the anion in the pH range in question and might be expected to be poorly absorbed.

At pH 5.1 and 6, essentially all cephalexin and cephradine molecules are present as zwitterions and uncharged molecules. If the rate constants in Tables III and IV are expressed as fractions of the average rate constants in this region and are plotted on the same graph with the fraction of molecules present as zwitterions plus uncharged molecules, the correlation is good (Figs. 3 and 4). This result is in accord with:

$$k_{ab} = k_n f_n \qquad \text{(Eq. 3)}$$

where k_n is the intrinsic rate constant for absorption of neutral species (zwitterions and uncharged molecules) and f_n is the fraction of molecules present as neutral species. The data for I also fit this equation, since there are no neutral species present and no measurable absorption takes place.

Cephaloglycin, although it has a species profile very similar to cephalexin and differs structurally only in the replacement of a hydrogen atom on the C-3 methyl group of cephalexin by an acetate group, shows very poor absorption. One contributing factor could be the different ratios of uncharged molecules to zwitterions in the two compounds. If these species are absorbed differently, Eq. 3 may be rewritten as:

$$k_{ab} = k_u f_u + k_z f_z \qquad \text{(Eq. 4)}$$

where k_z and k_u are the intrinsic absorption rate constants for the zwitterion and uncharged molecule, respectively, and f_z and f_u are the fractions of molecules present in these forms. Equation 4 can be rearranged to:

$$k_{ab}/f_z = k_u(f_u/f_z) + k_z \qquad \text{(Eq. 5)}$$

The ratio f_u/f_z is independent of pH and can be calculated from K_a values (14). For cephalexin, it is 10.5×10^{-5}; for cephaloglycin, it is 2.09×10^{-5}. At any pH values where f_z is the same for both compounds, the ratio of observed absorption rate constants is:

$$\frac{k_{ab}^{cx}}{k_{ab}^{cg}} = \frac{k_u^{cx}(10.5 \times 10^{-5}) + k_z^{cx}}{k_u^{cg}(2.09 \times 10^{-5}) + k_z^{cg}} \qquad \text{(Eq. 6)}$$

where superscripts cx and cg refer to cephalexin and cephaloglycin, respectively. If k_z for both compounds is small enough that virtually no zwitterions are absorbed and if k_u values are equal, Eq. 6 predicts that cephalexin will be absorbed about five times as fast as cephaloglycin. However, if k_z is assumed to be about zero, k_u^{cx} can be calculated from:

$$k_{ab}^{cx} = k_u^{cx} f_u^{cx} \qquad \text{(Eq. 7)}$$

In the pH 5–6 range, the average k_{ab}^{cx} is 10.4×10^{-3} min^{-1} and f_u^{cx} equals 10.5×10^{-5}. These values give k_u^{cx} equal to 99 min^{-1}, which is an exceedingly fast and probably unrealistic rate. This result suggests that properties of these molecules other than ionization are also important in their absorption behavior.

[7] A manuscript describing the calculation of pKa values for cephalexin and cephaloglycin is now in preparation; W. H. Streng, Merrell-National Laboratories.

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