Effect of Complex Formation on Drug Absorption II

Lipoid-Soluble Dye Complexes

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The intestinal absorption of certain water-soluble acidic dyes (bromthymol blue, methyl orange, and eosine-B) and some of their lipoid-soluble complexes has been investigated. The results of these studies show that the apparent lipoid–water partition coefficient does not reflect the intestinal absorption characteristics of the complexes investigated.

THE POTENTIALITIES of complex formation as a means of enhancing the gastrointestinal absorption of drugs are largely unexplored. The concept of a reversible reaction between a substrate and a carrier, resulting in the formation of a complex capable of moving relatively rapidly across a biological membrane, has proved useful in the quantitative interpretation of active transport processes (1). Attempts to isolate such carriers have resulted (in the case of investigations of the sugar-transfer system) in the isolation of phospholipids capable of forming lipid-soluble complexes with glucose and other mono-saccharides (2). Since the gastrointestinal absorption of most drugs proceeds by passive diffusion of nonionized molecules, and since their rates of absorption (from solution) usually increase with increasing lipoid–water partition coefficient (3), it is of interest to investigate the possibility of enhancing the absorption of certain drugs by formation of more lipid-soluble complexes. This report, one of a series on the effect of complex formation on drug absorption (4), deals with the intestinal absorption rate of certain water-soluble acidic dyes and their lipoid-soluble complexes.

EXPERIMENTAL

Materials.—Bromthymol blue (Nutritional Biochemicals Corp., lot No. 5152), methyl orange (Nutritional Biochemicals Corp., lot No. 4125), eosine-B (Allied Chemical Corp., certification No. 25), diphenhydramine hydrochloride,[1] 2,4-dinitrophenol (Mann Research Laboratories, Inc.). All other materials were of U.S.P. or N.F. grade.

Preparation of Solutions.—The buffer solutions used as the solvent for the dyes and complexing agents consisted of 12 parts, by volume, of Krebs-Ringer solution (5) and 1 part, by volume, of either 0.1 *M* phosphate or 0.1 *M* acetate buffer. The pH of the solutions and the buffer systems used were as follows: for bromthymol blue, pH 7.5 phosphate; for methyl orange, pH 5.0 acetate; for eosine-B, pH 7.0 phosphate. The solutions used for the intestinal absorption studies contained 1 m*M* dye and 10 m*M* complexing agent, unless solubility problems required the use of lower concentrations of one or both components. The final pH of these solutions was adjusted, if necessary, with hydrochloric acid or sodium hydroxide.

Intestinal Absorption Study.—Intestinal absorption of the dyes and dye complexes was studied by the cannulated everted intestine method developed by Crane and Wilson (6). Male Sprague-Dawley rats, weighing about 250 Gm., were fasted for 24 hr. but had access to drinking water at all times. The small intestine was removed under ether anesthesia and rinsed with Ringer solution. The intestine was then sleeved onto a glass rod and everted carefully. Two segments of 10 cm. length (when stretched slightly by attaching a 8 Gm. weight and suspending the segment vertically) were obtained, distal ends were tied, and proximal ends were attached to the cannula of the apparatus described by Crane and Wilson (6). An equal number of first (from the proximal end) and second intestinal segments was used in every part of each experiment. Each segment was suspended in 45 ml. of dye or dye-complex solution maintained at 37° and gassed continuously with a mixture of 95% oxygen and 5% carbon dioxide. The serosal (inner) solution consisted of 2 ml. buffer solution (for dye absorption studies) or buffer solution with complexing agent (for dye-complex absorption studies). At indicated times, the entire serosal solution was withdrawn by means of a hypodermic syringe with attached polyethylene cannula and the segment was rinsed twice with about 1 ml. of buffer solution. A fresh 2-ml. portion of buffer solution was then placed in the intestine segment. The initially withdrawn serosal solution and the washings were combined, and water was added to obtain a total volume of 5 ml. This solution was then filtered through membranes (Millipore, type HA) if necessary, and assayed as indicated.

Analytical Methods.—Bromthymol blue was determined spectrophotometrically at 617 mμ in aqueous solutions alkalized by addition of 0.1 *N* sodium hydroxide. Aminopyrine and codeine did not affect the assay. Bromthymol blue in organic solvent (used in determination of partition coefficients) was extracted into 0.1 *N* sodium hydroxide and determined as described above.

Methyl orange in aqueous solution was determined by employing a modification of Dill's method

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[1] Marketed as Benadryl Hydrochloride by Parke, Davis and Co.

TABLE I.—EFFECT OF COMPLEX FORMATION ON APPARENT PARTITION COEFFICIENT AND INTESTINAL ABSORPTION RATE OF BROMTHYMOL BLUE, METHYL ORANGE, AND EOSINE-B

Composition of Solution[a]	Lipoid-Water Partition: Organic Phase	Partition Coefficient	Intestinal Absorption[b]: Expt., No.	Absorption Rate, mg./hr.
1 m*M* bromthymol blue (BTB)	Benzene	0.01	4	<0.001
1 m*M* BTB, 10 m*M* aminopyrine	Benzene	0.1	2	<0.001
0.5 m*M* BTB, 5 m*M* codeine	Benzene	23.4	2	<0.001
0.085 m*M* methyl orange (MO)	Ethylene dichloride	0.008	4	0.002
0.085 m*M* MO, 0.85 m*M* diphenhydramine	Ethylene dichloride	112	4	0.002
0.202 m*M* MO	Ethylene dichloride	0.005	6	0.006
0.202 m*M* MO, 2.02 m*M* codeine	Ethylene dichloride	0.94	6	0.007
1 m*M* eosine-B (EB)	Chloroform	0.05	14	0.163 ± 0.045
1 m*M* EB, 5 m*M* atropine	Chloroform	12.0	10	0.159 ± 0.032
1 m*M* EB, 2.5 m*M* pheniramine	Chloroform	25.8	4	0.151

[a] The following solvents were used: pH 7.5 phosphate in Krebs-Ringer solution for bromthymol blue; pH 5.0 acetate in Krebs-Ringer solution for methyl orange; pH 7.0 phosphate in Krebs-Ringer solution for eosine-B. [b] Intestinal absorption was studied by the cannulated everted rat intestine technique.

(7) in reverse. Two milliliters of sample was placed in a glass-stoppered test tube, and 1 ml. of 1% diphenhydramine solution and 20 ml. of ethylene dichloride were added. After shaking vigorously for 10 min. and centrifuging, the aqueous phase was aspirated and discarded. Ten milliliters of the ethylene dichloride phase was removed and 1 ml. of sulfuric acid–ethanol mixture (2:98 by volume) was added to it. The absorbance of the mixture was determined spectrophotometrically at 525 mμ, using as a blank a solution obtained by extracting a buffer solution which did not contain dye. Methyl orange in organic solvent was determined directly after addition of sulfuric acid–ethanol mixture. Codeine did not interfere with the assay.

Eosine-B in aqueous solution was determined spectrophotometrically at 516 mμ after adjustment of pH to 7.0. Atropine, pheniramine, salicylamide, and 2,4-dinitrophenol did not interfere in the assay. Determinations of eosine-B in organic solvent used in partition experiments were not carried out (due to extraction difficulties); the concentration of the dye in the organic phase was determined by difference.

Salicylic acid was determined by the method of Brodie *et al.* (8), and salicylamide was determined by a modification (to obtain greater sensitivity) of the method of Crampton (9). Dyes and other additives did not interfere with the respective assays.

Determination of Apparent Partition Coefficient.—Forty milliliters of dye or dye-complex solution (in the same solvent system as used in the absorption experiments) and 10 ml. of organic solvent were placed in a glass-stoppered bottle. After being shaken vigorously for 20 min., the solutions were stored at room temperature for 24 hr. The solutions were then transferred to centrifuge tubes, centrifuged, and the phases were separated and assayed. In the determination of the apparent partition coefficient of eosine-B as a function of concentration, equal volumes (5 ml.) of aqueous and organic phase were used.

RESULTS AND DISCUSSION

The ability of certain acid dyes to combine with basic nitrogen compounds and thus to form complexes having good solubility in organic solvents is well known (10). This property has been utilized for the indirect colorimetric analysis of alkaloids and other nitrogenous bases. These drugs can carry an equivalent molar amount of dye from an aqueous into an organic phase, from which the dye can then be re-extracted into an alkaline aqueous phase (10). A number of the dyes and some of their complexes were chosen as model systems to determine if the absorption of these substances could be enhanced by the formation of lipoid-soluble complexes. Many of the dyes, particularly the sulfonic acid type compounds, are very poorly absorbed, due to their almost complete ionization under physiologic conditions. The dyes and dye-complex systems used in this study are described in the literature, and optimum conditions (pH, organic solvent) for complex formation and extraction have been determined (10). The particular systems used were chosen on the basis of their optimum pH (which had to be in the physiologic range) and ready availability of the materials. The cannulated everted intestine method of Crane and Wilson (6) was used in the absorption studies because it permitted the maintenance of essentially constant concentrations of dye or dye-complex on the mucosal side of the intestinal wall.[2]

As shown in Table I, the apparent partition coefficient of bromthymol blue was increased somewhat by complexation with aminopyrine, and markedly by complexation with codeine. No absorption of the dye could be detected during 1.5 hr. and, despite the relatively high partition coefficient, there was no detectable absorption of the bromthymol blue–codeine complex. Methyl orange was absorbed at a slow but measurable rate (Table I). Neither the diphenhydramine complex nor the codeine complex was more rapidly absorbed than the dye alone. Significantly, the apparent partition coefficient of methyl orange in the presence of diphenhydramine was 14,000 times greater than that of the dye alone.

To determine a possible membrane "blocking" effect of the sulfonic acid dyes (perhaps by being adsorbed on the membrane surface and thereby preventing their own absorption), the effect of these dyes on salicylic acid absorption was determined. Neither 1 m*M* bromthymol blue nor 0.067 m*M* methyl orange had any significant effect on the rate

[2] Intestinal perfusion procedures on living animals could not be used in view of the toxicity of most of the drugs and the high concentrations required. Preliminary experiments in which solutions were introduced into ligated intestinal segments of the anesthesized rat, and where dye absorption was determined by its appearance in the bile, proved unsuccessful.

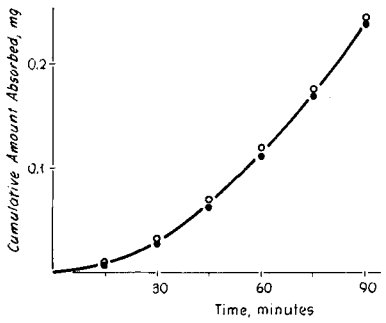

Fig. 1.—Effect of complex formation with atropine on intestinal absorption of eosine-B. Key: O, 1 m*M* eosine-B, average of 14 experiments; ●, 1 m*M* eosine-B and 5 m*M* atropine, average of 10 experiments.

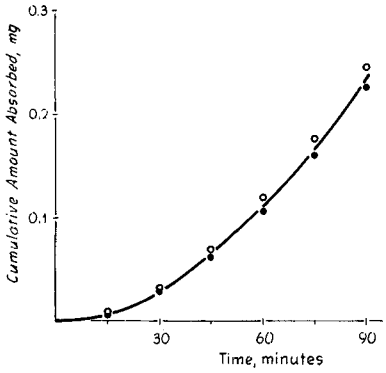

Fig. 2.—Effect of complex formation with pheniramine on intestinal absorption of eosine-B. Key: O, 1 m*M* eosine-B, average of 14 experiments; ●, 1 m*M* eosine-B and 2.5 m*M* pheniramine, average of four experiments.

of absorption of salicylic acid from 10 m*M* solution. This suggests, but does not prove, that membrane blocking did not occur.

In view of the negative results with sulfonic acid dyes, the weaker carboxylic acid dye, eosine-B, was investigated. Complexation with atropine or pheniramine markedly increases the apparent partition coefficient of this dye (Table I). Eosine-B was found to be absorbed considerably more rapidly than the sulfonic acid dyes, and a detailed study of the time course and concentration dependence of its absorption was therefore carried out. The effect of complexation with atropine and pheniramine, respectively, on the intestinal absorption of eosine-B is shown in Figs. 1 and 2. Despite the increased apparent partition coefficient, there was no effect on the rate of dye absorption. The time course of absorption was somewhat unusual compared with results of similar experiments with other types of compounds. Ordinarily, absorption proceeds at a constant rate after an initial short equilibration period (11); the present data indicated a continuing increase of absorption rate with time. This suggested the possibility of a progressive deterioration of the mucosa, perhaps due to toxic effects of the dye. Also, the possible toxic effects of atropine on the intestine were subject to concern. To investigate these possibilities, the absorption of salicylamide alone and in the presence of eosine-B, atropine, or eosine-B and atropine was determined (Fig. 3). Salicylamide was absorbed at a constant rate and the various additives did not affect the absorption process. These observations, and the well-known affinity of dyes to proteins (12), suggest that the continuing increase of absorption rate during the time of the experiment represents a relatively long equilibration period rather than deterioration of the intestine.

While the intestinal absorption of organic dyes is considered to be a passive process (13), the possibility existed that the failure of complex formation to enhance the absorption rate of the particular dyes studied was due to their being absorbed by an active process. However, the absorption of eosine-B exhibited no saturation phenomena over a 1000-fold concentration range, and the slope of a log–log plot of amount absorbed (in 1.5 hr.) *versus* concentration did not differ significantly from unity (Fig. 4). One other possibility was that the dye is absorbed by a mechanism involving solvent drag. The addi-

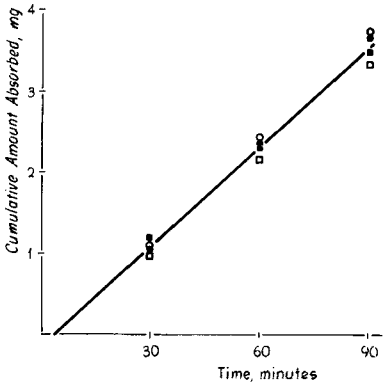

Fig. 3.—Intestinal absorption of salicylamide alone and in the presence of atropine, eosine-B, and eosine-B + atropine, respectively. Each point represents the average of two experiments. Key: O, 7.3 m*M* salicylamide; ●, 7.3 m*M* salicylamide and 5 m*M* atropine; □, 7.3 m*M* salicylamide and 1 m*M* eosine-B; ■, 7.3 m*M* salicylamide, 1 m*M* eosine-B, and 5 m*M* atropine.

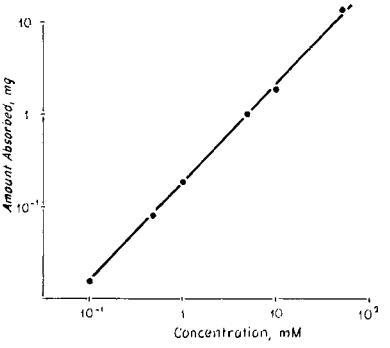

Fig. 4.—Effect of concentration on the intestinal absorption (in 1.5 hr.) of eosine-B. Each point represents the average of two experiments.

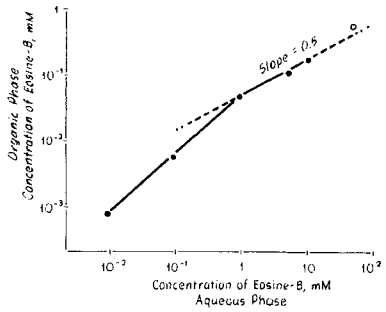

Fig. 5.—Effect of concentration on the distribution of eosine-B between equal volumes of chloroform and pH 7.0 aqueous phase. Each point represents the average of two determinations.

tion of 1 mM 2,4-dinitrophenol [a compound which inhibits active transport of water (14)] to the mucosal solution did not decrease the rate of absorption of eosine-B, and a solvent drag mechanism can therefore be excluded.

It has long been known that organic dye molecules tend to form dimers and larger aggregates in aqueous solutions (15). Dimerization is reflected by a change in the apparent partition coefficient of the dye. When the dye exists totally as a dimer in the aqueous phase, and only as the monomer in the organic phase

$$K = \frac{C_0}{C_w^{0.5}} \qquad \text{(Eq. 1)}$$

and

$$\log C_0 = \log K + 0.5 \log C_w \qquad \text{(Eq. 2)}$$

where K is the partition coefficient, C_0 is the equilibrium concentration of dye in the organic phase, and C_w is the equilibrium concentration of dye in the aqueous phase. A log–log plot of C_0 *versus* C_w should have a slope of 0.5 when dimerization in the aqueous phase is essentially complete. Figure 5 shows that dimerization of eosine-B is practically complete at a concentration of about 1 mM. (The circle in Fig. 5 represents a questionable value which was obtained at a concentration where some precipitation of dye occurred at the interphase of the chloroform–buffer system.) In view of the increased molecular weight and lower diffusivity of eosine-B dimers, it may be expected that the relative absorption rate of the dye will decrease as its concentration increases. No such effect was apparent (Fig. 4). Since the mucosal solution was well agitated by the constant bubbling of oxygen–carbon dioxide through it, and since the dye is intrinsically slowly absorbed, the effect of lower diffusivity should be on the rate of passage across the intestine rather than on the rate of diffusion of dye to the surface of the intestine. The absence of any effect shows that neither the formation of the hetero-complexes nor of homo-complexes modifies the intestinal absorption rate of eosine-B. Apparently, the intestinal membranes have a dissociating effect on the complexes studied. Considering the cell membrane as a bimolecular lipoid leaflet with adsorbed protein layers on both surfaces (16), it is conceivable that dye complexes are dissociated by interaction of either or both components with the outer protein layer. Consequently, only noncomplexed dye molecules may reach the absorption rate limiting lipoidal barrier within the membrane. It remains to be determined whether the proposed dissociating effect of cell membranes is limited to those complexes which consist of substances capable of interacting strongly with membrane constituents.

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