In conclusion, the serum parameters for enalaprilat and urine parameters for enalaprilat and total drug (enalapril-enalaprilat) were similar following administration of a single enalapril maleate 40-mg tablet to healthy volunteers under fasting and nonfasting conditions. Thus, food did not appreciably alter the absorption of enalapril or the bioavailability of enalaprilat in this study.

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Metabolism of Salsalate in Normal Subjects

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Abstract □ The metabolism of salsalate (I) was characterized in two normal volunteers. The drug was almost completely absorbed and was excreted primarily in the urine; only ~1% of the total dose was found in the stools. Although I is a salicylate derivative, which on hydrolysis yields two molecules of salicylic acid (II), ~7-10% of the dose was not hydrolyzed to salicylic acid and appeared in the urine either as unchanged drug or glucuronide conjugates. Thus, the incomplete availability of salicylate from salsalate that has been previously reported may not be due to incomplete absorption of the drug but to incomplete hydrolysis to salicylic acid.

Keyphrases □ Salsalate—metabolism, normal subjects □ Absorption—salsalate, normal subjects

Salsalate (I) is a salicylate derivative which on hydrolysis yields two molecules of salicylic acid (II). The results of several studies have indicated that the availability of salicylate from salsalate is lower than from either choline magnesium trisalicylate (1) or aspirin (2, 3). It has been suggested that the lower availability of salicylate from salsalate may be due to incomplete absorption (1). However, the recovery of total salicylate in the urine samples of patients receiving equivalent doses of salsalate and aspirin indicated that the absorption of the two salicylate formulations was essentially complete. The hydrolysis of salsalate to salicylate was incomplete, with <1% of the dose being excreted as unchanged salsalate and ≤13% of the dose being excreted in the urine as conjugates of salsalate (3). The only limitation of this latter study was the fact that the biological samples had been stored for several months prior to analysis, during which time salsalate (both nonconjugated and conjugated) may have partially hydrolyzed to salicylic acid.

The purpose of this study was to characterize the metabolism of salsalate in two normal volunteers following the administration of 2000 mg of salsalate containing ^{14}C-labeled salsalate.

EXPERIMENTAL SECTION

Two normal healthy male volunteers (age 29 and 35) entered into the 4-d study after initial screening procedures. The volunteers fasted for 12 h prior to and 4 h after drug administration. They ingested 2000 mg (four 500-mg capsules) of salsalate containing ^{14}C-labeled salsalate[1] (64 μCi) with water. Compound I was radiolabeled at both carboxyl positions.

Blood samples were taken just before drug ingestion, at hourly intervals up to 12 h, and then at 16, 20, 24, 30, 36, 48, 60, and 72 h following drug administration. Immediately after the blood samples were drawn into heparinized tubes, they were centrifuged, and the plasma was frozen until analysis. Urine samples were collected during the following intervals: 0-1, 1-2, 2-4, 4-6, 6-8, 8-12, 12-16, and 16-24 h. For the next 3 d, urinary output was collected as consecutive 12-h aliquots. The volume of urine and pH were recorded for each period, and aliquots were frozen in plastic containers until analysis. All stools were collected, weighed, and frozen until analysis. Due to the possibility of hydrolysis of salsalate and its conjugates to salicylic acid, all assays were completed within 2 weeks of collection.

Aliquots of plasma (0.1 mL with 0.9 mL of water) or urine (1.0 mL) were transferred into scintillation vials containing scintillation fluid[2] and counted in a scintillation counter[3]. Standards containing 500 μg of the radiolabeled salsalate[1] in propanol were also counted. All counts were corrected for quenching.

The plasma and urine samples were assayed for unchanged salsalate and salicylic acid by a previously reported HPLC technique (4). Salsalate[1], salicylic acid[4], and α-phenylcinnamic acid[4], the internal standard, were extracted from acidified plasma and urine samples. Methylene chloride[5] was used to extract plasma, whereas urine was extracted into hexane[6]. The organic phases were evaporated to dryness, redissolved in methanol (0.5 mL)[7], and analyzed by HPLC with an automatic sample injector accessory[8]. The mobile phase of methanol-1% acetic acid (60:40, v/v) was pumped at a rate of 2.0 mL/min through a 4.6 × 150-mm column[9]. Peaks were detected with a UV detector[10] (300 nm) coupled to a recorder[11] and peak integrator[12]. Plasma samples were assayed for total salicylate by HPLC after heating with HCl (18 M) overnight at 100°C. Extraction and chromatographic conditions were the same as described above for unchanged I.

Total urinary salicylate was determined by a modification of the colorimetric method described by Chiou and Onyemelukwe (5). Aliquots of urine

[1] Riker Laboratories, 3M Center, St. Paul, Minn.
[2] Ready-Sol EP; Beckman Instruments, Inc., Fullerton, Calif.
[3] Isocap 300; Scarle Analytical Inc., Des Plaines, Ill.
[4] Aldrich Chemical Co., Milwaukee, Wis.
[5] Spectroquality; Matheson, Coleman and Bell, Norwood, Ohio.
[6] Spectroquality; Mallinckrodt, St. Louis, Mo.
[7] Burdick & Jackson Laboratories, Muskegon, Mich.
[8] Model 1500; Altex Scientific, Berkeley, Calif.
[9] Ultrasphere ODS; Altex Scientific.
[10] Model 100-10 spectrophotometer; Hitachi Scientific Instruments, Mountain-View, Calif.
[11] Model 250-2, two channel; Curken Scientific, Danbury, Conn.
[12] Model 485; Varian Instrument Division, Palo Alto, Calif.

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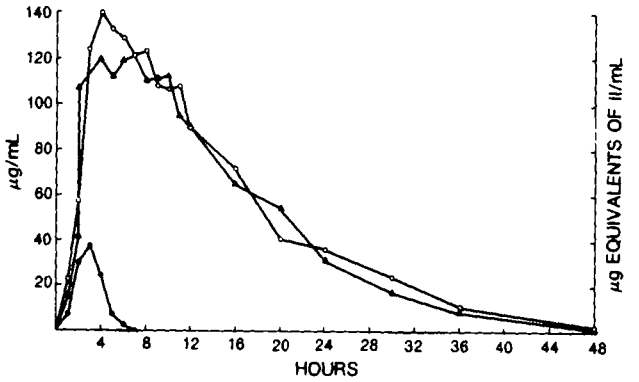

Figure 1—*Plasma levels of free salicylic acid (▲), salsalate (●), (in micrograms per milliliter), and radioactivity (○) (in microgram equivalents of salicylic acid per milliliter) in subject 1 following oral administration of 2000 mg of salsalate.*

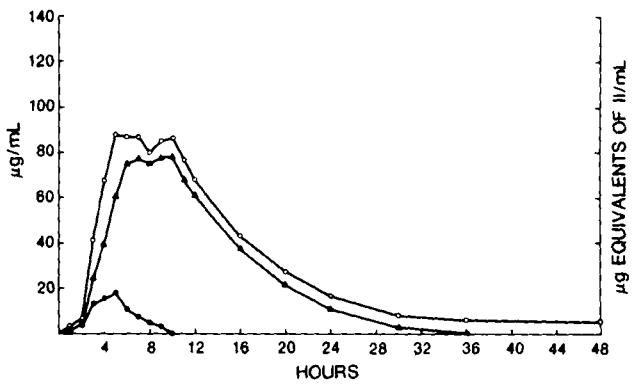

Figure 2—*Plasma levels of free salicylic acid (▲), salsalate (●), (in micrograms per milliliter), and radioactivity (○) (in microgram equivalents of salicylic acid per milliliter) in subject 2 following oral administration of 2000 mg of salsalate.*

were incubated overnight with concentrated HCl at 100°C. The tubes were then extracted with chloroform[13], an aliquot of the organic phase was shaken with the modified Trinder reagent (5), and the absorbance of the aqueous phase was measured at 540 nm. A standard curve was constructed by using known concentrations of sodium salicylate[4] from 0 to 100 mg %.

Plasma and urine samples were assayed for conjugated salsalate and salicylic acid by HPLC following incubation of the samples with β-glucuronidase[14] (5000 U/mL) in acetate buffer (pH 4.5) for 3 h at 37°C. Results of preliminary experiments indicated that partial hydrolysis of salsalate occurred during the incubation period; to correct for this hydrolysis, standards of salsalate, ranging from 20 to 300 μg/mL, were incubated and analyzed with each batch of samples. After incubation, the urine and plasma samples were analyzed for salsalate and salicylic acid by the HPLC method described above for unchanged I.

The urine samples were also assayed for nonconjugated gentisic, salicyluric, and salicylic acid levels by HPLC. Aliquots (1 mL) of standards and samples were transferred into screw-cap tubes, *o*-methoxybenzoic acid[4] (200 μg) was added as an internal standard in addition to 1.0 mL of HCl (0.6 M). The samples were extracted with ether[15] (6 mL). After centrifugation, the aqueous layer was frozen in dry ice–propanol. The ethereal phase was transferred to a tube and the solvent was removed under a stream of nitrogen at 10°C. The residue was then resuspended in 0.5 mL of mobile phase and injected into the HPLC *via* the automatic sampler. The mobile phase of methanol–1% acetic acid (15:85, v/v) was pumped at the rate of 2.0 mL/min through the reverse-phase column[9]. UV absorbance was measured at 300 nm[10]. The retention times for gentisic acid, salicyluric acid, anisic acid, and salicylic acid were 280, 600, 900, and 1190 s, respectively. A small peak was eluted between gentisic acid and salicyluric acid in the samples obtained at early times. This probably corresponds to the glucuronide(s) of salicylic acid and has been described by others (6). Under these chromatographic conditions, unchanged salsalate was not eluted.

Preliminary radioactive counting of aliquots of urine samples after HPLC indicated that not all the radioactivity could be accounted for following chromatography. This problem was resolved by using gradient elution chromatography.

Standards and urine samples (1.0 mL) were transferred into screw-cap tubes and extracted with ether[15] after acidification with 0.6 M HCl as described above. The ethereal layer was taken to dryness with nitrogen at 10°C, and the residue was resuspended in the initial mobile phase. The initial mobile phase was methanol–1% acetic acid (15:85, v/v). A solvent programmer[16] was used to change the relative pumping rates of two pumps to enable the initial phase to change linearly from its initial condition to a final composition of methanol–1% acetic acid (60:40, v/v). The gradient time was 30 min. This was followed by a 15-min elution while maintaining the final gradient conditions, after which the initial conditions were reestablished. UV absorbance was measured at 300 nm[10]. Under these conditions, the retention times were 280 s (gentisic acid), 500 s (salicyluric acid), 650 s (anisic acid), 780 s (salicylic acid), and 1800 s (salsalate). Two additional peaks were eluted at 1380 and 1590 s.

Each stool specimen was homogenized with a known amount of water. Weighed aliquots (0.5–1.0 g) were treated with the decolorizing agents perchloric acid[17] (0.4 mL) and hydrogen peroxide[18] (0.8 mL) and incubated for 24 h at 37°C, and four drops of 15% ascorbic acid[19] and 10 mL of scintillation fluid[2] were added. The treated specimens were then counted in a scintillation counter, corrected for quenching, and converted into micrograms of ^{14}C-labeled salsalate.

RESULTS AND DISCUSSION

Changes in total radioactivity of unconjugated salsalate and salicylic acid in plasma *versus* time for each subject is shown in Figs. 1 and 2. For subject 1, a peak plasma level of II (120 μg/mL) was reached after 4 h (Fig. 1), whereas with the results for subject 2, a lower peak level of 77.6 μg/mL 7 h after drug administration was attained (Fig. 3). Peak levels of I were seen in subject 1 at 3 h (37.9 μg/mL) and in subject 2 at 5 h (18.3 μg/mL).

The areas under the plasma level *versus* time curves for total radioactivity of salsalate and salicylic acid indicated that 86.6% of the area under the curve of total radioactivity was accounted for by the area under the curves of nonconjugated salsalate and salicylic acid in subject 1 and 67.3% in subject 2.

Acid hydrolysis of the plasma samples by incubation overnight at 100°C made the plasma concentrations of II congruent with the amount of radioactivity in the samples (expressed as microgram equivalents of salicylic acid per milliliter), indicating the presence of other metabolites of salicylic acid and/or salsalate in the plasma of these normal volunteers. The ratio of AUC radioactivity-$AUC_{total\ II}$ was 1.01 in subject 1 and 1.02 in subject 2.

The urinary excretion of radioactivity demonstrated that 45.8 and 56.6% of the dose was excreted after 12 h in subjects 1 and 2, respectively, whereas the amount of radioactivity in urine (expressed in microgram equivalents of salsalate) accounted for 95.5 and 101.8% of the drug dose after 84 h. Only ~1% of the total radioactivity was found in the stools of these subjects. The

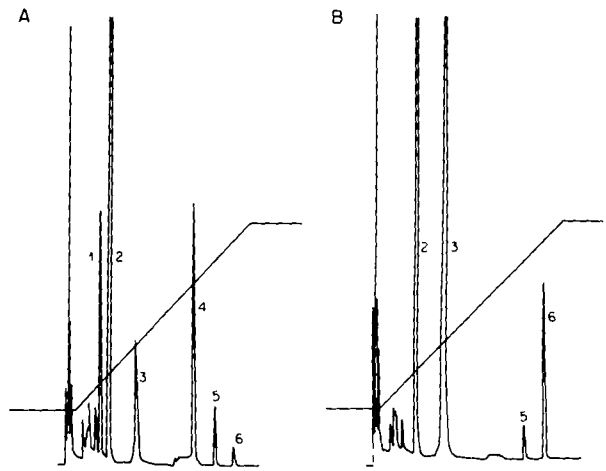

Figure 3—*A typical HPLC tracing of a urine sample before (A) and after (B) β-glucuronidase treatment (see text for explanation).*

[13] Analytical reagent; Mallinckrodt.
[14] Sigma Chemical Co., St. Louis, Mo.
[15] Nanograde; Mallinckrodt.
[16] Solvent Programmer; Altex Scientific.
[17] 70%; Eastman Kodak Co., Rochester, N.Y.
[18] 30%; Mallinckrodt.
[19] Baker Chemical Co., Phillipsburg, N.J.

Table I—Urinary Excretion of Salsalate and Its Metabolites

Compound	Urinary Excretion	
	Subject 1	Subject 2
Total radioactivity, mg equivalents of salsalate	1909.0 (95.4)[a]	2036.0 (101.8)[a]
Free salicylic acid, mg	90.5 (4.2)	50.7 (2.4)
Conjugated salicylic acid, mg	328.6 (15.4)	264.3 (12.4)
Free salsalate, mg	3.4 (0.2)	6.2 (0.3)
Conjugated salsalate, mg	136.0 (6.8)	200.0 (10.0)
Gentisic acid, mg	75.4 (3.2)	58.7 (2.5)
Salicyluric acid, mg	1514.7 (50.3)	1797.2 (59.6)
Total salicylate, mg	1961.4 (91.7)	2014.0 (96.2)

[a] Numbers in parentheses indicate the percentage of administered dose recovered in the urine.

urinary excretion of nonconjugated salsalate and salicylic acid during the study period is shown in Table I; nonconjugated salicylic acid accounted for 2–4% of the total dose, whereas <1% of the dose was excreted as nonconjugated salsalate. These results agree with those reported by Harrison *et al.* (2). Conjugates of both salsalate and salicylic acid were present in significant amounts in both volunteers (Table I). Approximately 12–15% of the dose was excreted as conjugates of salicylic acid, whereas the conjugates of salsalate accounted for 6.8 and 10% of the dose in subjects 1 and 2, respectively. The majority of the conjugates of salsalate were excreted within 12 h after the administration of the drug. A typical HPLC tracing of a urine sample before and after β-glucuronidase treatment is shown in Fig. 3. This chromatogram shows the disappearance of peak 4 and the increase in salsalate (peak 6) after incubation with glucuronidase. Peak 1 also disappeared on treatment with glucuronidase, whereas the salicylic acid peak (peak 3) increased, indicating that peak 1 was one or both of the glucuronides of salicylic acid.

The urinary excretion of nonconjugated gentisic acid, salicyluric acid, and salicylic acid is tabulated in Table I. The amount of nonconjugated gentisic acid excreted in the urine of these subjects was 2.5% (subject 1) and 3.2% (subject 2) of the total dose, whereas excretion as nonconjugated salicyluric acid accounted for 50.0% of the dose in subject 1 and 59.6% in subject 2. The total salicylate recovered in the urine (as percentage of the dose) was 91.7% in subject 1 and 96.2% in subject 2. These values do not include the gentisate excreted since this metabolite is not hydrolyzed to salicylic acid. Thus, urinary salicylate plus gentisic acid levels accounted for 94.9% (subject 1) and 98.7% (subject 2) of the administered dose of salsalate (Table I).

This study of the metabolism of salsalate in two normal volunteers clearly demonstrates the essentially complete absorption of the drug (as indicated by the almost complete recoveries of doses in the urine) and the incomplete hydrolysis of salsalate to salicylic acid (as indicated by the presence of glucuronide conjugates of salsalate in the urine). Formation of the glucuronide conjugates of salsalate may occur either during its passage through the GI tract (7) or in the liver. Regardless of the site of glucuronidation, however, in this study 7–10% of the dose was excreted as glucuronide conjugates of salsalate. Attempts to identify the structures of the glucuronide conjugates by mass spectrometry were unsuccessful. However, mass spectrometric analysis[20] of peak 4 (Fig. 3) following treatment with β-glucuronidase showed that the aglycone was salsalate.

The presence of the glucuronide conjugates, however, suggests that the lower plasma levels of salicylic acid achieved with salsalate, as compared with aspirin or choline magnesium salicylate in previous studies (1–3), are due to a decreased amount of salicylic acid in the body caused by the rapid conjugation of some salsalate (before its hydrolysis to salicylic acid) and its subsequent excretion into the urine.

Although the percentages of the dose excreted as conjugates of salsalate in the two volunteers in this study do not fully explain the 15–23% lower than expected plasma levels of salicylic acid observed in patients (3) and normal volunteers (1, 2) after the administration of salsalate, it should be pointed out that a 10% decrease in the dose of available salicylate may be accompanied by a disproportionate decrease in serum salicylate levels. Such disproportionality follows from the observation that a 50% increase in the dosage of aspirin can almost triple the serum salicylate levels (8).

It should be noted that the decreased availability is only with respect to salicylate, however, since salsalate is essentially completely absorbed and the rapid glucuronidation and excretion prevents complete hydrolysis of salsalate to salicylic acid *in vivo*, thus decreasing the amount of salicylic acid that reaches the systemic circulation.

The results of the present study in normal volunteers supports the results of our multiple dose study in patients with rheumatoid arthritis (3) and indicates that the reduced availability of salicylic acid from salsalate is due to the incomplete hydrolysis of the prodrug.

The clinical implication of these studies is that the dose of salsalate needed to achieve equal plasma salicylate levels will be greater than that predicted from an equivalent dose of aspirin. Preliminary data from multiple dose studies in normal volunteers (2, 9) suggest that the availability of salicylate from one 500-mg tablet of salsalate[21] is approximately equivalent to that available from two 325-mg tablets of aspirin. These doses contain 535 and 500 mg equivalents of salicylic acid, respectively. However, for optimal salicylate therapy of inflammatory arthritis, plasma salicylate concentrations should be determined periodically and used as the basis for dosage adjustment.

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[20] Carried out at the 3M Central Research Laboratories; Model 21-491B GC–MS equipped with a solid sample probe; DuPont de Nemours and Co., Inc., Wilmington, Del.

[21] Disalcid; Riker Laboratories, Northridge, Calif.