and 3. Nevertheless, the authors consider that Reaction 3 is properly classified as a Phase 1 conversion (11). Although 4′-hydroxydesalkylprazepam has not been identified, this compound, as well as oxazepam, may be a precursor to 4′-hydroxyoxazepam. Other routes to 4′-hydroxyoxazepam are also possible as stated in the legend of Scheme I.

Another of the unidentified metabolites may be 2′-hydroxyoxazepam. It seems that aromatic hydroxylation in the *ortho* and *para* positions is catalyzed by different enzymes (12) and that the dog generally produces more of the *ortho* isomer (13, 14). The authors' identification of the *para* hydroxy compound (4′-hydroxyoxazepam) in dog urine implies steric hindrance of the *ortho* (2′) position. On the other hand, it is quite possible that some 2′-hydroxy ACB was present with the 4′-hydroxy ACB. Since the 2′-hydroxy compound is not available for study, the possibility cannot be excluded that the four solvent systems did not resolve 2′- and 4′-hydroxy ACB.

In the dog, a single Phase 2 reaction was observed, namely, conjugation with glucuronic acid. As far as could be determined, all of the 3-hydroxyprazepam was conjugated. Thus, a competitive situation would exist if 3-hydroxyprazepam were also converted to oxazepam, as seems likely. The data show that most of the oxazepam was conjugated. The presence of some free oxazepam is interesting and suggests intermediate ranking of the dog among species capable of forming *O*-glucuronides. Although conclusive evidence is lacking, it is surmised that 4′-hydroxyoxazepam was also excreted mainly as a glucuronide.

Unlike diazepam (7, 8) and oxazepam (4), some unaltered prazepam was excreted into the urine by dogs. Thus, dogs treated with prazepam circulate at least three compounds with tranquilizer activity, namely prazepam (15–18), oxazepam, and desalkylprazepam (19).

CONCLUSIONS

The radioactivity from ^{14}C-prazepam administered to dogs was excreted slowly into the urine. Most of the drug was transformed, apparently by oxidative enzymes of the liver microsomes. The major drug metabolite was oxazepam glucuronide. Other glucuronides were formed from 3-hydroxyprazepam and 4′-hydroxyoxazepam. The urine collections also contained unaltered prazepam, desalkylprazepam, and unconjugated oxazepam. Prazepam is considered to serve as the precursor to a series of metabolites with tranquilizer activity.

REFERENCES

(1) F. J. DiCarlo, M. C. Crew, M. D. Melgar, and L. J. Haynes, *J. Pharm. Sci.*, **58**, 960(1969).

(2) G. Jommi, P. Manitto, and M. A. Silanos, *Arch. Biochem. Biophys.*, **108**, 568(1964).

(3) E. J. Merrill, *J. Labelled Compds.*, to be published.

(4) S. S. Walkenstein, R. Wiser, C. H. Gudmundsen, H. B. Kimmel, and R. A. Corradino, *J. Pharm. Sci.*, **53**, 1181(1964).

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

(6) H. Ganshirt, "Thin Layer Chromatography," E. Stahl, Ed., Springer-Verlag, Berlin, West Germany, 1965, p. 312.

(7) M. A. Schwartz, B. A. Koechlin, E. Postma, S. Palmer, and G. Krol, *J. Pharmacol. Exp. Ther.*, **149**, 424(1965).

(8) H. W. Ruelius, J. M. Lee, and H. E. Alburn, *Arch. Biochem. Biophys.*, **111**, 376(1965).

(9) J. A. F. DeSilva, B. A. Koechlin, and G. Bader, *J. Pharm. Sci.*, **55**, 692(1966).

(10) J. Kvetina, F. Marucci, and R. Fanelli, *J. Pharm. Pharmacol.*, **20**, 807(1968).

(11) R. T. Williams, *Federation Proc.*, **26**, 1029(1967).

(12) J. R. Gillette, *Advan. Pharmacol.*, **4**, 219(1966).

(13) D. V. Parke and R. T. Williams, *Biochem. J.*, **63**, 12P (1956).

(14) D. V. Parke, *ibid.*, **77**, 493(1960).

(15) E. Dunlop and J. Weisberg, *J. Psychopharmacol.*, **1**, 75 (1966).

(16) E. Dunlop and J. Weisberg, *Psychosomatics*, **9**, 235(1968).

(17) J. W. Shaffer, M. L Yeganeh, N. H. Foxwell, and A. A. Kurland, *J. Clin. Pharmacol.*, **8**, 392(1968).

(18) E. Kingstone, A. Villeneuve, and I. Kossatz, *Current Ther. Res.*, **8**, 159(1966).

(19) L. O. Randall, C. L. Scheckel, and R. F. Banziger, *ibid.*, **7**, 590(1965).

ACKNOWLEDGMENTS AND ADDRESSES

Received July 28, 1969, from the *Department of Biochemistry, Warner-Lambert Research Institute, Morris Plains, NJ 07950*

Accepted for publication September 29, 1969.

The authors thank Mr. Edward J. Merrill for the synthesis of ^{14}C-prazepam.

Gastric Absorption and Distribution of Acetylsalicylic Acid and Other Acidic Compounds in the Rat

CLARENCE H. MORRIS, JOHN E. CHRISTIAN, TOM S. MIYA, and WARREN G. HANSEN

Abstract □ A comparison was made of the gastric absorption and distribution of sodium acetylsalicylate-7-^{14}C with the absorption and distribution of sodium salts of other weakly acidic compounds. Each of the other compounds observed had an absorption pattern which was characteristic for the compound. Only sodium acetylsalicylate caused gastric lesions in the rat. The observations do not rule out the possibility that absorption characteristics of acetylsalicylic acid and its salts may be associated with their ability to cause gastric ulcers.

Keyphrases □ Acetylsalicylate-7-^{14}C, Na—absorption, distribution □ Acidic compounds—acetylsalicylate-7-^{14}C, Na—absorption, distribution comparison □ Gastric lesion production—acetylsalicylate-7-^{14}C, Na, acidic compounds □ TLC—analysis □ Autoradiography—analysis

It has been known for more than 50 years that oral administration of acetylsalicylic acid is followed by erosion of the gastric mucosa and bleeding from the site of the lesion (1). These aspirin-induced lesions have been observed in humans (1), dogs (2), cats (3), guinea pigs (4), and albino rats (5). A number of hypotheses have been suggested to explain the occurrence of these lesions. Davenport (6) and Martin (7) have suggested chemical models based upon the interaction of the compound with cellular constituents following its absorption

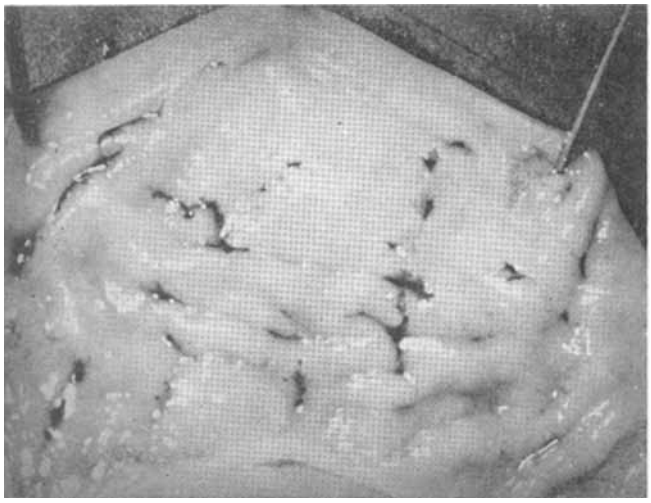

Figure 1—*Corpus mucosal surface showing lesions and areas of hemorrhage.*

into the cell. It became of interest to compare the absorption of aspirin, which causes ulceration and bleeding in the corpus portion of the rat stomach, with the absorption of other weakly acidic compounds which do not cause ulceration and bleeding.

MATERIALS AND METHODS

The radiochemical purity of sodium acetylsalicylate-7-^{14}C, sodium benzoate-7-^{14}C, sodium acetate-1-^{14}C, and sodium barbital-2-^{14}C (Tracer Labs, Waltham, Mass.) was established by TLC employing a stationary phase of silica gel G and a solvent system of low-boiling petroleum ether and 99% propionic acid. The location of the radioactive spots was determined by gross autoradiography.

All animals were fasted for 36 hr. prior to administration of the compound. Water was allowed *ad libitum* during the fast but was withdrawn after administration of the compound.

The compounds were administered orally to male Holtzman rats weighing between 150 and 275 g. (Holtzman Rat Co., Madison, Wis.) by means of a No. 16 curved steel oral catheter which had been dipped in mineral oil for lubrication. Each animal received a dose of 0.28 mmole of the selected compound per kg. of body weight. Each dose contained 30.5 μc. per kg. body weight of the ^{14}C-labeled compound diluted with the appropriate amount of carrier. The sodium acetylsalicylate was administered in 0.15 *M* citrate buffer, final pH 4.6. All other compounds were dissolved or suspended in a solution of 5% polyvinylpyrrolidone (PVP). Control animals received 0.5 ml. of the respective vehicle.

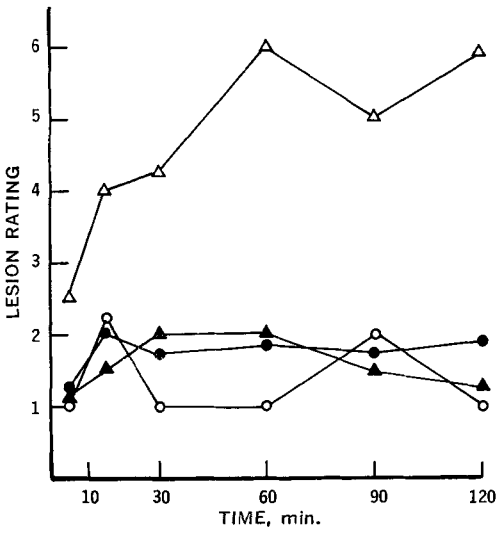

Figure 2—*Average gastric lesion rating of six animals per time interval following oral administration of: △, sodium acetylsalicylate; ○, sodium barbital; ●, sodium acetate; and ▲, sodium benzoate. All control animals had a rating of 1.*

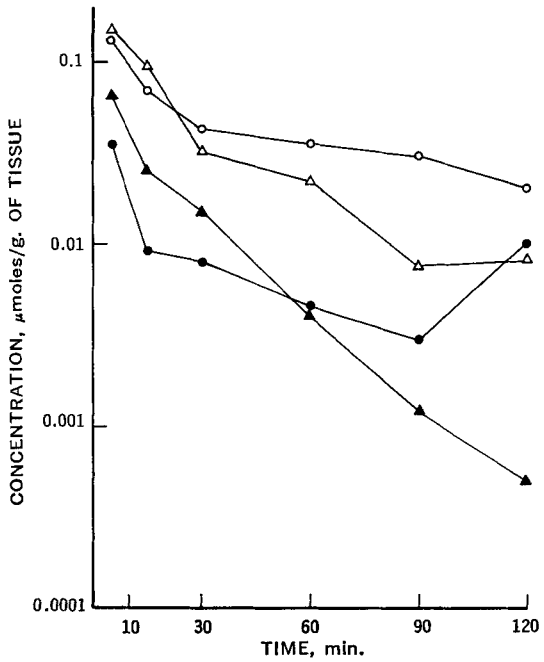

Figure 3—*Disappearance of labeled compounds from the corpus portion of the rat stomach. Key: △, sodium acetylsalicylate; ○, sodium barbital; ●, sodium acetate; and ▲, sodium benzoate. Each point represents the mean of three animals. To indicate the range of values around the averages shown in the plot, the minimum variation around any single point was 2.62 × 10^{-1}, 1.98 × 10^{-1}, and 2.24 × 10^{-1} (average 2.28 × 10^{-1}) μmoles per gram of tissue. The maximum variation around any single point was 1.94 × 10^{-3}, 1.06 × 10^{-2}, and 5.13 × 10^{-2} (average 4.95 × 10^{-2}) μmoles per gram of tissue.*

The rats were killed in groups of three by etherization at intervals of 5, 15, 30, 60, 90, and 120 min. following administration of each compound. The stomachs were removed, opened along the line of lesser curvature, stretched, and pinned on a large rubber stopper. The mucosal surface of each stomach was decontaminated with 0.9% NaCl, examined for the appearance of lesions, and rated on an 8-point scale developed by Morris *et al.* (5).

After being rated with respect to the severity of lesions, the stomachs were frozen in the stretched position with dry ice. Whole frozen stomachs were covered with plastic wrap (Saran), placed between two sheets of X-ray film (No Screen Medical, Eastman Kodak Co., Rochester, N. Y.), and stored in light, tight cassets at −20° for 60 days for gross autoradiography.

Following exposure of the film, samples of frozen rumen (nonglandular portion of the rat stomach) and corpus (glandular portion of the rat stomach) were prepared for liquid scintillation counting (Tri-Carb liquid scintillation counter model 3003, Packard Instrument Co., La Grange, Ill.) as described by Morris *et al.* (5). The amount of compound present per gram of tissue was calculated for each sample according to the following expression:

$$\mu\text{moles of compound/g. of tissue} = \frac{\text{observed d.p.m.}}{(W)\,(SA)\,(L)}$$

where: W = wet weight of the tissue in grams; SA = specific activity, d.p.m./mcg. of compound; and L = mcg. of compound/μmole.

RESULTS

Appearance of Lesions—Typical lesions as they appeared in the corpus portion of the rat stomach are shown in Fig. 1. It is of interest to note that the lesions appear in rows or furrows roughly paral-

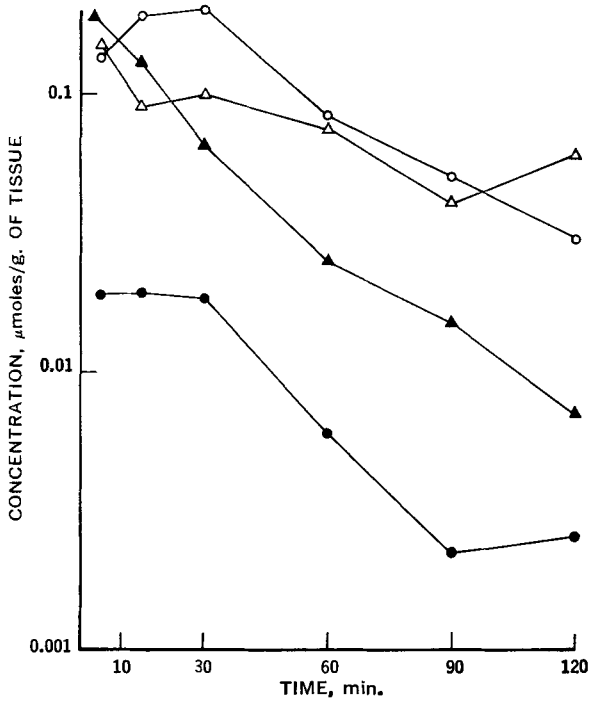

Figure 4—*Disappearance of labeled compounds from the rumen portion of the rat stomach. Key: △, sodium acetysalicylate; ○, sodium barbital; ●, sodium acetate; and ▲, sodium benzoate. Each point represents the mean of three animals. To indicate the range of values around the averages shown in the plot, the minimum variation around any single point was 4.27 × 10^{-2}, 3.94 × 10^{-2}, and 3.94 × 10^{-2} (average 4.95 × 10^{-2}) μmoles per gram of tissue. The maximum variation around any single point was 1.12 × 10^{-2}, 2.04 × 10^{-2}, and 4.6 × 10^{-3} (average 1.2 × 10^{-2}) μmoles per gram of tissue.*

lel to the longitudinal axis of the stomach. Under the conditions employed in these experiments, such lesions occurred only in the corpus portion of the rat gastric mucosa.

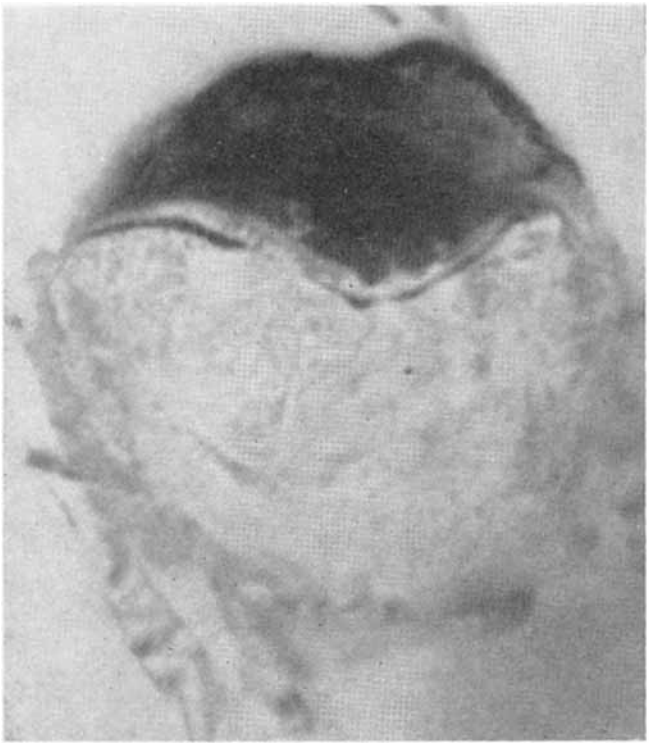

Figure 5—*Gross autoradiograph of musocal surface of rat stomach 120 min. following oral administration of ^{14}C-sodium acetylsalicylate.*

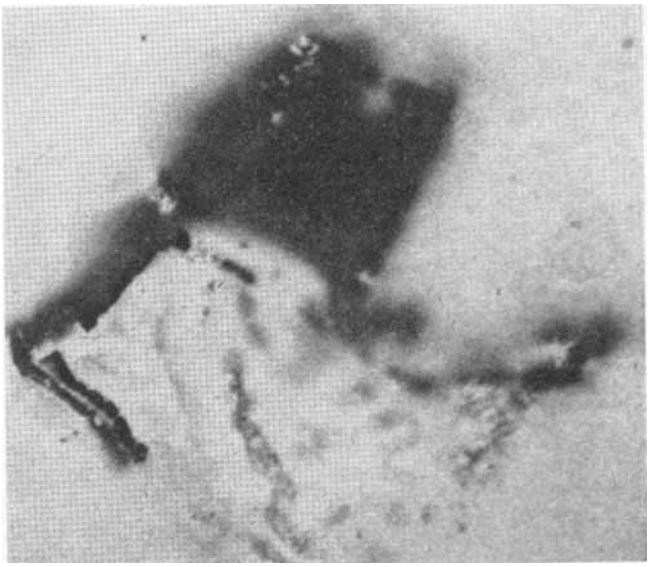

Figure 6—*Gross autoradiograph of mucosal surface of rat stomach 120 min. following oral administration of ^{14}C-sodium benzoate.*

It may be seen in Fig. 2 that, of the compounds studied, only sodium acetylsalicylate caused gastric lesions in the rat. A mild erythema, rated as 2 on the scale, occurred in some cases after the administration of the other compounds. Formation of lesions was essentially maximal 60 min. after administration of sodium acetylsalicylate.

Concentration Study—The concentration in micromoles per gram of tissue of each ^{14}C-labeled compound in gastric tissue was determined at each time interval. It is seen in Fig. 3 that the concentration of each compound in the corpus tissue diminishes rapidly with time.

The levels of these compounds in the rumen are remarkably different from the levels in the corpus (see Fig. 4). The concentration of sodium barbital in rumen tissue increases with time during the first 30 min. after its administration. Its concentration then drops

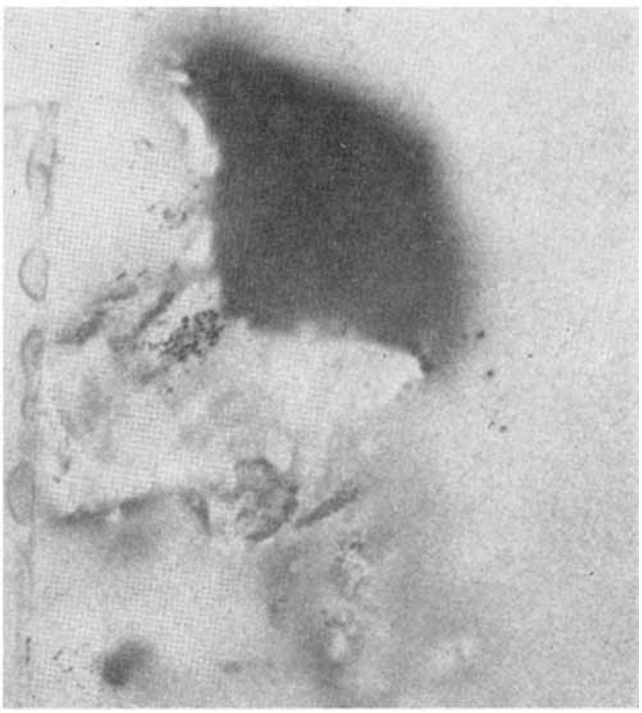

Figure 7—*Gross autoradiograph of mucosal surface of rat stomach 120 min. following oral administration of ^{14}C-sodium barbital.*

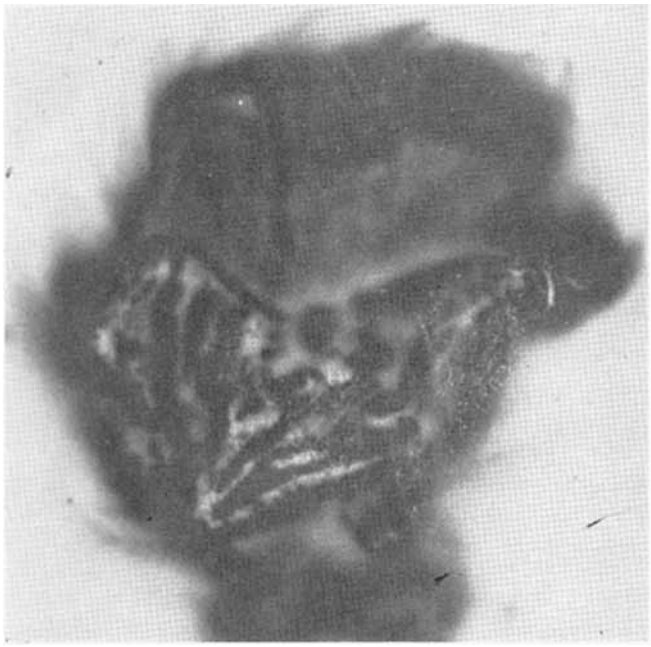

Figure 8—*Gross autoradiograph of mucosal surface of rat stomach 5 min. following oral administration of* ^{14}*C-sodium acetate.*

for the remaining time intervals. The concentration of sodium acetylsalicylate diminishes very slowly throughout the entire period of observation. The concentration of sodium acetate in rumen tissue remains essentially constant for the first 30 min. after its administration and then drops rapidly in a manner similar to that of sodium barbital. Sodium benzoate levels decrease rapidly with time.

Distribution Study—Figures 5 through 10 show selected autoradiographs of the mucosal surfaces. All stomachs are positioned such that the rumen is at the top of the figure. The compounds are localized in the rumen of all stomachs up to 120 min. following administration with the exception of sodium acetate. Sodium acetate disappears from both the rumen and the corpus within 60 min. following administration (Figs. 8–10). Sodium acetylsalicylate, sodium benzoate, and sodium barbital demonstrate gastric distribution patterns similar to the pattern o. acetylsalicylic acid reported by Morris *et al.* (5).

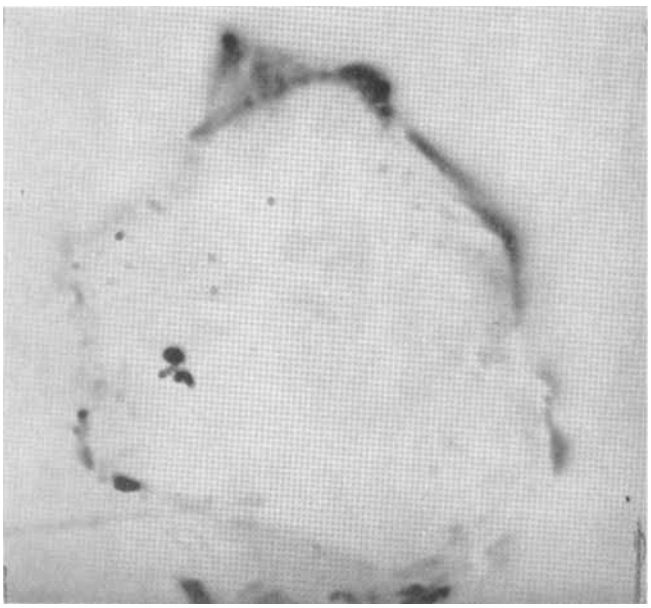

Figure 9—*Gross autoradiograph of mucosal surface of rat stomach 60 min. following oral administration of* ^{14}*C-sodium acetate.*

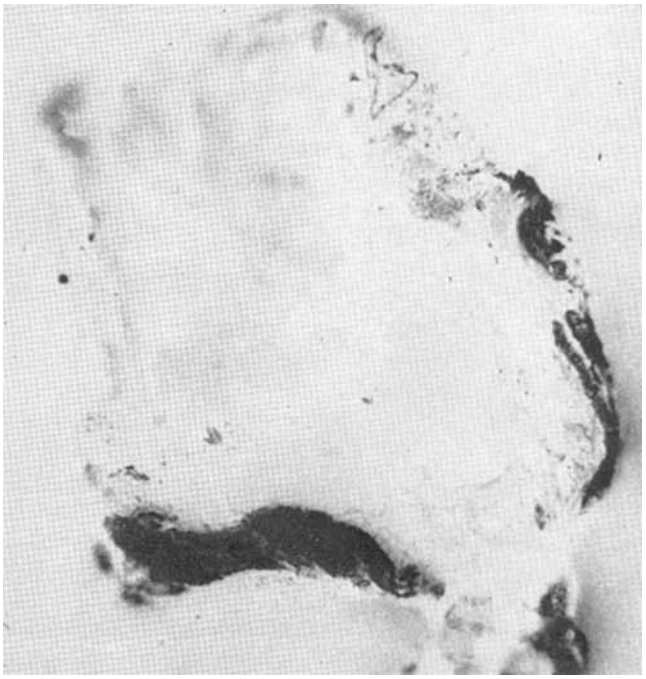

Figure 10—*Gross autoradiograph of mucosal surface of rat stomach 120 min. following oral administration of* ^{14}*C-sodium acetate.*

SUMMARY AND CONCLUSIONS

The data presented in Fig. 2 indicate a high incidence of lesions following the administration of sodium acetylsalicylate. These findings are in agreement with those reported by Morris *et al.* (5) who used acetylsalicylic acid. Sodium acetylsalicylate, sodium benzoate, and sodium barbital showed similar localization patterns. It is obvious from both the quantitative data and the gross autoradiographs that these three compounds disappear with time from the corpus but remain in relatively high concentrations at all observed time intervals in the rumen portion. By contrast, the results of the autoradiographic localization and the tissue analysis of sodium acetate indicate a relatively rapid disappearance from both the rumen and corpus.

It should be emphasized that the concentration values reported represent a specific concentration at the designated time following administration, and that this value is a function of the penetration of the compound from the lumen into the tissue, possible metabolism, binding, absorption into the bloodstream, reabsorption from the blood into the stomach tissue, and possible loss of activity from the tissue into the lumen following tissue damage. Although this study does not provide an explanation of gastric ulceration by aspirin, the observations do not rule out the possibility that absorption characteristics of acetylsalicylic acid and its salts may be associated with their ability to cause gastric ulcers. Since sodium acetylsalicylate administration results in lesion formation and administration of the other compounds studied did not result in lesion formation, it becomes difficult to escape the conclusion that absorbed sodium acetylsalicylate interacts with the corpus tissue or its secretions in some manner which is different from the interaction of the other weak acids. In addition, since lesions appeared only in the corpus, through which sodium acetylsalicylate penetrates and is absorbed, and not in the rumen, where the compound remains localized, it appears that the processes involved in penetration and absorption play a role in the mechanism of lesion formation. Investigations into the nature of the interactions between sodium acetylsalicylate and corpus and rumen tissue are presently in progress.

REFERENCES

(1) M. J. H. Smith and P. K. Smith, "The Salicylates. A Critical Bibliographic Review," Interscience, New York, N. Y., 1966, p. 236.

(2) J. W. Hurley and L. A. Crandall, *Gastroenterology*, **46**, 36(1964).
(3) L. A. Roth, "Salicylates, An International Symposium," A. St. J. Dixon *et al.*, Eds., Little, Brown, Boston, Mass., 1963, p. 224.
(4) *Ibid.*, p. 217.
(5) E. Morris, A. A. Noujaim, T. S. Miya, and J. E. Christian, *J. Pharm. Sci.*, **56**, 896(1967).
(6) H. W. Davenport, *Gastroenterology*, **46**, 245(1964).
(7) B. K. Martin, *Nature*, **198**, 896(1963).

ACKNOWLEDGMENTS AND ADDRESSES

Received July 25, 1969, from the *Bionucleonics and Pharmacology and Toxicology Departments, School of Pharmacy and Pharmacal Sciences, Purdue University, Lafayette, IN 47907*

Accepted for publication October 16, 1969.

Presented in part to the Pharmacology and Biochemistry Section, APhA Academy of Pharmaceutical Sciences, Miami Beach meeting, May 1968.

Research conducted under the auspices of the Institute for Environmental Health.

Quantification of the Binding Tendencies of Cholestyramine II: Mechanism of Interaction with Bile Salt and Fatty Acid Salt Anions

WILLIAM H. JOHNS and THEODORE R. BATES

Abstract □ The binding of a series of conjugated bile salt and fatty acid salt anions to cholestyramine from aqueous media was investigated and the data were plotted according to the Langmuir adsorption equation. Increases in affinity constants were noted as the number of hydroxy substituents on the bile salt-ring structure decreased. An increase in the chain length of the fatty acid salt caused a corresponding increase in the affinity constant, whereas an increase in the extent of unsaturation in the fatty acid chain produced a reduction in the affinity constants for the fatty acid–cholestyramine interaction. Apparent surface tension-lowering properties of the adsorbate molecules were found to parallel the affinities obtained for both classes of adsorbate molecules, with the exception of the fatty acid anion, linoleate. Based on the results of these studies, it is suggested that the binding mechanism involves a primary electrostatic component reinforced by a secondary nonelectrostatic interaction, the strength of the latter force being dependent on the degree of hydrophobicity of the adsorbate molecule.

Keyphrases □ Cholestyramine binding—quantification □ Bile salts, fatty acid salt anions—cholestyramine interaction mechanism □ Surface tension values–affinity constants, correlation—bile salts □ Hydrophobic character relationship, adsorbate—cholestyramine binding □ UV spectrophotometry—analysis

In previous work with the anionic exchange resin cholestyramine (1), the authors studied the effect of the physiologic electrolytes, sodium chloride and bicarbonate, on the binding process of bile salt anions to cholestyramine. The dihydroxy bile salt anions studied were noted to be insignificantly affected in their extent of interaction with the resin, while the trihydroxy bile salt anion–cholestyramine interactions were markedly reduced in the presence of an added electrolyte. These results suggested that a secondary, nonelectrostatic, type of interaction was taking place at the adsorption site.

The observation that structurally different bile salt anions exhibit varying types, as well as extents, of binding to cholestyramine is of biologic significance in that an appreciation of the possible modes of interaction could ultimately contribute to an enhancement in the efficiency of this pharmacologically important resin.

The purpose of this study was to elucidate the nature of this secondary binding mechanism and the effect of adsorbate structure thereon. In order to accomplish this, the binding tendencies of a selected series of glycine-conjugated bile salts and various physiologic fatty acid salts to cholestyramine were investigated.

EXPERIMENTAL

Materials—The sodium salts of glycocholic acid,[1] glycodeoxycholic acid,[1] glycodehydrocholic acid,[1] glycolithocholic acid,[1] lauric acid,[2] and oleic acid[3] were dried *in vacuo* for at least 48 hr. prior to use. The sodium salt of linoleic acid[3] was prepared by reacting equimolar quantities of the acid with sodium ethylate in absolute alcohol. The resulting salt was washed several times with absolute alcohol, dried at room temperature, and subjected to vacuum desiccation. The cholestyramine[4] employed in this study was of pharmaceutical grade (1). Reagent grade concentrated sulfuric acid, glacial acetic acid, hydrochloric acid, chloroform, sodium hydroxide, copper nitrate, *n*-butanol, and diethyldithiocarbamate were used as received.

Procedure for Adsorption Studies—A series of aqueous solutions of each bile salt and fatty acid salt was prepared over the concentration range of 0.75–5.0 millimolar (m*M*).[5] Twenty-five-milligram samples of cholestyramine were accurately weighed and placed into 50-ml. glass-stoppered conical flasks, together with a 25.0-ml. portion of the adsorbate solution. At each concentration, a control flask was prepared containing a similar quantity of the solution under study but no cholestyramine. These latter control solutions, which were assayed concomitantly with the solutions exposed to cholestyramine, were used to prepare the required Beer's law plots.

[1] Grade A. Obtained from Calbiochem Co., Los Angeles, Calif.
[2] Obtained from Eastman Organic Chemicals, Rochester, N. Y.
[3] Obtained from Fisher Scientific Co., Fair Lawn, N. J.
[4] Supplied by Merck and Co., Inc., Rahway, N. J.
[5] All systems exhibited complete solution over the concentration range studied, with the exception of the higher concentrations of glycolithocholate, which showed slight turbidity. Adsorption, being a dynamic process, would tend to increase the apparent solubility of a relatively insoluble material by removing molecules from solution and thereby promoting solution of any undissolved material.