The R_f values obtained in all solvent systems were identical with that of the commercial sample.

IR and Visible Spectra of Anthocyanidin—An IR spectrum was made of the red pigment and was found to be superimposable with that of a commercial sample of cyanidin chloride. Visible spectrum analysis of the pigment in ethanol–0.1% HCl solution was 545 nm. (11).

DISCUSSION

A tannin extract obtained from powdered cinnamon USP was examined phytochemically. The tannin extract consisted of polymeric leucoanthocyanidin units. Based on the conversion of the polymer to 3,5,7,3′,4′-pentahydroxyflavylium chloride and the identification of the same, it was concluded that the polymer consisted of polymeric units of 5,7,3′,4′-tetrahydroxyflavan-3,4-diol. No monomeric leucoanthocyanidins or other polyphenolic derivatives of benzoic and cinnamic acid were found in the tannin extract.

REFERENCES

(1) L. Buchalter, *J. Pharm. Sci.*, **58,** 10(1969).

(2) L. Buchalter and J. R. Cole, *ibid.*, **56,** 8(1967).

(3) H. W. Youngken, "Textbook of Pharmacognosy," 6th ed., Blakiston, Philadelphia, Pa., 1948, p. 368.

(4) G. M. Robinson and R. Robinson, *J. Chem. Soc.*, **1935,** 744.

(5) E. C. Bate-Smith and N. H. Lerner, *Biochem. J.*, **58,** 122 (1954).

(6) K. Venkataraman, in "Chemistry of Flavanoid Compounds," T. A. Geissman, Ed., Macmillan, New York, N. Y., 1962, p. 76.

(7) L. Hayashi, *Acta Phytochim. Japan*, **117,** 143(1933).

(8) K. Hayashi and Y. Abe, *Bot. Mag. Tokyo*, **69,** 577(1956).

(9) D. G. Roux, *Nature*, **183,** 890(1959).

(10) *Ibid.*, **180,** 973(1957).

(11) J. B. Harborne, *Biochem. J.*, **70,** 22(1958).

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Physiologic Surface-Active Agents and Drug Absorption VIII: Effect of Bile Flow on Sulfadiazine Absorption in the Rat

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Abstract □ The absorption of sulfadiazine was determined in rat intestinal loops *in situ* under four experimental conditions—*viz.*, control, bile duct ligation, sham bile duct ligation, and sodium dehydrocholate-stimulated bile flow. Enhanced bile flow increased the absorption of the drug about 50%, apparently by increasing the solubility and dissolution rate of sulfadiazine. This possibility is supported by the results of *in vitro* solubility studies. Rats with a ligated bile duct, on the average, showed significantly reduced absorption compared to control levels. The results suggest that bile plays an important, although not critical, role in the absorption of sulfadiazine under these experimental conditions.

Keyphrases □ Sulfadiazine—absorption, bile-flow effect, rats □ Absorption, sulfadiazine—effect of bile flow, rats □ Bile—role in sulfadiazine absorption, rats

Numerous studies have shown that surface-active agents may increase the gastrointestinal absorption of poorly water-soluble drugs by enhancing dissolution *via* an effect on the effective surface area or apparent solubility (1). Since bile manifests considerable surface activity, it is reasonable to consider that bile salts and certain phospholipids, which are normally present in the small intestine, solubilize and enhance the dissolution rate of poorly soluble drugs and should thereby promote the absorption of these compounds. It follows that where there is a diminished bile salt concentration in the proximal intestine, such drugs will be poorly absorbed. This possibility is supported by the findings of Bernhard *et al.* (2) who showed that in the biliary fistula of the rat, less than 1% of a dose of vitamin A was absorbed and that bile administration increased absorption significantly.

Greaves (3) also reported that bile salts are essential for the intestinal absorption of vitamin K in the rat. Adequate absorption of vitamin D by the rat was shown to require bile by Greaves and Schmidt (4). While the bile duct was anastomosed into the colon, the animals absorbed little or none of the vitamin. Oral administration of deoxycholic acid greatly improved absorption of the vitamin in these rats. Taylor *et al.* (5) confirmed the need of bile for adequate vitamin D absorption in dogs. Heymann (6) also found that dogs did not absorb crystalline vitamin D_2 when bile was not present in the small intestine.

Pekanmaki and Salmi (7) studied the gastrointestinal absorption of phenolphthalein and its glucuronide in the cat. Peak blood levels (portal vein) of 50 mcg./ml. were observed in control animals, in contrast to peak levels of 23 mcg./ml. in test animals (ligation of common bile duct) 1 hr. after gastric intubation of phenolphthalein. There was a marked decrease in the absorption of the poorly soluble drug when drainage of bile into the intestine was prevented. However, the absence of bile from the intestine had no effect on the absorptions of the highly water-soluble glucuronide.

More recently, Meli *et al.* (8) reported that endogenous bile influences the rate of intestinal absorption of ethynylestradiol-6,7-^{3}H-3-cyclopentyl ether in rats. The rate of absorption of the estrogen was considerably lower in bile duct-cannulated rats than in control

Table I—Sulfadiazine Absorption from Rat Intestinal Loops

Experimental Condition[a]	Volume of Intestinal Contents[b] (Range, ml.)	pH of Intestinal Contents (Range)	Length (cm.) of Loop (Mean ± *SD*)	Percent Absorbed[c] (Mean ± *SD*)
Normal (6)	0.5–1.0	7.2–7.6	23.0 ± 2.3	43 ± 8
Sham ligated (6)	—	—	24.9 ± 4.0	44 ± 7
Stimulated bile flow (6)	1.0–1.2	6.6–6.8	24.5 ± 2.9	63 ± 8[d]
Bile duct ligated (10)	0.1–0.2	7.2–7.4	22.3 ± 3.8	26 ± 17[d]

[a] Parenthetical values denote the number of rats. [b] Recovered from the loop 3 hr. after administration. [c] Calculated from residual levels in tissue and loop contents 3 hr. after administration of a 10-mg. dose. [d] Values are significantly different from those obtained with either normal or sham ligated rats, $p < 0.05$, one-tail t test.

animals. Since the drug is relatively water insoluble, it is reasonable to consider that the presence of bile increases the solubility of the drug in the intestinal lumen and thereby enhances the dissolution and absorption rate.

In view of the potential influence of endogenous bile on drug absorption, the purpose of this investigation was to study the role of bile flow on the absorption of a poorly water-soluble sulfa drug, sulfadiazine. Absorption was determined in the rat under four experimental conditions—*viz.*, in normal bile flow animals, in sham ligated animals, in bile duct ligated animals, and in bile flow stimulated animals.

EXPERIMENTAL

Sulfadiazine USP,[1] sodium dehydrocholate,[2] and Marshall's reagent [*N*-(1-naphthyl)-ethylenediamine dihydrochloride][3] were used. All other reagents and chemicals were of certified grade.[3] All compounds were used as received without further purification.

Male rats, Sprague-Dawley strain,[4] were deprived of food for 24 hr. prior to the absorption experiment but allowed water *ad libitum*. The rats used weighed approximately 260 g. (range 226–293 g.). They were divided into four groups (Table I): normal, bile flow stimulated, bile flow deprived, and sham ligated.

Normal Animals—The rats were anesthetized with ether, and the abdominal cavity was opened with a midline incision. A loop, approximately 25 cm. in length, was prepared by ligating the intestine approximately 2 cm. proximal and about 23 cm. distal to the bile duct. Prior to completing the ligation, a 20-gauge hypodermic needle was inserted into the proximal portion of the duodenum so that when the ligature was tightened the tip of the hypodermic needle was approximately 1 cm. proximal to the bile duct. At this time, a 10-mg. dose of sulfadiazine suspended in water was injected into the duodenum, the needle was removed, and the ligature was tightened simultaneously so that backflow of the drug suspension did not occur. The animal's abdominal cavity was closed with wound clips, and the anesthetic was removed so that the animal regained consciousness within 10–15 min. Three hours after administration of the drug, the animal was sacrificed by placing it in an ether tank.

Bile Stimulated Animals—These animals were treated in the same manner as the normal rats, except that 1 ml. of a 100 m*M* solution of sodium dehydrocholate (SDHC) was administered immediately prior to injecting the drug suspension. The SDHC was administered by placing the solution into the exposed peritoneal cavity, directly beneath the portal vein.

Bile Flow Deprived Animals—The abdominal cavity was opened as previously described and the bile duct exposed. Two ligatures were placed on the bile duct approximately 0.5 cm. apart and tightened to prevent bile flow or backflow from the intestine. The bile duct was then cut between the two ligatures. The abdominal cavity was closed by suture, and the animals were allowed to recover in individual cages for 48 hr. with food and water *ad libitum*. After 48 hr. the food was removed, and 24 hr. later the animal was anesthetized and treated in the same manner as described for the normal rats.

Sham Ligated Animals—These animals were treated in the same manner as the bile flow deprived rats, except that the bile duct was not ligated nor cut.

Preparation of Drug Suspension—To ensure minimum loss of drug in the preparation and transfer of the dosage form, the suspension was prepared *in situ* by placing the drug into the barrel of a dry 1-ml. hypodermic syringe, adding 0.5 ml. water, and injecting the resulting suspension. An additional 0.5 ml. water was added to the syringe in small increments and injected to ensure complete delivery of the drug from the syringe. Delivery of the suspension in this manner resulted in an approximate drug loss of 3–5%. Accordingly, a 5% excess of drug was added in each case.

Analytical Procedure—After sacrifice of the animal, the intestinal loop was excised and its contents were carefully milked into a collecting vessel to estimate volume and determine pH. The loop and the contents were then homogenized with about 10 ml. of 0.1 *N* NaOH in a Waring blender for 2 min. The blender was washed with 10-ml. increments of water until a total volume of 50 ml. was obtained. The homogenate and washings were mixed by inversion in a 50-ml. centrifuge tube and centrifuged for 20 min. at 2000 r.p.m. in an International centrifuge, model CS. An aliquot of the supernatant liquid was removed, treated with 30% (w/v) trichloroacetic acid to precipitate protein, and recentrifuged. The supernatant liquid was filtered, and an aliquot was assayed for drug content using the Bratton and Marshall method (9). All analyses were performed at 545 nm. using a Gilford model 222 photometer and power supply of the optical system of a Beckman DU spectrophotometer.

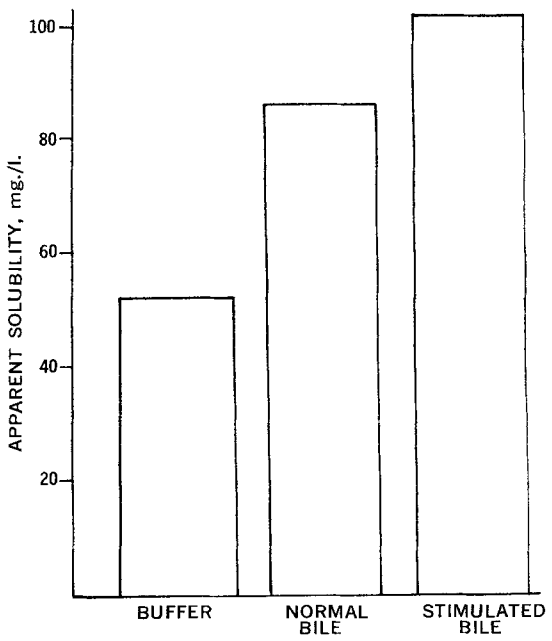

Figure 1—*Apparent solubility of sulfadiazine at 26° in pH 5.8 phosphate buffer and in buffer containing 40% (v/v) normal or SDHC stimulated bile.*

[1] Obtained from Rexall Drug Co., St. Louis, Mo.
[2] Obtained from General Biochemicals, Laboratory Park, Chagrin Falls, Ohio.
[3] Obtained from Fisher Scientific Co., Fair Lawn, N. J.
[4] Obtained from Blue Spruce Farms, Altamont, N. Y.

Tissue blanks were studied in animals using distilled water as the injected substance, and measurable absorbance values could not be detected.

Drug Recovery—Recoveries were performed by removing a 20-cm. segment of intestine from a freshly sacrificed animal, homogenizing in the presence of a known amount of drug (5–9 mg.), and extracting as previously described. A mean recovery (6 trials) of 87.8% was obtained. Further recovery studies were performed in a manner more analogous to experimental conditions. In these studies the abdominal cavity of the animal was opened and a loop was prepared as described for the control animals. Immediately prior to drug administration, the animals were sacrificed by ether. The drug was administered from suspension and allowed to remain in the loop for 3 hr. At this time, the loop was removed and assayed for drug content as described. A mean recovery of 83.9% (10 trials) was obtained using this technique. No statistical difference existed between the results of the two recovery studies. Accordingly the data were combined, which resulted in a mean recovery of 85.3%. All experimental results were corrected for this recovery value.

Solubility Study—To obtain an indication of the ability of bile to solubilize sulfadiazine, the following study was performed. An excess of sulfadiazine was placed in a 10-ml. graduated test tube. Either 2 ml. of distilled water, normal rat bile, or stimulated rat bile was added. Sufficient 0.5 *M* phosphate buffer (pH 5.8) was added to bring the system to a total volume of 5 ml. The suspension was vigorously agitated for 5 min. at room temperature. Agitation was accomplished with a Vortex-Genie model K-550-G mixer. After mixing, the suspension was filtered and an aliquot was diluted 10-fold and assayed according to the method of Bratton and Marshall (9). Blanks were negligible in all cases.

Normal or stimulated rat bile was obtained immediately prior to the solubility determination by the methods outlined previously (10). Bile was stimulated by intraperitoneal injection of 100 μmoles of SDHC. In each case, the bile of several rats was pooled to obtain a sufficient amount of material for the study.

RESULTS AND DISCUSSION

Results of the various absorption studies are summarized in Table I. It is apparent that sulfadiazine was rather poorly absorbed from this preparation. In control rats, only about 40% of a 10-mg. dose was absorbed over a 3-hr. period. It is also evident that the surgical procedure *per se*, to which bile duct ligated animals were subjected, had no influence on the absorption of the drug. Sham ligated rats absorbed sulfadiazine to the same extent as control animals.

Modification of bile flow produced several interesting findings. When bile flow was stimulated by coadministering SDHC, the absorption of sulfadiazine was increased about 50% compared to control values. In addition, the fluid recovered from the loop after the absorption period was of somewhat greater volume and distinctly more acidic than that observed in control rats.

The mean absorption of sulfadiazine in bile duct ligated rats was reduced to about 60% of control levels, but the results were highly variable. The coefficient of variation of percent drug absorbed in bile duct ligated animals was about 65%, in contrast to coefficients of variation of 13–19% observed under the other experimental conditions. Furthermore, in rats where bile flow was terminated, there was a marked decrease in the volume of fluid in the loop at the end of the experiment. The pH of the intestinal fluid, however, was comparable to control values.

The influence of rat bile on the solubility of sulfadiazine is shown in Fig. 1. As expected from the results of previous studies with components of bile, whole bile from both normal and stimulated (SDHC) rats exerted a solubilizing effect on sulfadiazine, presumably *via* micelle formation. Stimulated bile was a somewhat more effective solubilizing agent than unstimulated bile, producing a two-fold increase in the solubility of the drug.

The solubilizing effect of bile on sulfadiazine may well be responsible for the decreased absorption of the drug observed in bile duct ligated rats and for the enhanced absorption found upon stimulation of bile flow. While a strong argument can be developed for the latter observation, a more equivocal position must be taken for the former. The absence of bile in the proximal intestine would decrease the solubility of the drug in the intestinal fluids, and one would anticipate that dissolution-rate-limited absorption would also diminish. However, the volume change in ligated rats resulting from water absorption and the lack of replacement by bile introduces a complicating factor. Conceivably, the small volume present in the gut permitted the buildup of a high concentration of sulfadiazine in solution which would inhibit the dissolution rate of the drug.

The enhanced absorption of sulfadiazine upon coadministration of the hydrocholeretic (SDHC) appears to be more clearly related to the solubilization phenomenon. Although a difference in pH was noted when comparing intestinal fluid from rats where bile flow was stimulated with that from control rats, this difference cannot account for the increased absorption since the direction of the shift would result in a decreased solubility and dissolution rate of the weakly acidic drug. On the other hand, the significant solubilizing effect of stimulated bile on sulfadiazine, observed *in vitro*, could readily account for an enhancement in dissolution-rate-limited absorption.

REFERENCES

(1) M. Gibaldi and S. Feldman, *J. Pharm. Sci.*, **59,** 579(1970).

(2) K. Bernhard, G. Ritzel, and E. Scheitlin, *Helv. Physiol. Pharmacol. Acta*, **10,** C47(1952).

(3) J. D. Greaves, *Amer. J. Physiol.*, **125,** 423(1939).

(4) J. D. Greaves and C. L. A. Schmidt, *J. Biol. Chem.*, **102,** 101(1933).

(5) N. B. Taylor, C. B. Weld, and J. F. Sykes, *Brit. J. Exp. Pathol.*, **16,** 302(1935).

(6) W. Heymann, *J. Biol. Chem.*, **122,** 249(1937).

(7) K. Pekanmaki and H. A. Salmi, *Acta Pharmacol. Toxicol.*, **18,** 133(1961).

(8) A. Meli, D. I. Cargill, T. Giannina, and B. G. Steinetz, *Proc. Soc. Exp. Biol. Med.*, **129,** 937(1968).

(9) C. A. Bratton and E. K. Marshall, Jr., *J. Biol. Chem.*, **128,** 537(1939).

(10) C. H. Nightingale and M. Gibaldi, *Amer. J. Dig. Dis.*, **14,** 545(1969).

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