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Distribution of Aspirin in Rumen and Corpus Tissues of Rat Stomach during First Four Minutes after Administration

CLARENCE H. MORRIS▲, JOHN E. CHRISTIAN, ROBERT R. LANDOLT, and WARREN G. HANSEN

Abstract ☐ Autoradiography was used to determine the distribution of aspirin and/or its metabolites in the mucosa, submucosa, and muscularis of the rumen (nonglandular portion) and corpus (glandular portion) tissues of the rat stomach 4 min. after administration. A significant localization of aspirin and/or its metabolites was observed in the submucosa regions of both the rumen and corpus. In addition, the innermost portion of the rumen mucosa acted as a barrier toward absorption when compared to the corpus mucosa. This factor may be important in the mechanism of lesion production in the corpus tissue and the lack of lesion production in the rumen.

Keyphrases ☐ Aspirin (and metabolites), distribution—rumen and corpus tissues, rat stomach, 4 min. after administration ☐ Gastric lesions—aspirin (and metabolites) distribution 4 min. after administration, rumen and corpus tissues, rat stomach, histoautoradiography ☐ Autoradiography—aspirin (and metabolites) distribution 4 min. after administration, rumen and corpus tissues, rat stomach

Several mechanisms have been proposed to explain the production of gastric lesions by salicylates (1, 2). Davenport (3) and Martin (4) developed chemical models based on interaction of the compound with cellular constituents following absorption of the compound into the cell.

Previous studies (5) showed that some rats develop gastric lesions in the corpus portion of the stomach within 5 min. following oral administration of aspirin. Lesions have not been produced in the rumen under the same experimental conditions. Therefore, it was of interest to compare the relative quantities and distribution of aspirin and/or its metabolites in the mucosa, submucosa, and muscularis of the rumen and corpus tissues of the rat stomach shortly after oral administration of aspirin-7-^{14}C.

MATERIALS AND METHODS

Aspirin was administered to rats as previously described (6), with the exception that each animal received 0.01 mc. (0.93 mg.) of aspirin-7-^{14}C.

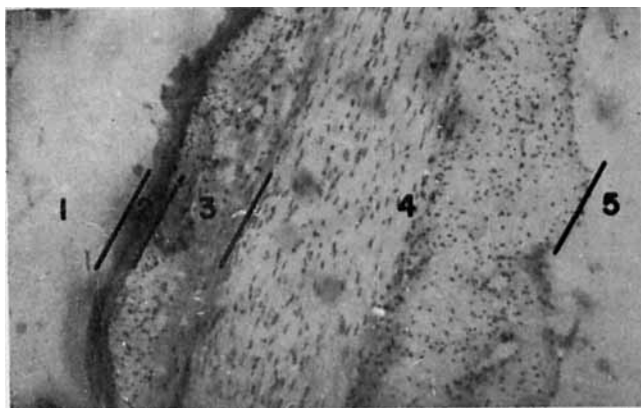
Preparation of Emulsion Slides—Standard size microscope slides were cleaned by soaking for 24 hr. in a 10% solution of dichromic acid. Following the acid bath, the slides were washed in flowing tap water for 1 hr. followed by two consecutive 30-min. rinses in distilled water. The slides were then dipped in a subbing solution (5 g. gelatin and 0.5 g. of chrom alum in enough water to make 1000 ml.) and dried vertically. Next, the slides were dipped into a liquid emulsion[1], previously warmed to 43°. This work was performed under a safelight[2]. The slides were withdrawn from the emulsion at a uniform rate and checked under the safelight to ensure that the emulsion was uniformly distributed and free from bubbles, and the moist emulsion was wiped from one side. Then the slides were placed in a test tube rack in a vertical position and allowed to dry. After drying, the slides were placed in light-tight boxes and stored at −20° until used.

Tissue Cutting and Mounting—The entire stomach was removed from the animal 2 min. after oral administration. Four minutes after oral administration, the entire stomach was frozen in liquid nitrogen. From this point until development of the autoradiograms, the tissues remained frozen to prevent relocation of the aspirin and/or its metabolites. Cross-sectional segments of rumen and corpus were cut from the stomach, with a cold scalpel, and mounted on a previously cooled brass block using a drop of mounting medium[3]. After equilibration at −15°, 10-μ sections of rumen and corpus were cut with a microtome mounted in a cryostat[4]. The sections were transferred under the safelight from the microtome blade to the emulsion slides (also maintained at −15°). The mounted tissue sections were stored in light-tight boxes at −20° for 9–18 days to allow exposure of the photographic emulsion.

Photographic Processing of Liquid Emulsion Slides—All solutions were maintained at 4° throughout the photographic processing to reduce the possibility of detachment of the tissue from the emulsion. The most satisfactory staining was obtained if the tissue was fixed in methanol. The slides were then developed[5] for 10 min., rinsed in distilled water for 1 min., fixed[6] for 15 min., and rinsed in distilled water for 30 sec.

Staining Techniques—The tissue sections were stained with lithium carmine followed by picric acid, as described by Witten and Holstrom (7). All solutions were maintained between 10 and 15°. After the slides were removed from the final xylene bath, coverslips were mounted with Canada balsam.

[1] NTB-2, Eastman Kodak Co., Rochester, N. Y.
[2] Wratten series 2.
[3] Lab Tak, Westmont, Ill.
[4] International Equipment Co., Needham Heights, Mass.
[5] In Kodak D-19.
[6] In Kodak acid fixer.



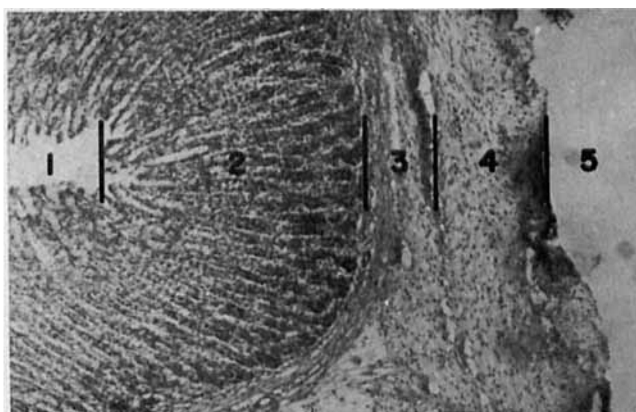


Figure 1—*Photomicrogram of cross section of rumen and corpus tissues, showing the (1) serosa, (2) muscularis, (3) submucosa, (4) mucosa, and (5) lumen.*

Grain Counting—The distribution of ^{14}C-compounds in the lumen, mucosa, submucosa, and muscularis regions of the rumen and corpus tissues (Fig. 1) was measured by counting grains of silver in the emulsion. A grid of 25 fields of 100 μ^2 each was optically superimposed over each region (Fig. 2) and the net number of grains per unit area was determined from each field in the emulsion below the following regions: (*a*) lumen, (*b*) mucosa along the mucosa-lumen interface, (*c*) mucosa along the mucosa–submucosa interface, (*d*) submucosa, and (*e*) muscularis. Grains were counted in two rumen and two corpus sections from each of two animals. In addition, the percentage of the net number of grains per unit area in each region relative to the net number of grains per unit area in the lumen was determined. Student's t test was used to determine the significance of the difference between the means of the net grains per unit area between the mucosa along the mucosa–submucosa interface and the submucosa. The F test was made prior to the t tests to test for homogenicity of variance. When the variances were not homogeneous, an adjusted t value was calculated from the formula developed by Cochran and Cox (8).

RESULTS

The distribution of aspirin and/or its metabolites in the mucosa, submucosa, and muscularis regions of the rumen and corpus tissues is presented in Table I. An increase in net grains per unit area is shown in the submucosa of each section from both rumen and corpus when compared to the mucosa. This increase is significant at the 99.9% confidence level as determined by the Student t test. In addition, by comparing rumen and corpus values of the average percent in each region relative to the lumen, it is evident that the rumen mucosa along the mucosa–lumen interface acts as

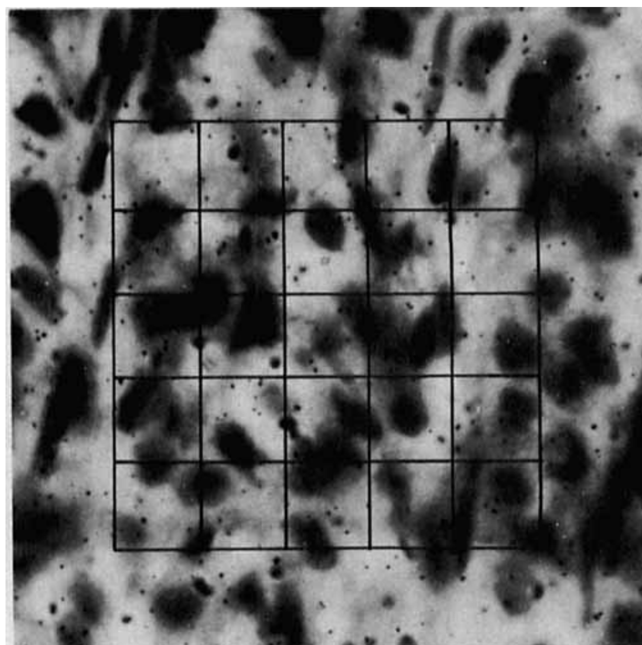


Figure 2—*Photomicrogram of a typical histoautoradiogram, showing silver grains in the emulsion below the corpus mucosa. Each small square of the grid has an area of 500 μ^2. Since the microscope was focused on the silver grains, the tissue appears to be slightly out of focus.*

a barrier against the absorption of aspirin and its metabolites. The quantities of labeled compounds found in the mucosal and submucosal regions of the corpus are significantly higher (at the 95% confidence level) than the quantities found in the corresponding regions of the rumen; however, quantities found in the muscularis regions of the rumen and corpus are not significantly different from each other.

DISCUSSION

Histoautoradiography showed that the rumen mucosa acted as a barrier toward absorption of aspirin and/or its metabolites, whereas the corpus tissue permitted much higher levels of penetration. In addition, there was a significant increase in aspirin (and/or metabolite) levels in the submucosa of the rumen and corpus tissue than in the other regions. In another study (6), significantly higher levels of salicylic acid were found in the corpus tissue than in the rumen tissue. Finally, it has been shown (5, 9) that lesions appear in the corpus portion but not in the rumen portion of the stomach following oral administration of aspirin. Taking these observations into consideration, one might speculate that the mechanism of lesion production is a function of absorption of aspirin and the subsequent

Table I—Average Net Grains per Field in Rumen and Corpus Tissue

Region	Corpus: Mean Grains per Unit Area	Corpus: Percent Relative to Lumen[a]	Rumen: Mean Grains per Unit Area	Rumen: Percent Relative to Lumen
Lumen	85.3	100	110.8	100
Mucosa at mucosa–lumen interface	22.0	25.7	2.5	2.2
Mucosa at mucosa–submucosa interface	6.8	7.9	2.0	1.8
Submucosa	23.3	27.3	11.2	10.1
Muscularis	3.7	4.3	2.5	2.2

[a] Average percent of the net number of grains in each region of the tissue as compared to the average net number present in the lumen.

hydrolysis of aspirin to salicylic acid within the corpus tissue. One might also speculate that the submucosa may be the site of the initial injury due to the apparent buildup of aspirin and metabolites at that level.

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Effect of Adsorbed Surfactant on Particle–Particle Interactions in Hydrophobic Suspensions

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Abstract ☐ The degree of flocculation of sulfamerazine suspensions as represented by relative sedimentation volume was determined as a function of both surfactant and electrolyte concentration. The results emphasize the importance of knowing the location as well as the concentration of the surfactant and are consistent with the Derjaguin, Landau, Verwey, and Overbeek theory.

Keyphrases ☐ Suspensions, hydrophobic (sulfamerazine)—degree of flocculation, relative sedimentation volume, effects of surfactant (sodium dodecyl polyoxyethylene sulfate) and electrolyte concentration ☐ Surfactants (sodium dodecyl polyoxyethylene sulfate)—effect on particle-particle interactions in hydrophobic (sulfamerazine) suspensions ☐ Sodium dodecyl polyoxyethylene sulfate—effect on flocculation of sulfamerazine suspensions ☐ Sulfamerazine suspensions—effect of adsorbed surfactant on particle-particle interactions ☐ Flocculation, sulfamerazine suspensions—effects of surfactant and electrolyte concentration

Controlled flocculation has been the subject of many papers over the past 15 years (1–5). Haines and Martin (1, 2) suggested that suspension flocculation can be controlled by modifying the charge surrounding suspended hydrophobic particles by the addition of electrolyte. For example, the addition of aluminum chloride to 2% sulfamerazine suspensions containing dioctyl sodium sulfosuccinate reduced the ζ-potential essentially to zero, producing pharmaceutically stable suspensions characterized by high final sedimentation volumes.

Wilson and Ecanow (3) took issue with the work of Haines and Martin for several reasons: (*a*) since flocculation was reported to have occurred spontaneously, the true ζ-potential could not have been measured; (*b*) it was unlikely that van der Waals' forces would be operative with particles greater than 2–5 μm.; and (*c*) the effect of electrolyte on the surfactant was not taken into account. During a subsequent investigation, they observed the formation of a precipitate when dioctyl sodium sulfosuccinate at concentrations used by Haines and Martin was mixed with aluminum chloride. Flocculation was interpreted as being due to a chemical reaction between the surfactant and the electrolyte instead of a reduction of the ζ-potential.

Matthews and Rhodes (4–6) studied suspensions of sulfamerazine and griseofulvin prepared with sodium lauryl sulfate, which is precipitated by aluminum chloride, and sodium dodecyl dioxylated sulfate, which apparently is not precipitated by aluminum chloride. The addition of aluminum chloride to both suspensions resulted in aggregation. Different sedimentation rates were observed; however, final sediment heights were identical. They interpreted the aggregation mechanism as "flocculation" for the suspension containing sodium lauryl sulfate and as "coagulation" for the suspension containing sodium dodecyl dioxylated sulfate. Ecanow *et al.* (7) claimed that both suspensions were "flocculated" on the basis of equal final sediment volumes and sediment compressibility. The difference in sedimentation rate was considered to be a function of aggregate structure, number, and geometry.

Two possible mechanisms have been proposed to explain flocculation (8–10): absorption bridging by the surfactant between suspension particles and cross-linking of suspension particles produced by metal-ion interactions with the adsorbed surfactant. Coagulation is an aggregation process resulting from a reduction in the ζ-potential to a point where attractive forces predominate. The aggregate is dense and nonporous and forms a caked sediment.