# *In Vitro* Assessment of *In Vivo* Absorption of Drug Complexes

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**Abstract** □ This study determined if *in vitro* transfer of drug complexes across the everted rat small intestine would reflect the *in vivo* absorption characteristics of the complex. The *in vitro* transfer of prednisolone in the presence of dimethyl-, diethyl-, dipropyl-, and dibutylpropionamide was in rank-order agreement with the increase in absorption reported previously from an *in situ* preparation. Excellent agreement was found between the *in vitro* and *in situ* absorption of prednisolone in the presence of various concentrations of dipropylpropionamide. The interaction of atropine with eosin-B, which has been reported not to increase the transfer of the dye across the everted rat intestine, was found to have no effect on absorption from an *in situ* preparation. The difference in effect of complexation on drug transfer across the everted rat intestine may be related to a difference in rate-limiting barriers for prednisolone and eosin-B.

**Keyphrases** □ Drug complexes—*in vitro* transfer as an indicator of *in vivo* absorption characteristics □ Dialkylpropionamides—effect on absorption of prednisolone, everted rat intestine, *in vitro* preparation as an indicator of absorption-enhancing characteristics of complex □ Complexes (dialkylpropionamides-prednisolone)—*in vitro* transfer as an indicator of *in vivo* absorption characteristics

The isolated everted small intestine has been used extensively to study the effects of drug interactions on GI absorption (1–5) as well as to study drug permeability across the intestinal membrane (6–8). More recently, it has been used as a model to classify drugs that may present potential absorption problems in the development of pharmaceutical dosage forms (9). The use of the everted rat intestine has also been evaluated as an animal model to predict antibiotic absorption in man (10).

The everted rat intestinal preparation has not been well studied as to whether the technique could be used to predict the *in vivo* absorption characteristics of the drug complex. Hayton and coworkers (11–13) examined the enhancement of the intestinal absorption of prednisone and prednisolone by dialkylpropionamides in rats using an *in situ* technique. It was therefore of interest to investigate the effect of the dialkylpropionamides on the absorption of prednisolone across the everted rat intestine to determine if the *in vitro* preparation would reflect the absorption-enhancing characteristics of the complex found *in vivo*.

## EXPERIMENTAL

**Materials**—The propionamides[1], prednisolone[2], eosin-B[3], and atropine[2] as well as all other chemicals were used as received.

**Drug Solutions**—Prednisolone and the propionamides were dissolved in 0.9% NaCl solution with the addition of 1% ethanol. Eosin-B and atropine were dissolved in a Krebs-Ringer solution buffered to pH 7.0 with phosphate (1).

**Test Animals**—Male, Sprague-Dawley descent rats[4], weighing 250–350 g and fasted for 20–24 hr, were used in all experiments. Drinking water was readily accessible.

***In Vitro* Absorption Studies**—The animals were anesthetized with ether and the small intestine was excised. The first 15 cm was discarded, and the intestine was everted over a glass rod as described previously (4). Two consecutive 10-cm segments were taken, attached to a cannula as described previously (4), and suspended in 80 ml of a mucosal solution consisting of 0.9% NaCl containing prednisolone alone or prednisolone in the presence of the dialkylpropionamides. Two-milliliter samples of the serosal fluid (0.9% NaCl) were withdrawn and replaced at appropriate intervals over 2 hr and assayed for drug content as previously described (4). In all cases, one segment served as the control (mucosal solution contained only prednisolone) while the other segment contained the steroid and dialkylpropionamide. No distinction was made as to the segments, and they were designated as the control or the complex segments on a random basis.

**Stripping Studies**—The experiment was set up as described in the preceding section with the following modifications. One intestinal segment was stripped of the mucosal layer by running forceps up and down its length. This resulted in essentially total removal of the mucosal layer. Identical solutions of 0.5 m*M* prednisolone or 0.5 m*M* prednisolone plus 28 m*M* dipropylpropionamide were used as the mucosal solution, and the amount transferred with time was measured.

***In Situ* Studies**—Each animal was anesthetized with ethyl carbamate (1.5 g/kg ip) and prepared as previously described (12, 14). A sufficient volume of drug solution at 37° containing either 1 m*M* eosin-B or 1 m*M* eosin-B and 5 m*M* atropine was passed from the duodenal syringe through the gut into the ileal syringe to yield a volume of 5 ml in the latter. The solution in the ileal syringe was then returned to the intestine. At appropriate intervals, the drug was transferred from the intestine into one of the syringes and a 0.1-ml sample was removed for assay. Prior to removing a sample, the volume of the drug solution in the syringe was adjusted to 5 ml with pH 7.0 Krebs-Ringer phosphate buffer.

**Assay Procedures**—Prednisolone concentrations were determined spectrophotometrically[5] by the colorimetric method of Porter and Silber (15). "Blank" serosal fluid (obtained from experiments where no drug was placed in the mucosal fluid) and the dialkylpropionamides did not interfere with the assay procedure.

Eosin-B concentrations were determined spectrophotometrical-

**Table I**—Effect of Dialkylpropionamides on the Transfer of 0.5 m*M* Prednisolone across the Everted Rat Small Intestine

| Amide | Number | Ratio of Amount Prednisolone Transferred in 2 hr (with Amide/Control)[a] |
|---|---|---|
| Dimethyl, 28 m*M* | 5 | 1.05 ± 0.07 |
| Diethyl, 28 m*M* | 5 | 1.32 ± 0.17 |
| Dipropyl, 28 m*M* | 5 | 1.55 ± 0.18 |
| Dibutyl, 5.6 m*M* | 5 | 2.08 ± 0.15 |

[a] Represents the average and standard deviation of the mean.

[1] Eastman Organic Chemicals, Rochester, N.Y.
[2] Sigma Chemical Co., St. Louis, Mo.
[3] J. T. Baker Co.
[4] Huntingdon Farms, Conshohocken, Pa.
[5] Coleman 124 spectrophotometer.

**Table II**—Effect of Various Concentrations of Dipropylpropionamide on the Transfer of 0.5 m*M* Prednisolone across the Everted Rat Small Intestine in 2 hr

| Concentration Amide, % | Number | Ratio (Amide/Control)[a] |
|---|---|---|
| 0.096 | 6 | 1.24 ± 0.08 |
| 0.24 | 5 | 1.39 ± 0.10 |
| 0.37 | 6 | 1.53 ± 0.10 |
| 0.44 | 5 | 1.55 ± 0.18 |

[a] Represents the average and standard deviation of the mean.

**Table III**—Effect of Stripping Mucosa on Amount of Prednisolone Transferred across the Everted Rat Small Intestine in 2 hr

| Drug Solution | Number | Ratio of Amount Transferred, Stripped/Intact[a] |
|---|---|---|
| 0.5 m*M* Prednisolone | 3 | 1.024 ± 0.071 |
| 0.5 m*M* Prednisolone + 28 m*M* dipropylpropionamide | 3 | 0.998 ± 0.029 |

[a] Represents the average and standard deviation of the mean.

ly[5] at 516 nm after appropriate dilution of the samples with pH 7.0 phosphate buffer. Atropine did not interfere with the assay procedure.

## RESULTS AND DISCUSSION

The effects of dimethyl-, diethyl-, dipropyl-, and dibutylpropionamide on the transfer of prednisolone across the everted rat small intestine are presented in Table I. The initial amide concentration in each case was 28 m*M* except for the butyl-amide concentration which was limited by solubility considerations to 5.6 m*M*. The total amount of steroid transferred in 2 hr was not significantly increased by the methyl-amide but was significantly affected by the ethyl-, propyl-, and butyl-amides. The results are qualitatively identical to those of Hayton and Levy (12) who examined the interaction of steroid and amide in an *in situ* preparation. A comparison of the *in vivo* and *in vitro* experiments is presented in Fig. 1. This figure illustrates the ratio of the amount of prednisolone transferred across the isolated gut in 2 hr in the presence of the amides to that in the absence of each amide *versus* the ratio of the percent absorbed in 1 hr from the *in situ* preparation. The *in situ* data were obtained from Hayton and Levy (12). As can be seen from the figure, the rank-order agreement between the two sets of data is good and the isolated preparation gives an adequate rank-order prediction as to the *in vivo* effect of the prednisolone–amide interaction on GI absorption.

The effect of various concentrations of dipropylpropionamide on the absorption of prednisolone from the *in situ* rat intestine was studied previously (13). To determine if the everted rat small intestine would predict the *in vivo* results, experiments were carried out using the *in vitro* preparation with various concentrations of the propyl-amide present in the mucosal solution (Table II). Increasing the concentration of propyl-amide in the mucosal solution results in an increase in the total amount of prednisolone transferred in 2 hr across the everted intestinal segments. The relationship between the effect of the complexing agent on the transfer of prednisolone both *in vitro* and *in vivo* is quite apparent in Fig. 2. The plot of the ratio of the amount of steroid transferred in 2 hr in the presence and absence of the amide *in vitro versus* the apparent absorption rate constant of prednisolone *in situ* is apparently linear, and an excellent quantitative prediction of the *in vivo* results can be obtained from the *in vitro* experiments.

In view of the excellent agreement between the *in vitro* and *in vivo* absorption results with the prednisolone–amide complexes, it was decided to examine the *in vivo* case where *in vitro* studies did not indicate increased intestinal transfer in the presence of a complexing agent. The data provided by Levy and Matsuzawa (1) on the absorption of water-soluble dye–drug complexes across the everted rat small intestine were examined as to the *in vivo* absorption characteristics of the dye and lipid-soluble complex. As representative of the group of dye–drug complexes, the complexation of eosin-B and atropine was examined using the *in situ* rat gut technique of Doluisio *et al.* (14). The *in vitro* data (1) showed no increase in the absorption rate of the eosin-B–atropine complex as compared to eosin-B alone, despite the fact that the chloroform–water partition coefficient of eosin-B increased 240-fold by the addition of atropine. The results from this part of the experiment appear in Fig. 3. After a rapid drop in the concentration of eosin-B in the intestinal lumen for the initial 10 min in the presence of atropine, both the esin-B and eosin-B complex had essentially identical half-lives for the disappearance of drug from the intestinal lumen. In each case, the half-life for "absorption" was approximately 40 min. No increase in the intestinal absorption rate of eosin-B was observed in the presence of atropine except at the initial 10-min period. This finding was in agreement with the *in vitro* results of Levy and Matsuzawa (1).

It is of interest to speculate as to why a lipid-soluble complex results in increased absorption in the case of prednisolone but has

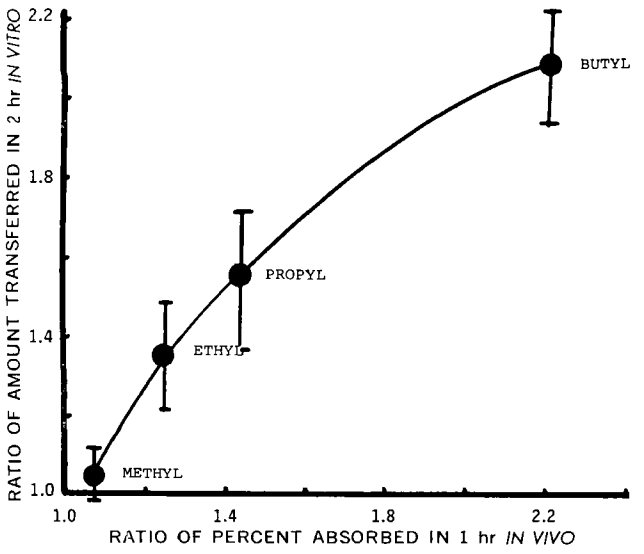


**Figure 1**—*Plot of the ratio (amide/control) of the amount of prednisolone transferred in 2 hr* versus *the ratio (amide/control) of the percent absorbed in 1 hr* in vivo. In vivo *data are from Ref. 12.*

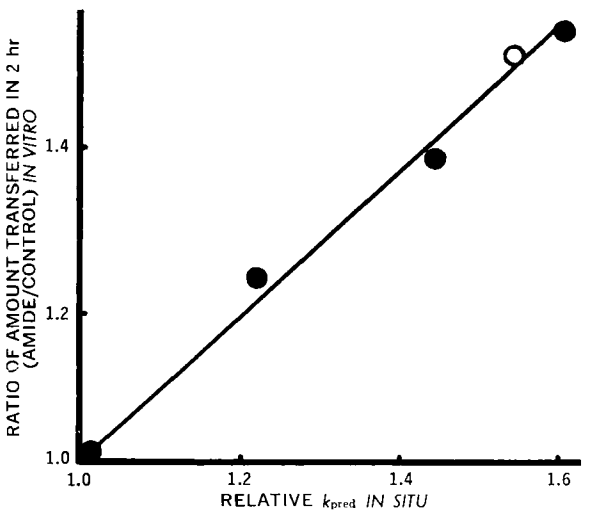


**Figure 2**—*Plot of the ratio (dipropylamide/control) transferred in 2 hr* in vitro versus *the relative absorption rate constant* ($k_{pred}$) in vivo. *The open circle represents an extrapolated point from Fig. 6 of Ref. 13.* In vivo *data are from Ref. 13.*

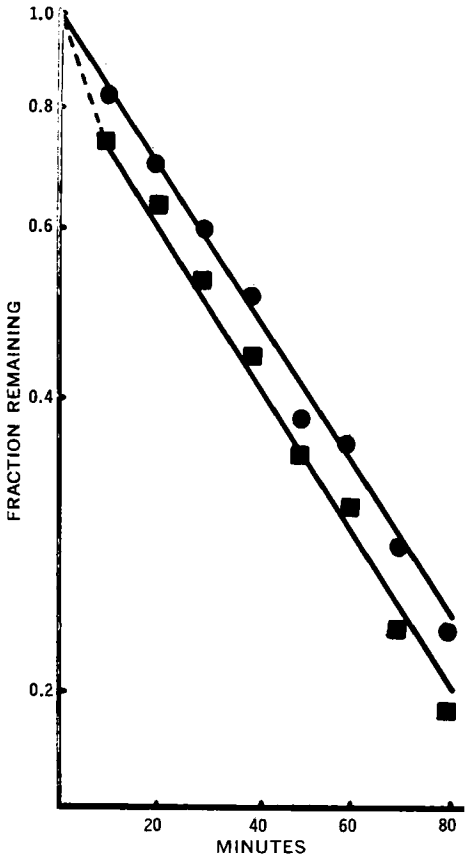


**Figure 3**—*Plot of fraction eosin-B remaining* versus *time for absorption from the* in situ *rat intestine. Key:* ●, *1 mM eosin-B; and* ■, *1 mM eosin-B and 5 mM atropine.*

no effect in the case of eosin-B. Prednisolone is a drug that has a relatively high clearance across the everted rat intestine (approximately 1 ml/hr for the initial 30 min). The clearance of eosin-B is much lower, around 0.04 ml/hr (16). Gibaldi and Grundhofer (16, 17) showed that a difference exists in the nature of the rate-limiting barrier to passive diffusion of poorly lipid-soluble and freely lipid-soluble compounds across the intestinal epithelium. Compounds with initial clearance values of >1 ml/hr fell into the category of rapidly absorbed drugs while those with less than 1 ml/hr clearance were the polar, more slowly absorbed drugs. It was found (17) that destruction of the epithelial barrier resulted in an increased rate of clearance for the poorly absorbed drugs while essentially no change occurred with the lipid-soluble compounds. This is presumably due to a difference in the rate-limiting barrier for the two different classes of drugs. To determine if this were the case with prednisolone and the amide complex, the stripping experiment was set up to examine the effect of removing the mucosa on the transfer of prednisolone and the prednisolone–amide complex across the everted rat intestine. The ratio of the amount transferred in 2 hr of the stripped to intact intestine was calculated (Table III). The ratio of unity suggests that the rate-limiting barrier to prednisolone transfer across the everted intestinal preparation is not the epithelial barrier but some other anatomical barrier in the preparation.

From the preceding discussion, it is obvious that in the cases of the prednisolone–amide and eosin-B–atropine complexes the results of the *in vitro* experiments utilizing the isolated everted rat small intestine would be a valid indicator of the *in vivo* intestinal absorption properties of the drug complex. The reason for the difference in absorption characteristics of the two drug complexes needs further investigation, but in the examples studied it is possible to predict the *in vivo* absorption of drug complexes from *in vitro* experiments utilizing the everted rat small intestine.

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## ACKNOWLEDGMENTS AND ADDRESSES

Received June 29, 1973, from the *Department of Pharmacy, School of Pharmacy, Temple University, Philadelphia, PA 19140*

Accepted for publication September 25, 1973.

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