Relationship Between Lesion Production, Absorption, and Distribution of ^{14}C-Labeled Acetylsalicylic Acid

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Carbon-14-labeled acetylsalicylic acid in conjunction with liquid scintillation counting and autoradiography was used to study the absorption and localization of the drug in the rat stomach. Lesions were produced in rats following oral administration of from 50 to 600 mg./Kg. The concentration of acetylsalicylic acid in the rumen and corpus portions of the stomach and in other tissues was determined for various time intervals after administration. The localization of the drug was studied in both the mucosal and muscularis surfaces of the stomach.

ALTHOUGH MANY divergent reports have been made on the blood loss and the gastric lesions caused by acetylsalicylic acid, it is clear that gastric bleeding does occur in humans and in experimental animals administered acetylsalicylic acid. As early as 1938, Douthwaite and Lintott (1) found severe mucosal reactions in 13 of 16 subjects following oral administration of acetylsalicylic acid.

These observations were extended by Hurst in 1943 (2). However, Wolf and Wolff in 1943 (3) were unable to produce gastric lesions by direct application of acetylsalicylic acid to the mucosa of a gastrostomy subject. Paul (4) observed no lesions in 107 subjects after acetylsalicylic acid administration. Caravati and Cosgrove in 1946 (5) repeated this work with 12 subjects with similar results. Since 1955, however, many investigations (6–10) have been reported agreeing, in general, with the earlier findings of Douthwaite and Lintott (1) and Hurst (2).

Davenport (7), in extensive studies of the production of mucosal damage by acetylsalicylic acid in vagally denervated dogs, suggested that the damage may be the result of the exchange of H^+ and Na^+ with a consequent efflux of Na^+, K^+, and Cl^- ions, eventually leading to damage of the capillary walls and subsequent hemorrhage.

Based on studies of gastric damage in guinea pigs, Anderson (8) concluded that the acetylsalicylic acid-induced gastric damage depended upon gastric absorption probably originating at the cellular level when the rate of entry into the mucosal cells exceeded some critical value. Initially the surface epithelial cells are shed, and this is followed by necrosis of the underlying glandular but not muscular tissue. If this is the case, the mechanism would therefore appear to involve an effect on the mucosal cells and is not directly related to gastric hyperacidity or peptic activity.

Martin (9) has suggested that the gastric mucosal injury is brought about by an appreciable accumulation of the drug anion in gastric mucosal cells because of the high pH gradient (stomach *versus* inside of cell; pH 1.4–6.8).

Croft (10) concluded that the gastric bleeding following repeated ingestion of acetylsalicylic acid appeared to be a result of gastric erosions brought about by the loss of surface cells. Davison (11) demonstrated that undissolved particles of aspirin are necessary for bleeding to occur in the dog.

From these and other reports, it is evident that the mechanism by which acetylsalicylic acid causes gastric lesions and subsequent blood loss is not well understood. The present study was carried out to uncover additional information about the concentration, localization, and penetration of the drug.

The terminology applied to the gross anatomy of the stomach in this paper was adapted from Shay (12).

EXPERIMENTAL

Materials and Methods—Carbon-14-carboxyl-labeled acetylsalicylic acid (Tracerlab, Waltham, Mass.), was used for all radiometric phases of this study. Radiocompound purity was confirmed by means of thin-layer chromatographic assay using a solvent system composed of benzene–ether–acetic acid–methanol (120:60:18:1). Polyvinylpyrrolidone (Antara Chemicals, Chicago, Ill.) was used as the suspending agent for oral administration. Kodak No-Screen medical X-ray film was used for the gross autoradiography. Liquid scintillation counting was accomplished using a model 3003 TriCarb liquid scintillation spectrometer (Packard Instrument Co., LaGrange, Ill.), with external standard attachment for quench correction. Tissue samples were prepared for counting by dissolving them in 2 ml. of hyamine–hydroxide solution (1 *M* methanolic hyamine HCl, 3 parts; 30% potassium hydroxide, 1 part). Excessive color was removed with 0.5 ml. of 30% hydrogen peroxide. Each sample was then acidified with 1.0 ml. of glacial acetic acid after which 15 ml. of XDC scintillation fluid consisting of xylene–cellusolve–dioxane (1:3:3), PPO (2,5-diphenyloxazole) 10 Gm./L., dimethyl POPOP (dimethyl 1,1-4-bis-2-(5-phenyloxazolyl) benzene, 0.5 Gm./L., and naphthalene, 80 Gm./L.

Production of Lesions—Preliminary experiments were carried out to confirm the production of lesions by oral administration of acetylsalicylic acid. U.S.P. grade acetylsalicylic acid was suspended in 5.0% polyvinylpyrrolidone and administered to 24 male rats of the Holtzman strain, weighing 200 to 260 Gm., in doses of 10, 50, 100, and 600 mg./Kg. The animals were fasted for 36 hr. prior to administration of the drug and were allowed no food or water during the experiment. Lesions were produced in the corpus in every case with doses of from 50 to 600 mg./Kg. No lesions were observed in the control animals which were

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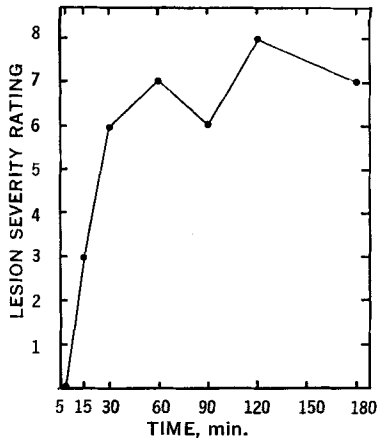

Fig. 1—Average gastric lesion ratings produced by oral 50 mg./Kg. acetylsalicylic acid. The condition of the stomachs was rated according to the following scale: No. 1, normal, no lesions, erythema or blood; No. 2, erythema only, no lesion or blood evident; No. 3, lesions present, less than 5, all of which are smaller than 1 mm. in diameter; No. 4, lesions present, less than 5 with one or more larger than 1 mm. in diameter; No. 5, lesions present, more than 5 but less than 10, all of which are less than 1 mm. in diameter; No. 6, lesions present, more than 5 but less than 10 with one or more larger than 1 mm. in diameter; No. 7, lesions present, more than 10, all of which are smaller than 1 mm. in diameter; No. 8, lesions present, more than 10 with one or more larger than 1 mm. in diameter.

given similar doses of polyvinylpyrrolidone alone in like volumes. The localization of lesions produced by aspirin to the corpus region has been previously noted by Gray (13).

Using time intervals between dose and sacrifice of 5, 15, 30, 60, 90, and 120 min., the time required to produce lesions and the severity of the lesions produced were studied. Three animals were used in each time group and a 50 mg./Kg. dose contained in 0.7 ml. of suspension was used in this and all subsequent experiments. The results are summarized in Fig. 1.

Surgical Techniques—The stomachs were removed, freed from all adhering tissue, and opened along the lesser curvature. Each stomach was pinned to a No. 14 rubber stopper such that the rugal folds were flattened out. All stomachs were washed with exactly 100 ml. of 0.9% saline solution by gently spraying from a plastic wash bottle. A previous experiment indicated that a 100-ml. wash was sufficient to remove surface contamination from the mucosa.

Determination of Drug Concentration in Selected Tissues—Twenty-one rats were administered a 50-mg./Kg. dose of ^{14}C-carboxyl-labeled acetylsalicylic acid having a specific activity of 0.625 μc./mg. Three control animals were given a similar dose of nonlabeled drug. The animals were sacrificed in groups of 3 at time intervals of 5, 15, 30, 60, 90, and 120 min. after dosing. After removal, the stomachs were washed with saline and random samples (50 to 100 mg.) were taken from the rumen and corpus portions, placed in a previously weighed Wheaton vial, and the wet weight of the tissue accurately determined. The samples were taken by cutting

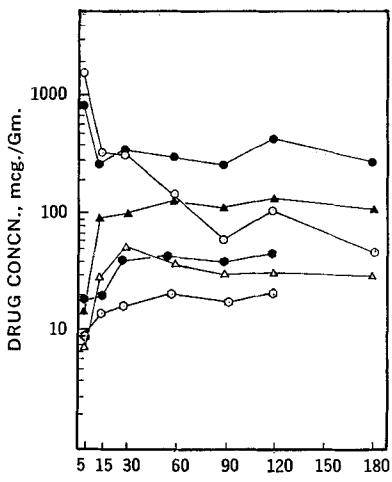

Fig. 2—Concentration of acetylsalicylic acid in the rumen and corpus portions of the stomach, skeletal muscle, kidney, blood, and adrenal gland of the rat. Key: ●, rumen portion; ○, corpus portion; △, skeletal muscle; ▲, kidney; ⬢, blood; ⬡, adrenal gland.

through the entire stomach wall with a hand cork borer. Samples were also taken from the liver, lung, kidney, adrenal gland, skeletal muscle, and blood (1 ml.). All samples were counted as previously described, and from the specific activity of the original dose, the amount of drug present per Gm. of tissue was determined for each sample (see Fig. 2).

Gross Autoradiography—Eighteen rats were dosed and sacrificed as previously described; 3 rats served as controls. After the stomachs had been removed, pinned, and washed, they were quick frozen with dry ice and removed from the rubber stoppers. In the dark room, the stomachs were covered on both sides with Saran wrap and arranged in groups according to the time intervals of 5, 30, and 120 min., respectively. Each group was then placed between 2 sheets of No-Screen medical X-ray film such that one film was in apposition to the mucosal surface and the other in apposition to the muscularis surface. The films with the stomachs in position were then placed in light tight cassets and stored at −20° for 60 days. The films were developed and photographic prints were made so that the dark areas corresponded to the areas of radioactivity where the drug was concentrated. Respresentative examples of these prints are shown in Figs. 3 through 8.

Intravenous Administration of Acetylsalicylic Acid—Eighteen animals were given *via* the caudal vein 50 mg./Kg. labeled drug dissolved in propylene glycol. Three control rats were used. Gross autoradiographs were prepared in the same manner as previously described (see Figs. 9 and 10). After exposure of the gross autoradiographs, samples were taken from the stomachs and counted to determine the drug concentrations. Drug concentration was found to be greatest 15 min. after intravenous administration in both the rumen and corpus portions.

DISCUSSION AND COMMENTS ON RESULTS

From the results of the drug concentration studies in selected tissues, it is apparent that very rapid

absorption occurs through the mucosa of the corpus portion of the stomach (Figs. 3–5). Five minutes after oral administration, the drug concentration in the corpus and rumen mucosa is greater by a factor of 10 than those concentrations found in any other tissue at any time. Furthermore, it was shown that the drug passes rapidly through the corpus mucosa into the muscularis where it is absorbed. Consequently, within 30 min. after administration, the concentration of drug

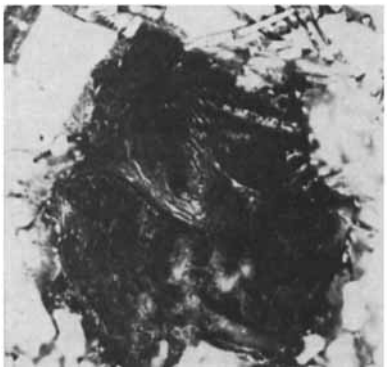

Fig. 3—Mucosal surface autoradiograph of a rat stomach 5 min. after oral administration. Note the high concentration of drug (acetylsalicylic acid and/or metabolites) in rumen and corpus portions.

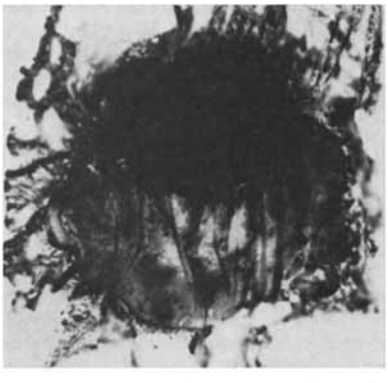

Fig. 4—Mucosal surface autoradiograph of a rat stomach 30 min. after oral administration. Note the decreasing concentration in the corpus portion with the rumen concentration remaining the same.

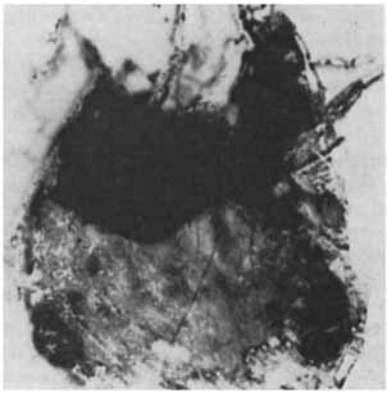

Fig. 5—Mucosal surface autoradiograph of a rat stomach 120 min. after oral administration. Note the lack of drug in the corpus portion.

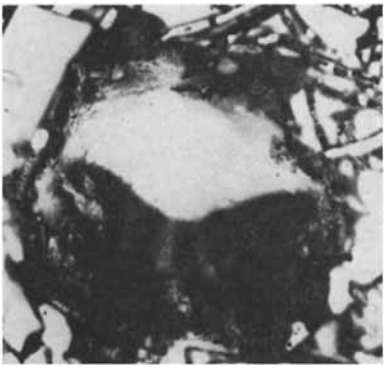

Fig. 6—Muscularis surface autoradiograph of a rat stomach 5 min. after administration. Note the high concentration of drug in the corpus portion and little or no concentration in the rumen portion.

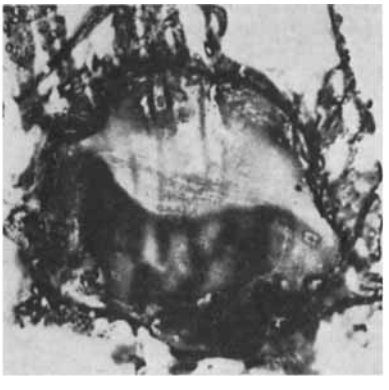

Fig. 7—Muscularis surface autoradiograph of a rat stomach 30 min. after administration. Note the drug in the corpus portion. The rumen concentration remains low.

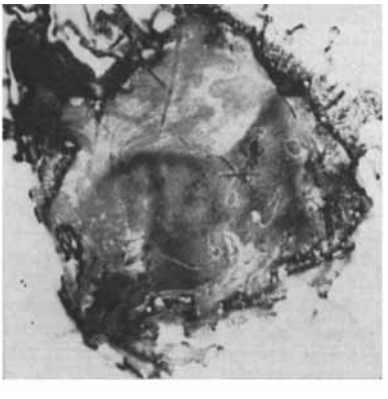

Fig. 8—Muscularis surface autoradiograph of a rat stomach 120 min. after administration. Note the greatly decreased drug in the corpus portion as compared to the 30-min. preparation. There is still little or no concentration of drug in the rumen portion.

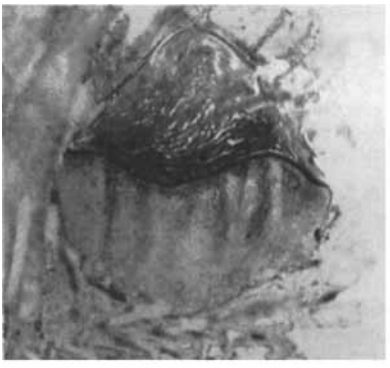

Fig. 9—Mucosal surface autoradiograph of a rat stomach 120 min. after intravenous administration.

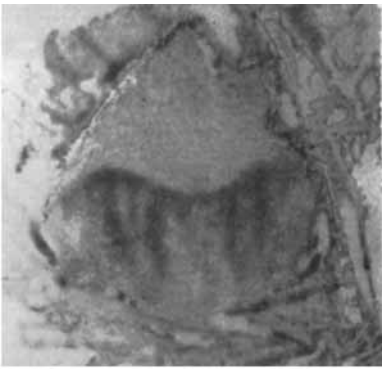

Fig. 10—Muscularis surface autoradiograph of a rat stomach 120 min. after intravenous administration.

remaining in the corpus mucosa is less than that found in the rumen mucosa. After 60 min. the drug levels in the corpus portion are essentially the same as those found in most other tissues. On the other hand, although the original absorption in the rumen portion is not nearly so great as in the corpus, there appears to be very little loss of drug from the rumen, so that 30 min. after administration until at least 3 hr., the concentration levels in the rumen are much greater than those found in any other tissue.

The gross autoradiographs clearly show the greater concentration of drug (acetylsalicylic acid and/or metabolites) in the corpus mucosa at 5 min. after administration and greater in the rumen mucosa after 120 min. The muscularis surface autoradiograms show increasing concentration in the corpus muscularis, followed by gradual decrease indicating absorption through this portion. No significant concentration is seen in the rumen portion of the muscularis indicating a lack of absorption through this portion.

SUMMARY AND CONCLUSIONS

The results may be summarized as follows. (*a*) Acetylsalicylic acid in doses of from 50 to 600 mg./Kg. produced gastric lesions in the corpus portion of the rat stomach. (*b*) Acetylsalicylic acid is absorbed through the mucosa of the corpus portion at a rapid rate with maximum concentration being reached within 5 min. after drug administration. (*c*) The drug concentration in the mucosa of the rumen portion remains relatively constant over a 3-hr. period following administration while the concentration in the corpus is initially high with steady decrease beginning 5 min. after oral administration. The drug concentration decreases in the corpus portion to a level comparable to that found in most other tissues after about 1 hr., while in the rumen it remains at a significantly higher level.

Although more work must be done before the entire mechanism of lesion production can be understood, the data presented here tend to refute the hypothesis of focal necrosis at least as the sole mechanism of gastric erosion. The data tend to support the concepts submitted by Davenport (7) as to the importance of tissue permeability and ion flux across the mucosa in the production of capillary wall damage eventually resulting in blood loss and erosion of the mucosa.

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