Effect of Complex Formation on Drug Absorption XII: Enhancement of Intestinal Absorption of Prednisone and Prednisolone by Dialkylpropionamides in Rats

WILLIAM L. HAYTON* and GERHARD LEVY▲

Abstract □ The effect of *N,N*-dimethyl-, *N,N*-diethyl-, *N,N*-di-*n*-propyl-, and *N,N*-di-*n*-butylpropionamide on the absorption of prednisone and prednisolone in aqueous solution from the rat small intestine was studied. The propyl and butyl compounds (initial concentration 28 m*M*) enhanced significantly the absorption of both steroids (initial concentration 0.5 m*M*). The ethyl compound had a less pronounced effect, while the methyl derivative did not significantly affect the absorption of the two steroids. The absorption-enhancing effect of the amides is concentration dependent and is rapidly and completely reversible. It appears to be due to the formation of a steroid–amide complex within the intestinal barrier.

Keyphrases □ Drug absorption, prednisone and prednisolone—effect of steroid–dialkylpropionamide complex formation, rats □ Complex formation, prednisone/prednisolone–dialkylpropionamide—enhancement of intestinal drug absorption, rats □ Intestinal drug absorption—effect of prednisone/prednisolone–dialkylpropionamide complex formation, rats □ Prednisone–dialkylpropionamide complex formation—effect on intestinal drug absorption, rats □ Prednisolone–dialkylpropionamide complex formation—effect on intestinal drug absorption, rats

Complex formation of *N,N*-dimethyl-, *N,N*-diethyl-, *N,N*-di-*n*-propyl-, and *N,N*-di-*n*-butylpropionamide (to be referred to as the methyl-, ethyl-, propyl-, and butylamide, respectively) with prednisone and prednisolone was reported in a previous paper of this series (1). The steroid–amide interaction was greater in an organic solvent (isopropyl myristate) than in water. The transfer of the steroids through an artificial lipoid barrier was enhanced in the presence of the propyl-amide, apparently due to formation of a steroid–amide complex within the barrier. Since biologic membranes have the characteristics of lipoid barriers, it is conceivable that the amides may complex with the steroids within such membranes and thereby enhance the absorption of the steroids. Preliminary experiments indicated that the propyl-amide enhanced the absorption of prednisone from the *in situ* rat intestine (2). A more extensive investigation of the effect of the amides on the absorption of the steroids was, therefore, carried out and is reported here.

EXPERIMENTAL

Materials—The propionamides[1] were purified by distillation at reduced pressure; prednisone USP[2], prednisolone USP[3], tritium-labeled prednisolone[4], and reagent grade caffeine[1] were used as received. 2,2-Dimethoxypropane[1] was distilled, and the 78.5–80.0° fraction was used. Reagent grade acetone was dried over barium oxide and distilled. Scintillation grade 2,5-diphenyloxazole[5] and 1,4-bis[2-(5-phenyloxazolyl)]benzene[5] and reagent grade naphthalene[5], dioxane[6], and other chemicals were used as received.

Table I—Details of the GLC Assay for Determination of Alkylpropionamide Concentrations

	Unknown[a]			
	DMP	DEP	DPP	DBP
Internal standard	DEP	DMP	DEP	DPP
Column temperature	130°	130°	160°	180°
Retention time, min.				
Unknown	4.0	6.2	5.0	5.6
Internal standard	6.2	4.0	2.4	2.8
Response[b], amp. × 10^{10}				
Unknown	1.4	1.2	2.8	2.3
Internal standard	1.2	1.4	4.3	4.4

[a] Abbreviations: DMP, *N,N*-dimethylpropionamide; DEP, *N,N*-diethylpropionamide; DPP, *N,N*-di-*n*-propylpropionamide; and DBP, *N,N*-di-*n*-butylpropionamide. [b] Maximum current generated at the indicated retention time by injection of a 0.5% v/v solution of the alkylpropionamide in acetone.

Drug Solutions—Prednisone, prednisolone, caffeine, and the propionamides were dissolved in 0.9% NaCl solution.

Preparation of Animals—Male Sprague-Dawley rats[7], weighing 225–350 g. and fasted for 14–22 hr., were prepared as described by Doluisio *et al.* (3) for studying drug absorption from the *in situ* small intestine. Each animal was anesthetized with ethyl carbamate (1.5 g./kg. i.p.), the abdomen was opened by a midline incision, and 5 ml. 0.9% NaCl at 37° was instilled into the peritoneal cavity. Glass cannulas were inserted through incisions in the small intestine and tied in place with a silk suture. One cannula was directed caudally 10 cm. from the stomach, and another was directed cephalad 5 cm. from the ileo-cecal junction. The duodenal cannula was attached with a 5-cm. length of tubing (Tygon) to a 10-ml. glass syringe fitted with a threeway stopcock. The intestine was washed by passing several 10-ml. portions of perfusion solution (3) at 37° from the syringe through the intestine.

Absorption Studies—The washed intestine was rinsed once with 10 ml. of drug solution, and the ileal cannula was attached with 5-cm. tubing (Tygon) to a 10-ml. glass syringe fitted with a threeway stopcock. Sufficient drug solution at 37° was passed from the duodenal syringe through the gut into the ileal syringe to yield a volume of 7 ml. in the latter. A small volume (0.1 or 0.2 ml.) of the drug solution was removed immediately for analysis, and the solution in the ileal syringe was then returned to the intestine. At appropriate intervals, the drug solution was transferred from the intestine alternately into one of the syringes, and 0.1- or 0.2-ml. samples were removed for assay. Prior to removing a sample, the volume of the drug solution in the syringe was adjusted to 7 ml. with 0.9% NaCl.

Approximately 45 sec. was required to transfer the drug solution from the intestine to the syringe, adjust the volume, mix, sample, and return the solution to the intestine. The plunger of at least one syringe was removed at all times to prevent excessive hydrostatic pressure in the intestine. At the end of each experiment, the segment of intestine between the cannulas was excised and its length was determined. The total volume of 0.9% NaCl added to the drug solution during the experiment was recorded. Steroid concentrations determined in the serial samples were corrected for the drug removed in preceding samples.

[1] Eastman Organic Chemicals, Rochester, N. Y.
[2] Parke, Davis and Co., Detroit, Mich.
[3] The Upjohn Co., Kalamazoo, Mich.
[4] Syntex Corp., Palo Alto, Calif.
[5] Amersham/Searle, Des Plaines, Ill.
[6] Fisher Scientific Co., Fair Lawn, N. J.
[7] Blue Spruce Farms, Altamont, N. Y.

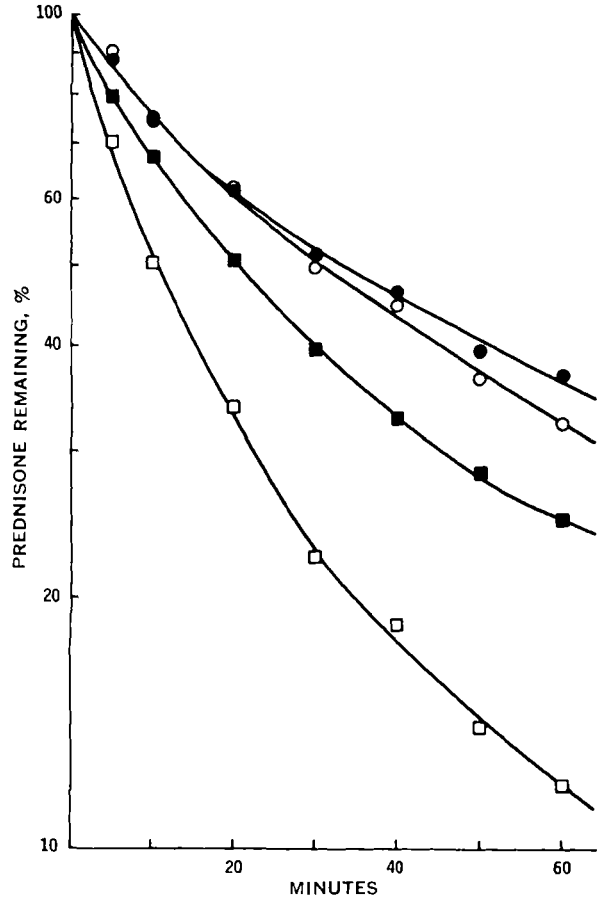

Figure 1—*Effect of dialkylpropionamides (initial concentration 28 mM) on the disappearance of prednisone (initial concentration 0.5 mM) from the* in situ *rat small intestine. Key: control, ●; methyl-amide, ○; ethyl-amide, ■; and propyl-amide, □. Each point represents the mean of four animals.*

Prednisolone Distribution Study—Exactly 7.0 ml. ^{3}H-prednisolone solution at 37° was transferred from the duodenal syringe into the washed intestine. After 30 min. the solution was removed through the ileal cannula into a volumetric flask. This was followed immediately by two successive 7-ml. portions of 0.9% NaCl, which were also collected in the volumetric flask. Blood was obtained from the heart by cardiac puncture, and the segment of intestine between the cannulas was excised and homogenized.

Assay Procedures—Prednisone and prednisolone concentrations were determined by the colorimetric method of Porter and Silber (4). Several "absorption" experiments were performed with solvent (0.9% NaCl) only, and the apparent steroid concentration was determined as a function of time. This blank, which increased with time from 0 to 0.03 m*M* apparent steroid at 60 min. for both steroids, was subtracted from the steroid concentrations determined in the actual drug absorption experiments. The propionamides did not interfere with the steroid assay and did not significantly affect the blank values.

Alkylpropionamide concentrations were determined by GLC as previously described (1), except that 15% polypropylene glycol was used as the liquid phase[8]. The column temperature, between 130 and 180°, was different for each amide; the injection port and detector temperatures were about 50° above the column temperature. Details of the assay for each amide are presented in Table I. To remove water which interfered with the determination of the amides, 0.7 ml. 2,2-dimethoxypropane[9] and 1 drop 0.93 *M* HCl in methanol were added to each 0.1-ml. aqueous amide sample in a 5-ml. centrifuge tube. An acetone solution of propionamide other than the one being determined was added as an internal standard. The volume of the sample was decreased to approximately 100 μl. under reduced pressure, and at least two 1–2-μl. portions were chromatographed at a full-scale sensitivity of 1, 2, or 5 × 10^{-10} amp. No apparent amide was detectable in samples of intestinal drug solution without amide.

Specific tritium activity (disintegrations per minute per milliliter) was determined by liquid scintillation spectrometry. Scintillation fluid was prepared by dissolving 0.3 g. 1,4-bis[2-(5-phenyloxazolyl)]-benzene, 7.0 g. 2,5-diphenyloxazole, and 100 g. naphthalene in sufficient dioxane to make 1 l. (5). One-tenth- or two-tenth-milliliter samples were mixed with 20 ml. scintillation fluid and counted[10] for 50 min. or 10^5 counts. At least 8000 counts were obtained from each sample; the background count rate was 28 c.p.m. Tritiated water[11] was used as an internal standard; the efficiency of the counting system was 40% for tritium in plasma, intestinal homogenate, and intestinal solution.

Caffeine was determined by two-component spectrophotometry at 272 and 299 nm. to overcome interference from unknown components which absorbed at 272 nm. in the intestinal solution (6).

RESULTS AND DISCUSSION

Effect of Amides on Steroid Absorption—The effect of 28 m*M* (initial concentration) methyl-, ethyl-, and propyl-amide, respectively, on the disappearance of prednisone and prednisolone from the intestinal drug solution is presented in Figs. 1 and 2. Since the solubility of butyl-amide in water is below 28 m*M*, the effect of this amide on the absorption of the steroids was determined at an initial concentration of 5.6 m*M* and compared to the effect produced by the same initial concentration of propyl-amide (Figs. 3 and 4). The rate at which the steroids disappeared from the intestinal solution was not significantly affected by methyl-amide, but it was increased somewhat by ethyl-amide and very much by the propyl- and butyl-amides. The effect of equimolar concentrations of the amides on the disappearance of the steroids from the intestine increased with the molecular weight of the amide.

To verify that the rate of disappearance of the steroids from the intestinal drug solution reflects their absorption, the activity of tri-

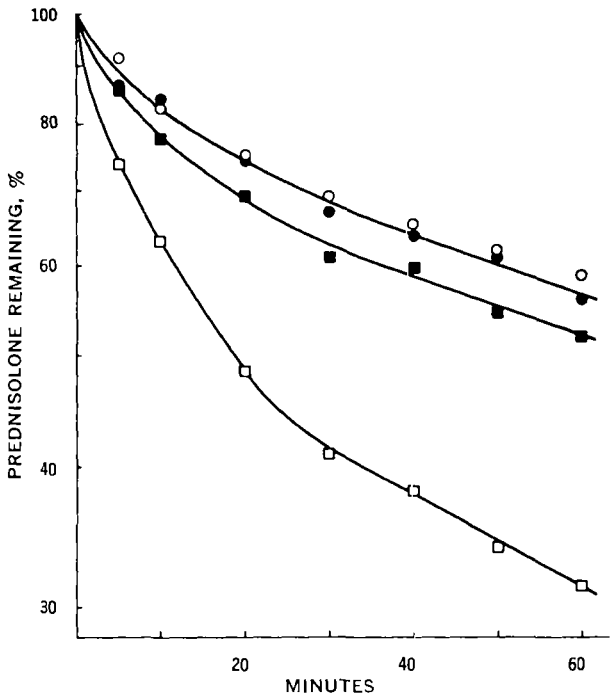

Figure 2—*Effect of dialkylpropionamides (initial concentration 28 mM) on the disappearance of prednisolone (initial concentration 0.5 mM) from the* in situ *rat small intestine. Key: see Fig. 1.*

[8] The column was prepared by Perkin-Elmer.
[9] Dimethoxypropane reacts with water under these conditions to form methanol and acetone.
[10] Packard Tricarb, model 3320, Packard Instrument Co., Downers Grove, Ill.
[11] New England Nuclear tritium standard, No. NES-003.

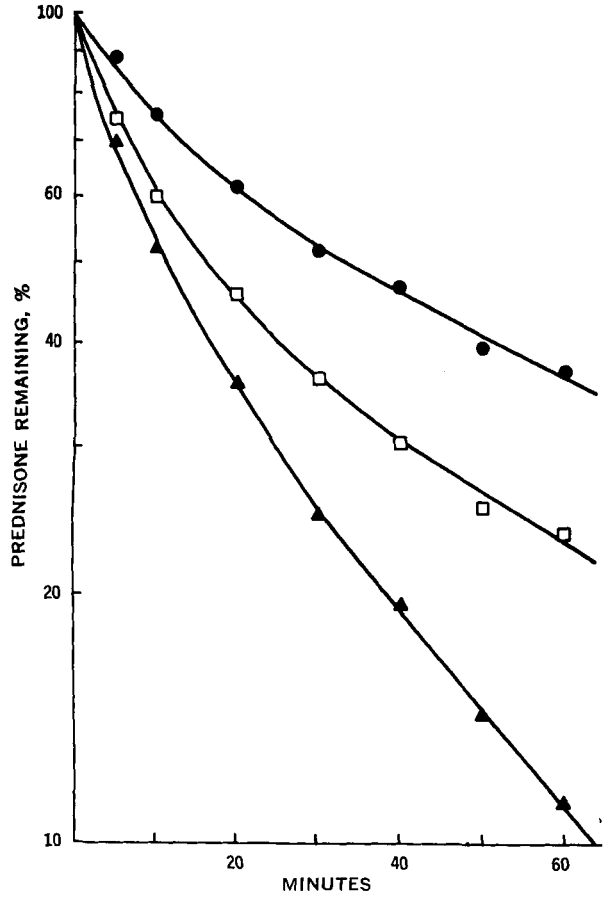

Figure 3—*Effect of 5.6 m*M *(initial concentration) propyl- and butyl-amide on the disappearance of prednisone (initial concentration 0.5 m*M*) from the* in situ *rat small intestine. Key: control, ●; propyl-amide, □; and butyl-amide, ▲. Each point represents the mean of four animals.*

tium in the plasma was determined 30 min. after a solution of tritiated prednisolone was placed in the rat intestine with and without 28 m*M* propylamide. The concentration of tritium in the plasma was significantly increased and the fraction of the administered dose of tritium remaining in the intestine was significantly decreased in the presence of the amide (Table II). These findings show that the decline in steroid concentration in the intestinal drug solution reflects absorption of the steroids and that the amides enhance this process.

Amide Absorption—The four amides are absorbed by apparent first-order kinetics, with absorption half-lives ranging from 10 min. for the butyl-amide to 15 min. for the methyl-amide (Fig. 5). This is considerably more rapid than the absorption of prednisone and prednisolone.

Steroid Absorption Kinetics—The absorption of the steroids alone and in the presence of the amides was not a simple exponential process as is evident from the curvature of the plots in Figs. 1–4. Similar kinetics were reported by Doluisio *et al.* (7) for the absorption of prochlorperazine, haloperidol, and other drugs from the *in situ* rat intestine and were attributed to drug accumulation in the

Table II—Effect of 0.028 *M* Di-*n*-propylpropionamide on Distribution of Radioactivity 30 min. after Intestinal Administration of ^{3}H-Prednisolone in the Rat

	Percent of Dose[a]			
	Luminal Content	Intestinal Tissue	Body[b]	Plasma Concentration[c]
Control	49.3 (2.34)	20.7 (1.21)	30.0	1.08 (0.12)
Amide	33.8 (0.66)[d]	18.1 (0.78)	48.1	2.05 (0.13)[d]

[a] Mean of four animals; *SEM* in parentheses. [b] By difference. [c] (d.p.m./ml./d.p.m. administered) × 10^3. [d] Statistically significantly different from control ($p < 0.01$).

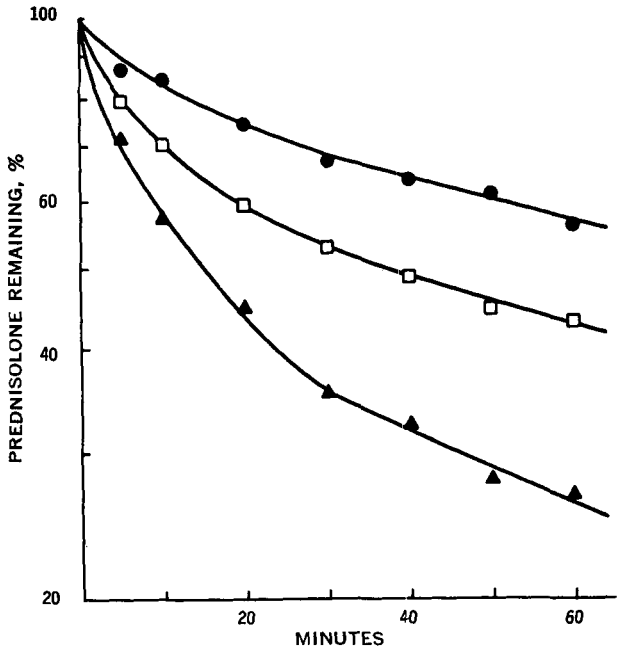

Figure 4—*Effect of 5.6 m*M *(initial concentration) propyl- and butyl-amide on the disappearance of prednisolone (initial concentration 0.5 m*M*) from the* in situ *rat small intestine. Key: see Fig. 3.*

intestinal wall. With the colorimetric steroid assay, the steroid absorption experiments could not be continued beyond 60 min. due to high blank values at the later times and insufficient assay sensitivity. With tritiated prednisolone, however, it was possible to continue the experiments for 120 min. These experiments showed that after 60 min., steroid absorption followed single exponential kinetics (Fig. 6).

It is possible that the curvatures in the control experiments shown in Figs. 1–4 and 6 are due to accumulation of the steroids in the intestinal wall (7). However, a determination of the possible effect

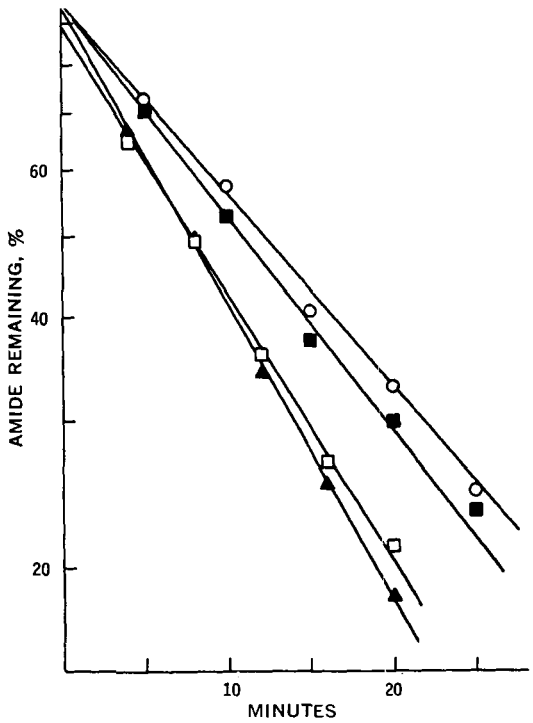

Figure 5—*Disappearance of 0.5% (initial concentration) methyl-amide (○), ethyl-amide (■), and propyl-amide (□) and 0.2% butyl-amide (▲) from the* in situ *rat small intestine. Each point represents the mean of four animals.*

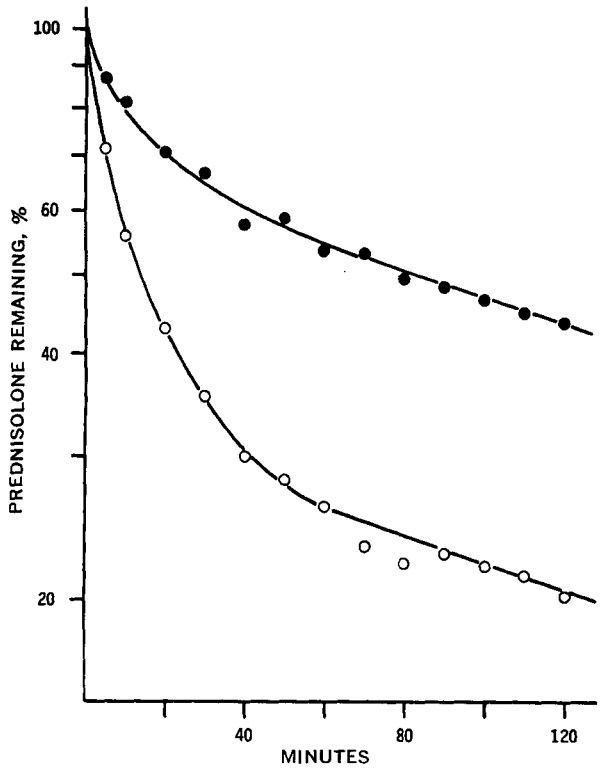

Figure 6—*Disappearance of ^{3}H-prednisolone (initial concentration made to 0.5 mM with unlabeled prednisolone) from the* in situ *rat small intestine. Key: control, ●; and in the presence of 28 mM (initial concentration) propyl-amide, ○.*

of the amides on the transfer constants for steroid movement into and out of the intestinal wall could not be carried out under the experimental conditions, because the amides are rapidly absorbed and their absorption-enhancing effect is concentration dependent (Figs. 7 and 8) and rapidly reversible (see following section). Thus, the curvatures in the amide data in Figs. 1–4 and 6 reflect not only the initial distribution of steroid in the intestinal barrier but also the time-dependent effect of the amide.

Reversibility of Absorption-Enhancing Effect—The reversibility of the absorption-enhancing effect of the amides was investigated by determining the absorption of prednisone for 30 min. in the presence of the propyl-amide, rinsing the intestine with two 10-ml. portions of 0.9% NaCl, and then determining the absorption of prednisone in the absence of the amide. Control experiments were carried out without amide in either time period. Prednisone absorption was enhanced significantly by the propyl-amide in the first time period, but the absorption of steroid in the second 30-min. period (when amide was not present) was not significantly different from that in either of the 30-min. periods of the control experiments (Fig. 9). The absorption-enhancing effect of the propyl-amide is, therefore, rapidly reversible. This reversibility is also apparent in Fig. 6, where the permeability of the intestine to prednisolone in propyl-amide solution decreases with time to that of the control as the amide is absorbed from the solution (after 60 min., less than 2% of the initial concentration of the amide remains in the intestinal solution).

Mechanism of Absorption-Enhancing Effect—There is considerable evidence that the absorption-enhancing effect of the amides is not due to mucosal damage and that it does not reflect a general effect of the amides on the permeability characteristics of the intestinal barrier. First, the absorption of caffeine is not affected by the propyl-amide (Fig. 10). Solubility and partition experiments (1) showed no detectable interaction between the amide and caffeine in isopropyl myristate and only very slight interaction in water. Second, the amount of 0.9% NaCl solution required to maintain a constant volume of intestinal drug solution in the absorption experiments was not affected by the amide. An average of 4.79 ml. (SD = 1.00) 0.9% NaCl was required in the presence of 28 mM propyl-amide, and 4.97 ml. (SD = 0.67) was required when no amide was present in studies on nine rats each. Third, the absorption half-life of the propyl-amide is concentration independent over a 3.3-fold range

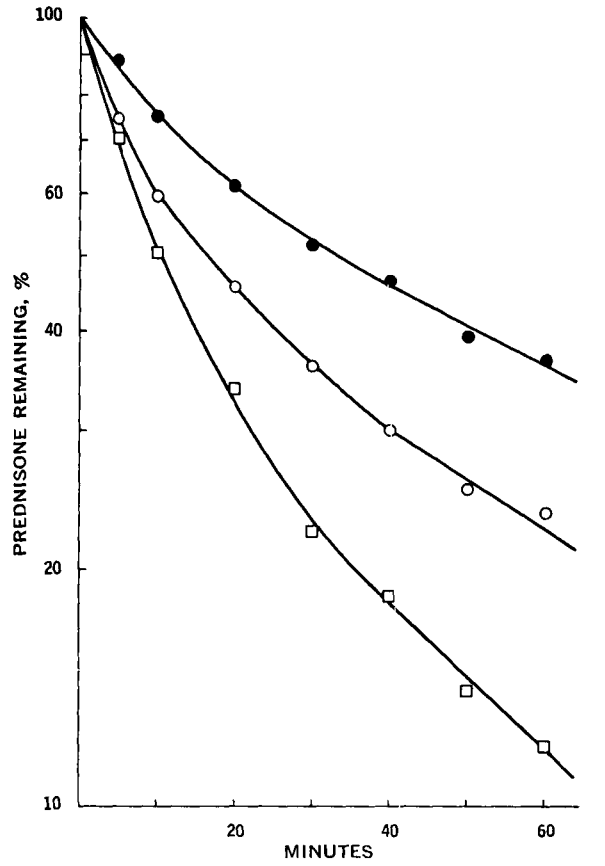

Figure 7—*Effect of* N,N-*di*-n-*propylpropionamide concentration on the disappearance of prednisone from the* in situ *rat small intestine. Key: control, ●; 5.6 mM propyl-amide, ○; and 28 mM propyl-amide, □. Each point represents the mean of four animals.*

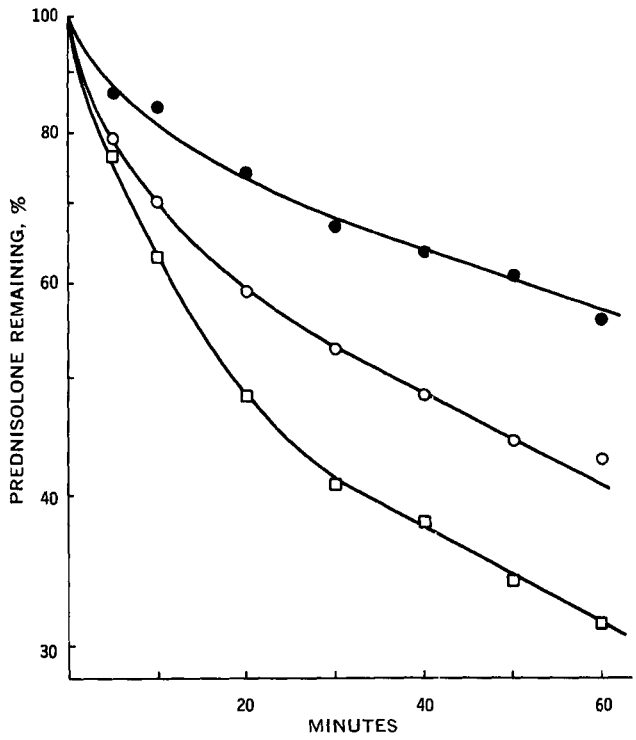

Figure 8—*Effect of propyl-amide concentration on the disappearance of prednisolone from the* in situ *rat small intestine. Key: see Fig. 7.*

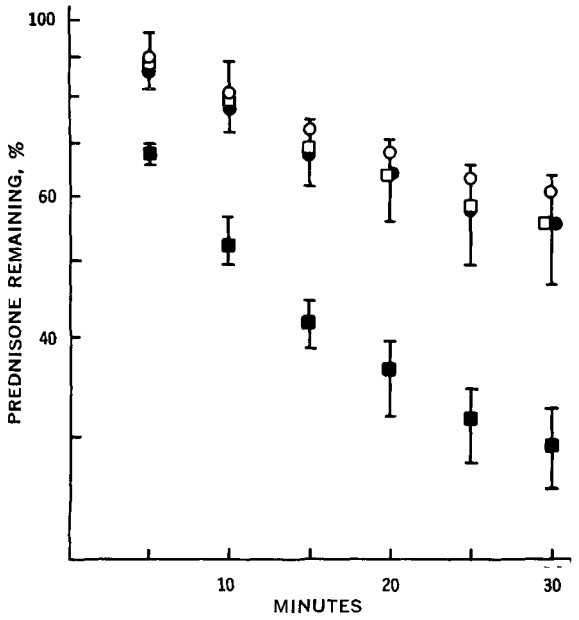

Figure 9—*Reversibility of the effect of propyl-amide on prednisone absorption from the* in situ *rat small intestine. Key: control, initial experiment, ●; control, repeat experiment, ○; with 28 mM amide, ■; and repeat experiment without amide, □. Each point represents the mean of four animals. The bars show the range of data from either four (■) or 12 (○, □, and ●) animals.*

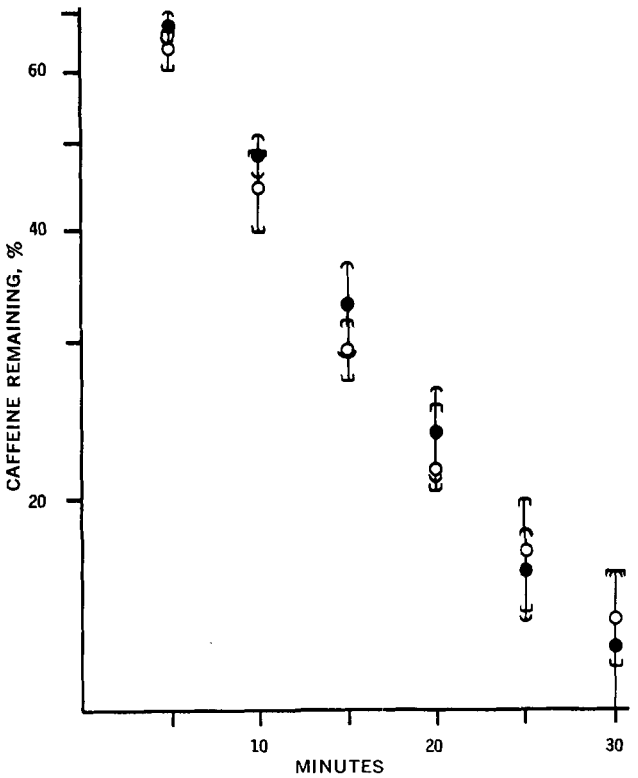

Figure 10—*Lack of effect of propyl-amide (initial concentration 28 mM) on the disappearance of caffeine (initial concentration 1 mM) from the* in situ *rat small intestine. Key: control, ●; and 28 mM amide, ○. Each point represents the mean of four animals; the brackets indicate standard deviations.*

(0.15–0.5%). If the enhanced absorption of the steroids in the presence of the amides resulted from damage to the mucosa, it would be anticipated that the propyl-amide would also enhance the absorption of caffeine and water and that its own absorption half-life would be shorter at higher initial concentrations. Fourth, the absorption-enhancing effect is rapidly and completely reversible. This is not consistent with a mechanism based on damage to the intestinal mucosa since physiologic repair of such damage would require time.

Less than 11% of the steroid in the intestinal drug solution was complexed with amide at the beginning of the experiment; this amount decreased to less than 6% after 10–15 min. when half the amide was already absorbed. It is, therefore, most unlikely that the effect of the amide on the absorption of the steroids is due to a more rapid transfer of a steroid–amide complex from the aqueous drug solution into the mucosal barrier as compared to that of the free steroid. For this mechanism to account for the absorption-enhancing effect of the amides, the absorption half-life of the complex would have to be less than 2 min. This is not realistic, and such rapid absorption is probably not achievable under the experimental conditions since diffusion to the mucosal surface would become rate limiting.

The alkylpropionamides used in these studies form complexes with prednisone and prednisolone in an organic solvent (1). It has also been shown that the rate of transfer of these steroids through an artificial lipoid barrier is enhanced by the propyl-amide, apparently due to the formation of a steroid–amide complex within the barrier (1). A similar mechanism may account for the absorption-enhancing effects of alkylpropionamides as revealed in the present investigation. The stability constants of complexes of each of the four amides used in this study with one of the steroids are equal in organic solvent (1). The concentration of the steroid–amide complex in a lipoid barrier depends on the amide concentration in the barrier which, in turn, is a function of the lipoid–water partition coefficient of the amide. The larger the partition coefficient, the more pronounced should be the effect of the amide on steroid transfer through lipoid barriers. The results of this investigation show such correlation, consistent with the proposed mechanism.

It is not possible to determine directly the extent of partitioning of amides into the lipoid phase of the intestinal barrier and the degree of complex formation within that barrier. It is feasible, however, to assess these characteristics indirectly by maintaining known and constant concentration gradients of amide across the intestinal barrier. Such studies are presently in progress. The intestinal absorption characteristics of the steroid–alkylpropionamide system are of considerable fundamental interest because the interaction occurs primarily within the biologic barrier and the concentration of complexing agent (amide) in the intestinal fluids required to enhance absorption of the steroids is very low (about 0.1%). While the system described here is not suitable for clinical use, it does represent some of the characteristics needed for using complex formation to enhance drug absorption in man since only a low concentration of complexing agent is required and the effect is relatively specific and not due to a breakdown of the biologic barrier.

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* Present address: College of Pharmacy, Washington State University, Pullman, WA 99163

▲ To whom inquiries should be directed.