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

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Abstract □ TLC and liquid scintillation spectrometry were used to determine the identities and relative quantities of aspirin and its metabolites in the gastric juice, blood plasma, rumen (nonglandular portion), and corpus (glandular portion) of the rat stomach 4 min. after the oral administration of aspirin-7-^{14}C. It was found that aspirin and salicylic acid are the major components in the gastric juice, blood plasma, and rumen and corpus tissues. Trace levels of a compound with an R_f value corresponding to that of salicyluric acid was found in the fluids and tissues analyzed. No glucuronide conjugates of salicylic acid or polyhydroxy metabolites of aspirin were found. The level of salicylic acid was fourfold higher in the corpus tissue than in the rumen tissue.

Keyphrases □ Aspirin, radiolabeled—metabolism in rumen and corpus tissues, during first 4 min. after oral administration, rats, TLC and liquid scintillation spectrometric determination of metabolites □ Salicylic acid—determination as metabolite in rumen and corpus tissues of rat 4 min. after oral administration of radiolabeled aspirin □ Metabolism—radiolabeled aspirin, determination of metabolites in rat 4 min. after oral administration, rumen and corpus tissues

It is well known that gastric irritation leading to the production of lesions and occult bleeding occurs in laboratory animals and man following oral administration of salicylates (1, 2). Aspirin has been reported (3, 4) to produce gastric lesions in the corpus portion of the albino rat when administered orally at a level of 50 mg./kg. of body weight.

The production of gastric lesions following intravenous administration of salicylates was reported by Hurley and Crandall (5), Grossman *et al.* (6), and Morris (7), but the lesions were much less severe than those produced by oral administration.

Although systematic mechanisms have been proposed to explain the production of lesions, the present authors felt that the initial mechanism of lesion formation might be a localized chemical phenomenon since previous studies (3) had shown that some rats develop gastric lesions in the corpus (glandular) portion of the stomach within 5 min. after administration of aspirin. Lesions have not been produced in the rumen (nonglandular portion) under the same experimental conditions.

It, therefore, became of interest to identify and compare the relative quantities of aspirin and/or its metabolites in the rumen and corpus tissues of the rat shortly after administration of aspirin-7-^{14}C.

MATERIALS AND METHODS

Radiochemical purity of aspirin-7-^{14}C was established by TLC in conjunction with autoradiography and liquid scintillation spectrometry. The chemical identity and purity of standards of aspirin and its metabolites were established by melting point and TLC.

Four male albino laboratory rats[1], weighing between 100 and 200 g., were fasted for 12 hr. prior to the administration of aspirin-7-^{14}C but were allowed water *ad libitum* until the time of drug administration.

Drug Administration—To achieve the most rapid and uniform absorption, 0.1 mc. (9.3 mg.) of aspirin-7-^{14}C was administered orally as the sodium salt in 1.0 ml. of citrate buffer solution (pH 4.6).

Processing of Tissue—Two minutes after administration of the labeled aspirin, the rats were anesthesized with ether and the stomachs were removed. The stomachs were opened along the line of lesser curvature and the contents were collected in a small vial. Each stomach was stretched and pinned on a large rubber stopper, and the mucosal surface was rinsed with 100 ml. of 0.9% saline. Four minutes following administration, the stomachs were rapidly frozen with dry ice. The chest cavities of the rats were opened, and blood samples were obtained by cardiac puncture using syringes which had been previously swabbed with heparin[2]. The blood was placed in a centrifuge tube and centrifuged for 10 min. at 4600×g. While frozen, the rumen and corpus portions were separated. Each portion was minced and homogenized with 5 ml. of distilled water in a ground-glass tissue grinder, which was kept in an ice bath at 4° to inhibit enzymatic action and spontaneous hydrolysis of aspirin.

Identification of Metabolites—Following homogenization, the tissue samples were centrifuged at 4600×g for 15 min. Ten microliters of the aqueous supernate was spotted on thin-layer plates and analyzed for aspirin, salicylic acid, salicyluric acid, 2,3-dihydroxybenzoic acid, 2,5-dihydroxybenzoic acid, and 2,3,5-trihydroxybenzoic acid, utilizing the solvent systems described later. Ten-microliter samples of blood plasma and gastric juice were analyzed for these compounds in a similar manner. The remaining tissue was resuspended in the supernate, and the suspension was analyzed for the presence of salicyl acyl- and salicyl phenolic glucuronides using an extraction method described by Schachter (8) and Schachter and Manis (9), followed by TLC, autoradiography, and liquid scintillation spectrometry.

To determine the amount of spontaneous hydrolysis of the aspirin occurring during storage and analytical procedures, an aliquot of the dose solution was added to homogenized rumen and corpus tissue taken from four untreated animals. These samples were subsequently treated exactly as the experimental samples. An aliquot of the dose solution itself was also analyzed.

TLC—A stationary phase of standard thickness (250 μ) silica gel G mixed with fluorescent indicator on a thin aluminum backing was used for all thin-layer chromatographs[3]. The following solvent systems were used to identify aspirin and its metabolites in the gastric juice, blood plasma, and rumen and corpus tissues. Petroleum ether (boiling range 30–60°) and propionic acid (10:1 v/v) were used to separate aspirin and salicylic acid from each other and from the rest of the metabolites. A benzene, ether, glacial acetic acid, and methanol (120:60:18:1 v/v) system was used to separate salicyluric acid from the rest of the metabolites. A system of benzene, glacial acetic acid, and water (2:2:1 v/v) was used to separate the polyhydroxy metabolites from each other and from the rest of the metabolites. In all cases, unlabeled, authentic standards of the compounds were used to identify the labeled compounds found.

In the final stages of the work, two additional solvent systems were used to confirm the identities of aspirin and salicylic acid.

[1] Holtzman Rat Co., Madison, Wis.
[2] Abbot Laboratories, North Chicago, Ill.
[3] Brinkmann Instruments Co., Westbury, N. Y.

Table I—Mean Percent[a] and Ranges of Total Activity of Aspirin and Its Metabolites in Rat Tissues 4 min. after Administration of Aspirin-7-^{14}C

Tissue Analyzed	Aspirin		Salicylic Acid		Salicyluric Acid		Percentage of Total Radioactivity Identified, Mean
	Mean	Range	Mean	Range	Mean	Range	
Gastric juice	91.1	71.3–97.9	6.9	1.5–22.0	0.1	0.09–0.19	98.1
Blood plasma	65.1	60.3–73.9	18.8	0.0–31.6	11.5	3.4–19.6	95.4
Rumen tissue	91.8	87.8–94.0	6.6	0.0–10.5	0.6	0–1.19	99.0
Corpus tissue	74.0	64.7–88.2	25.4	11.3–35.0	0.5	0.0–1.59	99.9

[a] All percentages are averages taken from four animals.

These systems were hexane, glacial acetic acid, and chloroform (4:1:1 v/v) and carbon tetrachloride, ether, glacial acetic acid, and methanol (120:60:18:1 v/v).

After developing and drying the TLC plates, autoradiograms were made using no-screen medical X-ray film. Following exposure and development of the film, the R_f values of the standards (observed with UV radiation) were compared with the R_f values of the labeled compounds (observed by autoradiography). Subsequently, the areas corresponding to the R_f values of the labeled compounds, the origin, and the solvent front were removed and placed in counting vials, and the amount of radioactivity in each sample was determined by liquid scintillation spectrometry[4]. The internal method of standardization (10) was used to determine the disintegration rate of each sample. The scintillation solution consisted of 300 ml. xylene, 900 ml. dioxane, 900 ml. 2-ethoxylethanol[5], 21 g. diphenyloxazole, and 168 g. naphthalene.

RESULTS AND DISCUSSION

The results of the analyses for aspirin and its metabolites in the gastric juice, blood plasma, and rumen and corpus tissues of the rat stomachs are presented in Table I. The percentage activity reported represents the mean percentage activity calculated from individual percentage activities from four animals. Each individual percentage activity represents the percentage of the activity for the compound in question relative to the total amount of activity spotted on the thin-layer plate. No glucuronide conjugates of salicylic acid or polyhydroxy metabolites of aspirin were detected.

Trace amounts of a compound having the same R_f value as salicyluric acid were found in all samples examined for salicyluric acid, but it occurred as a significant percentage of total activity only in blood. The absolute amounts were so small (calculated to be approximately 10^{-11} g.) that further verification of identity was not warranted. In addition, very low levels of labeled but unidentified compounds were detected at the origin and solvent front in all systems studied.

The major components found in the gastric juice, blood plasma, and rumen and corpus tissues were aspirin and salicylic acid. The amounts of aspirin found (corrected for spontaneous hydrolysis), expressed as percentage of total radioactivity, were as follows: gastric juice, 91.1%; blood plasma, 65.9%; rumen, 92.1%; and corpus, 74.0%. Spontaneous hydrolysis of aspirin equaled 1.1% in the gastric juice and blood, 6% in the rumen tissue, and 8.4% in the corpus tissue. The amount of salicylic acid found (corrected for spontaneous hydrolysis of aspirin) was as follows: gastric juice, 6.9%; blood plasma, 18.8%; rumen, 6.6%; and corpus, 25.4%. The amount of salicylic acid found in the corpus tissue was fourfold higher than the amount found in the rumen tissue (25.4 *versus* 6.6%). Analysis of variance showed no significant difference between: (*a*) gastric juice and rumen tissue, (*b*) blood plasma and corpus tissue, and (*c*) animals, and it showed a significant difference between: (*a*) aspirin, salicylic acid, and salicyluric levels in all tissues, and (*b*) rumen and corpus tissue with respect to salicylic acid and aspirin levels ($p = 0.05$).

Since lesions occur only in corpus tissue under the conditions of this study, it is tempting to speculate that the higher levels of salicylic acid in that tissue may be related to lesion production. Historically, one undesirable side effect of sodium salicylate was gastric irritation, which was partly overcome by substitution on the phenolic hydroxyl group (11). It is possible to speculate that higher levels of esterase activity in corpus cells may account for the increased amounts of free salicylic acid in that tissue. The significance of this finding will be the object of future research.

[4] Tri-Carb liquid scintillation counter, model 3003, Packard Instrument Co., Downers Grove, Ill.

[5] Cellosol.

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