Some Aspects of Salicylate Distribution and Metabolism in Man

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This study is concerned with the pharmacokinetics of salicylate elimination in man, with special reference to the possible effect of absorption rate and the effect of normal variations in urinary pH. Data are presented concerning the quantitative composition of salicylate metabolites in the urine and the rate constants for the major salicylate elimination processes. Apparent volumes of distribution are reported, and their possible relation to individual serum protein profiles and salicylate elimination kinetics are discussed.

A RECENT investigation of aspirin absorption from compressed tablets and from an experimental prolonged-release dosage form (1), which involved relatively intensive sampling of blood and terminal collections of urine, has afforded us the opportunity to study the kinetics of salicylate elimination in some detail. Although salicylate has been used extensively in human therapy for a long time, the pharmacokinetics of its elimination in man have been elucidated only recently (2, 3). In addition to extending previous studies by Levy (2, 3) to a larger sample of subjects, the purpose of the present investigation was to determine the effect, if any, of differences in absorption pattern on the metabolism of salicylate in man. Such differences may occur for three reasons: (*a*) a drug may be metabolized (conjugated) to some degree in the gastrointestinal wall (4) and slow absorption may permit relatively more extensive conjugation since the conjugating capacity of enzyme systems in the gastrointestinal wall is limited, (*b*) conjugating capacity may be greater in the intestine than in the stomach so that site of absorption may affect the extent of conjugation, and (*c*) the rate of formation of salicyluric acid (the major metabolite of salicylate in humans) reaches a maximum when body salicylate levels exceed about 2 mmoles (equivalent to about 0.3 Gm. salicylic acid) in the adult human (2). For these reasons, slower gastrointestinal absorption of aspirin may cause more extensive glucuronide ester formation during passage of drug across the gastrointestinal membranes,[1] more extensive salicylurate formation (since slower absorption will reduce the magnitude of peak salicylate levels), and more rapid elimination of salicylate.[2]

The possible effects of absorption rate on drug metabolism are well illustrated by the results of a recent study of the acetylation of *p*-aminobenzoic acid in man (5). A dose of 110 μmoles/Kg. administered orally on an empty stomach was 51% acetylated; when given together with fat or glucose (which decrease absorption rate by retarding gastric emptying) the extent of acetylation increased to 80–90%. When the drug was administered in divided doses given at 0.5-hr. intervals, acetylation was practically complete. Although the authors did not suggest a kinetic basis for their findings, the observed effects are probably due to the limited acetylating capacity (with respect to PAB and/or its conjugates) of the human body (2).

Pertinent studies concerning biotransformation of drugs during gastrointestinal absorption have been reviewed and discussed by Levy (6). In addition to these, it is appropriate to cite a recent paper by Naito (7), who found more extensive acetylation of sulfamethylthiadiazole (SMTD) when the latter was administered in the form of a slowly absorbed polyvinylpyrrolidone granulation (as compared with the more rapidly absorbed SMTD powder). While results of the latter study require verification, they do suggest a possible effect of absorption rate on the metabolism of SMTD.

In the present report, data are presented concerning (*a*) the quantitative composition of salicylate metabolites excreted in the urine after oral administration of aspirin in rapidly absorbed tablets and in a prolonged-release dosage form, (*b*) the effect of normal variations in urine pH on salicylate metabolism, (*c*) first-order rate constants for the major salicylate elimination processes, and (*d*) apparent volumes of distribution of salicylate, with special reference to serum protein

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[1] Glucuronide conjugation of salicylic acid in human gastrointestinal mucosa (mainly the small intestine) has been demonstrated by Schachter *et al.* (17).

[2] A detailed discussion of the relationship between salicylate levels and elimination rate is included in *Reference 2*.

TABLE I.—METABOLIC FATE OF 1 GM. ASPIRIN ADMINISTERED ORALLY IN RAPIDLY AND SLOWLY ABSORBED FORM

Subject	Age, Yr.	% of Total Salicylates Excreted as Salicylic Acid C.T.[a]	Salicylic Acid P.R.[b]	Salicyluric Acid C.T.	Salicyluric Acid P.R.	Salicyl Glucuronides C.T.	Salicyl Glucuronides P.R.
A	43	10.9	10.6	73.1	73.3	16.0	16.1
B	39	3.3	2.2	78.4	75.4	18.3	22.4
C	55	2.7	1.5	64.4	68.0	32.9	30.5
D	47	25.7	23.9	61.8	54.9	12.5	21.2
E	42	11.5	11.7	71.9	70.4	16.6	17.9
F	36	10.5	10.8	61.3	62.5	28.2	26.7
G	39	7.2	7.5	76.7	74.4	16.1	18.1
Mean	..	10.3	9.7	69.6	68.4	20.1	21.8

[a] Aspirin administered in compressed tablets. [b] Aspirin administered in prolonged-release dosage form.

profiles. Finally, there is a general discussion of the possible effects of variations in release properties of a dosage form on the elimination of salicylate.

EXPERIMENTAL

Details of the clinical study have been described in the preceding paper (1). Quantitative determinations of salicylic acid, salicyluric acid, salicylic glucuronides, and total salicylates in the urine were carried out by the method of Smith *et al.* (8) as modified by Levy (9). Urine pH was determined immediately after voiding. Elimination rate constants were determined from the slope of the "true" linear portion of semilogarithmic plots of serum salicylate concentrations as a function of time; rate constants for salicylate excretion, salicylurate formation, and salicyl glucuronide conjugation were calculated as described previously (2) (on the basis of the composition of the last 200–300 mg. of salicylic acid and metabolites excreted and the fraction of the dose recovered in the urine); apparent volumes of distribution were calculated from A_∞/V values (10). Serum protein profiles were determined by electrophoresis with the Beckman model R 101 Microzone Electrophoresis Cell (11).

RESULTS AND DISCUSSION

The metabolic fate of 1 Gm. of aspirin administered orally in compressed tablets and in prolonged-release dosage form, respectively, is shown in Table I for seven subjects. It has been shown in the preceding paper that drug absorption was as complete from the prolonged-release form as from the more rapidly absorbed compressed tablets (1). There was no significant difference in the metabolic fate of aspirin given in the two forms. The excellent reproducibility of these data would be most gratifying ordinarily, but was not expected at the time when this study was initiated because use of the prolonged-release dosage form was expected to result in considerably slower absorption. However, the major effect of the prolonged-release dosage form was to essentially delay absorption for an average of 1.23 hr. (1); the subsequent absorption occurred as rapidly as from compressed tablets. Stated in another way, drug release from the compressed tablets was rapid and apparently occurred mainly in the stomach, while drug release from the prolonged-release form was practically negligible in the stomach but rapid in the small intestine. Although the

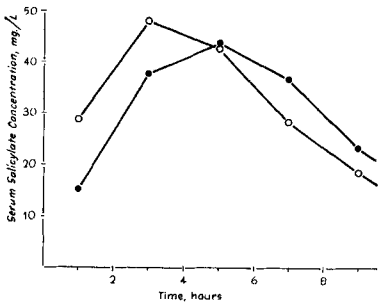

Fig. 1.—Average serum salicylate concentrations as a function of time after oral administration of 1 Gm. of aspirin in compressed tablets (○) and in prolonged-release dosage form (●) to seven subjects.

release rate from the prolonged-release form was lower than from compressed tablets *under equal in vitro conditions*, the respective release rates were comparable when drug in compressed tablets dissolved in the stomach (low pH, low agitation intensity) and drug in prolonged-release form dissolved in the small intestine (higher pH, greater agitation intensity). This is illustrated also by the serum salicylate concentration *versus* time curves shown in Fig. 1; average peak levels differed only slightly and the curve for the prolonged-release dosage form is essentially similar to the compressed tablets curve except for being displaced with respect to time.[3] Under these conditions (and if site of absorption has no effect on drug metabolism) there should be no difference in metabolic fate of aspirin administered in the two dosage forms, and the excellent reproducibility of the data is therefore very gratifying.

Two of the subjects, *B* and *C*, excreted an unusually small fraction of total salicylates as free salicylate, while one subject (*D*) excreted an unusually large fraction as free salicylate. This was due to a consistently low urine pH in the case of subjects *B* and *C* and a consistently high urine pH in the case of subject *D* (Table II). All other subjects excreted urine with pH values around 6. Since the renal clearance of free salicylate is extremely sensitive to urine pH [it increases more than tenfold when urine pH changes from below 6.0 to above 7.0 (12)], this effect is to be expected. It is of interest, however, that there were subjects with

[3] The authors have cautioned in previous reports (1, 10) against basing interpretations only on averaged data. However, Fig. 1 is representative of individual data, in this particular respect, as established by examination of the individual data.

TABLE II.—EFFECT OF URINE pH ON SALICYLATE METABOLISM

Subject	Dosage Form[a]	pH of Urines Collected at Different Times (hr.) after Drug Administration 2	4	6	8	10	12	16	24	28	% of Total Salicylates Excreted as Salicylic Acid
C	P.R.	...	5.1	...	5.2	...	5.2	5.3	5.1	5.3	1.5
	C.T.	5.1	5.0	4.8	5.3	5.2	5.0	5.1	4.8	5.1	2.7
B	P.R.	...	5.6	...	5.6	...	5.3	5.4	5.8	5.6	2.2
	C.T.	5.6	5.6	5.8	6.2	5.6	5.6	5.2	5.7	5.6	3.3
D	P.R.	...	6.4	...	6.7	...	7.0	6.5	6.4	6.3	23.9
	C.T.	7.7	6.4	6.4	6.7	7.0	7.0	6.1	5.3	6.1	25.7

[a] P.R., prolonged-release dosage form; C.T., compressed tablets.

TABLE III.—RATE CONSTANTS[a] FOR SALICYLATE ELIMINATION

Subjects	K	K_{SA}	K_{SG}	K_{SU}
A	0.212	0.010	0.040	0.135
B	0.355	0.007[c]	0.074	0.224
C	0.256	0.004[c]	0.080	0.150
D	0.239	0.042[d]	0.037	0.124
E	0.338	0.010	0.076	0.214
F	0.198	0.010	0.052	0.112
G	0.291	0.009	0.057	0.184
Mean[b]	0.258	0.008	0.055	0.153

[a] In reciprocal hours. [b] Harmonic mean. [c] Subject with low urinary pH. [d] Subject with high urinary pH.

consistently high or low urine pH. This was apparently not due to differences in diet, since all of the subjects were on the same standard diet.

The average maximum salicylurate formation rate in the seven subjects was 68 mg./hr., well within the range of 58 to 78 mg./hr. reported previously (2). Rate constants for salicylate elimination (K), renal excretion of salicylate (K_{SA}), formation of salicylic glucuronides (K_{SG}), and formation of salicylurate (K_{SU}) were determined from blood level and urinary excretion data obtained during the terminal (exponential) phase of elimination (*i.e.*, when only about 300 mg. aspirin equivalent remained to be eliminated). These constants are listed in Table III and are based on the average of two experiments for each test subject.[4] The values obtained are in good agreement with similar values reported in a previous communication (2). The effect of urine pH on K_{SA} is readily recognizable; the magnitude of the effect approximates that which could be predicted theoretically on the basis of pH-partition considerations. Rate constants for over-all elimination of salicylate (K) ranged from 0.198 to 0.355 reciprocal hr., equivalent to half-lives of 3.5 and 2.0 hr., respectively. Since K is practically the sum of K_{SA}, K_{SG}, and K_{SU}, it can be noted that pH does *not* have an appreciable effect on salicylate elimination when doses are sufficiently small so that first-order kinetics apply. This is so because K_{SA} is only a small fraction of K. On the other hand, elimination rate is very sensitive to pH when doses are high [since, in the latter case, K approximates $K_{SA} + K_{SU}$, because salicylurate formation contributes only slightly to salicylate elimination (2), and K_{SA} will then represent a considerable fraction of the apparent K]. In the previous study (2), K_{SA} and K_{SG} were combined[5] and reported as K_{SA+SG}, since it was considered possible that partial hydrolysis of salicylic glucuronides *in vivo* might yield erroneous values. Examination of the data obtained in the present study, particularly in relation to pH, showed that such precautions were apparently unnecessary.

Apparent volumes of distribution for salicylate were calculated[5] for each subject from serum salicylate concentration data obtained in three separate experiments (Table IV). The values are reasonably reproducible, although this is difficult to judge since the authors are not aware of similar studies involving multiple determinations of individual apparent volumes of distribution and thus have no standard for comparison. The average data agree well with those found previously in another study by Levy and Hollister (2). There was one consistently unusually low value—namely, the apparent volume of distribution in subject *B*. To establish, if possible, the reason for this low value, plasma protein profiles were determined for all subjects (Table V). Subject *B* had an appreciably higher albumin level than the other test subjects. Since a major fraction of salicylate in plasma is protein bound and since this complexation involves only albumin (13), it can be assumed that the low apparent volume of salicylate distribution in subject *B* is due to the high level of plasma albumin.[6] A plot of apparent volume of distribution *versus* albumin level for all subjects (Fig. 2) also suggests an inverse relationship, although interpretation is difficult in view of the limited range of plasma albumin levels in the other subjects. Values for the several salicylate elimination rate constants obtained with subject *B* were examined closely, but these were not unusual as compared to values obtained with subjects having average serum protein profiles. (Note, for example, the values for subjects *E* and *G*.) However, data published recently by Wiegand and Sanders (14) suggest an inverse relationship between salicylate elimination rate constant and apparent volume of distribution (Fig. 3).

Results of the present study, when evaluated in conjunction with results of a previously reported study on the pharmacokinetics of salicylate elimination in man (2), yield some important information

[4] The small difference between K and the sum of K_{SA}, K_{SG}, and K_{SU} probably represents several minor pathways of elimination.

[5] These volumes were calculated from respective A_∞/V values (16), body weights, and doses administered (1 Gm.). Such calculations were possible because the drug was found to be fully absorbed (1).

[6] To establish this relationship definitely, it is necessary to determine the total quantity of albumin in the body.

TABLE IV.—A_∞/V VALUES, BODY WEIGHTS, AND RELATIVE APPARENT VOLUMES OF DISTRIBUTION OF SALICYLATE IN SEVEN ADULT MALE SUBJECTS

Subject	A_∞/V Values, mg./L. First Expt.[a]	Second Expt.[b]	Third Expt.[a]	Body Wt., Kg.	Relative Vol. of Distribution,[c] ml./Kg. Mean[d]	Best Value[e]
A	75	74	(84)	83	154	161
B	129	123	(103)	83	102	96
C	(68)	57	59	103	159	168
D	99	99	(97)	68	147	148
E	77	(83)	78	73	172	177
F	80	(87)	83	79	152	156
G	(116)	84	93	64	159	176
Mean	92	87	85	79	149	155
					157[f]	164[f]

[a] One gram of aspirin administered in prolonged-release dosage form. [b] One gram of aspirin administered in compressed tablets. [c] Based on A_∞/V values obtained from serum salicylate concentrations. [d] Based on average A_∞/V values from three experiments. [e] Based on best two of three A_∞/V values; excluded A_∞/value in parentheses. [f] This mean value does not include the volume of distribution of subject *B*.

TABLE V.—SERUM PROTEIN PROFILES OF TEST SUBJECTS

Subject	Albumin	Relative Concn. of Serum Proteins: Globulins α_1	α_2	β	γ
A	52.1	6.4	13.3	9.6	18.6
B	69.7	3.5	6.6	8.6	11.5
C	55.9	4.0	7.4	13.9	18.8
D	64.8	3.2	7.6	9.8	14.6
E	54.8	4.8	10.1	12.0	18.3
F	56.3	5.3	10.0	13.2	15.3
G	59.8	4.4	9.3	9.3	17.2

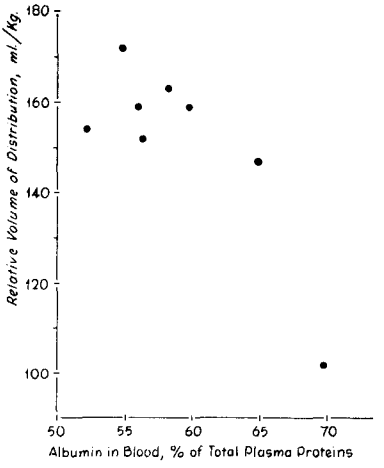

Fig. 2.—Relationship between relative apparent volume of distribution of salicylate and plasma albumin level in eight subjects. (The fifth data point from the left represents values obtained from a subject who received aspirin in compressed tablets only.)

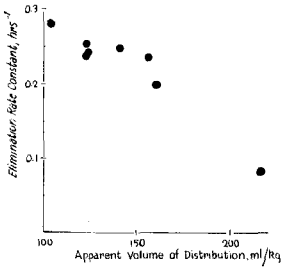

Fig. 3.—Apparent inverse relationship between salicylate elimination rate constant and apparent volume of distribution. Based on data from *Reference 14*.

concerning the possible effect of dosage form on the time course of salicylate levels in the body. The relative elimination rate[7] of salicylate decreases with increasing dose (2). Thus, high doses administered in rapidly absorbed form have their own "sustaining" effect relative to the elimination rate of lower doses. This makes the utility of prolonged-release preparations of salicylates somewhat questionable. In this connection, it is of interest that a recent study of the analgesic effect of aspirin administered in prolonged-release dosage form and in conventional tablets, respectively, showed that there was no demonstrable difference in duration of pain relief elicited by these two forms of the drug (15). Administration of salicylates in dosage forms with delayed (rather than prolonged) absorption characteristics does not affect salicylate metabolism or relative elimination rate, as long as absorption is only delayed with respect to time and is as rapid as from conventional tablets when it occurs.

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[7] Relative rate of elimination refers to per cent of dose eliminated per unit of time, comparing rates at similar times after drug administration.