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NOTES

Concurrent Water and Drug Absorption in the Rat Intestine

KATHLEEN S. PELZMANN▲ and RUTH N. HAVEMEYER

Abstract □ The absorption of water from the rat small intestine during drug absorption experiments was studied. The data indicated that approximately 4 ml. of water was lost from the intestine during 60 min.

Keyphrases □ Drug absorption—concurrent loss of water, in rat intestine □ Water absorption, rat intestine—effect on drug absorption profiles □ Absorption, water—effect on drug absorption rates in the intestine

In these studies involving drug absorption and pharmacokinetics, an unusual trend was noted which may be a consequence of water absorption. Approximately 1 hr. into the experiment, a rise in the lumenal drug concentration was observed. Enterohepatic circulation was considered, but cannulation of the bile duct failed to prevent the drug concentration rise. Return from circulation of metabolized steroid was investigated and found to be extremely low. Water absorption was evaluated next.

In 1932, Heller and Smirk (1) showed that approximately 5 ml. of water/hr. was absorbed from the small intestine of an unanesthetized rat. More recently, Miller and Schedl (2), using phenol red, polyethylene glycol 4000, and inulin-^{14}C indicators with the intestinal perfusion method, found approximately 13% (6 ml.) net water absorption in the rat intestine during 60 min. In drug absorption studies on the rat intestine, Doluisio *et al.* (3) reported ±10% (1 ml.) changes in phenol red concentration and concluded that water absorption was not significant in their studies. However, Bates and Gibaldi (4), using the Doluisio intestinal preparation, found particularly significant water absorption in experiments extending beyond 30 min. Our drug absorption data indicated approximately 30–40% (4 ml.) net water absorption per hour. Phenol red and tritiated water experiments confirmed the 4-ml. water loss from the intestine to be the major contributing factor to the rise in drug concentration in the lumen.

EXPERIMENTAL

Solution—The instillation fluid was a 10% solution of ethanol and isotonic Sørensen's buffer at pH 6.24. The drug, norethindrone, was made radioactive by coprecipitation in cold acetone with tritiated norethindrone. This steroid was dissolved in the buffer solution; then phenol red was added to a concentration of 5 mg./ml. of buffered drug solution. Ten milliliters of the solution (100 mcg. norethindrone) was then instilled into the duodenum of each rat.

The second instillation fluid was isotonic Sørensen's buffer (pH 6.24) made with tritiated water; 2.5- and 5-ml. samples were instilled into the rat's duodenum.

Animal Preparation—Sprague-Dawley female rats, weighing 200–250 g., were fasted 18 hr. with water *ad libitum* prior to the experiment. The animals were anesthetized with sodium pentobarbital[1], 50 mg./kg. An abdominal incision exposed the intestine and bile duct. While paying particular attention not to tie off major vessels of the gut, the pylorus and the jejunoileal junction were ligated. The bile duct was cannulated with polyethylene tubing[2]. The buffered solution was injected into the duodenum using a 23-gauge needle.

The bile samples taken were the total bile flow over 5-min. periods (~0.1 ml.). Intestinal samples of 0.1 ml. were taken along the length of the ligated section with a 27-gauge needle and disposable syringe. Blood samples were taken from the carotid artery, and plasma was obtained after centrifugation at 4000 r.p.m.

Assay Method—For phenol red solution data, lumen and bile samples were divided into 50-μl. portions: 50 μl. was placed in 10 ml. of Bray's solution (5) and counted[3], and 50 μl. was diluted with 1 *N* NaOH and the absorbance was read at 500–560 nm. on a scanning spectrophotometer[4]. Numerically, the intestinal lumen sample was added to the bile sample as though the phenol red had been returned to the intestine. The radioactive norethindrone was used to follow the absorption of the drug. The absorbance was used to estimate water movement using the following equation:

[1] Nembutal.
[2] PE 50 Intramedic, Clay Adams.
[3] On a Nuclear Chicago Unilux II.
[4] Beckman model DU.

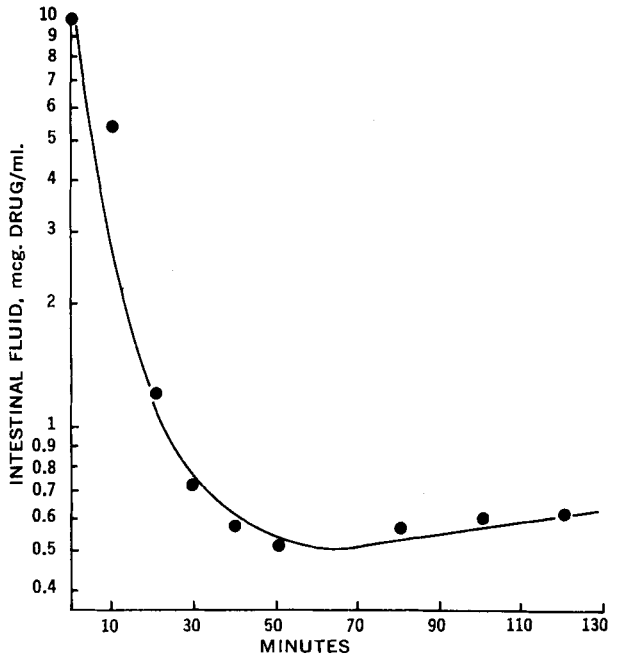

Figure 1—*Norethindrone intestinal absorption curve.*

$$\left(1.00 - \frac{C_i}{C_f}\right) \times 100 = \% \text{ water absorbed} \qquad \text{(Eq. 1)}$$

where C_i and C_f are the initial and final concentrations of phenol red solution, respectively. In preparations using tritiated water, the plasma, bile, and intestinal contents were assayed by scintillation counting. The 0.1-ml. samples were counted in 15 ml. of Bray's scintillation fluid. Quench was determined with a known ^{3}H-toluene spike delivered to each vial. The water volume remaining in the gut was determined by comparing the activity of the initial tritium solution with the activity of the sample solution:

$$\left(1.00 - \frac{A_i}{A_f}\right) \times 100 = \% \text{ water absorbed} \qquad \text{(Eq. 2)}$$

where A_i and A_f are the initial and final disintegrations per minute of tritium, respectively. Bile was assayed, but the activity was negligible. The plasma accounted for virtually all of the radioactivity not found in the intestine. Since tritiated water is rapidly equilibrated in the body, the first few plasma samples are the most meaningful.

RESULTS AND DISCUSSION

The results that initiated these experiments are shown in Fig. 1. A summary of the data from phenol red and tritiated water experiments is shown in Figs. 2 and 3.

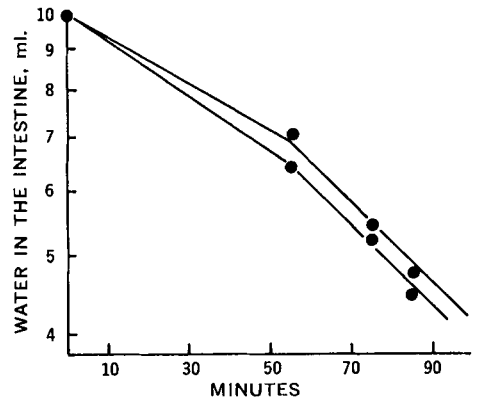

Figure 2—*Phenol red determination of water absorption.*

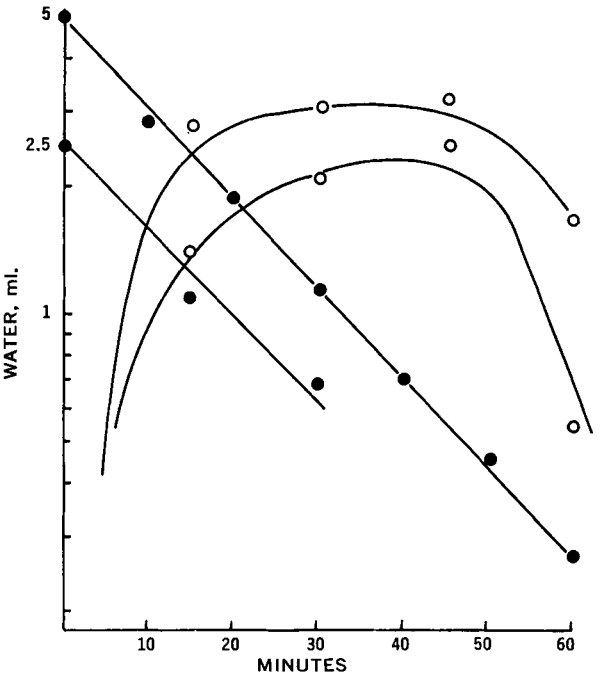

Figure 3—*Tritiated water determination. Key: ●, milliliters of water in the intestine; and ○, milliliters of water in the plasma.*

The data from the phenol red and tritiated water experiments indicated that, like Heller and Smirk's (1) results, approximately 4 ml./hr. was absorbed from the rat intestine. This rate was not constant, but appeared to increase as the lumen water volume decreased. A volume-to-surface area phenomenon may well account for the observed increase.

In the phenol red experiments, 10 ml. of solution was instilled into the intestine and 6 ml. remained after 60 min. With tritiated water, 2.5 and 5 ml. were instilled. After 30 min., the 2.5-ml. sample was virtually gone, and the 5-ml. sample proved difficult to sample. Lumen data from the tritiated water experiments do not provide a good basis for a conclusion. However, plasma levels of tritiated water are much more indicative of actual water movement. Figure 3 is also a graph of the activity found in the blood.

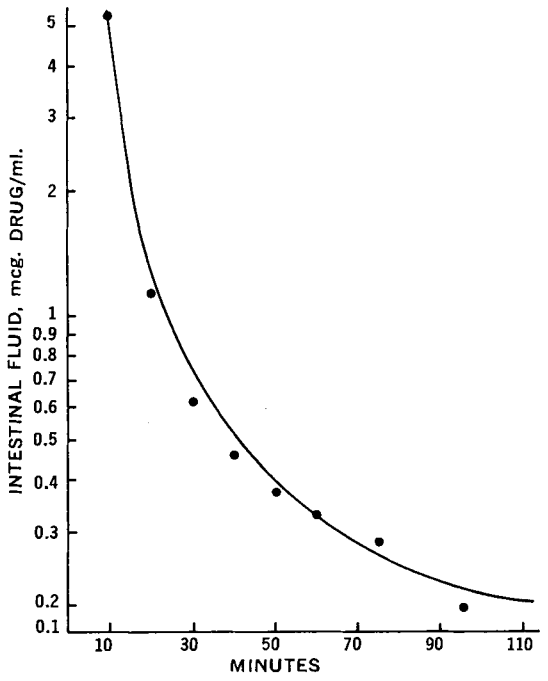

Figure 4—*Drug absorption curve corrected for water absorption.*

It can be seen that, after 30 min., virtually all of the counts administered in 2.5 ml. were found in the plasma; approximately one-half of the counts administered in 5 ml. were found in the plasma.

During the phenol red experiment, large quantities of indicator were found in the bile. Approximately 32% of the initial 500 mcg./ml. was found in the bile after 1 hr. The total amount of indicator recovered by the liver from the blood was 98%, with very little remaining in the hepatic vein plasma. If enterohepatic circulation had not been interrupted, the phenol red concentration in the intestinal lumen would have remained fairly constant after the initial loss due to the amount circulating through the liver. Evidence of phenol red absorption from the rat jejunum was also found by Kunze (6) and indirectly mentioned by Miller and Schedl (2, 7).

No significant effect of water movement was observed in the first part of the drug absorption experiments. It is probable that as the steroid absorption process progressed from the lumen-to-membrane phase to the membrane-to-blood phase, the water effect was magnified by the membrane-to-blood rate constant, which is approximately a factor of 10 less than the initial lumen-to-membrane rate constant. From the water outflow rate, the drug absorption data were corrected for water loss. These corrected results are shown in Fig. 4. After correcting the data for water influence, the drug absorption curve no longer shows a rise in concentration. The resulting graph is very similar to the expected curve for a three-compartment drug absorption model. These expected data were generated from the corrected first-phase data of the absorption curve and the Metzler (8) curve-fit computer program modified for the pharmacokinetic treatment of steroid drug absorption studies presently being done in this laboratory (9). If absorption studies are prolonged, water movement must not be ignored, especially if the compounds investigated show biphasic absorption as described here and by Doluisio *et al.* (10). Misleading concentrations of compounds in the lumen may lead to incorrect conclusions concerning the drug absorption mechanisms and the rate constants.

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Substituted 3-Aminomethylbenzoxazoline-2-thiones as Potential Antibacterial Agents

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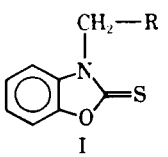
Abstract □ A series of 3-aminomethylbenzoxazoline-2-thiones was tested for possible antibacterial activity. Out of the 17 compounds screened, 11 exhibited some degree of activity.

Keyphrases □ 3-Aminomethylbenzoxazoline-2-thiones, substituted —screened as potential antibacterial agents □ Antibacterial agents, potential—substituted 3-aminomethylbenzoxazoline-2-thiones screened □ Agar diffusion technique—screening of 3-aminomethylbenzoxazoline-2-thiones as antibacterial agents

Several benzoxazoline-2-thiones (I), substituted in position 3, were synthesized (1) for biological evaluation. In this publication, the results of the screening of these compounds by the agar diffusion technique (2) against four different organisms are described.

EXPERIMENTAL

The following test organisms (3) were included in this study: A, *Staphylococcus aureus* K 257; B, *Pseudomonas aeruginosa;* C, *Klebsiella pneumoniae* ATCC 8052; and D, *Mycobacterium smegmatis.* The agar medium was heavily inoculated with the test organism; then filter paper disks (6.35 mm.) saturated with two drops of the solution of the test compound (20 mg./ml. in ethanol) were placed on the agar. The zones of inhibition



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